Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (4): 335-342, 2023
DOI: 10.9775/kvfd.2023.29167

Research Article

The Efficacy of Thyme, Peppermint, Eucalyptus Essential Oils, and Nanoparticle Ozone on Nosemosis in Honey Bees

Mehmet ÖZÜİÇLİ [1 (*)] Ahmet Onur GİRİŞGİN [2] Ali İhsan DİKER [1] Yasin BAYKALIR [3] İhsan KISADERE [4] Levent AYDIN [2]

[1] Balıkesir University, Faculty of Veterinary Medicine, Department of Parasitology, 10000, Balıkesir, TÜRKİYE
[2] Bursa Uludag University, Faculty of Veterinary Medicine, Department of Parasitology, 16059, Bursa, TÜRKİYE
[3] Balıkesir University, Faculty of Veterinary Medicine, Department of Biostatistics, 10000, Balıkesir, TÜRKİYE
[4] Balıkesir University, Faculty of Veterinary Medicine, Department of Physiology, 10000, Balıkesir, TÜRKİYE

ABSTRACT

Nosemosis is an intracellular fungus that seriously affects honeybees' health globally. This study aimed to evaluate and compare the efficacy of thyme, peppermint, eucalyptus essential oils, and nanoparticle ozone applied by spray on the frames against Nosemosis in adult honey bees under field conditions. Five treatment groups and two (negative and positive) control groups were assigned for this study. In treatment groups; the 3% solutions of thyme, peppermint, and eucalyptus, and the 1.000 and 2.000 ppm solutions of nanoparticle ozone, were applied four times each week, 125 mL per hive (500 mL solution in total). Average *Nosema* spore counts before and after the treatment were calculated on a Neubauer hemocytometer slide by the digestion method, and the percent reduction test determined the efficacy of the combinations. According to the results, the highest reduction in the number of *Nosema* spores was observed in the thyme essential oil group at 84%. Peppermint essential oil, eucalyptus essential oil, and 1.000 and 2.000 ppm nanoparticle ozone efficacies were found at 77.45%, 76.10%, 72.41%, and 71.21%, respectively. Findings from this study revealed that essential oils and nanoparticle ozone can reduce the *Nosema* spore load to a point under field conditions. Plant extracts would offer a non-antibiotic alternative for *Nosema* control and further studies of herbal extracts are required as potential *Nosema* control agents in honey bees.

Keywords: Honey bee, Essential oil, Eucalyptus, Peppermint, Thyme, Nanoparticle ozone, Nosemosis

(*) **Corresponding author:** Mehmet ÖZÜİÇLİ
Phone: +90 266 613 6692
Cellular phone: +90 554 334 1870
E-mail: mehmet.ozuicli@balikesir.edu.tr

How to cite this article?
Özüiçli M, Girişgin AO, Diker Aİ, Baykalır Y, Kısadere İ, Aydın L: The efficacy of thyme, peppermint, eucalyptus essential oils, and nanoparticle ozone on nosemosis in honey bees. *Kafkas Univ Vet Fak Derg,* 29 (4): 335-342, 2023.
DOI: 10.9775/kvfd.2023.29167

Article ID: KVFD-2023-29167
Received: 10.02.2023
Accepted: 21.07.2023
Published Online: 24.07.2023

Introduction

Pollinator insects present essential contributions to agricultural production and the ecosystem. Among the insects, managed bees are known as the most important pollinators of crops worldwide. In addition, honey bees offer essential products such as honey, pollen, propolis, royal jelly, and bee venom [1-3]. In the USA, pollination provides an annual economic contribution of 16 billion dollars, and 12 billion dollars of this economy is obtained from honey bees [4]. Several factors cause the decline of honey bee colonies worldwide. Among these factors, parasitic mites, pathogens, poor nutrition, and pesticide exposure are in the foreground. [5,6]. Nosemosis in honey bees is caused by two species of microsporidian parasites, *Nosema apis* and *N. ceranae*. Although *N. ceranae* was originally discovered in the Asian honey bee *(Apis cerana),* it has outcompeted *N. apis* in the Western honey bee *(Apis mellifera)* in many regions [7]. Nosemosis is spread orally within the honey bee colony through contaminated food, pollen, and water. The disease is then spread by spores in the excrement of infected bees [8,9]. Nosemosis causes fatigue, a shorter lifespan, poor foraging, delayed immune response, and pheromone and hormone production problems. These findings lead to significant mortality, adult population loss, reduced honey production, and potential colony failure [10].

