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Breakthroughs in Honey Bee Health, Continuous-Release Mist Diffusion of Thymol-Based Essential Oils: Part II - The Field Study

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Abstract

Honey bees (*Apis mellifera*) are pivotal pollinators in agricultural and natural ecosystems; however, since the winter of 2006-2007, honey bee colonies have been disappearing. The greatest single contributor to the decline of honey bee health is the *Varroa* mite. Synthetic chemicals are used to control *Varroa*, but the mites are developing resistance. Essential oils (EO) may be a viable alternative. EO are cheaper, environmentally-friendly, pose fewer health risks to bees and consumers, and *Varroa* have not developed resistance to the oils. Currently, all commercially available thymol-centered systems are gel-based and work by direct contact with the mite. These systems are also highly dependent on temperature and humidity for effectiveness. Following the laboratory investigation (Part I), this field study (Part II) examined the use of thymol-based EO for miticide efficacy as well as the use of mist diffusers to eliminate any dependence of the essential oils on temperature and humidity conditions. Miticide efficacy was recorded as: thyme>oregano>rosemary>control (vegetable glycerin). Across all tested EO, the highest miticide activity occurred during the first two weeks of treatment. A brief cost analysis demonstrated using mist diffusers was more cost-effective than commercially available thymol-based systems (US$3.20 versus US$15-$18 per application). Continuous-release mist diffusion permits the disbursement of EO throughout the entire hive and effectively provided early elimination of mites as they emerged from the brood cell, while remaining safe for honey bees.

Keywords: Varroa Destructor; Apis Mellifera; Mites; Honey Bees; Essential Oils; Mist Diffusion

1. Introduction

Honey bees, *Apis mellifera,* are crucial pollinators for agriculture, responsible for over 80% of all cultivated crops (Randall, 2020). In fact, bee pollination accounts for approximately US$15 billion in added crop value (USDA, 2021). Additionally, bees also produce honey, pollen, royal jelly, beeswax, propolis, and venom for nutritional and medicinal uses for an additional US$300 million annually (Calovi, 2021). In the United States, there are approximately 2.5 million commercially farmed honey bee hives and around 500,000 colonies kept by hobbyists and semi-professional bee keepers (Penn State Extension, 2013). Unfortunately, these numbers are declining at a rapid rate. Losses are attributed to Colony Collapse Disorder which occurs when there is a sudden loss of a colony's worker bee population yet the queen, brood and a relatively abundant amount of honey and pollen reserves remain. Various reports have suggested losses between 30% to 50% of winter bee colonies in the US, (EPA, 2021); its lowest point in the past 50 years.

Honey bee colonies do not remain dormant during the winter and remain active to maintain the hive temperature between 24-34 degrees Celsius

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(75.2-93.2 degrees Fahrenheit) by forming a thermoregulating cluster (Calvoli, 2021) which enables them to survive long periods of cold temperature. The colony relies on its existing honey stores and stops foraging for nectar and pollen and brood rearing ceases. While summer worker bees live only for a few weeks, winter bees live for several months. A critical component to increasing the lifespan of winter bees is the control of *Varroa destructor* mites. The greatest single contributor to the decline of bee health is the *Varroa* mite (Rosencranz, 2010). A single *Varroa* mite can shorten the lifespan of a bee by one-third, and two mites can shorten it by one-half (Bryant, 2006). *Varroa* weakens and ultimately kills colonies by out-reproducing their host.

Varroa is an ectoparasitic mite that feeds on the fat bodies of developing honey bee larvae and adult bees and aggressively reproduces within an infected bee colony. Recent research by Ramsey et al. brings to light the *Varroa* mite's focus on the fat body tissue, and not the hemolymph as previously believed, rendering the honey bee susceptible to harm from pesticides (Ramsey, 2019). Furthermore, parasitism by *Varroa* decreases the body weight and water content of young emerging bees (Noel, 2020). The lowered weight of the future adult bee increases with the number of mites. Specifically, by decreasing the size of drones, *Varroa* induce a deficit in sperm production (Noel, 2020). *Varroa* also alters flying, homing and orientation abilities in foragers thereby limiting the efficiency of honey bees collect resources needed for colony development.

