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Potential Of Nanoscale Elements To Control Fusarium Wilt Disease In Tomato (solanum Lycopersicum), Enhance Macronutrient Use Efficiency, And Increase Its Yield

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I dedicate this work

TO ALLAH (SWT)

for his abundant blessings,

and

TO MY LOVING PARENTS

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Abstract

Nanotechnology has a great potential in ensuring food production, security and safety globally. Over the past decade, research on the use of nanomaterials to supply nutrient elements and protect plants from pest and diseases has significantly increased. Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables in the world and United State is one of its largest producers globally generating billions of dollars annually in revenue.. Tomato plants are affected worldwide by Fusarium wilt caused by *Fusarium oxysporum f. sp. Lycopersici*. There is growing concern about excessive use of conventional pesticides in controlling Fusarium and other diseases in tomato production. Nanoparticles have been reported to potentially increase plant growth and yield, and improve the nutritional value by enhancement of essential micronutrient required by the plants. However, little is known about the impact of nanoparticle elements on disease suppression, in tomato. This research was aimed at evaluating the potential of nanoscale elements in suppression of Fusarium wilt disease in tomato, enhance macronutrient use efficiency, and increase its yield.

The research was developed in two phases. In the first phase, three week-old Bonny Best cultivar seedlings were exposed, by root or foliar pathways, to CeO_2 nanoparticles and cerium acetate at 50 and 250 mg/L prior to transplant into sterilized soil. One week later, the soil was inoculated with the fungal pathogen *F.oxysporum* f. sp. *lycopersici* (1 g/kg) and plants were cultivated to maturity in a greenhouse.. Disease severity was significantly reduced by 250 mg/L of nano-CeO_2 and CeAc applied to the soil (53% and 35%, respectively) or foliage (57% and 41%, respectively), compared with non-treated infested controls. In addition, Fusarium infection decreased fruit height (10%), dry weight (42%) and lycopene (17%), and increased the total sugar (60%) and Ca content (140%) in infested untreated control, compared with the non-infested

untreated control ($p \leq 0.05$). Foliar exposure to NP CeO_2 at 250 increased the fruit dry weight (67%) and lycopene content (9%) in infested plant, compared with the infested untreated control. Foliar exposure to CeAc at 50 mg/L reduced fruit fresh weight (46%), and water content (46%), and at 250 mg/L increased fruit dry weight (94%), compared with infested untreated control. Fruit lycopene content also increased by 11% in infested plants exposed to CeAc at 50 mg/kg via root, compared with untreated infested control. Total sugar contents decreased in fruits of infested plants exposed via roots to NP CeO_2 at 50 mg/kg (63%), at 250 mg/kg (54%), CeAc at 50 mg/kg (46%), and foliarly at 50 mg/L (50%) and 250 mg/L (50%), compared with infested untreated control. Overall, the findings show that nano-CeO_2 has potential to suppress Fusarium wilt, improve the chlorophyll content in tomato plants and has negligible effects on the nutritional value of tomato fruit.

In the second phase, we investigated the physiological and biochemical effect of copper oxide nanoparticles on tomato plant grown in *F. oxysporum* infested soil. Bonny Best tomato seedlings (three weeks old) were exposed to copper oxide nanoparticles (nCuO at 250 or 500 mg/L, root and foliar), $CuSO_4$ (25 or 50 mg/L, foliar) and commercial fungicide, Kocide 3000, and transplanted into pots containing 1 kg sterilized soil mixture (1 natural: 2 potting mix). Seven days after the transplant, a group was inoculated with *Fusarium* (1 g/kg soil ~100,000 colonies) and cultivated in a greenhouse until the flowering stage (5 weeks after transplant). The root and shoot physiological parameters, biomass, plant height, chlorophyll content, enzyme activities (polyphenol oxidases and catalase), total proteins, micro, and macro elements were evaluated. Chlorophyll content reduced by 11% in infested control, relative non-infested control but increased in plants exposed to $CuSO_4$ at 25 mg/L (8%) and 50 mg/L (9%), compared with infested untreated control ($p \leq 0.05$). Chlorophyll content was elevated in plants treated foliarly with nCuO at 250

(10%), 500 (14%), and $CuSO_4$ (15%), and via root to nCuO at 500 mg/kg (14%), compared with plant treated with Kocide 3000. Root exposure to nCuO at 500 mg/kg increased Shoot fresh weight by 18%. Root fresh weight increased in plant exposed to foliar treatment with nCuO at 250 mg/L (36%), and root exposure at 250 and 500 mg/kg by 33%, compared with untreated infested control. Root polyphenol oxidase and catalase activities increased plant exposed via root to nCuO at 500 mg/L (178%), and foliarly with CuO at 250 mg/L (138%), respectively, compared with untreated infested control. Overall, nCuO improved the chlorophyll content, increased plant biomass, and improve defense mechanism against the pathogen.

This study revealed that the tested nanoparticles (CeO_2 and CuO) has the ability to suppress Fusarium wilt disease in tomato, improve its chlorophyll content, and increase its yield and alter the nutritional content, and rely on antioxidant and microbial properties of Ce and Cu. These findings opens an opportunity for utilization of these nanoparticle as fungicides. Therefore, formulations containing nanoparticle micronutrients may proffer a new strategy that can suppress plant diseases and increase the yield. However, more research work needs to be done to fully understand the mechanism behind the nanoparticle-pathogen interaction in plants.

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Chapter 1

Introduction

The rise in global population, combined with improved income and dietary changes, is driving an ever-increasing food demand that is expected to rise by 70% in 2050 (Bindraban *et al.*, 2018). Agriculture is the major source of food and feed for humans and domestic animals. However, agricultural crop pests, climate change events such as drought, and low nutrient use efficiency are significant hindrances to achieving global food security (Kegan, 2016). Over 22,000 species of plant pathogens, weeds, insects, and mites are attacking farm produce, globally (Zhang, 2018). Annually, China and the United States utilize approximately 1,806 and 386 million kilograms of pesticides, respectively. Yet, economic losses caused by crop diseases and pests in the United States are estimated at several billions of dollars annually. In the United States, efforts to combat fungal pathogens alone exceed $600 million annually (Oerke and Dehne, 2014). This level of economic loss and inefficiency in food production continue to confound efforts aimed at achieving and maintaining food security (Oerke and Dehne, 2014). The management of plant diseases and pests is particularly challenging, both in terms of timely identification of disease and due to the limited number of management options. There is an increased interest in the application of nanotechnology to enhance the growth, improve the yield and nutritional quality of crops. This is due to its unique ultra-small sizes and large surface areas, which enhance its biological functions in a living system (Kah *et al*., 2016).

Nanotechnology can play a critical role in ensuring global food production, security and safety. Over a period, few isolated systems nanoparticles have been demonstrated to improve growth, suppress disease, and increase yield. These applications have been shown to increased crop production, control pests and disease and ensure proper management of soil quality and plant

health (Servin *et al.*, 2015). Projected socioeconomic prosperity of nanotechnology has increased the global investments by governments, companies, and individuals. The United States, through U.S. National Nanotechnology Initiative (NNI), has invested almost $20 billion in nanotechnology between fiscal years 2001 and 2014 (Sargent, 2013).