Different approaches are applied against Nosemosis infection [11]. Fumagillin, an antibiotic, has been used for over 50 years to treat Nosemosis [12]. Although this antibiotic is widely used in the USA, it is prohibited in Europe because the maximum residue limits have not been determined [13]. The use of antibiotics causes residue

problems in honey bee products and has negative effects such as destroying intestinal bacteria that reduce immune function and increases vulnerability to *Nosema* infection [14]. Due to these problems arising from synthetic drugs, studies on natural origin treatment methods are carried out recently. Natural products have advantages such as regional availability, low resistance, and minimal toxicity. Microorganisms, phytotherapeutics, essential oils, and organic acids are the main natural products used to treat Nosemosis [15]. Among these natural products, essential oils are the most used organic compounds against Nosemosis. They are mixtures of fragrant and odorless substances extracted by steam distillation from raw plant materials [16]. Essential oils are mainly composed of terpenoids (isoprenoids), aromatic compounds (aldehydes, alcohols, phenols, methoxy derivatives, and others), and terpenes (monoterpenes and sesquiterpenes) [17]. Due to these components, essential oils have repellent and insecticidal properties [18]. Ozone (O_3), a highly reactive molecule, is one of the most potent oxidizers after fluorine and persulfate [19]. Ozone is an allotropic form of oxygen with many applications in medical and industrial fields. In addition, it has immunomodulatory, analgesic and anti-inflammatory properties [20], and is used as a supplement in treating various degenerative, infectious and autoimmune disorders. Ozone remains stable in oils for many years. Studies have shown that the anti-bacterial effects of ozonated oils with their liquefied formulas with nanotechnological methods remain the same for two years [21].

Ozone has been tested with the fumigant method on *Galleria mellonella*, *Ascosphaera apis*, and *Paenibacillus larvae*. Ozone killed *G. mellonella* adults within five h, and the egg form was destroyed by fumigation of 460-920 mg O_3/m^3 for 48 h. *A. apis* and *P. larvae* were removed with 3.200 O_3/m^3 and 8.650 O_3/m^3 ozone fumigation, respectively [22]. It was reported that applying ozone against *Nosema* spores on the honeycomb reduced the number of *Nosema* spores by 20.25% [23].

In the present study, alternative approaches to control *Nosema* disease were investigated by testing the anti-microsporidian activity of essential oils (thyme, peppermint, and eucalyptus), and nanoparticle ozone on *Nosema* spp. under field conditions.

Material and Methods

Ethical Statement

Insect studies do not require ethics committee approval.

Study Area

This study was carried out in Balıkesir province in August, September, and October 2022. There were 100 hives in the apiary where the study was carried out. Balıkesir is located in the southern part of the Marmara Region of Türkiye (39°40'N-26°28'E). August, September, and October 2022 weather information is shown in *Table 1*.

Table 1. *August, September, and October 2022 temperature, humidity, and rain condition*

Weather Parameters	August 2022	September 2022	October 2022
Minimum-maximum temperature values	19°C-31°C	15°C-29°C	21°C-14°C
Average humidity	50%	56%	67%
Sunny day	31 days	29 days	16 days
Rain condition	No rain	One day (heavy rain)	Four days (heavy rain)

Field Experiment

One-hundred hives were included in the study. Samples were collected individually from each hive from the outermost frames. The samples were placed into sterile containers, and the numbers of the hives and other necessary information were written on the containers. The honey bee samples were collected between 6:00 and 7:00 p.m.

Honey Bee Information

The Anatolian honey bee breed *(Apis mellifera anatoliaca)* was studied. The queen bees in all hives were one year old. During the period of the study, honey bee colonies average between 45.000 and 50.000 individuals. Before the study, the honey bees' owner confirmed that no chemical or plant extract was used to treat any pathogens, in that season.

Detection of Nosemosis

To detect Nosemosis, 50 honey bees were collected from the outer frame of 100 hives. To ensure the immobilization of the honey bees, the samples were kept in a deep freezer in the laboratory for one day. Then, 10 immobile honey bees per hive were examined for the existence of *Nosema* spp. spore (positive or negative) by the digestion method. *Nosema* spore forms are shown in *Fig. 1*. In this method, first, the abdomens of 10 honey bees were separated from their body with the help of a scalpel. The abdomens were put into a mortar, where they were crushed. One ml of distilled water was added per abdomen in the mortar. The honey bees' abdomens were crushed with the help of a baguette for about five min. The solution was homogenized via a Pasteur pipette, and one drop was examined at ×400 magnification under a light microscope (Nikon Eclipse E100, Japan) to detect *Nosema* spp. spores.

Counting of *Nosema* spp. Spores

Nosema spp. spores were counted in positive honey bee samples from 81 hives. Adult honey bees of each colony