To control the spread of *Varroa*, bee keepers initially used acaricides, pyrethroids, and organophosphates pesticides (Bahreni, 2020). The frequent use of these synthetic miticides to control *Varroa* infestations has resulted in the development of resistance to many of the chemical components of these miticides (Bahreni, 2020, Traynor, 2016).

Essential oils may be an alternative to chemical pesticides. They are cheaper, environmental-friendly, and pose fewer risks to the health of bees and consumers. Most importantly, *Varroa* have not developed resistance to essential oils for honey bee mite control (Ghasemi, 2011; Damiani, 2009).

Currently, numerous essential oil compounds have been evaluated for miticidal activity. One of the proven successful essential oils is thymol. It works by disorienting the mite and blocking its pores (Tennessee's Honey Bees, 2021). Thymol is the only compound of essential oils widely used in beekeeping with 70%-90% efficacy against *Varroa* (Garrido, 2018) Thymol also has the added advantage of being active against fungus (chalkbrood) and some efficacy in tracheal mites (Davis, 2003). The most widely used and popular beekeeping products with thymol as a main ingredient are Apiguard®, ApiLifeVar® and Thymovar® (Garrido, 2018). None of these currently available systems utilizing thymol reach reproducing *Varroa* mites in the brood cell (Garrido, 2018). All of these delivery systems only kill the mites on the adult bees (Garrido, 2018).

Although a wide variety of essential oils (over 150) have been tested as potential miticides; very few of them have proven successful when tested in field trials (Sabahi, 2017). Unfortunately, a key problem, evidenced in field trials to evaluate the miticide activity of essential oils, is that results are not consistent; there is tremendous variability between studies due to local temperatures and the relative humidity affecting EO efficacy (Sabahi, 2017). All commercially available thymol-based miticide systems are gel-based and the effectiveness of the thymol is highly dependent upon the ambient temperature and relative humidity. Temperature and humidity affect the rate of essential oil evaporation (Sabahi, 2017). Furthermore, because, these systems are gel-based, they are only effective when there is direct contact with the mite (Garrido, 2018). Following the laboratory investigation (Part I), this field study (Part II) examines the use of thymol-based EO for miticide efficacy as well as the use of mist diffusers to eliminate any dependence of the essential oils on temperature and humidity conditions for EO efficacy.

2. Materials and Methods

2.1. Apiary and Colonies

Following a laboratory investigation demonstrating the potential utility of battery-operated mist diffusion of thymol-based essential oils, a field study was conducted in *A. mellifera* colonies with bee hives naturally infested by *Varroa destructor* mites located at Cherry Hill, New Jersey. The level of *Varroa* mite infestation of colonies, headed by Italian queens and housed in Langstroth hives, was determined by measuring the rate of mite drop before starting the field study. The hives were equipped with screened bottom boards and 3mm mesh hardware cloth and sticky paper coated with vegetable shortening (Crisco®, B&G Foods, Parsippany, NJ, USA) to capture falling mites. Four colonies were selected for having similar mite infestation rates. Each colony was assigned to one of four treatment groups. Trials commenced October 2, 2021 for a 4-week period and concluded October 30, 2021.

2.2 Essential Oils and Continuous-Release Battery -Operated Mist Diffusers

For this experiment, three USDA, certified organic, premium food-grade (highest grade) essential oils (Zongle Therapeutics, Norcross, GA, USA) consisting of thyme (Thymus linearis), oregano (Lippia berlandieri), and rosemary (Rosmarinus officinalis) were evaluated along with a control consisting of organic, premium food-grade, vegetable glycerin (Plant Guru, Plainfield, NJ, USA).

To provide continuous-release, two alternating battery-powered mist diffusers (AirWick® Essential Mist Essential Oil Diffuser, Reckitt Benckiser, Slough, England) were used in each hive to provide 24/7 release because each mist diffuser automatically shuts down after eight hours of continuous use. Battery-powered mist diffusers were selected due to the lack of electrical power available at the hive location. The AirWick Mobile App was utilized to ensure that all diffusers were operating properly to provide 24/7 continuous-release. Hive monitors (Broodminder T2SM Internal Hive Monitors, Stoughton, Wisconsin, USA) were utilized to monitor temperature and relative humidity in the hive. The mist diffusers and hive monitor set-up in the hive super are depicted in Figure 1.

