RESEARCH GRANT PROPOSAL FINAL REPORT

PROJECT TITLE: New methodology for the application of plant essential oils to control *Varroa* mite infestation in honey bee colonies.

Project Number: 05-GD-01

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Problem and its Significance

The ectoparasitc mite *Varroa jacobsoni* is the most destructive pest of honey bees *Apis mellifera* (10,11). The rapid emergence and spread of the parasite in the last 10 years has drastically changed beekeeping practices. To avoid the devastating effects the mites have on honey bee colonies, beekeepers are forced to treat mite infestation with pesticides. The repeated application of such products can lead to a potential problem of residues in apiculture products, to a lesser extent in honey and also the elevated chance of environmental contamination (6). The rapid development time of *Varroa,* the large population and the massive use of chemicals are contributing to the development of resistance, especially if challenged repeatedly with the same pesticide (4). With the increasing resistance of mites to registered acaricides, there is an immediate need for alternative methods to control honey bee parasites. Preliminary data utilizing essential oils was able to demonstrate that some of the oils were effective for mite control. Under an Almond Board Project # 04-GW-01, we studied the inclusion of essential oils in our liquid protein diet fed to the bees in colonies with mite infestation.

In this project, we are proposing the development of a new and novel essential oil delivery system based on a starch microencapsulation. In this process, cornstarch will be used to encapsulate microscopic droplets of the oils about the size of a pollen grain. The long-term goal of this research is to find three essential oils that are effective in controlling *Varroa*, so in the end, we will be able to provide products that can be alternated to minimize the chance of resistance.

Objectives:

1. Select essential oils for their ability to control *Varroa* mites.
2. Determine the optimum concentration of essential oils to be used in the delivery system.
3. Determine the effects of the essential oil formulation on mite infestation and reproduction rates.

Objective 1: Select essential oils for their ability to control *Varroa* mites.

The essential oils were selected for starch encapsulation based on their ability to control mites. Preliminary data obtained under an Almond Board Project # 04-GW-01, showed efficacy of some oils to control mites in colony trials.
The essential oils we proposed to test were: Thymol, Origanum, Clove and Cinnamon.

The microencapsulation of the essential oils was done by ARS chemists at the USDA Bioproducts Laboratory in Albany, CA. The chemists have developed a

technique for microencapsulation that we used for our essential oils as a delivery system. The active ingredient (AI) of the oil in the capsules was 25%.

Objective 2: Determine the optimum concentration of essential oils to be used in the delivery system.

To make the microencapsulated essential oils more attractive to the bees, we mixed them with powdered sugar, a commonly known method used by beekeepers to apply antibiotics in the colonies called dusting. Each starch encapsulated essential oil was mixed in the laboratory with powdered sugar at different concentrations such as: 1%; 2.5%; 5% and 7% AI. Preference feeding trials were performed in various nucleus colonies by applying 5 grams of oil-sugar mix on the top bars of the frames. Plain powdered sugar was used as a control and applied at the same time with the treatments. Consumption and preference were recorded over time. Feeding preference trial is shown in Table 1.

Feeding Preferences Table 1

Essential Oil	Concentration				Overall
	1%	2.5%	5%	7%	
Origanum	+	+	-/+	-/+	2.5%
Clove	+	+	-/+	-/+	2.5%
Cinnamon	+	+	-/+	-/+	2.5%
Thymol	+	+	-/+	-/+	2.5%
Control (Sugar)	+	+	+	+	All

The Optimum Concentration was determined at 2.5% AI

Objective 3: Determine the effects of the essential oil formulation on mite infestation and reproduction rates.

Once the acceptable concentration of the oil was determined at 2.5%, a mixture of each microencapsulated essential oil and powdered sugar was fed to 5 nucleus colonies (nucs) for each treatment period. A total of 15 grams of oil mixture was applied to each nuc and the treatments were replenished as needed. Control nucs were fed powdered sugar without the essential oil starch encapsulations. Pre-treatment mite population levels in 25 5-frame nucleus colonies were determined by the sticky board technique. In addition, we sampled sealed brood to estimate mites per cell. To determine the efficacy of the essential oils treatments, we monitored the rates of mite invasion and reproduction in the tests nucs. The duration of the treatment with the microencapsulated oils was 8 weeks followed by 1 week of Apistan, a commercially available miticide used as a known treatment to kill the remaining mites in the colonies. The results and data analysis from these experiments are shown in Figures 1 through 4.