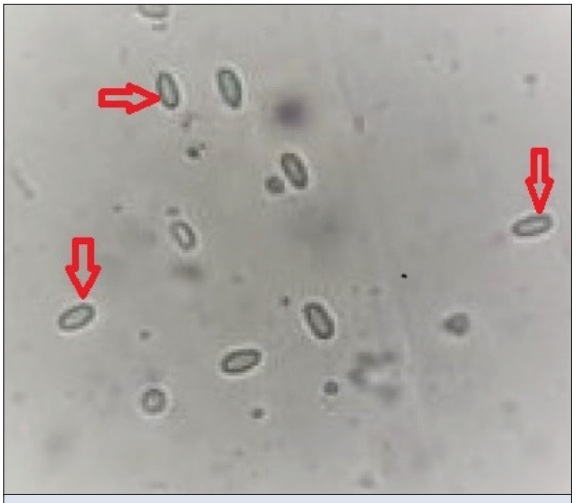

Fig 1. Nosemosis agents are shown red arrow

Fig 2. Spray application

were collected and counted at 0, 15, 30, 50, 65, and 90 days after initial treatment [24]. The digestion method evaluated the *Nosema* infection level from a pooled sample of 50 bees from each colony. A Neubauer hemocytometer slide was used for counting *Nosema* spp. spore loads. The results of these six days were assessed with the formula: $N=S\times4\times10^6/80$, where S is the spore count from all 80 cells of the hemocytometer (one cell represents $1/4\times10^6$ of the total volume) [25].

Treatment Groups

Before the field studies, no tests were made in vitro. This study was designed to determine the effectiveness of spray forms of compounds from thyme, peppermint, eucalyptus essential oils, and nanoparticle ozone against Nosemosis infection in live honey bees. Also, the spray application route is shown in *Fig. 2*.

Preliminary field studies were carried out with 1.000, 2.000, 3.000, 4.000, and 8.000 ppm nanoparticle ozone. A sharp odor formed in concentrations of the nanoparticle ozone of more than 2.000 ppm. Due to this sharp odor, the honey bees were irritated and failed to perform the associated self-licking cleaning process, which is in their normal biology, and the combinations were not fully consumed. Therefore, 1.000 and 2.000 ppm nanoparticle ozone solutions were included in the main field trials.

The information about the thyme essential oil; 100% purity, active ingredients: p-cymene (8.25%), γ-terpinene (31.35%) and thymol (48.50%), linear formula: $2\text{-}[(CH_3)2CH]C_6H_3\text{-}5\text{-}(CH_3)OH$, molecular weight: 150.20 g/mol, peppermint essential oil; 100% purity, active ingredients: menthol (32%), mentone (16.40%), menthofuran (10.6%), 1,8- cineole (6.5%), trans-ferulic acid (11.3 mg/g), hesperidin (8.2 mg/g), ellagic acid (7.5 mg/g), and sinapic acid (5.3 mg/g), linear formula: C10H20O|CID 1254, molecular weight: 960.5 g/mol, eucalyptus essential oil; 100% purity, active ingredients: eucalyptol (50.22%), α-pinene (24.78%), p-cymene (9%), and β-cymene (9.24%), linear formula: C10H18O| ID 2758, molecular weight: 153.20 g/mol.

Thyme, peppermint, and eucalyptus essential oils (Yeşilvadi Botanical Products, Türkiye) were homogenized with Poly Ethylene Glycol 400 (PEG-400: Alpha Lab®, United States) as an emulsifier in sugar syrup. A previous study has proven the fungicidal effect of ozone [23]. On this basis, whether it affects the Nosema spore development in spray form was investigated. Liquid nanoparticle ozone (Genoxyn Nanotech®, Farmoksi Drug, Türkiye) was used at 1.000 and 2000 ppm concentrations. Treatment groups are shown in *Table 2*. Thyme, peppermint, and eucalyptus essential oils were dissolved by mixing them with PEG-400 in a 1:1 ratio. The desired concentrations were obtained by adding sugar syrup to the essential oils dissolved in PEG-400. The preparation of essential oils is shown in *Table 3*. Eight hives containing seven or nine frames were used in

Table 2. *Treatment groups and the number of colonies included in the treatment*

Treatment Groups	Colony Number
125 mL-3% thyme (for each colony)	8
125 mL-3% peppermint (for each colony)	8
125 mL-3% eucalyptus (for each colony)	8
1.000 ppm-1.000 mL nanoparticle ozone (for each colony)	8
2.000 ppm-1.000 mL nanoparticle ozone (for each colony)	8

***Table 3**. Preparation of stock solutions of essential oils for treatment groups*

Percentage	Thyme	Peppermint	Eucalyptus
3%	30 mL thyme oil + 30 mL PEG-400 + 940 mL sugar syrup	30 mL peppermint oil + 30 mL PEG-400 + 940 mL sugar syrup	30 mL eucalyptus oil + 30 mL PEG-400 + 940 mL sugar syrup

PEG-400: Poli Etilen Glikol 400

each treatment combination. These combinations were applied at a ratio of 1000/8 mL (125 mL) to each hive on days 0, 7, 14, and 21.

Control Groups

The *Nosema*-positive and *Nosema*-negative control groups consisted of 8 hives, as in the treatment groups. Honey bee samples of positive and negative control groups were collected at 0, 15, 30, 50, 65, and 90 days as in the treatment groups and *Nosema* spore loads were determined.