2.3 Mite Mortality/Bee Mortality

To assess treatment efficacy and rate of mite fall, sticky papers were placed underneath the screen of the hives' bottom boards to capture falling mites as described in previous field studies (Asha, 2015; Sabahi, 2017). Furthermore, honeybees are known to remove fallen or diseased nestmates. Removing corpses protects against infection. As depicted in Figure 2, the papers were collected twice a week and fallen mites on them counted and divided by 3 or 4 (depending on day of week) to obtain an average rate of mites dropped per day. For example, a mite collection and count conducted on Wednesday, would be divided by 4 to obtain a daily average mite fall for Sunday, Monday, Tuesday, Wednesday whereas a mite collection and count conducted on Saturday would be divided by 3 for an average daily average mite fall for Thursday, Friday, Saturday. After 4 weeks of trials, two plastic strips containing amitraz (Apivar, Ve´to-Pharma, Villebon-sur-Yvette, France) were placed in each hive as finisher treatments.

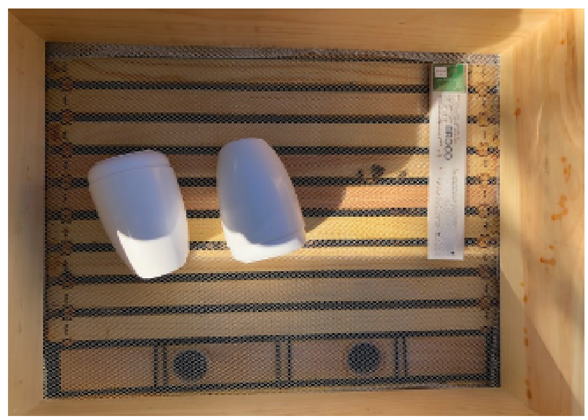

Figure 1. Essential Oil/Mist Diffusers Set-Up in the Hive Super

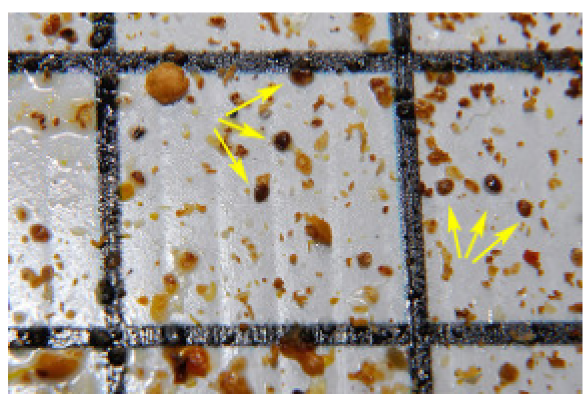

Figure 2: Fallen Mites on Sticky Paper

2.4 Treatment Efficacy

The efficacy of each treatment was determined using the following equation:

$$\textbf{Efficacy (\%)} = \Sigma[N1/(N1 + N2)] \times 100$$

where, N1 is the number of mites that fell during the 4 weeks of test treatments, and N2 is the number of mites that fell during the final treatment with amitraz.

Early *Varroa* mite control in the fall season is important to improve the health of colonies and to increase their likelihood of winter survival. Therefore, the proportion of mites that were killed by the treatments each week, during the treatment period, was calculated. The Relative Mite Weekly Fall Rates (MFWRs) were calculated for each treatment using the following equation:

$$\textbf{MFWR} = [(a + b)] / c \times 100$$

"a" is the number of mites that fell during the first observation of a week, b, is the number of mites that fell during the second observation of the same week, and c, is the total number of mites that fell during the 4 weeks of treatment.

To evaluate the effect of compounds on bee mortality, Todd dead bee entrance traps (Stoner 1979) were installed at the entrance of hives as depicted in Figure 3. Dead bees were counted twice a week during the trial's 4-week period.