Nanotechnology plays a significant role among the latest emerging technological advancements in agriculture because of its verse applications across all stages of production (Ali *et al*., 2014). It is widely used in agriculture due to its potential to enhance plant growth, increase nutrient absorption by plants, reduce agricultural input, increase crop production, and improve food quality and safety, among others. The primary purposes of utilization of nanoscale elements in agriculture are to minimize the use of non-environmental friendly chemicals, reduce the leaching of plant nutrients to fertilizers, and improve crop productivity via disease and pest control (Prasad *et al*., 2017). The overall goal is to ensure food safety and security.

The primary goal of this doctoral research is to investigate the potential of nanoscale elements CeO_2, and CuO to suppress Fusarium wilt in tomato, enhance macronutrient use efficiency, and increase the crop production.

1.1 Engineered Nanomaterials (ENMs)

Nanotechnology can simply be described as the manipulation and utilization of nanoscale elements (nanomaterials or nanoparticles) within the dimension of 1 to 100 nanometers (Hong *et al.*, 2013) taking advantage of its unique physical, chemical, and biological properties. It is widely applied across many disciplines including agriculture, medicine, pharmaceuticals, electronics, communication, energy, cosmetics, water treatment, and environmental remediation. The building blocks of nanotechnology are nanoparticles. One of the unique properties of nanomaterials is the

greater surface area to volume ratio, which makes it highly reactive when compared to the bulk materials. This has greatly increased the application of nanoparticles across virtually all scientific disciplines most especially in technologies (Hong *et al.,* 2013; Reddy *et al.,* 2016).

1.2 Nanotechnology and Agriculture

Nanotechnology is increasingly changing the phase of integrated pest management in agriculture and, if fully explored, it has the potential to revolutionize agriculture (Maynard *et al.,* 2006). As previously mentioned, the general aims of utilization of nanoscale elements in agriculture include reduction of agricultural chemical inputs, improvement of crop productivity, and reduction of agricultural pollution, contamination, and waste. Conventional agricultural practices, which include the use of fertilizers and pesticides, as well as weed control, are expensive and not labor efficient, coupled with the possible health hazard for the farm workers (Gruere *et al*., 2011; Joseph and Morrison, 2006). Different types of products and devices have been developed and currently used to simplify product application to boost commercial agricultural production, which are cost efficient and environmental friendly. These products include nanofertilizers, nanopesticides, nanosensors and transgenic varieties (Hong *et al.,* 2013). The following highlight the impacts of current conventional nanotechnology applications on agricultural practices.

Several types of nanofertilizers can deliver nutrients to the plant crops based on the need for growth and development (Scott and Chen, 2012; Kottegoda *et al.,* 2011; Dimkpa *et al.,* 2012). Though large scale industrial production and utilization of nanofertilizers is yet to be achieved. However, it has been established that nanotechnology can stimulate crop production and minimize the nutrient losses (Dimkpa & Bindraban, 2017). Nanoparticles can also enhance plant growth. Elmer and White (2016) sprayed the foliage of young tomato plants with sonicated suspensions of

NP and grew them in soil infested with *Fusarium oxysporum* and found that NP of CuO increased the fresh weight by 33%. Moreover, Elmer *et al.* (2018) demonstrated in both greenhouse and field experiments that foliar exposure to NP CuO (500-1000 mg/L) reduced Fusarium wilt severity in watermelon by 29% and increased fruit yield in the two field experiments by 39 and 53% as compare with the untreated control. Priester *et al*. (2012) revealed that sandy-silt soil amended with bare ZnO NP at concentrations between 50-500 mg per kg of soil stimulated growth yield and Zn uptake in bean. In addition, different surface coated ZnO NP increased the biomass production of green pea when applied in the soil (Mukherjee *et al.*, 2016). Fungus-synthesized ZnO NPs, when foliarly applied to cluster bean increased the growth and biomass (Raliya & Tarafdar, 2013) and increased the root and shoot growth and nodule development of mung bean rhizosphere (Raliya *et al.*, 2016). Bare Fe_2O_3 NPs were reported to increase the growth, biomass, and Zn content of peanut (Rui *et al.*, 2016).

Over the past decade, the use of nanopesticides in agricultural practices have marginally increased. The second approach for nano-enabled agriculture is nanopesticide. Prevalent pathogenic diseases against plant crops need to be tackled to ensure adequate food production in the world and the use of conventional pesticides is, thus, necessary. Since these chemical pesticides contaminate our immediate environment, and they very expensive, there is a need for alternatives that will be environmental friendly, and cost effective. Nanopesticides are the best available alternative that can minimize these adverse effects because they are bioactive, mostly soluble in water and heat stable than conventional molecules (Bergeson, 2010; Bouwmeester *et al.*, 2009; Bordes *et al*., 2009).

1.3 Tomato crop and *Fusarium oxysporum*

The United States is one of the largest world producers of tomato. The tomato crop (*Solanum lycopersicum*) is the most significant horticultural crop in the world and it is the second most consumed vegetable in the US, producing over $2 billion annual revenue (USDA). Tomato belongs to the Solanaceae family and *Solanum* genus and they are the most cultivated vegetable in the world (4.7 million ha). The fruits vary in size, shape, and color across different cultivars. In addition, tomato is a widely studied fleshy fruit because it is easy to grow and is mostly used as a model plant (Schwarz *et al.*, 2014).

Fusarium wilt is the most common destructive soil borne disease that reduces the growth and production of tomato plants in the world. The disease is caused by the fungi, *Fusarium oxysporum f. sp. lycopersici* which is common to both field and greenhouse cultivations, resulting in great economic losses annually (Bawa, 2016; Girhepuje and Shinde, 2011). The control of Fusarium wilt disease is an uphill task due to the ability of the fungus to remain in the soil dormant for a long period of time in the form of spores (Zeller *et al.*, 2003). The most successful control strategy has been host resistance. Host resistance has been difficult due to a lack of resistant genes and consumer driven preference for susceptible heirloom cultivars. Other traditional methods of controlling this disease is the use of chemical fungicides, which has been proven to be non-environmental friendly and cost ineffective (Servin *et al.*, 2015).

1.4. Conventional Approach to Disease Control and Treatment in Plants

There are number of traditional methods of controlling plant pathogenic diseases, which include cultural practices with sanitation, host indexing, solarization, genetic breeding, use of new pesticides, improved eradication methods, and integrated pest management (IPM) (USDA-ARS).

Genetic breeding has resulted in the development of disease resistant types of cultivar, and is obviously the most successful method of controlling plant diseases. However, not all plant crops have resistant genes available and the controversial public perspectives of genetically modified food stocks are concerning issues. Most of all the conventional strategies are either not ecofriendly, have socio controversies, or very expensive. Hence, there is a need to develop better approaches that will be environmentally friendly, less controversial, and cost effective. One of the most promising strategy is the manipulation of the nutritional status of the plant to boost its defense against pathogenic diseases. One of the major factors that limit adequate nutrient supply to plants is the variation in their nutrient requirements, which affects the range of plant diseases in various ways. Moreover, formulation methods required to improve plant health usually vary with the degree of infection or absence of the pathogen (Servin *et al.,* 2015). For example, visible lack of micronutrients can be mitigated by foliar application of small amounts of micronutrients (B, Cu, Fe, Mn, Zn), but the maintenance of whole plant or root health requires greater element uptake and accumulation.

