# INVESTIGATION INTO THE EFFECTS OF COPPER BASED NANOPARTICLES ON SUGARCANE (*SACCHARUM OFFICINARUM*) AND ZUCCHINI (*CUCURBITA PEPO*)

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# Dedication

To Mom and Dad, I think I'm finally done with school.

*"Science is a way of thinking much more than it is a body of knowledge."*
*– Carl Sagan*

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by

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## Abstract

The widespread use of copper-based nanomaterials has been accompanied by an increasing interest to understand their potential risks. Due to high surface area to volume properties, nanomaterials are more reactive than their bulk counterparts. Copper nanoparticles are used in numerous products, and could enter the environment through their synthesis or incidental use. It is essential to understand the effects of nanoparticles on edible crops by performing both short-term and long-term experiments at relevant exposure concentrations. In order to evaluate biochemical and physiological effects crops, sugarcane and zucchini, were grown in soil amended with: Kocide 3000 (copper-based fungicide), nano-sized CuO (nCuO), a bulk micron-sized CuO (bCuO), copper nanoparticles (Cu NP), and $CuCl_2$. Briefly, our results show immature sugarcane plants increased their activity of stress enzymes ascorbic peroxidase (APX) and catalase (CAT), at varying concentrations. Concentrations of Cu in roots increased with treatment, with minimal translocation into aerial tissues. Mature sugarcane plants showed no changes in Cu concentrations in root and leaf tissues, but superoxide dismutase activity in sugarcane roots treated with Cu NP and $CuCl_2$ decreased by 55%. Zucchini grown for 3 weeks saw Cu concentrations in root, stem, and leaf tissues increase with rising treatment. APX activity in zucchini roots treated with Kocide, nCuO, and bCuO decreased 45%, while CAT activity in roots treated with Cu NP decreased 77%. Similarly, mature zucchini showed Cu increases in root, leaf, and flower tissues, with all applied treatments. CAT activity in roots increased only in plants treated with Cu NP at 400 mg $kg^{-1}$. In all studies, plant growth was unaffected by the applied treatments. Based on the results observed, sugarcane and zucchini displayed minimal negative effects upon exposure to copper-base nanoparticles at the tested concentrations.

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# Chapter 1: Introduction

Nanotechnology is changing every aspect of science. Old technologies and ways of thought have been reinvigorated, thanks in part to the embrace of nanotechnology. Within that emerging world engineered nanoparticles have found a highly productive niche (Behzadnasab et al., 2011; Espitia et al., 2012; Horcajada et al., 2010; Hua et al., 2012; Kim et al., 2012; Koponen et al., 2011; Krishnaraj et al., 2010; Longano et al., 2012; Veiseh et al., 2010). Engineered nanoparticles are materials with at least two dimensions less than 100 nm (Bhatt and Tripathi, 2011). As the dimensions of a particle decrease, the surface area increases; an increased surface area allows a material to be more reactive, or otherwise display properties not found in their larger sized counterparts (Ma et al., 2010). Conventionally engineered nanoparticles are separate into two distinct categories, organic and inorganic (Peralta-Videa et al., 2011). Organic nanoparticles include: Carbon Nanotubes (both single walled and multi-walled) and fullerenes. Inorganic nanoparticles are further divided into: metals, metal oxides, and quantum dots.

Currently engineered nanoparticles such as: $TiO_2$, ZnO, CuO, $CeO_2$, Ag, and Au are found in a wide variety of commercial and industrial products. Sunscreens, food packaging, automotive paint coatings, and electronics are just a few of the everyday products that contain these vital materials. Of these metal-based nanoparticles only Ag, $TiO_2$ and ZnO have applications approved by the FDA (anti-microbial bandages and sunscreens, respectively) (Sadrieh, 2005).

