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Plant Extracts as Alternative Agents to Control *Nosema Ceranae* in Honeybees (*Apis Mellifera*)

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Abstract—*Nosema ceranae* is a serious microsporidian parasite that threatens honeybee (*Apis mellifera*) colonies, and contributes to worldwide losses. Although the antibiotic fumagillin has been the primary treatment, its toxicity, potential for honey contamination, and the emergence of resistant *Nosema* strains have raised significant regulatory concerns, prompting the search for alternative agents to control *Nosema*. This study investigated the efficacy of aqueous extracts of *Thymus capitatus*, *Rosmarinus officinalis*, and *Artemisia herba-alba* in reducing *N. ceranae* infection and improving honeybee health over two seasons (2021–2022). Infected colonies (n = 32) were treated with plant extracts (20 mL/L sugar solution) in three weekly doses. Our results showed significant reductions in *N. ceranae* spore counts, where the aqueous extract mixture (*Thymus*, *Artemisia*, and *Rosmarinus*) was the most effective, with a mean of 0.25–0.5 infected bees per 25-bee sample vs. 22.5–23 in the control group. Additionally, the mixture enhanced brood area (24.5–26.75 in²) and hive density (9–9.75 frames/hive) compared to individual extracts. These findings highlight the potential of plant derived compounds as natural alternatives for managing *Nosema* infections, possibly owing to their synergistic antimicrobial and immune-boosting properties. Further research is required to identify the active compounds and optimize the formulations for field applications.

Keywords—Honeybees; *Nosema ceranae*; Plant extracts; Natural control

1. Introduction

Honeybees (*Apis mellifera L.*) are key pollinators essential for maintaining global biodiversity and agricultural productivity, as they contribute to the reproduction of more than 75% of flowering plants and 35% of food crops [1]. In addition to their ecological contributions, they produce valuable hive products, such as honey and beeswax, which have nutritional and medicinal uses [2]. However, various factors have been implicated in the alarming decline of honeybee populations worldwide, including pathogens such as *Nosema ceranae* [3], pesticide exposure [4], and habitat loss [5]. Among these factors, *Nosema ceranae*, a microsporidian parasite, poses a severe threat to the health of honeybee colonies [6]. Initially identified in the Asian honeybee *Apis cerana*, it has now become a prevalent pathogen in *A. mellifera* colonies [7]. This obligate intracellular parasite infects midgut epithelial cells and disrupts nutrient absorption. Fumagillin is an effective antibiotic used to control nosemosis; however, despite its efficacy, it can be toxic, potentially contaminate honey, and lead to the emergence of resistant *Nosema* strains [8-10]. These limitations have spurred interest in plant-derived alternatives with antimicrobial properties and minimal environmental impact [11]. Furthermore, essential oils and plant extracts, such as those from *Thymus vulgaris* [12] and *Rosmarinus officinalis* [13], have shown promise against *N. ceranae* while being safe for bees and humans [14]. Additionally, methanolic extracts of *Acacia nilotica* reduced spore counts by 37.8% at 0.1% concentration, with no adverse effects on bees [15]. The development of sustainable and accessible alternatives to chemical treatments would significantly contribute to global pollinator conservation efforts. Therefore, the main aim of this study was to investigate the effectiveness of aqueous extracts from three Libyan plants (*Thymus capitatus*, *Rosmarinus officinalis*, and *Artemisia herba-alba*), individually and in combination, against *N. ceranae* infection in honeybee colonies.

2. Materials and Methods

2.1. Plant Material

Three plant species were used to prepare the aqueous extracts: *Thymus capitatus* (L.) (wild thyme), *Rosmarinus officinalis* L. (rosemary), and *Artemisia herba-alba* (white wormwood). The study was conducted over two seasons (2021 and 2022) in August and September.

2.2. Preparation of Plant Extracts

Leaves of *T. capitatus*, *R. officinalis*, and *A. herba-alba* were collected from the El-Jabal El-Akhdar region in northeastern Libya (32°30'N, 21°30'E). The samples were then dried (room temperature) for 7-10 days. Dried plant material (20 g) was added to 80 mL of distilled water in a glass beaker. The mixture was stirred using a magnetic stirrer for 12 h and then filtered through filter paper. The extracts were stored in sterile airtight glass containers at 4°C until use.