Figure 3: Todd Dead Bee Entrance Trap

3. Results and Discussion

3.1 Miticide Efficacy

As shown in Figure 4, the efficacy rates for *Varroa* mite control among the essential oil treatments were significantly higher than the vegetable glycerin control. Treatment 2 (thyme essential oil) had the highest percent efficacy rate (97.4% ± 0.68), which was followed closely by Treatment 1 (oregano essential oil) with a comparable percent efficacy rate (93.8% ± 0.72) and more distantly by Treatment 3 (rosemary essential oil with an efficacy rate of 78.2% ± .89). Conversely, the lowest percent efficacy rate was observed for vegetable glycerin control (42.8% ± .81). Statistical significance was determined using regression analysis. Statistical significance is the probability of an observation not being caused by a sampling error. When the p-value is equal to or less than 0.05 the statistical result is believed to be accurate. All p-values ≤ 0.05 and were found to be statistically significant [*thyme (p=.00000742); oregano (p=.0000593); rosemary (p=.000487)]*.

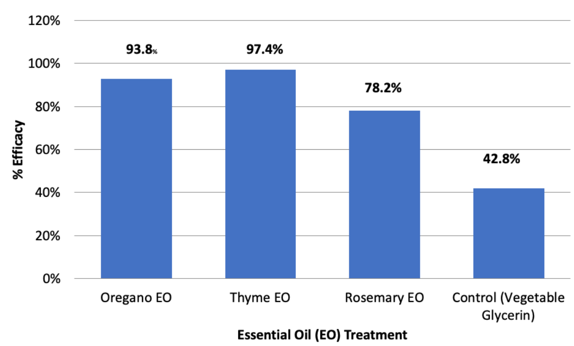

Figure 4: Mean Varroa Mite Control % Efficacy of Tested Essential Oil Treatments in Hives

3.2 Mite Fall Weekly Rates (MFWR)

As depicted in Figure 5, the mite fall weekly rates (MFWR) was dramatically higher for all essential oil treatments in the first two weeks of treatments in comparison to the vegetable glycerin control. The control had greater mite fall percentage in the last two weeks whereas thyme exhibited the highest proportion of mites killed during weeks 1 and 2 (82.6%) followed by oregano (73.2%) and rosemary (61.8%) in comparison to the control (45.7%). In this scenario, where there is a continuous release of essential oils at a sufficient dose over an extended period, it appears that the mites are killed as they emerged from brood cells. As such, a large proportion of the mites were killed within the first 2 weeks of treatment (particularly during the second week of treatment) due to miticide efficacy of the essential oils. A significant difference should be noted that in the last week of treatment across all the essential oils. During the last week of treatment, the percent of mite fall was between 4.1% to 12.3% for all EOs in comparison to the percent of mite fall of the control at 26.2%. Miticide efficacy of thymol-based essential oils was greatest during the first two weeks of treatment in contrast to the vegetable glycerin control which exhibited greater percentage of falls during the last two weeks of treatment. Statistical significance was determined using regression analysis. Statistical significance is the probability of an observation not being caused by a sampling error. When the p-value is equal to or less than 0.05 the statistical result is believed to be accurate. All p-values ≤ 0.05 and were found to be statistically significant [*thyme (p=.00048), oregano (p=.00929); and rosemary (p=.00658)]. n=1,281.*

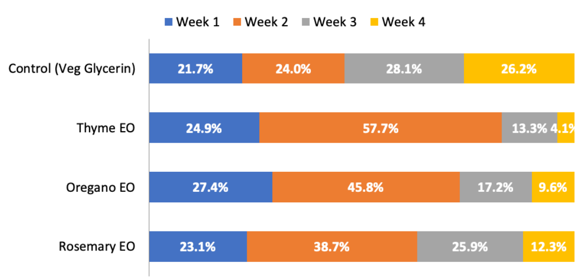

Figure 5: Mite Fall Weekly Rates of Tested Essential Oil Treatments in Hives

3.3 Worker Bee Mortality

As illustrated in Figure 6, the average per day worker bee mortality was similar across all tested essential oils and the vegetable glycerin control indicating the tested essential oils were safe for honey bees. The greatest daily mortality was found in thyme (29.3), followed by oregano (28.8), and rosemary (27.5). All of which was comparable to the vegetable glycerin control (26.8). Statistical significance was determined using regression analysis. Statistical significance was determined using regression analysis. Statistical significance is the probability of an observation not being caused by a sampling error. When the p-value is equal to or less than 0.05 the statistical result is believed to be accurate. All p-values > 0.05 and not statistical significant [thyme (p=.876); oregano (p=.594); rosemary (p=.452)]. n=836.