The ubiquity of nanoparticles poses distinct environmental challenges, especially at the massive quantities being produced. Keller et al. estimates the production of nano-copper and nano-copper oxides at 200 metric tons in 2010 (Keller et al., 2013). The fate of these manufactured materials was traced through the environment and was shown that approximately 25% of these materials were located in the soil, air, or bodies of water. The contamination of

soil and water with nanoparticles leaves plants, wildlife, and humans at risk of exposure with potentially dangerous side effects (Baek and An, 2011; Buffet et al., 2013, 2011; Gomes et al., 2011; Manusadžianas et al., 2012; Saison et al., 2010; Shaw et al., 2012). In addition to contamination via waste disposal, there is also an increased demand for authorization to apply copper-based nanoparticles onto agricultural lands and crops (Naderi and Danesh-Shahraki, 2013). These demands bring urgency to the need to study copper-based nanoparticles such as: Copper metal nanoparticles (Cu NPs), nano-sized CuO (nCuO), a bulk micron-sized CuO (bCuO), and the commercially available fungicide Kocide 3000 ($Cu(OH)_2$). Currently there is not sufficient evidence to determine the implications of these nanoparticle's application onto food crops over the entire life cycle of the treated plants.

Interactions between nanoparticles and plants have been well documented, but the research area has a sizeable knowledge gap when it comes to copper-based nanoparticles(Clément et al., 2013a; Hong et al., 2014; Lee et al., 2012; Schwabe et al., 2013; Wang et al., 2015). Previously conducted studies using copper metal nanoparticles (Cu NPs) in hydroponic environments have shown a 77% reduction in root emergence and a 90% decrease in the biomass of squash (Stampoulis et al., 2009). A similar study also found that the growth and transpiration of squash was reduced by 60-70% in the presence of Cu NPs (Musante and White, 2010). Both of these studies found similar albeit, less toxic effects when non-nanosized (bulk) copper was exposed to the plants. Cucumber seedlings grown in suspensions of CuO, Cu metal, ZnO, or Zn metal NPs saw a significant decrease in biomass (Kim et al., 2012). Plants exposed to Zn or ZnO NPs experienced a 56% and 42% decrease in biomass, respectively. Alternatively, cucumber seedlings exposed to nCuO experienced a higher decrease in biomass, 75%, compared to 33% in plants exposed to Cu NPs. Atha et al. found significant amounts of DNA damage to radish sprouts grown in the presence of nano-sized copper oxide (nCuO). This same study also found decreased growth in radish, perennial ryegrass, and annual ryegrass (Atha et al., 2012).

Hydroponic studies have also been published using core/shell nanoparticles. Core/shell nanoparticles are materials that combine a metal core with a metal oxide shell, or vice versa. Trujillo-Reyes et al. sprouted lettuce in suspensions of Cu/CuO NPs and found diminished chlorophyll levels, up to 14%, as well as lower biomass and up to 50% shorter roots (Trujillo-Reyes et al., 2014). These results were mirrored in lettuce plants grown with copper ions from $CuSO_4$. Numerous hydroponic studies have been published using copper-based nanoparticles on different plants, ranging from aquatic weeds to maize, all obtaining similar results of diminished biomass, increased oxidative stress, and reduced chlorophyll content and photosynthetic activity (Hong et al., 2015; Johnson et al., 2011; Lee et al., 2013; Nekrasova et al., 2011; Perreault et al., 2010; Shaw and Hossain, 2013; Shi et al., 2011; Wang et al., 2012).

Servin et al. have demonstrated the uptake and translocation of intact nanoparticles, using hydroponically grown cucumber exposed to nano-sized $TiO_2$ (Servin et al., 2012). Using synchrotron and micro X-ray absorption near edge structure (micro-XANES) spectroscopy, Servin was able to confirm the chemical speciation of the translocated titanium to be consistent with $TiO_2$ nanoparticles. Translocation of nCuO has also been demonstrated using maize grown in a suspension of nanoparticles. Transmission Electron Microscopy (TEM) images confirmed transport of nCuO from root to shoot in the plant phloem (Wang et al., 2012). Other studies completed with wheat grown in sterilized sand have further confirmed the uptake of nCuO into plant shoots (Dimkpa et al., 2013, 2012).