1.5 Nanoparticles and Disease Control

Micronutrients are essential for plant growth and development, as well as defense against infections and diseases. Infections caused by pathogens can trigger a cascade response in many inhibitory secondary metabolites. The secretion of these secondary metabolites is driven by enzymes activated by micronutrient cofactors. For example, phenylalanine ammonia lyase and polyphenol oxidases, which are plant defense enzymes, can be activated by micro elements Cu, Mn, and Zn in the presence of any injury or infection (Huber and Thompson, 2007; Evans *et al.,* 2007; Duffy, 2007). Many times the rate at which a plant is able to respond to injury or infection

by secretion of the secondary/defense metabolites is a function of its susceptibility or resistance against such infection. Availability of micronutrients in key tissues can play a critical role in building a defense mechanism against any pathogen. However, the availability of many micronutrients is limited by soil pH and translocation of the micronutrients, among other factors. For example, less available microelements like Zn, Mn and Fe in alkaline soil, limit plant defense against any possible infection (Sim, 1986). In addition, translocation of most micronutrients becomes difficult when applied through the leaves because they cannot be transferred basipetally, unlike nitrogen, phosphorous, and potassium (Bukovac and Wittwer, 1957).

Biswas *et al.* (2012) reported that amendment of $CaCl_2$ and orthophosphate enhanced the level of phenolic compounds that suppressed the damage caused by Fusarium wilt disease in tomato. There is a possibility that the ions from the metallic oxides could trigger identical responses to suppress the disease but inadequate availability in soil and shoot to root absorption and translocation remain a challenge. However, there is growing interest in the application of nanotechnology to suppress pathogenic diseases in plants, enhance growth, improve the yield, and nutritional quality of crops. This is because of its unique ultra-small sizes and large surface areas, which enhance its biological functions in a living system (Kah *et al.*, 2016). Moreover, one of the most crucial features of nanoscale elements and their oxides is that the availability and translocation is greatly mediated by their unique, ultra-small sizes. Therefore, formulations containing nanoparticle micronutrients may proffer a new strategy that can suppress plant diseases and increase the yield.

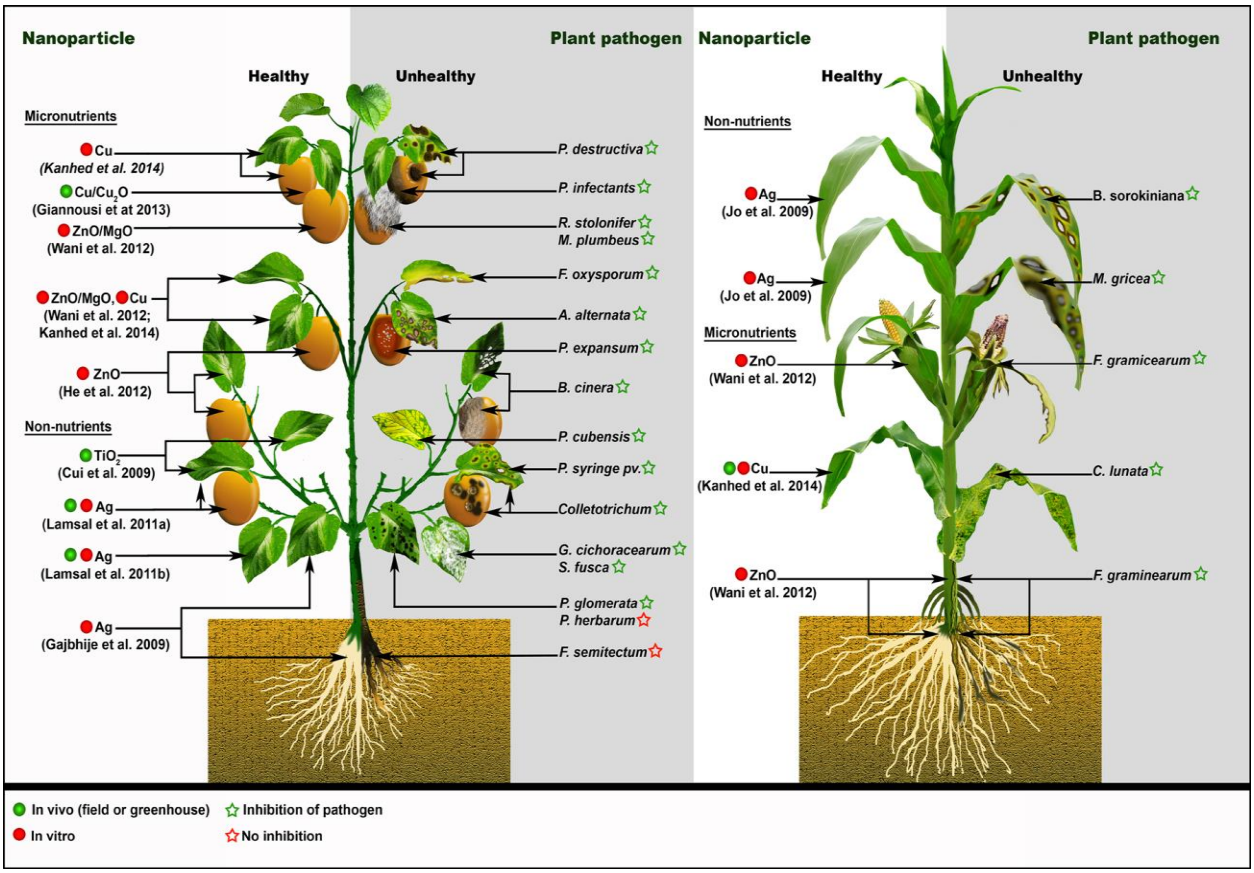


Fig. 1.1 Effect of nanoparticle nutrients and non-nutrients on crop disease. Reprinted from Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J. C., Bindraban P. & Dimkpa, C. (2015). A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *Journal of Nanoparticle Research, 17*(2), 92.

Figure 1.1 illustrates the current literature of the variable effects of nanoparticle types across plant species. The potentials of different nanoparticles in the control of various pathogenic diseases and enhancement of plant growth have been reported. Antimicrobial properties of particles such as Ag, Mg, Si, TiO_2, and ZnO have been reported to be likely responsible for suppression of diseases in plants (Ram Prasad and Prasad, 2014). ZnO NPs have been reported to reduce *Fusarium graminearum* in mung bean broth by 26% compared with the bulk oxide and control (Dimka *et al.*, 2013). ZnO NPs at 3-12 mmol also significantly suppressed the growth of

Penicillium expansum and *Botrytis cinerea* significantly by 61-91% and 63-80%, respectively (He *et al.,* 2011). The mechanism included a hyphal malfunctioning and eventual fungi cell death resulted from physiological disruption of the biological system of the pathogens, according to the authors. This ability to successfully reduce plant pathogenic disease and improve growth result from thier low toxicity and secondary benefits on soil fertility, giving it an advantage over Ag in the fight against fungal infection (Dimka *et al.,* 2013). Moreover, Giannnousi *et al*. (2013) revealed that application of Cu NPs are 75% effective, when compared with the currently available non-nano Cu formulation, which is 57% effective in a field study where tomato (*Lycopersicon esculentum*) was infested with *Phytophthoral infestans*.