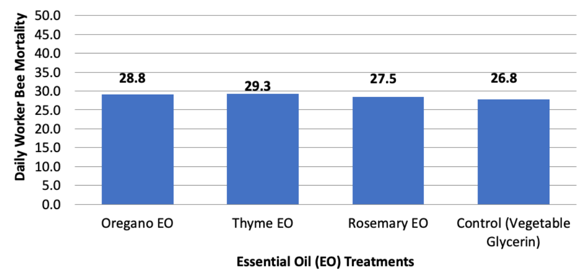

Figure 6: Per Day Worker Bee Mortality of Tested Essential Oil Treatments in Hives

4. Discussion

This investigation of various essential oils demonstrated the effectiveness of select thymol-based essential oils for the control of *Varroa* mites during the fall in the hive. All tested essential oils were shown to be effective miticides with no effect on worker bee mortality. A critical advantage of using essential oils in hives is that essential oils are generally recognized as safe and environmentally-friendly and the mites have not developed resistance to the oils. All the essential oils utilized for this field study were premium food-grade quality. Worker bee mortality was found to be the same across all essential oils and comparable in safety to the vegetable glycerin control.

This study also demonstrated the clinical utility of using a battery-operated continuous-release delivery system. Our results demonstrate that thymol-based essential oils delivered with battery-operated mist diffusers can achieve a high level of *Varroa* mite control. The most effective miticide control came from thyme (97.4% efficacy), followed by oregano (93.8% efficacy) and rosemary (78.2% efficacy) in comparison to the vegetable glycerin control (42.8%). The miticidal activity of thymol-based essential oils is attributed to terpenes like carvacrol (Sabahi, 2017). In our study, the tested oils contained between 60-69% carvacrol; however, the concentration of this component may vary from source to source and can potentially affect the essential oil's varroacidal efficacy.

The use of a battery-operated mist diffusion system provided a continuous-release mist diffusion of thymol-based essential oils throughout the hive. In contrast, currently available thymol-based gel systems are limited by their need to be in direct contact with the mite as well as a “goldilocks” range of temperature and humidity for efficacy. The proportion of mites killed at different treatment periods can be attributed to the mist diffusion thymol-based essential oil technology. For thyme, 82.6% of the total number of mites that died during the 4-week trial period, fell during the first 2 weeks. For oregano, 73.2% and rosemary, 61.8%. However, only 45.7% of the mites fell during this time period in the vegetable glycerin control. In this scenario, where there is a continuous release of essential oils at a sufficient dose over an extended period, the mites are killed as they emerge from brood cells. As such, a large proportion of the mites were killed within the first 2 weeks of treatment (particularly during the second week of treatment) due to miticide efficacy of the essential oils. Only between 4.1%-12.3% of mites had fallen during the last week of treatment indicating the majority of mites were killed as they emerged from the brood cell. The early elimination of mites is critical as it results in longer bee lifespan and higher colony survival after winter (van Dooremalen, 2012)

Lastly, a cost-effectiveness analysis found the average cost per application of the thymol-based essential oil continuous-release mist diffuser technology is approximately US$3.20 per hive per application (assuming the use of 2 mist diffusers) in comparison to currently available thymol-based commercial products at US$15-US$18 per hive per application.

5. Conclusion

In light of the current crisis surrounding honey bees and the *Varroa* mite, new solutions are desperately needed. The goal of this field study was to demonstrate that battery-operated, continuous -release mist diffusion of thymol-based essential oils can serve as a cost-effective miticide while also being safe for honey bees. Thymol-based essential oils delivered with battery-operated mist diffusers achieved a high level of *Varroa* mite control (thyme > oregano > rosemary > vegetable glycerin control) particularly in the first two weeks of treatment allowing for a longer bee lifespan and higher colony survival after winter. Continuous-release mist diffusion of thymol-based essential oils may effectively, safely, and cost-effectively be incorporated as part of a natural miticide control plan to enhance the chances of honey bee colony survival.

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