Figure 1

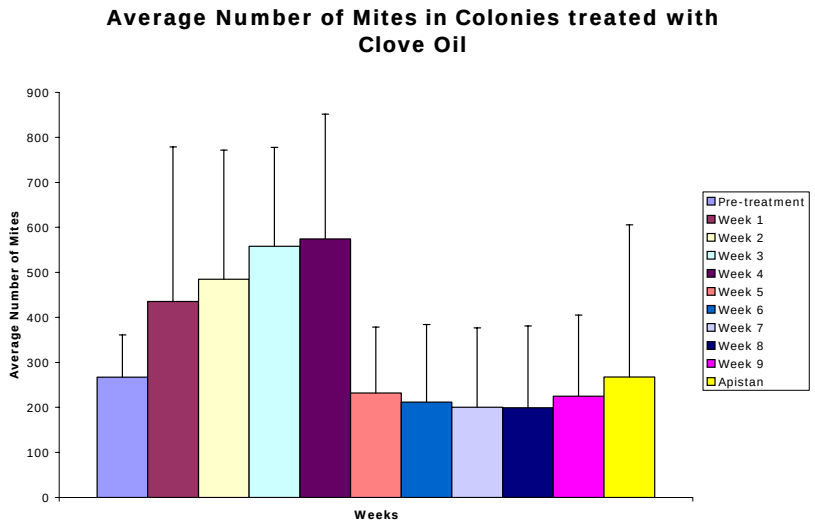

Figure 2

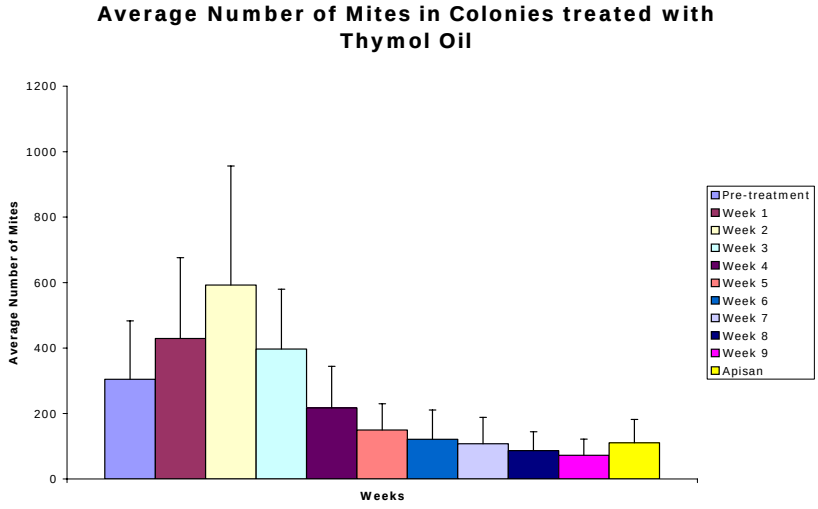

Figure 1 and Figure 2. The number of mites decreased significantly in both oils after 4 weeks of treatment, and remained at that level for the rest of the treatment. There is no significant difference on mite population when the miticide was applied at week 10.