Elmer and White (2016) demonstrated that the foliar application of the micronutrient nanoparticles, such as CuO, MnO, and ZnO could reduce disease incidence in tomato grown in soil infested with *Fusarium oxysporum.* The authors revealed that CuO NPs can be used to boost vigor and yield in crops cultivated in disease infested soil as it increased the growth and yield of both tomato and eggplants in the field experiments. Although silver was reported not to be as injurious to microorganisms as silver nanoparticles. It has been widely reported that Ag nanoparticles can inhibit the colonization of *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Khan *et al.,* 2014).

A. Objectives

The following are the general objectives of this research work;

- To determine if root or foliar applications of the nanoparticles (CeO_2 and CuO) are effective in suppressing Fusarium wilt of tomato;
- To evaluate the impact of the nanoparticles on the yield of the tomato plants and nutritional value of tomatoes harvested from plants grown in soil infested with the pathogen;

- To investigate the biochemical response of the plant to the treatments evaluating the stress and defence enzyme activities in the plant tissues.

B. Hypothesis

This research is tailored to test the following hypothesis:

- Nanoparticles (CeO_2 and CuO) can be used to suppress Fusarium disease in tomato, improve its nutritional quality and enhance its growth and yield.

Chapter 2

Role of cerium compounds in Fusarium wilt suppression and growth enhancement in tomato (*Solanum lycopersicum*)

2.1 Introduction

It has been estimated that the agricultural field in the United States, loses hundreds of millions of dollars annually due to soil borne diseases, resulting in displacement of industries and discontinuation of product lines (Kagan, 2016; FAO report, 2015). Soil borne diseases are difficult to manage and can potentially reduce crop yields by 20% (Kagan, 2016). Fungal pathogens alone reduce economic return on yield by approximately $200 million, in spite of the more than $600 million spent per year on control efforts (Tuite and Lacey, 2013). Fusarium wilt is one of the most destructive fungal diseases, decreasing agricultural yield and nutritional value of crops such as soybean, watermelon, eggplant, and tomato, resulting in billions of dollars in annual losses (Servin *et al*., 2015). This scourge, coupled with increasing human population, drastic climate change, and loss of arable land for agriculture, will make the need to double food production by 2050 extremely difficult (Kagan, 2016). Hence, there is urgent need for novel approaches to tackle this menace.

The United States is one of the largest global producers of tomato, the second most consumed vegetable in the country, which generates over $2 billion in annual revenue (Minor and Bond, 2018). Several diseases affect tomato production in the US, but Fusarium wilt is recognized as the most destructive soil borne disease of this plant. The disease is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*, which can affect tomato both in the field and under protected cultivation (Bawa, 2016).

The control of Fusarium wilt is difficult because the fungus may remain dormant in the soil in the form of chlamydospores for a long period of time (Bawa, 2016). The most successful control

strategy for plant pathogens has been host resistance. However, this technique has been limited for tomato due to a lack of resistant genes, consumer-driven preference for susceptible heirloom cultivars, and social unease surrounding the use of genetically modified food. Another traditional control method is the use of fungicides, but this approach is environmentally unsustainable and cost ineffective (Servin *et al*., 2015). Hence, there is significant need to develop novel and more effective strategies for fungal pathogen control.

It has been reported that an improvement in a plant's nutritional status can increase defense against pathogenic diseases (Servin *et al*., 2015). Nitrogen fertilization has been shown to improve plants' defenses against pathogenic infection (Mur *et al.,* 2017). However, continuous nitrogen fertilization causes imbalances in soil microbial communities and is not sustainable (Zhou *et al.,* 2017). Currently, there is great interest in the application of nanotechnology to enhance the growth, yield, and nutritional quality of crops (Dimkpa *et al.,* 2017). This is because of the unique ultra-small size and large surface area of nanoparticles (NPs), which significantly enhances biological activity and functions in biological living systems.

Little is known about the impact of NPs on the suppression of plant pathogenic diseases; recent results highlight increased crop production, pest\disease control, and plant health (Servin *et al.,* 2015). The antimicrobial properties of particles such as Ag, Mg, Si, TiO_2, and ZnO can directly reduce fungal pathogen activity (Servin *et al.,* 2015). For instance, ZnO NPs reduced *F. graminearum* growth in mung bean (*Vigna Radiata*) broth by 26%, as compared with the bulk oxide and controls (Dimkpa *et al.,* 2013). ZnO NPs at 3-12 mmol also suppressed the growth of *Penicillium expansum* and *Botrytis cinerea* by 61-91% and 63-80%, respectively (He *et al.,* 2011). This ability to successfully reduce pathogen activity and to improve growth suggests that nanoscale

nutrients such as ZnO may be a better control option than antimicrobials such as AgNPs to manage fungal infection (Dimkpa *et al.,* 2013).

Foliar application of micronutrient NPs such as CuO, MnO, and ZnO reduced disease symptoms (such as yellowing and browning of older leaves, and stunted growth) in tomato grown in soil infested with *F. oxysporum* (Elmer and White, 2016). Elmer and White (2016) also reported that CuO NPs increased the growth and yield of both tomato and eggplants (*Solanum melongena L.*) cultivated in infested soils. Unlike Cu and Mn, Ce is not a nutritional element for plants; however, it has been reported that nano-CeO_2 enhances plant growth, although the mechanism is still unclear (Servin *et al.,* 2015; Gomez *et al.,* 2017). Additionally, Ce is the major component of "Changle," a rare earth element (REE) fertilizer that contains about 50% Ce and is used in rice, wheat (*Triticum aestivum* L.), and other vegetables (Hu *et al.,* 2004). Nano-CeO_2 was reported to stimulate soybean (*Glycine max* (L.) Merr.) growth (Cao *et al.,* 2017), increasing both shoot and root lengths and chlorophyll content in tomato (Barrios *et al.,* 2016). Moreover, Ce was reported to enhance photosynthetic activity and reduced the inhibition of UV-b radiation in soybean seedlings (Liang *et al.,* 2006). Nonetheless, to the best of the authors' knowledge, there is no information on the role of nano-CeO_2 in the suppression of Fusarium wilt in plants. The objective of this study was to evaluate the potential of nano-CeO_2 to suppress Fusarium wilt disease and to enhance tomato production. Cerium acetate was used as ionic control for comparison. UV-Vis spectrophotometer was used for catalase and polyphenol oxidase assays, single photon avalanche diode (SPAD) for chlorophyll measurement, and inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used to quantify Ce and micro/macro element contents.

2.2. Materials and Methods

2.2.1 Nanoparticle suspension preparation

Nano-CeO_2 (Meliorum Technologies) was obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to Keller *et al.* (2010), nano-CeO_2 have a primary size of 8 ± 1 nm, aggregate to 231 ± 16 nm in deionized (DI) water, have a surface area of 93.8 m^2 g^{-1} and are 95.14% pure. Cerium acetate (CeAc, Sigma-Aldrich) has a size of about 5 μm. Following the procedure previously described by Barrios *et al*. (2016), NP suspensions and CeAc solutions were prepared in DI water at 0, 50 and 250 mg/kg, compound-based concentrations relative to 3 kg of soil (Barrios *et al.,* 2016).