2.3. Evaluation of Treatment Efficacy Against N. ceranae in Honeybee Colonies

To assess the efficacy of the plant extracts, thirty-two honeybee colonies (*Apis mellifera*) with approximately equal bee density and queens of similar age, all naturally infected with *Nosema ceranae*, were used. The colonies were divided into eight groups (four colonies per group) in a completely randomized design (CRD). The treatment solution consisted of 20 mL of aqueous extract mixed with 1 L of sugar syrup (2:1 sugar: water, w/v). The groups received:

- Individual extracts: *Thymus*, *Artemisia*, *Rosmarinus*
- Binary mixtures: *Thymus+Artemisia*, *Thymus+Rosmarinus*, *Artemisia+Rosmarinus*
- Ternary mixture: *Thymus+Artemisia+Rosmarinus*
- Control: sugar solution only

Each colony received 250 mL of the treatment solution per dose for three consecutive weeks. Evaluations were conducted 21 days after the initial treatment. For each colony, the following parameters were measured: (1). Number of infected workers: Individual bee abdomens were crushed in 1 mL of distilled water, and 10 µL of the homogenate was examined per bee under light microscopy at 400×. Spores were counted in at least 10 fields per sample; bees with ≥1 spore were considered infected. (2). Brood area: The area of the capped brood was measured in square inches using a grid template. (3). Hive density: The number of frames fully covered with bees was recorded (frames/hive)

2.4. Data Analysis

Statistical analyses of all experiments were performed using Minitab software. Data were analyzed using analysis of variance (ANOVA) following a completely randomized design (CRD) with four replications per treatment. Mean separation was performed using Fisher's Least Significant Difference (LSD) test at a significance level of $p < 0.05$. Duncan's multiple range test was also applied for comparison of means.

3. Results

3.1. Effect of Plant Extracts on Nosema ceranae Infection

The results revealed that all plant extract treatments significantly reduced *N. ceranae* infection levels in honeybee workers compared to the control, with varying degrees of efficacy (Table 1). The ternary mixture (*Thymus+Artemisia+Rosmarinus*) was the most effective, resulting in only 0.25 infected bees per 25-bee sample in 2021 and 0.5 in 2022. Binary mixtures also significantly reduced the infection levels, ranging from 1.25 to 2.5 infected bees per sample. In contrast, the individual extracts exhibited moderate efficacy, with a mean of 2.25–4.25 infected bees. In contrast, the control group had 22.5–23 infected bees per 25-bee samples.

Table 1. Effect of plant extracts against *Nosema ceranae* infection in *Apis mellifera* workers (mean infected bees per 25-bee sample)

Treatment	Mean numbers of infected bees in the sample (25 bees)	
	2021	2022
Thymus	3.5^{bc}	3^{bc}
Artemisia	2.75^{cd}	2.25^{cd}
Rosmarinus	4.25^{b}	3.25^{b}
Thymus + Artemisia	1.75^{e}	1.25^{ef}
Thymus + Rosmarinus	2.25^{de}	2.5^{bcd}
Artemisia + Rosmarinus	2.25^{de}	1.75^{de}
Thymus +Artemisia + Rosmarinus	0.25^{f}	0.5^{f}
Control (untreated)	23^{a}	22.5^{a}
LSD 0.05	0.818	0.921
Significant	***	***

Note: Values followed by different letters within the same column are significantly different at $p < 0.05$. n = 4 colonies per treatment group. *** $p < 0.001$.

3.2. Effect of Plant Extracts on Brood Area

The data indicated that all treatments increased the brood area compared with the control (Table 2). The ternary mixture produced the largest brood area (26.75 in^2 in 2021, 24.5 in^2 in 2022), followed by the binary mixtures (21.0–24.5 in^2) and individual extracts (16.75–20.25 in^2). The control group had the smallest brood area (7.5–8.0 in^2).

Table 2. Effect of plant extracts on capped brood area (in²)

Treatment	**Mean of the brood area (inch^2)**	
	2021	2022
Thymus	18.25^{d}	19^{d}
Artemisia	19.75^{c}	20.25^{c}
Rosmarinus	16.75^{e}	17.5^{e}
Thymus +Artemisia	24.5^{b}	23.25^{b}
Thymus+ Rosmarinus	21^{c}	22.5^{b}
Artemisia + Rosmarinus	22.75^{b}	23.5^{ab}
Thymus +Artemisia +Rosmarinus	26.75^{a}	24.5^{a}
Control (untreated)	7.5^{f}	8^{f}
LSD 0.05	**1.263**	**1.151**
Significant	***	***

Note: Values followed by different letters within the same column are significantly different at $p < 0.05$. n = 4 colonies per treatment group. *** $p < 0.001$.

3.3 Effect of Plant Extracts on Hive Density

The results showed that all treatments increased the frames covered with bees compared with the control (Table 3). The ternary mixture resulted in the highest hive density (9.0 frames/hive in 2021 and 9.75 frames/hive in 2022). Artemisia alone also showed a high density (8.5 frames/hive in 2021), which was not significantly different from the ternary mixture in the same year. The control group had the lowest density (2.75–3.0 frames/hive).

