CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD March 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Fixed-Quat: A novel alternative to Cu fungicide/bactericide for preventing citrus canker

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
04/19/2016	Citrus Research and Development Foundation	Unclassified

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

This research project aims to develop an alternative to Cu biocides in the form of Quaternary ammonium compounds (Quat) as an antimicrobial agent. While Quat compounds are powerful antimicrobial agents they are not known to be used directly on plants because of potential toxicity to plant tissue. However, combining Quat with other inactive ingredients such as our silica gel delivery makes it safer by producing Fixed-Quat.

In the previous reporting period, a new nanoformulation, Fixed-Quat E nanogel was synthesized with a quat concentration of 9,000 ppm ("For Food Use" Quat used). The formulation composition and interactions between the components (silica and Quat) was confirmed using Fourier Transform Infrared Spectroscopy (FTIR), with Si-O stretching and SiO-H stretching confirming the presence of silica. The Si-O stretching frequency changed from 1039 cm (-1) to 1031 cm (-1) whereas the SiO-H frequency changed from 3391 cm (-1) to 3388 cm (-1), suggesting interaction of positively charged Quat with the silica gel. The Quat N-H bending frequency changed from 1468 cm (-1) to 1431 cm (-1). This change also supports the interaction of Quat with silica gel. In this reporting period, Fixed-Quat E was optimized to a quat concentration of 13,500 ppm ("For Food Use" Quat used). FTIR was used to confirm silica and Quat interaction of the concentrated formulation and similar peak shifts were observed. The Fixed-Quat E nanogel's safety was tested by phytotoxicity studies carried out in a Panasonic Environmental Test Chamber (Model MLR- 352H) which allowed for controlled day/night cycling temperatures, light intensity and humidity to simulate summer weather conditions (biocide application season). Studies conducted on Cleopatra orange, a common citrus variety and Tomato sp, an ornamental plant revealed no sign of plant injury when tested with Quat concentration as high as 1000 ppm. It is noted that EPA maximum concentration for Quat industrial use (as surfactant/flocculating agent) is 200 ppm, indicating a large therapeutic window. Antimicrobial studies of optimized Fixed-Quat E nanogel was conducted against several model bacterial species, Xanthomonas alfalfae subsp. citrumelonis (Citrus Canker Surrogate), Pseudomonas syringae pv syringae, a gram negative causative agent of bacterial speck in citrus and tomato sp and Clavibacter michiganensis subsp michiganensis, a gram positive causative agent of canker and systemic infections in tomato. Studies were conducted to determine the Minimum Inhibitory Concentration (MIC) and compared against Kocide 3000 and copper sulfate. MICs of Fixed-Quat E were found to be ≤ 1.0 µg/mL for X. alfalfae, ≤ 1.0 µg/mL for P. syringae and ≤ 1.0 µg/mL for C. michiganensis. Optimized versions of Fixed-Quat A-II and Fixed-Quat E have been prepared for 2016 Citrus Canker trials on Ruby Ray grapefruit.

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.