2.2.2 Experimental design, plant materials and inoculation with *F. oxysporum*

Seeds of tomato (*Solanum lycopersicum*), Bonny Best variety, were obtained from Totally Tomato, Randolph, WI. The seeds were washed and rinsed with 4% sodium perchlorate and DI water, respectively, and were germinated in a sterile soilless media (vermiculite) for 21 days. The seedlings were gently washed to remove attached vermiculite and were transplanted into 6.4-liter plastic pots (21.27 cm × 22.86 cm) filled with three (3) kg of natural soil and commercial potting mix at a ratio 1:2. The natural soil had been autoclaved at 121 ^{0}C for 1 h to eliminate microbial and pathogen activity. The potting soil was not sterilized but has minimal microbial activity.

The nano-CeO_2 suspensions and CeAc solutions were applied to the roots/soil or leaves of the tomato plants. For the root application, the three (3) kg soil mixture was homogeneously amended with the prepared suspensions/solutions prior to seedling transplant. For the foliar application, the shoots of 21-day old seedlings were sprayed with 5 ml of the nano-CeO_2 and CeAc suspensions/solutions that had been amended with one (1) drop of a non-ionic surface active agent

(Lesco Spreader-Sticker) to allow retention to the leaf surface. The shoots were allowed to dry, keeping the suspensions/solutions off the roots prior to transplant into the pots containing the soil mixture.

The *F. oxysporum* f. sp. *lycopersici* Race 2 inoculum, isolated from an heirloom tomato cultivar, was obtained from the Scratch Farm, Cranston, RI. Procedures for producing inoculum were as described by Elmer and White (2016). After seven days of the NP/ionic exposure, six treatment replicates were divided into two groups. To infest the soil, triplicates of each treatment were inoculated with *F. oxysporum* by carefully removing the plants and thoroughly mixing the soil with three (3) g of the inoculum per pot (1 g/kg soil ~100,000 colonies) to ensure homogeneity; the seedlings were then re-transplanted. The remaining triplicates were treated as non-infested controls. Plants were watered with 150 ml of water as needed for plant growth. Peter's soluble 20:20:20, nitrogen: phosphorous: potassium (NPK), fertilizer was applied on a weekly basis and the plants were cultivated until full maturity (126 days).

Table 2.1 Treatment name abbreviation

Abbreviations	Treatment/meaning
CTRL/INF	Untreated infested control
CTRL/NI	Untreated non-infested control
Root 50/INF CeO_2	Root 50 mg/kg Nano-CeO_2 Infested
Root 50/INF CeAc	Root 50 mg/kg CeAc Infested
Root 50/NI CeO_2	Root 50 mg/kg Nano-CeO_2 Non-Infested
Root 50/NI CeAc	Root 50 mg/kg CeAc Non-Infested
Root 250/INF CeO_2	Root 250 mg/kg Nano-CeO_2 Infested
Root 250/INF CeAc	Root 250 mg/kg CeAc Infested
Root 250/NI CeO_2	Root 250 mg/kg Nano-CeO_2 Non-Infested
Root 250/NI CeAc	Root 250 mg/kg CeAc Non-Infested
Foliar 50/INF CeO_2	Foliar 50 mg/L Nano-CeO_2 Infested

Foliar 50/INF CeAc	Foliar 50 mg/L CeAc Infested
Foliar 50/NI CeO_2	Foliar 50 mg/L Nano-CeO_2 Non-Infested
Foliar 50/NI CeAc	Foliar 50 mg/L CeAc Non-Infested
Foliar 250/INF CeO_2	Foliar 250 mg/L Nano-CeO_2 Infested
Foliar 250/INF CeAc	Foliar 250 mg/L CeAc Infested
Foliar 250/NI CeO_2	Foliar 250 mg/L Nano-CeO_2 Non-Infested
Foliar 250/NI CeAc	Foliar 250 mg/L CeAc Non-Infested

2.2.3 Disease severity

Disease severity in each triplicate pot was assessed weekly for 18 weeks, as the symptoms manifested using a 1-6 scale, where 1 = no disease, 2 = 1-10 % disease, 3 = 11-25 %, 4 = 26-50 % disease, 5 = 51-75 % and 6 = > 75 % or dead (Jeger and Viljanen-Rollinson, 2001). The disease progress was plotted against time and the area-under-the-disease-progress-curve (AUDPC) was calculated using the trapezoid rule:

AUDPC $= \sum(Y_i + Y_i)/2 \times (t_{i+1} - t_i)$, where Y_i = disease rating at time t_i (Jeger and Viljanen-Rollinson, 2001).

2.2.4 In vitro antifungal activity test

Potato dextrose agar (PDA) was used for in vitro inhibitory test of nano-CeO2 against *F. oxysporum*, following Fraternale *et al*. (2003) with some modification. Nanopaticle suspensions were prepared at 0, 50, 100, and 250 mg/L with DI water, which was then amended with 25% PDA. The mixtures were autoclaved, poured into 10-cm diameter petri dish, and were allowed to solidify by cooling. Mycelial plugs of 4 mm diameter size were cut from the edge of the *Fusarium* isolates grown on PDA for 7 days and were placed at the center of triplicate petri dish containing the nano-CeO_2 suspensions. The inoculated dishes were then incubated at 28 ºC for 7 days. The

inhibitory potential of nano-CeO_2 was determined by mycelial expansion (cm), measuring the diameter of the spore germination at 2-, 4-, and 6-d intervals (Fraternale *et al*., 2003).

2.2.5 Chlorophyll content

The chlorophyll content was determined by using hand held single photon avalanche diode (SPAD, Minolta Camera, Japan) (Dimkpa *et al.,* 2017). Six leaves per plant were randomly selected and average chlorophyll content was determined using SPAD, 5 weeks after transplant, when the symptoms of Fusarium wilt had developed, and at harvest (18^{th} week).

2.2.6 Plant harvest and agronomical parameters

At full maturity (126 days), the plant tissues (roots and shoots) were washed and rinsed 3 times with a 5% $CaCl_2$ and Millipore water (MPW) (Hong *et al.,* 2016). The length and weight of individual fresh plant tissues were recorded. The fresh root samples were collected for enzyme assays; the leaf, stem, and root samples were also separated for elemental analysis. The remaining plants were oven dried for 72 h at 60ºC to determine the total biomass. The fruit from each plant was collected and weighed upon ripening until day 126. The size, total mass, and total number of fruit produced by each plant was determined at harvest.

2.2.7 Enzyme Assays

Activities of a typical defense enzyme (polyphenol oxidase; E.C.1.14.18.1)) and stress enzyme (catalase; EC 1.11.1.6) were examined in the plant roots. Root extracts following the procedure described by Barrios *et. al.* (2016) were used for enzyme analysis. The extracts were centrifuged at 9600 X g for 10 min at -4 ºC (Eppendorf AG bench centrifuge 5417 R, Hamburg,

Germany), and the supernatants were collected in 2 mL Eppendorf tubes for analysis (Barrios *et. al.,* 2016).

2.2.7.1 Catalase (CAT; EC 1.11.1.6) activity

Following the method described by Gallego *et al.* (1996) a reaction mixture containing 950 µL of 10 mM H_2O_2 and 50 µL of the enzyme extract was shaken three times in a quartz cuvette. The absorbance of the mixture was read and recorded for three min at 240 nm using a Perkin Elmer Lambda 14 UV/Vis Spectrophotometer (single-beam mode, Perkin Elmer, Uberlingen, Germany). Catalase activity was expressed as the amount of enzyme required to degrade 1 µmol of H_2O_2 per minutes.