Determination of Efficiency of Combinations

The efficiency of combinations was determined with the following formula: Percent reduction test = 100-($\frac{Final\ Number\ of\ Nosema\ spores}{Initial\ number\ of\ Nosema\ spores}$×100) [25]. Day 90 was accepted as the final count of the number of *Nosema* spores, while day 0 was accepted as the initial count (baseline) of *Nosema* spores.

Statistical Analysis

All data were evaluated by IBM®SPSS 20 software. The data were subjected to a Chi-square test of independence due to the number of *Nosema* spores in hives as observed counts. A 2x2 cross-tabulation chi-square test was applied to multiple comparisons for the agents. The days of the *Nosema* loads are compared with a paired-sample t-test. The data are presented as the number of *Nosema* counts in hives. Statistical significance level was considered when $P \leq 0.05$. [26]

Results

Eightyone of 100 hives were found to be positive, and 19 of them were negative. *Nosema* spp. spores were counted on days 0, 15, 30, 50, 65, and 90 after 5 different compounds had been applied to the treatment groups. The percent efficacy values of the treatment groups were calculated by applying the percent reduction test to the results of the counts on days 0 and 90. Treatment efficacies and the total *Nosema* spp. spore loads on the different days are given in *Table 4*. Eight negative hives at the first examination, separated from the positive hives, were found to be positive due to the microscopic examination fifteen days later. In addition, the *Nosema* spore load increased from day 0 to day 90 in the positive and negative control groups. Positive, negative, and treatment groups daily *Nosema* spp. spore loads, and SPSS results are given in *Table 5*. Statistical significance was determined by comparing the agents and *Nosema* loads ($P<0.001$). However, after 2 × 2 multiple comparisons, it was determined that the difference was sourced only days. All the agents effectively reduced the *Nosema* load in the hives up to day 50. The decrease was also found to be significant on days 65 and 90. The *Nosema* load on the 50th day was not statistically significant between the agents ($P>0.05$). According to the percent decrease test results, the highest decrease in *Nosema* spore load was observed in the thyme treatment group with 84%; however, this result was not statistically significant ($P>0.05$). The percentage increase and decrease in Nosemosis spore load daily are given in *Table 6*. The highest decrease in *Nosema* spore load was determined as 40.29% between days 65 and 90 in the thyme treatment group. The minimum decrease in *Nosema* spore load was determined as 8.10% between 15-30 days in the peppermint treatment group. In addition, an increase of 154.11% was observed in the Nosemosis spore load between days 30 and 50 in the peppermint treatment group.

The effectiveness of nanoparticle ozone applied to the frames by spray was investigated against Nosemosis in

***Table 4.** Positive, negative, and treatment groups Nosema spp. spore loads and treatments efficacies*

Treatment Groups	Day 0	Day 15	Day 30	Day 50	Day 65	Day 90	Efficiency (%)
Thyme	6.525×10^3	5.125×10^3	3.500×10^3	2.425×10^3	1.675×10^3	1.000×10^3	84
Peppermint	6.875×10^3	4.625×10^3	4.250×10^3	10.800×10^3	2.050×10^3	1.550×10^3	77.45
Eucalyptus	5.650×10^3	4.350×10^3	3.500×10^3	2.750×10^3	1.950×10^3	1.350×10^3	76.10
1.000 ppm nanoparticle ozone	7.250×10^3	5.200×10^3	3.600×10^3	3.800×10^3	2.850×10^3	2.000×10^3	72.41
2.000 ppm nanoparticle ozone	6.600×10^3	5.100×10^3	4.000×10^3	3.575×10^3	2.700×10^3	1.900×10^3	71.21
Positive	4.350×10^3	5.550×10^3	6.600×10^3	7.600×10^3	8.900×10^3	9.800×10^3	
Negative	0	3.200×10^3	3.750×10^3	4.600×10^3	5.850×10^3	7.000×10^3	

Table 5. Positive, negative, and treatment groups daily Nosema spp. spore loads, and SPSS results

Treatment Groups	Day 0[a]	Day 15[b]	Day 30[c]	Day 50[abcdef]	Day 65[e]	Day 90[f]	df	X^2	P Value
Thyme	6.525.000	5.125.000	3.500.000	2.425.000	1.675.000	1.000.000	20	7481.854	<0.001
Peppermint	6.875.000	4.625.000	4.250.000	10.800.000	2.050.000	1.550.000			
Eucalyptus	5.650.000	4.350.000	3.500.000	2.750.000	1.950.000	1.350.000			
1.000 ppm nanoparticle ozone	7.250.000	5.200.000	3.600.000	3.800.000	2.850.000	2.000.000			
2.000 ppm nanoparticle ozone	6.600.000	5.100.000	4.000.000	3.575.000	2.700.000	1.900.000			
Positive	4.350.000	5.550.000	6.600.000	7.600.000	8.900.000	9.800.000			
Negative	0	3.200.000	3.750.000	4.600.000	5.850.000	7.000.000			

The data presented is the number of Nosema counts in hives. df: Degree of freedom; X^2: Table chi-square value. Superscripts of the columns indicate differences between the days