From the literary search conducted here, few studies were performed using soil as the growth medium. In one report lettuce was sprouted in soil amended with Cu NPs; the authors found no difference in shoot-root ratios, when planted immediately after exposure (Shah and Belozerova, 2009). After a 15-day incubation period, higher concentrations of Cu NPs produced seedlings with high shoot/root ratios, indicating a reduction in root growth. Ebbs et al. looked at the effects of nCuO exposure to carrots grown in sand (Ebbs et al., 2016). Following 16 weeks of growth, the treated carrots showed no effects on biomass or root/shoot ratios when

compared to controls. The authors also found higher copper concentrations in the aerial tissues as opposed to the edible tap root.

It is clear that the vast majority of studies on the interactions of copper nanoparticles and plants are lacking in two serious ways: (1) The studies focus on plants grown in either a nutrient solution suspension or in soil medium that has been washed and/or sterilized. (2) The studies monitor only the effects that manifest during the early growth stages. This illustrates the need for experiments to be completed under more realistic conditions, using natural soil that has not been sterilized, with the effects of nanoparticle exposure going beyond the developmental stages and through the entire life cycle. It is also apparent for the literary search conducted, that more exploration of copper-based nanoparticle interaction with food crops is needed.

Sugarcane (*Saccharum officinarum*) is a perennial grass with a life cycle of approximately 12 to 20 months, and is grown predominately in tropical to subtropical regions. According to the FAO cane sugar accounts for 70% of the world's sugar supply, over $1.9 \times 10^{12}$ kg, making it the top commodity in 2016 (Food and Agriculture Organization, 2016) . The United States is the 10th largest grower of sugarcane, approximately $2.8 \times 10^{10}$ kg, with the majority of cultivation occurring in: Florida, Louisiana, Texas, and Hawaii. Although the US is one of the top 10 producers of sugar cane, the vast majority of cane production occurs in newly industrialized countries such as: Brazil, China, India, and Mexico. These four countries alone account for 68% of total production, making sugarcane an important crop on both the national and global stages.

In 2017 the average American consumed nearly 18 kg of refined cane or beet sugar (USDA ERS, 2018). The potential for the translocation of copper nanoparticles into the aerial tissues of the sugarcane plant could present two potential problems: (1) the presence of copper nanoparticles in the edible tissues of sugarcane provides a direct route into the food chain, and (2) nanoparticles accumulated in the leaf tissue of sugarcane could be redistributed in the surrounding environment. In many parts of the world the edible parts of sugarcane are directly consumed, either by chewing of the stalk to extract the sugary juices or by pressing the stalk to

make sugarcane drinks. Mechanized harvesting of sugarcane requires the removal of all leaf tissue; setting an entire field on fire prior to harvest does this. The incineration of leaf tissue with copper nanoparticles can contaminate the surrounding air, exposing field workers and surrounding communities.

Grey Zucchini is a variety of summer squash belonging to the species *Cucurbita pepo*. Domestic cultivation of all squash varieties in the US is $2.6 \times 10^{11}$ kg, representing a product value of 1.92 million dollars (USDA NASS, 2015). Zucchini is consumed worldwide and can be a significant source of minerals (e.g. magnesium, potassium, and phosphorus) and vitamin C (USDA ARS, 2015). Because there are many varieties of zucchini within the *C. pepo* species, Grey Zucchini could possibly serve as a model for nanoparticle exposure. If it holds true that closely related plant species would show similar effects, then it can be expected that zucchini will behave similar to the closely related cucumber, which has already demonstrated uptake and translocation of metal oxide nanoparticles (Servin et al., 2012).



