Silver suppression of bacteria: Potential treatment to reduce insect vectors and *Liberibacter* in citrus trees

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ABSTRACT:

The citrus industry is in dire need of solutions for Huanglongbing (HLB) disease that is cause by the bacteria Candidatus Liberibacter asiaticus (CLas). A gram-negative organism, it is both difficult to detect and treat within citrus trees. The disease typically results in reduction of fruit yield and ultimately the loss of infected trees, putting a strain on growers to maintain their crop. Oxytetracycline, an antibiotic, was recently approved for use within citrus to control CLas. However, there is a chance for antibiotic resistance to develop, necessitating the discovery of additional treatments.

Metal nanoparticles have been shown to have antimicrobial effects on both gram-negative and positive bacteria[1,2], as well as toxicity against agricultural insect pests[3]. Screening these nano-metals against CLas is difficult as it cannot be cultured. A surrogate gram-negative bacterium, *Agrobacterium tumefaciens,* was used to screen treatments for efficacy. Nano-preparations of metal molecules (Ag, Zn) and Ag combined with Oxytetracycline were screened in a 24-hr growth assay to determine the Minimum Inhibitory Concentration (MIC). The silver+Oxy had significant suppression activity when both were mixed at sub-antimicrobial concentrations.

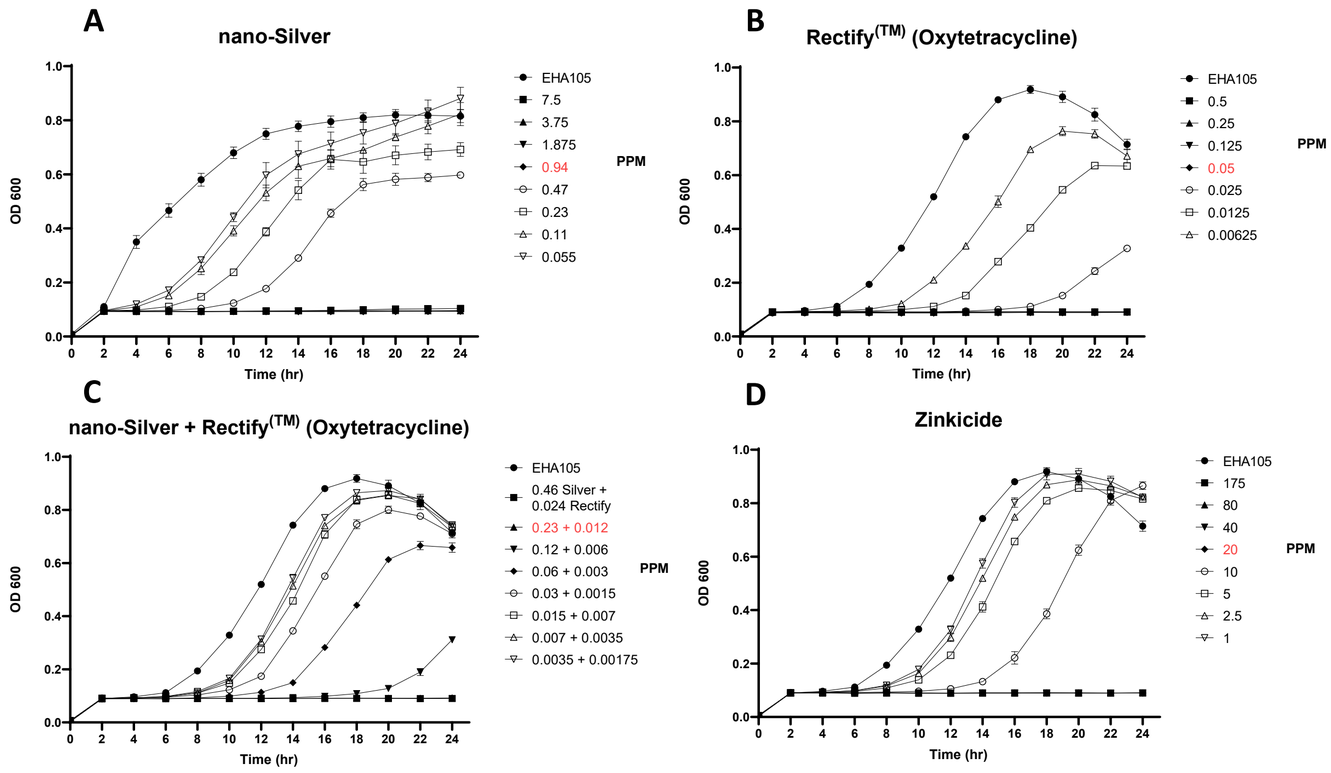
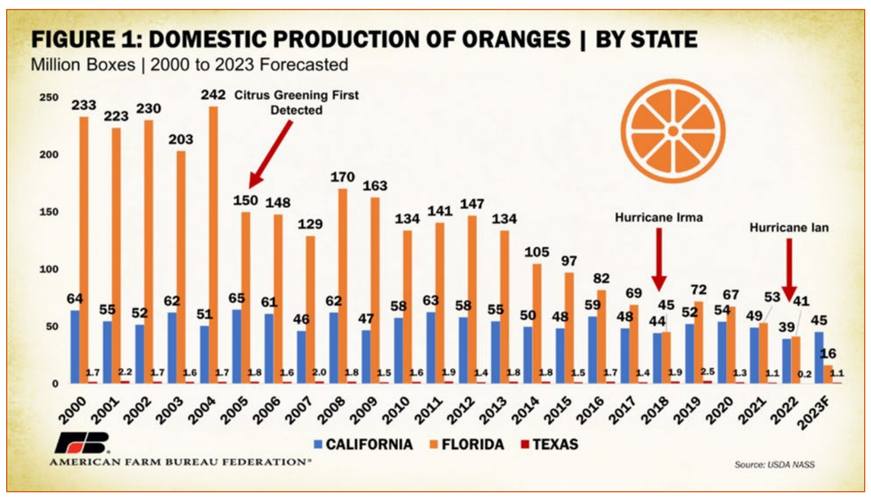