Table 6. Percent increase and decrease of Nosemosis spore load daily

Day	Thyme	Peppermint	Eucalyptus	1.000 ppm Nanoparticle Ozone	2.000 ppm Nanoparticle Ozone	Positive	Negative
0-15	21.45% ↓	32.72% ↓	23% ↓	28.27% ↓	22.72% ↓	27.58% ↑	3.200.000 total increase
15-30	31.70% ↓	8.10% ↓	19.54% ↓	30.76% ↓	21.56% ↓	18.91% ↑	17.18% ↑
30-50	30.71% ↓	154.11% ↑	21.42% ↓	5.55% ↑	10.62% ↓	15.15% ↑	22.66% ↑
50-65	30.92% ↓	81.01% ↓	29.09% ↓	25% ↓	24.47%↓	30.26% ↑	27.17% ↑
65-90	40.29% ↓	24.39% ↓	30.76% ↓	29.82% ↓	29.62% ↓	10.11% ↑	19.65% ↑

The increase between days 0-15 was given as a number in the negative control group. ↓: Decrease; ↑: Increase

field conditions, and a decrease of 72.41% Nosemosis spore load with 1.000 ppm nanoparticle ozone, and a decrease of 71.21% Nosemosis spore load with 2.000 ppm nanoparticle ozone was found on the day 90 compared to the day 0. The ozone value in the solutions was calculated as gr/ml and expressed as a ppm value. The preparation methods of the ozonated nano solutions are specified in the patent numbered PCT/TR2022/050177.

Discussion

Essential oils (EOs) are also used as bee diet supplements. Honeybees actively collect several phytochemicals that are also dominant components in essential oils, and many of these compounds have antibiotic activity against pathogens and parasites [27]. Honey bees fed plant-derived phytochemicals survive longer and have a high capacity to overcome infections [28]. Essential oils or single compounds isolated from them such as camphor, carvacrol, eucalyptol, menthol, thymol, and several sesquiterpenes have been used as topical medication to control *Varroa destructor* mites and as nutraceutical compounds against *Nosema* spp. spores [29]. The anti-nosemosis properties of essential oils may result from their lipophilic nature and the low molecular weight of terpenes/terpenoids. They can cause cell death or inhibit the sporulation and germination of fungi by disrupting the cell membrane structure or inhibiting chitin polymerization of the cell wall [30].

Various studies have been conducted on the antiparasitic activities of essential oils against Nosemosis. Thymol (3-hydroxy-p-cymene) is one of the most abundant active ingredients derived mainly from *Thymus vulgaris*. Thymol was used against Nosemosis by adding 0.1 g/kg sugar syrup. Fewer spore loads were found in the thymol-treated group than in the Nosemosis-positive group [31]. A study conducted treatment trials with 100 ppm thymol with the honey bees experimentally infected with *N. ceranae in vitro*. Significant differences were found between the treatment and the control group, which was a significant result (60±9 million spores/bee in the treatment group, 138±7 million spores/bee in the control group) [32]. Both studies were carried out in vitro. In a study conducted in vivo, thymol was given to each colony 6 times at 3-day intervals of 3 mL/L to 1:1 ratio sugar syrup, and an undesirable increase of *Nosema* spore load was observed in the thymol group, whereas there was a partial decrease in nettle, especially garlic [33]. Contrary to the increase mentioned in that study due to thymol usage, our study's *Nosema* spore load decreased from day 0 to day 90 *(Table 6)*.

Menthol is derived from peppermint oil and was previously used for some honey bee parasites. It has been tested with thymol to combat *Varroa in vivo* and promising results have been obtained (infestation rate reduced from 21.86% to 1.32% at the end of treatments). It has also been reported that using menthol at a dose of 2.5 mL is effective in tracheal mite infestations [34]. In our study of peppermint oil, the effectiveness of which was tested in other bee pests, it was used for the first time against Nosemosis in honey bees *in vivo*. Peppermint oil was found to be a crucial agent that suppresses *Nosema* spore load after thyme oil. According to the percent reduction test, its effectiveness was determined as 77.45%.

Eucalyptus essential oil has been studied in vitro for Varroosis [35] and Nosemosis [36]. In both, mites were reduced at 71.06% and spore burden at 23%. In a field study with eucalyptus, although a decrease was observed in the first four applications compared to the control group, an increase in Nosemosis spore load was observed in the last two applications. This increase has been described as the intestinal flora may have been damaged due to eucalyptus with the last two applications [33]. In our field study with eucalyptus essential oil, the Nosemosis spore load decreased by 76.10% at the end of the treatment. During our trials, no clinical findings, especially diarrhoea, which may occur due to damage to the intestinal flora, were not encountered.

