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	4/21/10

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

Principal Investigator Swadeshmukul Santra	*PI Last Name* Santra
Email ssantra@mail.ucf.edu	*FDACS Contract Number* 186
Phone 407-882-2848	*Project Duration (years)* 1 *Year of Project* 1
Organization University of Central Florida	*Total Direct Funds (current year)* 90,000

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

Deliverables are summarized below (research results are being compiled and will be published in the near future):
Task 1 (CuSiNP/NG design, synthesis, characterization and protocol optimization): A series of copper loaded silica nanoparticle (NP)/nanogel (NG) formulations (CuSiNG 100, CuSiNG 200, CuSiNG 300, CuSiNP 100 and CuSiNP 200) were synthesized during protocol optimization process. CuSiNP 200 and CuSiNG 300 are two optimized nanoformulations (objectives) that were studied extensively. Silica nanogel (SiNG) without copper was synthesized as negative control. Systematic materials characterization was performed on two optimized nanoformulations (i.e. CuSiNP 200 and CuSiNG 300). The HRTEM images confirmed formation of crystalline copper oxide nanoparticles (<10 nm size) embedded in silica matrix. HRTEM based elemental mapping confirmed presence of Si, Cu and O, showing that Cu is distributed uniformly throughout the matrix. The X-ray diffraction (XRD) study showed that the material is overall amorphous. The presence of ultra-small (<10 nm) crystalline copper oxides could not be detected within the resolution of XRD. X-ray photoelectron spectroscopy (XPS) indicates the co-existence of Cu(I) and Cu(II) within the same silica matrix. HRTEM study (measurement of crystallite line spacing) indicates formation of Cu(I) oxide nanocrystals, thus supporting XPS results. The BET measurements (based on nitrogen adsorption/desorption isotherms) was done for CuSiNG300 material. Data showed that the material is highly porous (pore size 7.4 nm and pore volume 1.023 cc/g) and possess enormous surface area (336.1 m2/g). We expect similar porosity and surface area will be obtained for other formulations as their HRTEM based morphologies are similar. FT-IR studies were performed on Cu salt, SiNG, CuSiNP and CuSiNG. Characteristic FT-IR peak shift in the frequency range between 800 and 1200 cm-1 indicated binding of Cu to silica. EDTA (a strong Cu chelator) extracted all Cu (amorphous and crystalline) from the CuSiNP and CuSiNG materials when treated overnight (confirmed by the HRTEM study). Atomic absorption spectroscopy (AAS) was performed (instead of ICP and UV-VIS) to estimate Cu loading in the formulations. The maximum Cu loading in the optimized formulations, CuSiNG 300 and CuSiNP200 was found to be ~33 w/w % and 45 w/w% respectively. The copper ion release from CuSiNG and CuSiNP was tested for a period of four weeks in rain water and DI water. No detectable amounts of copper ion release from silica matrix was observed by AAS, indicating that CuSiNP/CuSiNG material is likely interacting directly with the bacteria cell surface. The strong adherence property of nanoformulation was assessed at the microscopic level using fluorescently labeled SiNG material (FLSiNG, resistant to 16 mins continuous wash under running water due to high surface area and strong intermolecular forces). The viscosity of the CuSiNG 300 (20-22 cps) and CuSiNP 200 (11-13 cps) was measured using a Brooke field viscometer.
Task 2 (Assessment of the antimicrobial activity of the CuSiNP/NG nanoformulation): The anti-bacterial efficacy of all the CuSiNG/NP formulations was evaluated with E. coli as well as Xanthomonas Alfalfea (a citrus canker surrogate) as model systems using a disc diffusion assay. The CuSiNG/NP formulations exhibited reproducible and improved results over the positive controls, Kocide 3000 and Copper sulfate against both E. coli and X. alfalfae. In collaboration with James H. Graham, preliminary field trials were conducted last season with CuSiNG 100, CuSiNG 200 and CuSiNP100 nanoformulations (Kocide 3000 and SiNG served as controls). Improved protection against canker incidence was obtained from all nanoformulations. Field trial is being continued this year.
Task 3 (Develop large-scale nanoformulation production set-up): All formulations were synthesized both in small scale (up to 1.0 L volume) and large scale (up to 4.5 gallons). We have also established a synthesis set-up with 20 gallons capacity.