Table 3. Effect of plant extracts on honeybee colony density (frames covered with bees per hive)

Treatment	Mean number of covered frames with bees (frame/hive)	
	2021	2022
Thymus	5.5^{de}	4.75^{e}
Artemisia	8.5^{a}	7.25^{c}
Rosmarinus	5^{e}	6^{d}
Thymus + Artemisia	7.5^{b}	8.75^{b}
Thymus + Rosmarinus	6.25^{cd}	5.75^{d}
Artemisia+ Rosmarinus	6.5^{c}	6.25^{d}
Thymus + Artemisia + Rosmarinus	9^{a}	9.75^{a}
Control (untreated)	3^{f}	2.75^{f}
LSD 0.05	0.97	0.80
Significant	***	***

Note: Values followed by different letters within the same column are significantly different at $p < 0.05$. n = 4 colonies per treatment group. *** $p < 0.001$.

4. Discussion

The present study highlights the potential of plant-derived extracts, particularly the combination of *Thymus capitatus*, *Artemisia herba-alba*, and *Rosmarinus officinalis*, in mitigating *Nosema ceranae* infection and enhancing colony health, brood production, and bee density in *Apis mellifera*.

The ternary extract mixture achieved remarkable reductions in infected bees (0.25–0.5 per sample; 22.5–23 in the control), outperforming the individual extracts. These findings align with the accumulated evidence that plant extracts can improve honey bee health and mitigate various diseases, including fungi (*Ascosphaera apis*), mites (*Varroa* spp., *Tropilaelaps* sp.), bacteria (*Melissococcus plutonius*, *Paenibacillus* larvae), and microsporidia (*Nosema apis*, *N. ceranae*) [15]. The strong effect of the mixture may be attributed to the complementarity of the combined chemical composition of these herbal extracts. *Thymus capitatus* is rich in thymol and carvacrol, which have well-documented antimicrobial and antiparasitic properties [16]. *Artemisia herba-alba* contains artemisinin and other sesquiterpene lactones known for their antiparasitic activity [17]. *Rosmarinus officinalis* provides rosmarinic acid, carnosic acid, and other phenolic diterpenes with potent antioxidant and antimicrobial properties [18]. When combined, these diverse active compounds (phenolic compounds, terpenes, terpenoids, alkaloids, and polysaccharides) may act synergistically against the parasite through multiple mechanisms, including direct inhibition of spore germination, disruption of the parasite's life cycle, and enhancement of the honeybee's immune response [19]. All plant extract treatments (20 mL of extract per litter of sugar solution) inhibited *N. ceranae*, with the ternary mixture being the most effective. Our findings suggest that this mixture can significantly enhance the activity of the honeybee immune system against microsporidian infections or inhibit the replication of vegetative forms of *Nosema* spp. [20]. These results are consistent with earlier research by Özkırım and Küçüközmen [21], who demonstrated that herbal extract mixture containing *Rumex acetosella*, *Achillea millefolium*, *Plantago lanceolata*, *Salvia officinalis*, *Thymus vulgaris*, *Rosmarinus officinalis*, and *Laurus nobilis* was more effective against *N. ceranae* as compared to individual extracts.

The significant improvements in brood surface area and hive density following treatment can be attributed to reduced infection pressure, which allows colonies to allocate more resources to brood rearing and population growth. The immune-stimulating compounds present in the extracts (phenolic compounds, terpenes, and polysaccharides) likely contributed to reduced *N. ceranae* infection and facilitated colony recovery. This is consistent with the ability of natural compounds to inhibit *N. ceranae* development and enhance colony productivity. A limitation of this study is the absence of a fumagillin-positive control group, as fumagillin was not available due to regulatory and supply constraints in Libya. While future studies should include fumagillin for direct comparison, the significant reduction in spore counts and improved colony health observed with the triple extract mixture support its potential as a natural alternative.

Conclusion

The present study demonstrated that aqueous extracts of *Thymus capitatus*, *Artemisia herba-alba*, and *Rosmarinus officinalis* show significant potential for controlling *N. ceranae* infection in honeybee colonies. The ternary extract mixture was highly effective in reducing infection levels and improving colony health parameters. While the bioactivity of these extracts is likely attributable to secondary metabolites such as phenolic compounds, terpenes, and flavonoids, the specific active compounds responsible for the observed efficacy were not identified in this study. This represents an important direction for future research. Additionally, further studies are required to characterize the bioactive compounds, optimize extract formulations, and evaluate their performance across diverse environmental conditions. This study demonstrates the potential of plant-based mixtures as a sustainable, eco-friendly alternative to synthetic antibiotics for managing nosemosis in honeybees.

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