Figure 3. Twenty four hour minimum inhibitory concentration (MIC) Assay. The control =*Agrobacterium tumefaciens* (EHA105) was incubated for 24 hrs in 96-well plates with nano-metal and antimicrobial treatments. Spectrophotometer and optical density readings were taken every 2 hrs. Highlighted concentrations in **RED** indicate the MIC concentration. (**A**) Growth curve for EHA105 treated with nano-silver. (**B**) Growth curves for EHA105 treated with an oxytetracycline commercial product. (**C**) Growth curves for EHA105 treated with the combination nano-silver and oxytetracycline. (**D**) Growth curves for EHA105 treated with nano-zinc. The curves labeled EHA105 in each figure represent the control bacteria with no other treatment added.



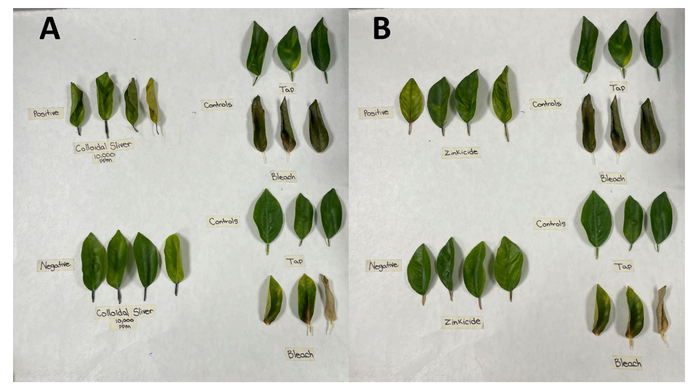


Figure 4. Seven day excised leaf phytotoxicity assay. (A) *Candidatus* Liberibacter asiaticus (CLas) positive and negative citrus leaves exposed to 10,000 ppm nano-silver for 7 days. Positive control is Bleach (1% w/v). Negative control is tap water. (B) *Candidatus* Liberibacter asiaticus (CLas) positive and negative citrus leaves exposed to 1% Zinkicide ®for 7 days. Positive control is Bleach (1% w/v). Negative control is tap water.