Thyme, peppermint and eucalyptus essential oil were used by dissolving them in different volumes of PEG-400. PEG-400 was found to be inexpensive and non-toxic [37]. No adverse effects were observed in the honey bees due to using PEG-400. Various studies have been carried out on ozone. One of these studies on the medical use of ozone found that during ozone application, hydrogen peroxide, which is formed due to oxidative stress and lipid oxidation, acts as a secondary messenger. As a result of repeated ozone applications, the antioxidant system is stimulated and resistance against oxidative stress develops [38]. As seen in *Table 1*, although the temperature, humidity average, and the number of rainy days in October caused a suitable environment for the development of the *Nosema* fungal form, an increase in Nosemosis spore load was observed in the peppermint and 1.000 ppm nanoparticle ozone groups only on the 50th day in the treatment groups. The reason for this increase can be explained by the fact that the honey bee colonies in these hives are clinically weaker compared to other honey bee colonies. In addition, the negative control group was found to be positive for Nosemosis on the 15th day, and the Nosemosis load increased until the 90th day. This is because nectar, pollen, and water contaminated with *Nosema* spores are transferred to healthy hives. In the positive control group, the Nosemosis spore load increased from day 0 to day 90. This increase can be explained by the contamination of food and water sources with *Nosema* spore forms as in the negative control group. According to the results of the current study, although all agents were found to be similarly effective in reducing the burden of *Nosema* spp, the proportional effect of thyme oil was determined more effective than the other agents. Essential oils are tried by most researchers in the laboratory environment, but not under field conditions. In addition, the active ingredients with proven effectiveness in the laboratory environment have not been tested under field conditions. The reason why the active substances were given by spray is the hygienic behavior of honey bees by licking each other. With this behavior, the honey bee took the active substances given by the spray more quickly. During the study, abnormal bee death and plundering were not encountered in the colonies.

As a result, thyme, peppermint, eucalyptus essential oils, and nanoparticle ozone, which constitute our study subject, were applied to the frames by spraying in field conditions and their effectiveness values against Nosemosis were revealed. Promising results were obtained in this study and more comprehensive studies are needed in treating with natural extracts. The health of honey bee colonies and bee products can be guaranteed with these studies' results if the natural extract is applied in a suitable dose. Our field study may allow bee products that are healthier and have no drug residue problems to be offered to the public.

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.

Acknowledgements

I would like to thank the Assist Prof. Dr. Barış Güner for writing and editing manuscirpt.

Funding Support

The study in this article was supported by the Balıkesir University Scientific Research Projects with the number 2021/074.

Competing Interest

The author reports no declarations of interest.

Author Contributions

MÖ planned the study and designed the experiments, LA, AOG and AİD helped write the article and laboratory process, İK helped collect samples during fieldwork and wrote the manuscript, and YB helped with data analyses. All authors read and approved the final version of the manuscript.

Ethical Approval

Ethics committee approval is not required for this study.

References

1. Yosri N, Abd El-Wahed AA, Ghonaim R, Khattab OM, Sabry A, Ibrahim MAA, Moustafa MF, Guo Z, Zou X, Algethami AFM, Masry SHD, AlAjmi MF, Afifi HS, Khalifa SAM, El-Seedi HR: Anti-viral

and immunomodulatory properties of propolis: Chemical diversity, pharmacological properties, preclinical and clinical applications, and in silico potential against SARS-CoV-2. *Foods*, 10 (8):1776, 2021. DOI: 10.3390/foods10081776

2. El-Seedi HR, Eid N, Abd El-Wahed AA, Rateb ME, Afifi HS, Algethami AF, Zhao C, Al Naggar Y, Alsharif SM, Tahir HE, Xu B, Wang K, Khalifa SAM: Honey bee products: Preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties. *Front Nutr*, 8:761267, 2022. DOI: 10.3389/fnut.2021.761267

3. El-Seedi H, El-Wahed AA, Yosri N, Musharraf SG, Chen L, Moustafa M, Zou X, Al-Mousawi S, Guo Z, Khatib A, Khalifa S: Antimicrobial properties of *Apis mellifera*'s bee venom. *Toxins*, 12 (7):451, 2020. DOI: 10.3390/toxins12070451

4. Rader R, Bartomeus I, Garibaldi LA, Garratt MP, Howlett BG, Winfree R, Cunningham SA, Mayfield MM, Arthur AD, Andersson GK, Bommarco R, Brittain C, Carvalheiro LG, Chacoff NP, Entling MH, Foully B, Freitas BM, Gemmill-Herren B, Ghazoul J, Griffin SR, Gross CL, Herbertsson L, Herzog F, Hipólito J, Jaggar S, Jauker F, Klein AM, Kleijn D, Krishnan S, Lemos CQ, Lindström SA, Mandelik Y, Monteiro VM, Nelson W, Nilsson L, Pattemore DE, Pereira Nde O, Pisanty G, Potts SG, Reemer M, Rundlöf M, Sheffield CS, Scheper J, Schüepp C, Smith HG, Stanley DA, Stout JC, Szentgyörgyi H, Taki H, Vergara CH, Viana BF, Woyciechowski M: Non-bee insects are important contributors to global crop pollination. *Proc Natl Acad Sci USA*, 113, 146-151, 2016. DOI: 10.1073/pnas.1517092112