2.2.7.2 Polyphenol oxidase (PPO; E.C.1.14.18.1) activity

The PPO activity was determined following Mayer *et al.* (1965) with slight modification, as previously reported by Anusuya and Sathiyabama (2015). The reaction mixture containing 1.5 ml of 0.1 M potassium phosphate buffer at pH 6.5 and 0.2 ml of the enzyme extract was initiated by addition of 0.2 ml of 0.01 M catechol. The absorbance was recorded at 495 nm using a Perkin Elmer Lambda 14 UV/Vis Spectrophotometer (single-beam mode, Perkin Elmer, Uberlingen, Germany) to determine the enzyme activity. The PPO activity was defined as change in absorbance at 495 nm per minute per milligram protein (Mayer *et al.*, 1965).

2.2.8 Accumulation of cerium, micro and macro elements in plant

Cerium and selected micro/macro element (Ca, Fe, Zn, Cu, Mn, Al, P and K) concentrations were determined in the plant tissues. At harvest, portions of roots, stems, and leaves

tissues were rinsed three (3) times using a 5 % $CaCl_2$ and Millipore water (MPW), and were oven dried at 70 ºC for 72 h. Plants tissues were acid digested for elemental analysis following an EPA method as described by Ebbs *et al*. (2016). The Ce and micro/macro element content was quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer, Optima 4300 DV, Shelton, CT). To validate the digestion and the analytical methods employed, blanks, spikes, and a standard reference material (NIST 1547, Gaithersburg, MD, peach leaves) were used. To ensure quality control and quality assurance, ICP readings of the blank and the standard were repeated after every 15 samples (95% recovery).

2.2.9 Statistical analysis

Triplicate samples were used for all treatments. All data sets were subjected to one-way ANOVA to determine the level of significance of means differences and a Tukey's HSD test at confidence level ($p \leq 0.05$) using SPSS 22 software support. Data were presented as mean ± standard errors (SE).

2.3 Results and discussion

2.3.1 Disease Severity

The symptoms of Fusarium wilt became evident on the infested plants at the fourth week after soil inoculation; disease progression was monitored until harvest and was estimated using AUDPC (Figure 2.1). The root or foliar application of nano-CeO_2 at 50 mg/L had no impact on disease suppression of the disease as compared with nontreated infested control (Figure 2.1). However, at 250 mg/L both root and foliar applications significantly decreased the disease severity by 53% and 57%, respectively, compared to the control ($p \leq 0.05$). Similar results were also

observed with CeAc. There was no effect at 50 mg/L, whereas, 250 mg/L of foliar or root application reduced the disease progression by 41 % and 35 %, respectively ($p \leq 0.05$) compared to the infested control (Figure 2.1). The potential of Ce compounds to enhance plant growth and improve resistance against infection could be attributed to characteristics of lanthanide group of elements (such as antioxidant and photosynthetic enhancement), which cerium belongs to (Liang *et al.,* 2006). Micro-fertilizers containing rare elements have been extensively used in China since the 1970s to promote plant growth, productivity, and improve resistance against stress (Liang *et al.,* 2006; Huang *et al.,* 2005). A rare earth nitrate fertilizer known as "Changle," with more than 50 % CeO_2 in composition, is commonly used in China to fertilize rice, wheat, soybean, and peanuts (Hu *et al.,* 2004). However, since a similar effect was observed in infested plants treated with CeAc, the antifungal activity could be attributed to the antioxidant property of Ce in general. Cerium coexists in Ce^{3+} and Ce^{4+} oxidation states (Ma *et al.,* 2016), which enhances its antioxidant properties. Liang *et al*. (2006) reported that Ce improves photosynthetic parameters, reducing the inhibition of UV-b radiation in soybean seedlings. The mechanism by which the cerium compounds suppress disease is unknown; however, previous reports indicated that CeO_2 NPs inhibit the growth of *Escherichia coli* and *Bacillus subtilis*. (Pelletier *et al.,* 2010). Yan (1999) revealed the protective potential of rare earth elements on the growth and physiological metabolism of wheat under acid rain stress. Huang *et al.* (2005) also reported that Ce can reduce the inhibitory effects of acid rain on the growth and germination of barley by quenching excessive free radicals generated by the acid stress and by promoting chlorophyll synthesis and root growth. It is possible that reactive oxygen species (ROS) generated by pathogen infection can be mitigated by the cerium compounds (Rico *et al.,* 2013).

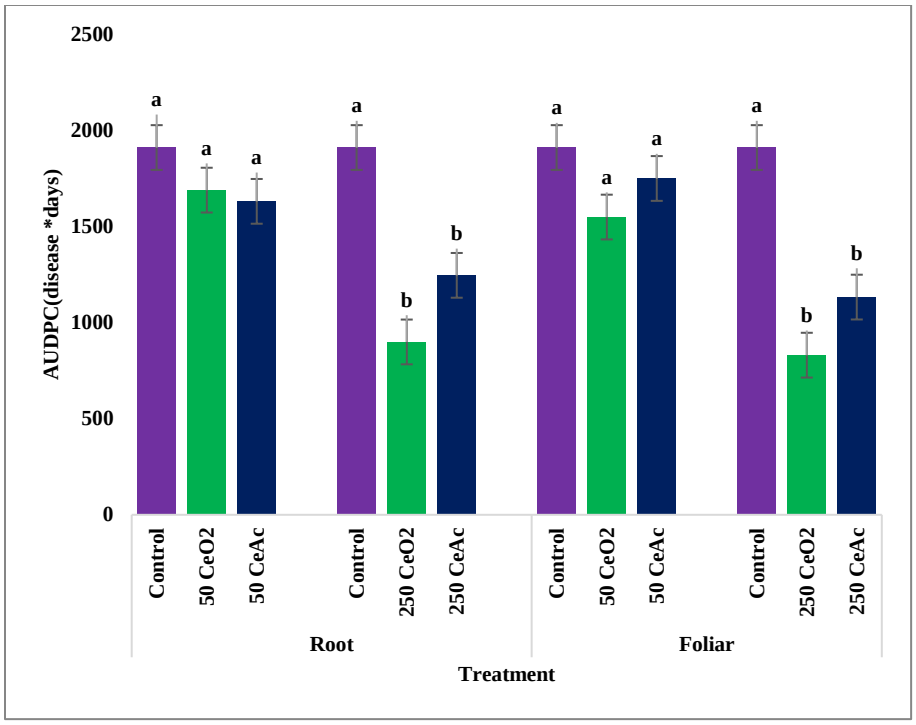


Figure 2.1 Effect of root and foliar applications of nano-CeO_2 and CeAc at 0, 50 and 250 mg/L on Fusarium wilt infested tomato plants grown for 18 weeks. The disease progression was monitored and estimated over time using AUDPC between 5^{th} to 18^{th} weeks. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

2.3.2 Antifungal activity test

There were no significant changes in the diameter of spore germination at two, four, and six days upon exposure to 50, 100 and 250 mg/L as compared with the control ($p \leq 0.05$). This demonstrates that nano-CeO_2 is not acting as a direct inhibitor on the pathogen, at least under in vitro conditions. Previous studies have demonstrated anti-microbial properties of nano-CeO_2. Pelletier *et al.* (2010) revealed that CeO_2 NPs (at 0.5 % wt/vol) can inhibit bacteria and reduce

overall viability. The reasons for this discrepancy are not known but could be related to differences in the nature of the exposure or the pathogen (bacteria vs fungi).