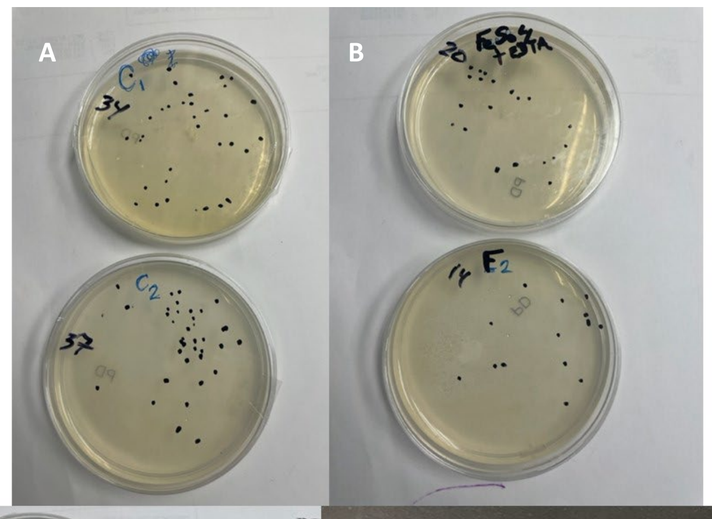


Figure 2. Demonstration of reduced bacterial colony survival post-inoculation with nano-silver. (**A**) Control plates that had *E. sonchifolia* homogenate applied to the media. (**B**) Negative control $FeSO_4$. treated plates *E. sonchifolia* homogenate. (**C**) Treated plates *E. sonchifolia* homogenate with colloidal silver (10nm nanoparticle size). (**D**) Growth on Plates 4 days post-inoculation. (Ag conc. ~1000 ppm)

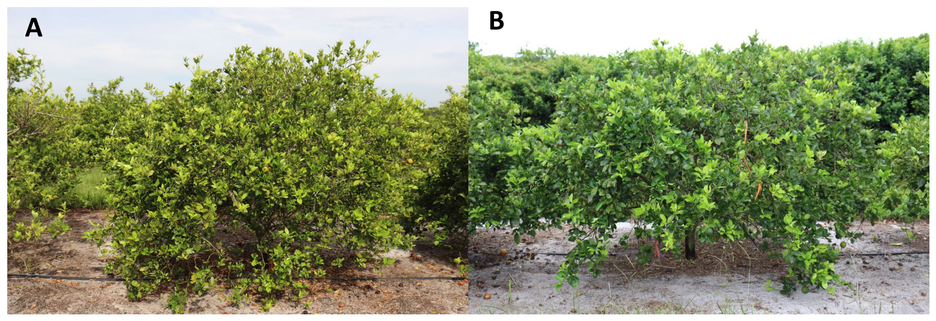


Figure 5. Thirty day post-injection of nano-metals into Valencia citrus. (**A**) Valencia tree treated with 100mL 1% colloidal silver (nano-silver). (**B**) Valencia tree treated with 100mL 1% Zinkicide (nano-zinc).

SUMMARY

- Silver nanoparticles are highly effective in inhibiting bacterial growth.
- Nanometals show significant antimicrobial activity against gram-negative bacteria *Agrobacterium tumefaciens* in lab plate assays.
- Combining the commercially available oxytetracycline (OTC) product, Rectify™, with nano-silver increases the efficacy of both molecules 4-fold on CLas suppression .
- Nano-metals induce slight toxicity in excised leaf petioles after 7 days, however no phytotoxicity is seen in full grown citrus after 30 days post-injection of nano-metals.
- Current studies being conducted in CLas-infected field trees will determine the efficacy as antibacterial treatments and insecticidal efficacy of nano-metal combinations with oxytetracycline.

Acknowledgments

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