5. Stanimirovic Z, Glavinic U, Ristanic M, Aleksic N, Jovanovic N, Vejnovic B, Stevanovic J: Looking for the causes of and solutions to the issue of honey bee colony losses. *Acta Vet Beogr*, 69 (1): 1-31, 2019. DOI: 10.2478/acve-2019-0001

6. Richardson LL, Adler LS, Leonard AS, Andicoechea J, Regan KH, Anthony WE, Manson JS, Irwin RE: Secondary metabolites in floral nectar reduce parasite infections in bumble bees. *Proc R Soc B*, 282:20142471, 2015. DOI: 10.1098/rspb.2014.2471

7. Higes M, Meana A, Bartolome C, Botías C, Martín-Hernandez R: *Nosema ceranae* (Microsporidia), a controversial 21st-century honey bee pathogen. *Environ Microbiol Rep*, 5 (1): 17-29, 2013. DOI: 10.1111/1758-2229.12024

8. MacInnis CI, Keddie BA, Pernal SF: Honey bees with a drinking problem: Potential routes of *Nosema ceranae* spore transmission. *Parasitology*, 149 (5): 573-580, 2022. DOI: 10.1017/S0031182021001827

9. Neidel V, Steyer C, Schafellner C, Hoch G: Simulation of rain enhances horizontal transmission of the microsporidium *Nosema lymantriae* via infective feces. *J Invertebr Pathol*, 149, 56-58, 2017. DOI: 10.1016/j.jip.2017.07.007

10. Kurze C, Le Conte Y, Kryger P, Lewkowski O, Müller T, Moritz RFA: Infection dynamics of *Nosema ceranae* in honey bee midgut and host cell apoptosis. *J Invertebr Pathol*, 154, 1-4, 2018. DOI: 10.1016/j.jip.2018.03.008

11. Pietropaoli M, Ribarits A, Moosbeckhofer R, Köglberger H, Alber O, Gregorc A, Smodis- Skerl MI, Presern J, Bubonic J, Necati-Muz M, Higes M, Tiozzo B, Jannoni-Sebastianini F, Lubroth J, Cazier J, Raizman E, Zilli R, Bagni M, Marta U, Formato G: Biosecurity measures in European beekeeping. *Rev Sci Tech*, 39 (3): 725-735, 2020. DOI: 10.20506/rst.39.3.3174

12. Johnson RM, Dahlgren L, Siegfried BD, Ellis MD: Acaricide, fungicide and drug interactions in honey bees *(Apis mellifera)*. *PLoS ONE*, 8 (1):e54092, 2013. DOI: 10.1371/journal.pone.0054092

13. Huang WF, Solter LF, Yau PM, Imai BS: *Nosema ceranae* escapes fumagillin control in honey bees. *PLoS Pathog*, 9 (3):e1003185, 2013. DOI: 10.1371/journal.ppat.1003185

14. Li JH, Evans JD, Li WF, Zhao YZ, DeGrandi-Hoffman G, Huang SK, Li ZG, Hamilton M, Chen YP: New evidence showing that the destruction of gut bacteria by antibiotic treatment could increase the honey bee's vulnerability to *Nosema* infection. *PLoS ONE*, 12 (11):e0187505, 2019. DOI: 10.1371/journal.pone.0187505

15. Botías C, Martín-Hernández R, Meana A, Higes M: Screening alternative therapies to control Nosemosis type C in honey bee *(Apis mellifera iberiensis)* colonies. *Res Vet Sci*, 95 (3): 1041-1045, 2013. DOI: 10.1016/j.rvsc.2013.09.012

16. Rios JL: Essential oils: What they are and how the terms are used and defined. **In**, Preedy VR (Ed): Essential Oils in Food Preservation, Flavor, and Safety. 3-10, Elsevier, Holland, 2016.

17. Bakkali F, Averbeck S, Averbeck D, Idaomar M: Biological effects of essential oils-A review. *Food Chem Toxicol*, 46 (2): 446-475, 2008. DOI: 10.1016/j.fct.2007.09.106

18. Karahroodi ZR, Moharramipour S, Rahbarpour A: Investigated repellency effect of some essential oils of 17 native medicinal plants on adults *Plodia interpunctella*. *Am Eurasian J Sustain Agric*, 3 (2): 181-184, 2009.

19. Ratzlyko A, Arct J, Pytkowska K: Moisturizing and anti-inflammatory properties of cosmetic formulations containing *Centella asiatica* extract. *Indian J Pharm Sci*, 78 (1): 27-33, 2016. DOI: 10.4103/0250-474x.180247

20. Mawsouf MN, El-Sawalhi MM, Martinez-Sanchez G, Darwish HA, Shaheen AA, Re L: Effect of ozone therapy on redox status in experimentally induced arthritis. *Int J Ozone Ther*, 9 (1): 7-13, 2010.