2.3.3 Effect of cerium compounds on chlorophyll content

Figures 2.2 and 2.3 display the chlorophyll content in leaves of tomato plants exposed to nano-CeO_2 and CeAc with or without *F. oxysporum* infestation at weeks 5 and 18 after transplant, respectively. At week 5, the relative chlorophyll content of the plants was not affected by the root and foliar applications of nano-CeO_2 and CeAc, regardless of the concentration or infestation (Fig. 1). This could be a result of the early stage of infection and plant growth. Cao *et al*. (2017) reported that uncoated nano-CeO_2 at 10, 100 and 500 mg/kg soil had no significant impact on total chlorophyll in soybean. At week 18, the chlorophyll content of Ce treated, non-infested plants, was similar to that of non-infested control (Fig. 2). However, the chlorophyll content of infested control reduced by 32 % ($p \leq 0.05$) compared with the non-infested control. This is an indication that the Fusarium infestation affected the photosynthetic system of the infested plants. Similarly, the chlorophyll content of infested plants exposed with nano-CeO_2 at 50 mg/kg via roots reduced by 29 % ($p \leq 0.05$) compared with the non-infested plants treated to nano-CeO_2 at 50 mg/kg via roots (Fig. 2). However, none of the treatments in the non-infested plants affected the chlorophyll content at week 18, compared with the non-infested control. Plants grown in infested soil treated with CeAc at 50 mg/kg exhibited a 36 % increase in chlorophyll content compared with the infested control ($p \leq 0.05$) (Fig. 2). Infested plants foliarly exposed to 250 mg/L of nano-CeO_2 also exhibited significant increases chlorophyll content (28 %, $p \leq 0.05$) compared with the infested control (Figure 2). Conversely, exposure of infested plants to 250 mg/L of nano-CeO_2 or CeAc via the roots, and CeAc at 250 mg/L via the leaves did affect the chlorophyll content. Leaf pigments,

including chlorophyll, are known to change in response to stress (Du *et al.*, 2017). It has been previously reported that nano-CeO_2 and other NPs alter chlorophyll content in plants (Du *et al.*, 2017; Cao *et al.*, 2017). Cao *et al.* (2017) reported that PVC-coated CeO_2 NP at 10 mg/kg increased the total chlorophyll content in soybeans. However, Du *et al*. (2017) found that CeO_2 NP at 400 mg/kg decreased total chlorophyll content in wheat. The significant increase in chlorophyll content, and likely photosynthetic output at week 18, could be an indication that, relative to infested controls, the treated plants had enhanced tolerance to infection. The stress generated from infection could inhibit the movement of water and nutrients required for photosynthetic activities through the xylem. The data suggest that Ce mitigates the negative impacts of infection, perhaps due to its antioxidant activity. This is in agreement with Rossi *et al*. (2016) which reported a significant increase in chlorophyll content in *Brassica napus* exposed to CeO_2 NPs when grown under stress conditions. Conversely, Rico *et al.* (2013) reported that in non-stressed rice plants, nano-CeO_2, at 125 mg/L reduced the chlorophyll content. Clearly additional investigation is needed to determine the conditions under which Ce (NP or otherwise) impact photosynthesis under a range of stressed and non-stressed conditions.

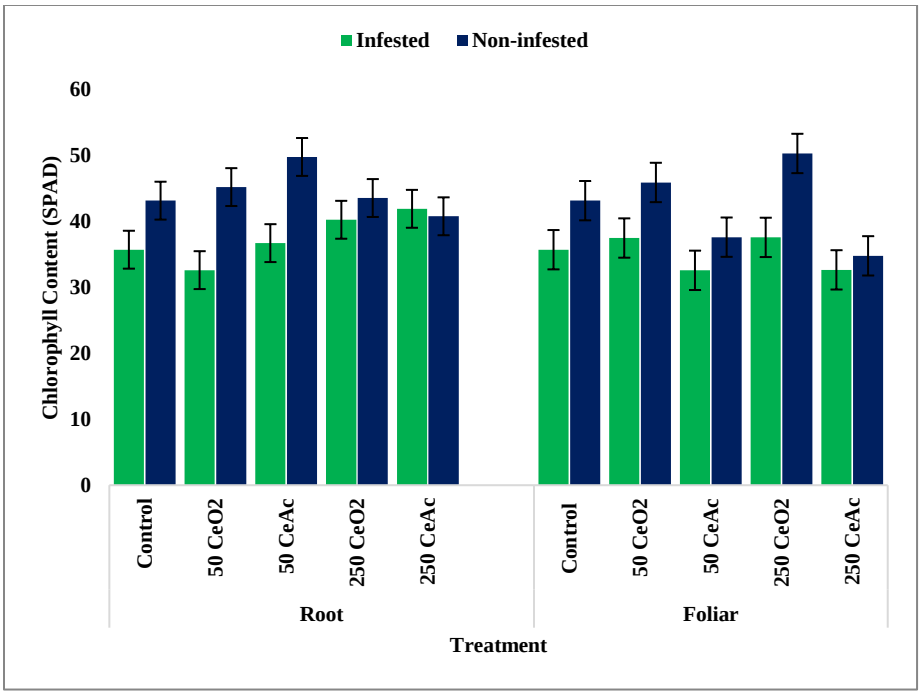


Figure 2.2 Effect on the leaf chlorophyll content of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L, at 5^{th} week. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

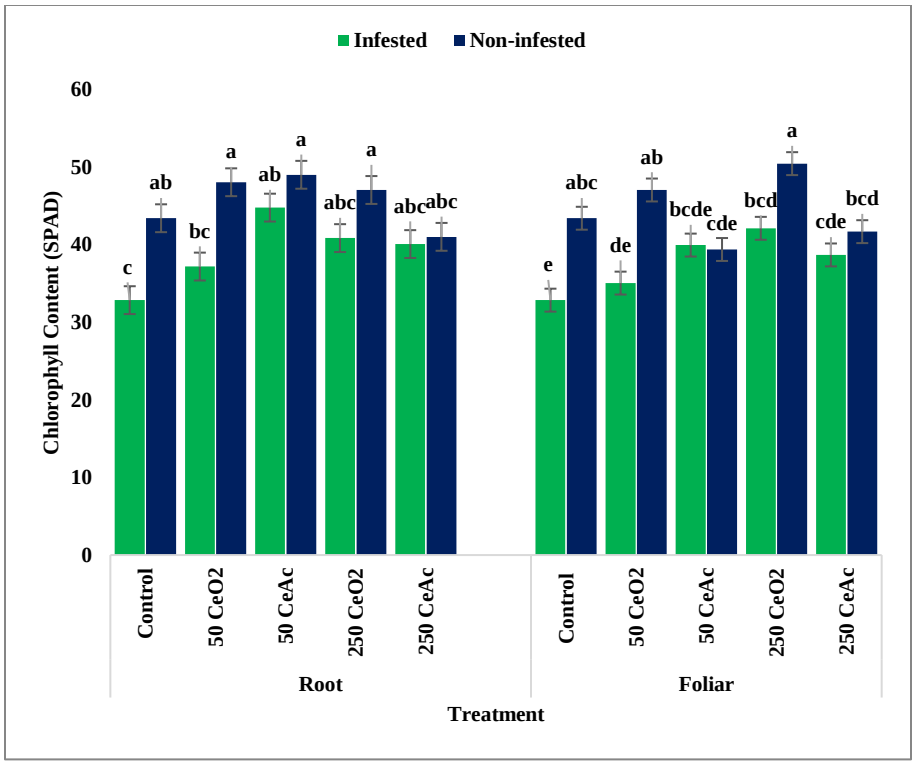


Figure 2.3. Effect on the leaf chlorophyll content of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L, at 18th week. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