This study aims to understand the effects and implications of copper-based nanoparticle exposure to both sugarcane and zucchini. To the best of the author's knowledge research into the effects of nanoparticle exposure to sugarcane is completely lacking, and more investigation with zucchini done under soil-based conditions is warranted. Understanding the effects of copper nanoparticle can be carried out by conducting both short term (over a few weeks) and complete life cycle studies on sugarcane and zucchini exposed to varying concentrations of: Kocide 3000 nCuO, bCuO, and $CuCl_2$. Overall plant health will be evaluated by determining nutrient content, via inductively coupled plasma optical emission spectroscopy (ICP-OES), monitoring plant growth, and biochemical analysis for chlorophyll content and enzymatic activity. The uptake of Cu into the root, stem and leaf tissues can be established using ICP-OES.

**<u>Research Objectives</u>**

1. Investigate the potential detrimental effects of Cu NPs, nCuO, bCuO, Kocide 3000, and $CuCl_2$ exposure on sugarcane and zucchini.

2. Determine if nanoparticles will translocate to the edible tissues of sugarcane or zucchini.

**Specific Aims**

- To measure physical changes (plant height, root length, and tissue mass) in sugarcane and zucchini exposed to copper-based nanoparticles.

- Determine biological effects of nanoparticle exposure by measuring biochemical indications of plant health such as: photosynthetic activity, oxidative stress indicators, and nutrient levels.

- Quantify uptake of copper in treated plants in comparison to control plants, confirm that copper speciation is consistent with that of the appropriate nanoparticle.

- Evaluate fruit quality, measure translocation of copper, and determine any correlation between quantity/quality and nanoparticle treatment.

**Hypothesis**

1. Plants exposed to copper treatments will be less productive than control plants.

2. Nanoparticles will be translocated from the roots to the aerial plant tissues, and possibly edible portions.

3. nCuO will be more toxic than bCuO.

4. Fruit production and quality will be negatively affected when exposed to nanoparticle treatments.

# Chapter 2: Biochemical and physiological effects of copper compounds/nanoparticles on sugarcane (*Saccharum officinarum*)[1]

## 1. Introduction

The unique properties of engineered nanomaterials has led to its widespread applications in sectors ranging from medicine and energy to cosmetics and textiles (Jeevanandam et al., 2018). Apart from their increased surface area, copper-based nanoparticles (NPs) have unique electrical, chemical and thermal properties that makes them attractive for commercial purposes (Dang et al., 2011; Kim et al., 2009; Magdassi et al., 2010). Keller et al. estimates the production of nano-copper and nano-copper oxides at 200 metric tons in 2010 (Keller et al., 2013). The fate of these manufactured materials was traced through the environment and was shown that approximately 25% of these materials were located in the soil, air, or bodies of water. The contamination of soil and water with nanoparticles leaves plants, wildlife, and humans at risk of exposure with potentially dangerous side effects (Baek and An, 2011; Buffet et al., 2013, 2011; Gomes et al., 2011; Manusadžianas et al., 2012; Saison et al., 2010; Shaw et al., 2012). In addition to contamination via waste disposal, there is also an increased demand for authorization to apply copper-based nanoparticles onto agricultural lands and crops (Naderi and Danesh-Shahraki, 2013). These demands highlight the urgency to study copper-based nanoparticles such as: Copper metal nanoparticles (Cu NPs), nano-sized CuO (nCuO), a bulk micron-sized CuO (bCuO), and commercially available Kocide 3000 ($Cu(OH)_2$).

Interactions between nanoparticles and plants have been well documented, but the research area has a sizeable knowledge gap when it comes to copper-based nanoparticles (Clément et al., 2013b; Hong et al., 2014; Lee et al., 2012; Schwabe et al., 2013; Wang et al., 2015). Studies have been published showing the effects of Cu NP and nCuO exposure on lettuce, cucumber, wheat, rice, alfalfa, cilantro, maize, and yellow squash (Dimkpa et al., 2013,

[1]Reprinted from Tamez, C., Morelius, E.W., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J., 2019. Biochemical and physiological effects of copper compounds/nanoparticles on sugarcane (Saccharum officinarum). Sci. Total Environ. 649, 554–562. ©2019 Elsevier B.V.