# RESEARCH GRANT PROPOSAL FINAL REPORT

## PROJECT TITLE: Utilization of plant essential oils for the control of the American Foulbrood Pathogen *Paenibacillus larvae*: Susceptibility of Oxytetracycline-Resistant strains and development of delivery system

**Project Number:04-FA-01**

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**Problem and its Significance:**

American Foulbrood disease (AFB) is a highly contagious disease of honey bee brood and is caused by the bacterium *Paenibacillus larvae*. This pathogen is extremely virulent and can be spread in a number of ways, including drifting bees and the interchange of contaminated bee equipment between colonies (1). The disease is controlled by either burning the infected colonies or by the use of antibiotics.

Oxytetracycline hydrochloride (OTC= Terramycin) remains the only approved drug treatment available in the United States for the prevention and control of AFB (2,4).

The rapid emergence of the pathogenic bacteria resistant to antibiotics has led to problems, and many attempts have been made to develop new antibiotics. Last year under Almond Board grant project number 03-GD-02 we successfully screened and identified plant essential oils that are effective in controlling OTC-susceptible and resistant strains of AFB. A summary of the results obtained is shown in Table 1.

The current research is a continuation of the investigation described above.

The long-term goal of this research is to develop a system for the control of AFB based on the utilization of the essential oils as alternative antimicrobial agents. The oils will control pathogens resistant to oxytetracycline and also can be used as prophylaxis.

**Objectives:**

**1. Isolate oxytetracycline-resistant strains of AFB from different geographic regions and test the efficacy of the essential oils.**

**2. Develop an effective delivery system for the essential oils.**

**3. Perform field tests with the essential oils on colonies infected with oxytetracycline-resistant strains of AFB.**

**Objective 1: Isolate oxytetracycline-resistant strains of AFB from different geographic regions and test the efficacy of the essential oils.**

Oxytetracycline-resistant strains field samples were provided by beekeepers that had infected colonies with AFB suspected to be resistant to OTC. These field samples were isolated and screened in our laboratory against OTC and resistance to the antibiotic was observed. We conducted the first set of experiments to determine the dosage levels of each of the oils needed to control the field isolates from different geographic origins. The bioassays were performed using the existing emulsified oils. As standard controls, we used the OTC-susceptible strain of *Paenibacillus larvae*, subsp. *larvae* (American Type Culture Collection strain # 9545) as well as OTC-resistant strain field isolates: **#1a, #3a, # 3b.**

The sensitivity of the microorganisms (OTC-susceptible and resistant strains) to the active agent was determined by the Minimum Inhibitory Concentration (MIC)-Agar dilution method. A summary of the results obtained is shown in Table 1.
The results obtained with the emulsified formulations indicated that Thymol and Origanum are the most effective in controlling the pathogen *in vitro*. Results from these experiments are shown in Figures 1 & 2.

**Table 1.** MIC of essential oils utilized in the proposal

| Essential Oil | MIC Slow Release (%) | MIC Emulsified (%) |
|---|---|---|
| Thymol | 0.02 | 0.01 |
| Origanum | 0.03 | 0.01 |
| Cinnamon | 0.06 | 0.03 |
| Bay | * | 0.06 |
| Clove | * | 0.06 |

- **For these treatments no inhibition was detected even at the higher concentration tested (0.06%)**

**Objective 2: Develop an effective delivery system for the essential oils.**

The results from the laboratory trials provided us with enough information to be used in the field. An efficient delivery method of the emulsified oils played an important role in the project. We performed systematic feeding trials using in site colonies at the bee yard in the facility to determine the range of concentrations of the oils that can be tolerated by the bees. The oils were incorporated in different nutritional supplements such as pollen patties, pollen substitute patties and the artificial liquid diet developed in our laboratory. The advantage of the emulsified oils is that the particle size is very small which allows a homogeneous combination with the other solid components of the diet. From all the diets tested, we were able to observe a better distribution of the oils in the liquid diet. The optimum feeding concentration of oils acceptable by the bees was 0.06%.

**Objective 3: Perform field tests with the essential oils on colonies infected with oxytetracycline-resistant strains of AFB.**

Once the acceptable treatments levels were established and the oils well incorporated into a compatible diet we were ready to test the oils in infected colonies.

Due to the nature of the pathogen, infected colonies need to be isolated in order to avoid the spread of the disease into healthy colonies. An enclosed square cloth structure is located on one corner of the bee yard on site to keep colonies isolated and that would allow us to perform the field test treatment using infected colonies.

During the spring before we started our field trial, a big AFB outbreak disease took place in the bee yard and spread rapidly in the colonies. The colonies needed to be treated with the OTC to avoid colony losses. It was not safe to use the bee yard for our project, so we needed to look for another yard. We were able to find a yard and adjustments were made in order to fit our needs. The yard holds eight infected colonies and the treatment with the emulsified oils will begin shortly.

Unfortunately, we were not able to have the results of the field trial in time for this final report. We will continue to do the research and analyze the data in order to accomplish our objective. We are hoping that the treatment with these essential oils will provide the bee industry an alternative source to fight the disease.

**REFERENCES:**


1 Henrik Hansen, Camilla Juul Brodsgaard (1999). American foulbrood: a review of its biology, diagnosis and control. Bee World 80 (1): 5-23.

2 Hachiro Shimanuki, David A. Knox (February 1994). Susceptibility of *Bacillus larvae* to Terramycin. American Bee Journal 125-126.

3 David A. Knox, H. Shimanuki, Dewey M. Caron (1977). Susceptibility of *Bacillus larvae* to Ethylene Oxide and Tetracycline HCl. Journal of Apicultural Research 16 (4): 201-203.

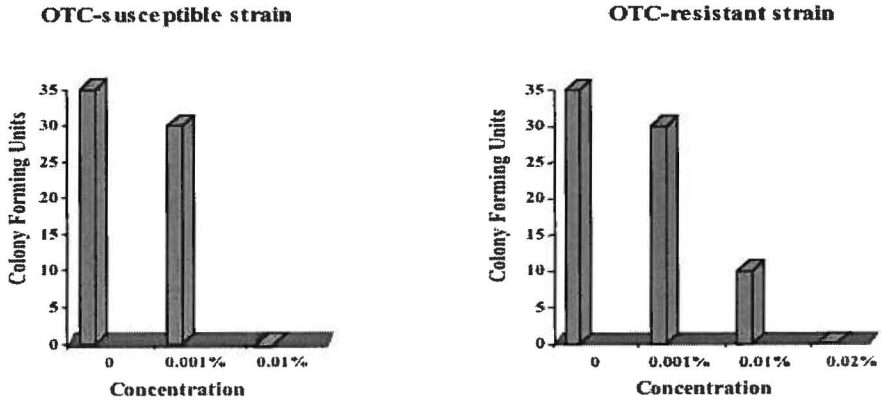
4 Jan Kochansky, David A. Knox, Mark Feldlaufer, Jeffery S. Pettis (2001). Screening alternative antibiotics against oxytetracycline-susceptible and –resistant *Paenibacillus larvae*. Apidologie 32, 215-222.

5 Panuwan Chantawannakul, Brian N. Dancer (2001). American foulbrood in honey bees. Bee World 82 (4): 168-180.

6 Andrew Matheson, Murray Reid (July 1992). Strategies for the Prevention and Control of American Foulbrood. American Bee Journal, Part II of a Three-part Series, 471-475.

# Figure 1

**Effect of Thymol on the growth of *P. larvae in vitro***




# Figure 2

**Effect of Origanum on the growth of *P. larvae in vitro***