Figure 3

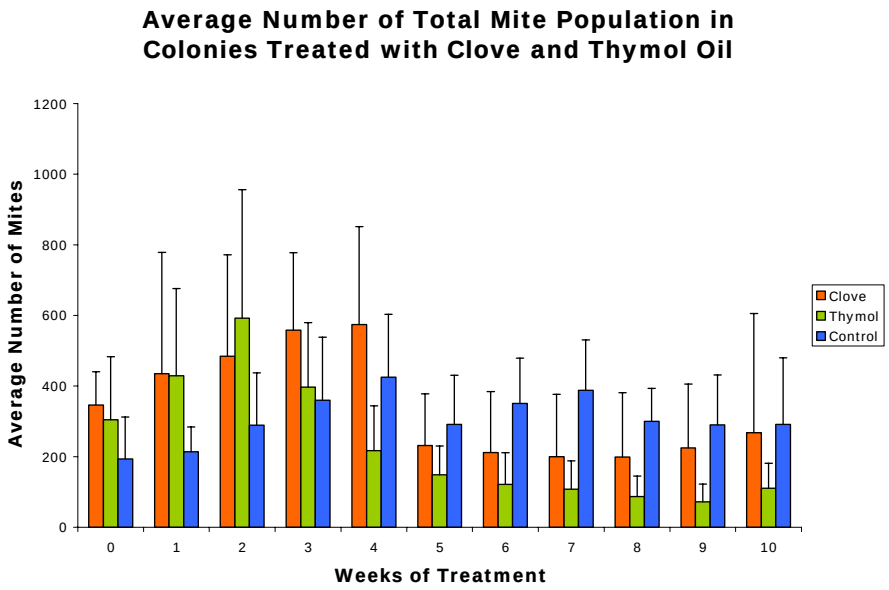

Figure 3. This graph is the combination of Fig.1 and 2 and the control was added. Average of total mite population recorded after 9 weeks of treatment. Week 0 represents mite population before treatment. Week 1-9 treatment period, Week 10 a commercial miticide was applied to knock down the remaining mites in the colonies. The mite population was recorded using the sticky board technique. Boards were replaced weekly. The oils were used at 2.5% concentration from a 25% stock.

Figure 4

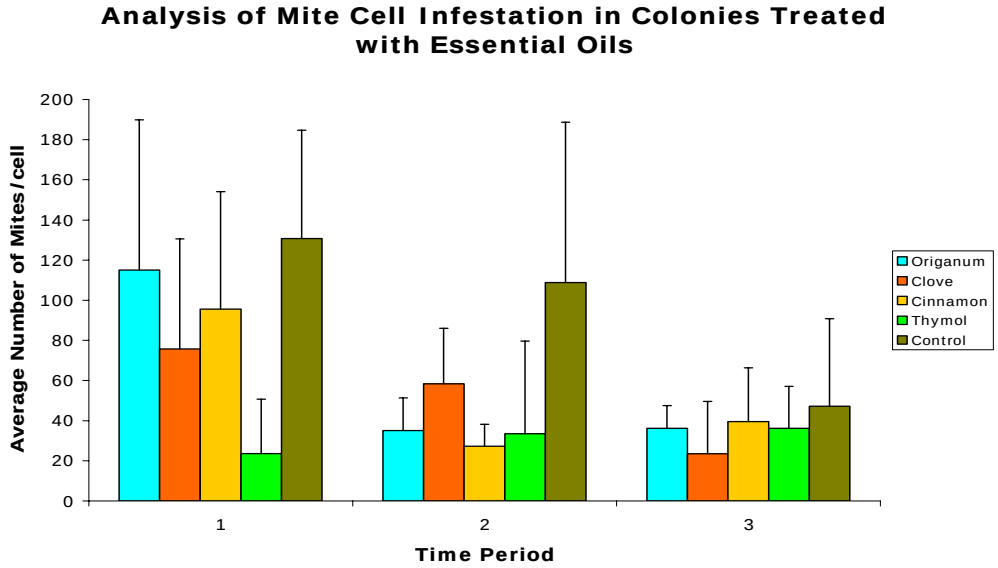

Figure 4. Brood cells were examined for mite invasion and reproduction. Each time period represents 1 brood cycle of 21 days. In period 2 generally all the oils were able to reduce the number of mites.

Conclusions

- From all the oils tested, Clove and Thymol were the most effective in controlling invasion and reproduction in the cells as well as mite drop rates.
- The optimum concentration to be used in the treatment was 2.5%.
- Easy treatment application that shows to be efficient and at the same time reduce human exposure to toxic compounds.
- Even though the delivery system works well, we need to improve the release rate of the oils and we plan to do that as an extension of this research project.

We strongly believe that this new technology will enable us to test a wide range of essential oils and derivatives.

Results of Phase II of this project will be presented at the annual Almond Board Conference.

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