21. Sabancı AU, Erkan-Alkan P, Mujde C, Polat HU, Ornek-Erguzeloglu C, Bisgin A, Temel SG: Nanobubble ozone stored in hyaluronic acid decorated liposomes: Antibacterial, Anti-SARS-CoV-2 effect and biocompatibility tests. *Int J Nanomed*, 17, 351-379, 2022. DOI: 10.2147/IJN.S328090

22. James RR: Potential of ozone as a fumigant to control pests in honey bees (Hymenoptera: Apidae) hives. *J Econ Entomol*, 104 (2): 353-359, 2011. DOI: 10.1603/EC10385

23. Zanet S, Elena B, Roberto A, Trisciuoglio A, Cauda C, Ferroglio C: *Nosema ceranae* contamination in beekeeping material: The use of ozone as a disinfection method. *J Apic Res*, 58, 62-66, 2018. DOI: 10.1080/00218839.2018.1517989

24. Chaimanee V, Kasem A, Nuanjohn T, Boonmee T, Siangsuepchart A, Malaithong W, Sinpoo C, Disayathanoowat T, Pettis JS: Natural extracts as potential control agents for Nosema ceranae infection in honeybees, *Apis mellifera*. *J Invertebr Pathol*, 186:107688, 2021. DOI: 10.1016/j.jip.2021.107688

25. Cantwell GE: Standard methods for counting *Nosema* spores. *Am Bee J*, 110, 222-223, 1970.

26. SPSS® for Windows: Rel. 17.0.0. Incv. 23, Chicago: SPSS Inc, 2018.

27. Erler S, Moritz RFA: Pharmacophagy and pharmacophory: Mechanisms of self-medication and disease prevention in the honey bee colony *(Apis mellifera)*. *Apidologie*, 47 (3): 389-411, 2016. DOI: 10.1007/s13592-015-0400-z

28. Bernklau E, Bjostad L, Hogeboom A, Carlisle A, Arathi HS: Dietary phytochemicals, honey bee longevity and pathogen tolerance. *Insects*, 10 (1): 1-14, 2019. DOI: 10.3390/insects10010014

29. Borges D, Guzman-Novoa E, Goodwin PH: Control of the microsporidian parasite *Nosema ceranae* in honey bees *(Apis mellifera)* using nutraceutical and immunostimulatory compounds. *PLoS ONE*, 15:e0227484, 2020. DOI: 10.1371/journal.pone.0227484

30. Nazaaro F, Fratianni F, Coppola R, Feo V: Essential oils and antifungal activity. *Pharmaceuticals*, 10 (4):86, 2017. DOI: 10.3390/ph10040086

31. Glavinic U, Blagojevic J, Ristanic M, Stevanovic J, Lakic N, Mirilovic M, Stanimirovic Z: Use of thymol in *Nosema ceranae* control and health improvement of infected honey bees. *Insects*, 13:574, 2022. DOI: 10.3390/insects13070574

32. Costa C, Lodesani M, Maistrello L: Effect of thymol and resveratrol administered with candy or syrup on the development of *Nosema ceranae* and the longevity of honeybees *(Apis mellifera* L.) in laboratory conditions. *Apidologie*, 41, 141-150, 2009. DOI: 10.1051/apido/2009070

33. Yılmaz F, Kuvancı A, Konak F, Öztürk H, Şahin AE: The effects of some essential oils against Nosemosis. *Bee Studies*, 12 (2): 37-41, 2020. DOI: 10.51458/BSTD.2021.7

34. Topal E, Cornea-Cipcigan M, Tunca Rİ, Kösoğlu M, Margaoan R: The use of medicinal aromatic plants against bee diseases and pests. *Bee Studies*, 12 (1): 5-11, 2020. DOI: 10.51458/BSTD.2021.2

35. Ghasemi V, Moharramipour S, Tahmasbi G: Biological activity of some plant essential oils against *Varroa destructor* (Acari: Varroidae), an ectoparasitic mite of *Apis mellifera* (Hymenoptera: Apidae). *Exp Appl Acarol*, 55, 147-154, 2011. DOI: 10.1007/s10493-011-9457-1

36. Chen X, Wang S, Xu Y, Gong H, Wu Y, Chen Y, Hu F, Zheng H: Protective potential of Chinese herbal extracts against microsporidian Nosema ceranae, an emergent pathogen of western honey bees, *Apis mellifera* L. *J Asia Pac Entomol*, 24, 502-512, 2021. DOI: 10.1016/j.aspen.2019.08.006

37. Mekala RS, Balam SK, Harinath JPS, Gajjal RRG, Cirandur SR: Polyethylene glycol (PEG-400): An efficient medium for the synthesis of 1,2-disubstituted benzimidazoles. *Cogent Chem*, 1:1049932, 2015. DOI: 10.1080/23312009.2015.1049932

38. Özler M, Öter Ş, Korkmaz A: The use of ozone gas for medical purposes. *TAF Prev Med Bull*, 8 (1): 59-64, 2009.