Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Canker Pathology	10/15/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Copper loaded silica nanoparticle/nanogel - a new generation of nanobiocide for the prevention of citrus canker disease

TITLE and CONTACT INFORMATION

Proposal Title Development of nanoparticle/nanogel formulation for the prevention of citrus canker disease

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Organization University of Central Florida	*Total Direct Funds (current year)* 90,000

REPORT UPDATE (650 words; provide details about your headline)

The overall goal of this project is to apply nanotechnology to develop Cu loaded silica nanoparticle/nanogel (CuSiNP/NG) formulation that will improve Cu bioavailability and longevity and prevent citrus canker disease infection in long-term. During this reporting period we have accomplished the following.

Task 1 (CuSiNP/NG design, synthesis, characterization and protocol optimization):

(a) Performed systematic characterization of CuSiNG material (optimized nanoformulation) using high-resolution transmission electron microscopic (HRTEM) technique. We discovered that Cu is present in two different forms, amorphous and crystalline. The selected area electron diffraction (SEAD) and X-ray diffraction (XRD) analysis confirmed that the CuSiNG material is primarily amorphous in nature. Detailed investigation of the crystalline form of Cu by the HRTEM revealed that they are nanocrystalline Cu(I) oxide (crystal size 1-3 nm). X-ray photoelectron spectroscopic (XPS) study confirmed the presence of Cu both in Cu (II) and Cu(I) forms. Cu(II) ions are complexed with the amorphous silica matrix (characteristic blue color appearance).

(b) Performed optimization of CuSiNP synthesis protocol with the focus on Cu loading. Cu-sulfate amount was varied from 236 mg to 1900 mg keeping the tetraethyl orthosilicate (TEOS, a silane precursor of silica nanoparticle/nanogel matrix) amount fixed at 0.659 gm. Optimum Cu loading was quantified by the atomic absorption spectroscopy (AAS). Sample preparation for the AAS involved extraction of Cu from the sample using EDTA chelates. The Cu loading was 401 ppm as measured by AAS.

(c) Studied adherence property of CuSiNP nanoformulation. Unlike CuSiNG, CuSiNP formulation is 100% water based. When sprayed on the citrus plants (variety - orange and grapefruit; potted) using a regular spray-bottle, the CuSiNP nanoformulation formed droplets on to the citrus leaf surface (as expected). Once droplets dried up it formed whitish-blue spots of CuSiNP material on the leaf surface. Plants were them placed in open air for about a weak that received afternoon rain showers (up to 1 inch of rain fall). We clearly observed the characteristic spots of CuSiNP material, showing that CuSiNP adhered well onto the leaf surface.

Future Plan: We have completed first round optimization of CuSiNP and CuSiNG synthesis protocols. We will continue materials characterization of CuSiNP/NG as proposed. Cu release study will be performed as well.

Task 2 (Assessment of the antimicrobial activity of the CuSiNP/NG nanoformulation): We have performed preliminary disk-diffusion assay (DDA) to evaluate the anti-bacterial efficacy of several CuSiNG as well as CuSiNP samples (synthesized in the Task 1) using E. coli as well as Xanthomonas Alfalfea (a citrus canker surrogate) as model systems. Both the CuSiNG and CuSiNP formulations reproducibly exhibited improved anti-microbial activity with respect to Kocide 3000 as well as Cu-sulfate (positive controls). Note that metallic Cu concentration in all the samples and controls were identical. As expected, SiNP/SiNG material without Cu did not show any anti-microbial property. The anti-microbial activity of CuSiNP/NG was comparable for both E. coli and X. alfalfea.

Future Plan: We will repeat DDA to obtain statistically significant results.

Task 3 (Develop large-scale nanoformulation production set-up): Large-scale synthesis setup (up to 20 gallons) of CuSiNP/NG formulation is ready.

Future Plan: We will synthesize a batch of CuSiNG as well as CuSiNP materials using this setup and characterize them.