2.3.4 Effects of cerium compounds on enzyme activity

2.3.4.1 Catalase (CAT) activity in the roots

Root catalase activity was not affected when the infested control was compared with the non-infested control (Figure 4). Root exposure to both nano-CeO_2 and CeAc at 50 and 250 mg/kg did not alter the root CAT activity in infested plants compared with the non-infested treatments. Also, none of the treatments affected the CAT activity, compared with the infested control. This indicated that the infestation may not effect on CAT activity in the root treatments. Similar results

were found in foliar exposure to CeAc at 50 mg/L and CeO_2 at 250 mg/L in infested treated plants compared with non-infested treated plants. However, foliarly treated infested plants with nano-CeO_2 at 50 mg/L and CeAc at 250 mg/L significantly increased the catalase activities by 65 % and 91 % ($p \leq 0.05$), respectively, compared with the relative treated non-infested plants. However, the root catalase activity significantly increased (137 %, $p \leq 0.05$) after foliar exposure to nano-CeO_2 at 50 mg/L, compared with the untreated infested control (Fig. 4). Nano-CeO_2 is considered an excellent antioxidant because of its role in scavenging free radicals (Ma *et al.,* 2016; Rico *et al.,* 2013). Plants have evolved complex defensive systems against pathogens and oxidative stress, which include the production of antioxidant enzymes such as catalase (Ma *et al.,* 2016). The antioxidant potential of nano-CeO_2 is due to the presence of Ce^{3+} and Ce^{4+} oxidation stages (Ma *et al.,* 2016; Rico *et al.,* 2013). Though disease severity was not significantly reduced by foliar exposure to 50 mg/L nano-CeO_2, an increase in catalase activity for this treatment can likely be attributed to the antioxidant properties of nano-CeO_2 in response to oxidative stress resulting from infection. It is thought that the stress imposed by the pathogens can trigger the generation of H_2O_2, which could possibly be mitigated by the presence of Ce. However, additional investigation is needed to understand the potential antioxidant behavior of foliarly applied nano-CeO_2. Previous studies have shown contradictory roles of CeO_2 NPs as either potential scavenger of free radicals (Yan, 1999), or an inducer of oxidative stress (Ma *et al.,* 2016). These roles depend on the size and surface charge of the NPs, exposure duration, plant species, and age (Ma *et al.,* 2016). However, surprisingly the CAT activity did not increase in plants exposed to 250 mg/L of nano-CeO_2 or CeAc. Perhaps at this concentration, Ce controlled the excess ROS and the plant cells did not need to increase CAT activity since no additional stress was evident.

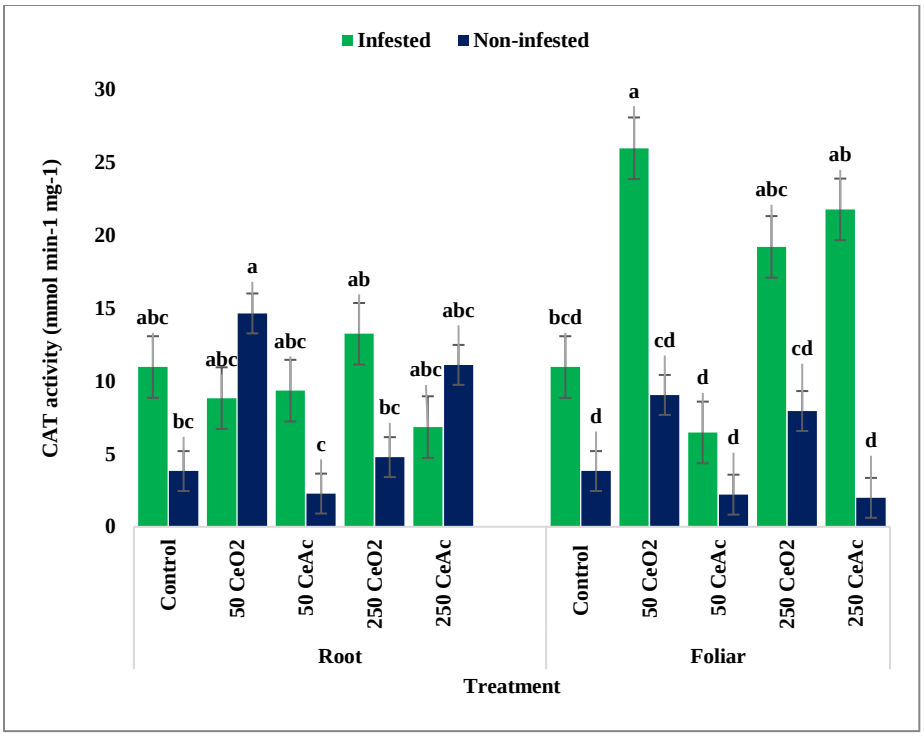


Figure 2.4 Effect on root catalase activity of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

2.3.4.2 Polyphenol oxidase (PPO) activity in the roots

As shown in Figure 5, the root polyphenol oxidase activity increased significantly (81 %, $p \leq 0.05$) in the untreated infested control, compared with the untreated non-infested control. In root applications, only CeAc at 250 mg/kg increased the polyphenol oxidase activity (92 %, $p \leq 0.05$) in treated infested plants, compared with treated non-infested plants. Other root treatments did not altered the polyphenol oxidase activity in treated infested plants, compared with treated non-infested plants (Figure 2.5). However, polyphenol oxidase activity decreased significantly in

infested plants exposed through root to nano-CeO_2 at 50 and 250 mg/kg (59 % and 60 %, respectively; $p \leq 0.05$), or CeAc at 50 mg/kg (49 %, $p \leq 0.05$), compared with infested control. Polyphenol oxidase activity in non-infested plants was unaffected by root or foliar exposure to nano-CeO_2 or CeAc, at both concentrations. Polyphenol oxidases are copper containing enzymes that catalyze the oxidation of phenolic compounds to highly reactive quinones. Quinones may confer resistance to the host plant against pathogenic invasion (Isaac, 1991). Several studies have demonstrated that PPO plays a vital role in the defense response against pathogens, although there is no clear mechanistic evidence for this role (Mayer, 1965; Isaac, 1991). In this study, PPO in roots of all infested Ce treated adult plants, showed no increased activity, which contrasts the possible defense response by the enzymatic activity. It is possible that antioxidant properties of the Ce compounds minimized the plants' PPO response.

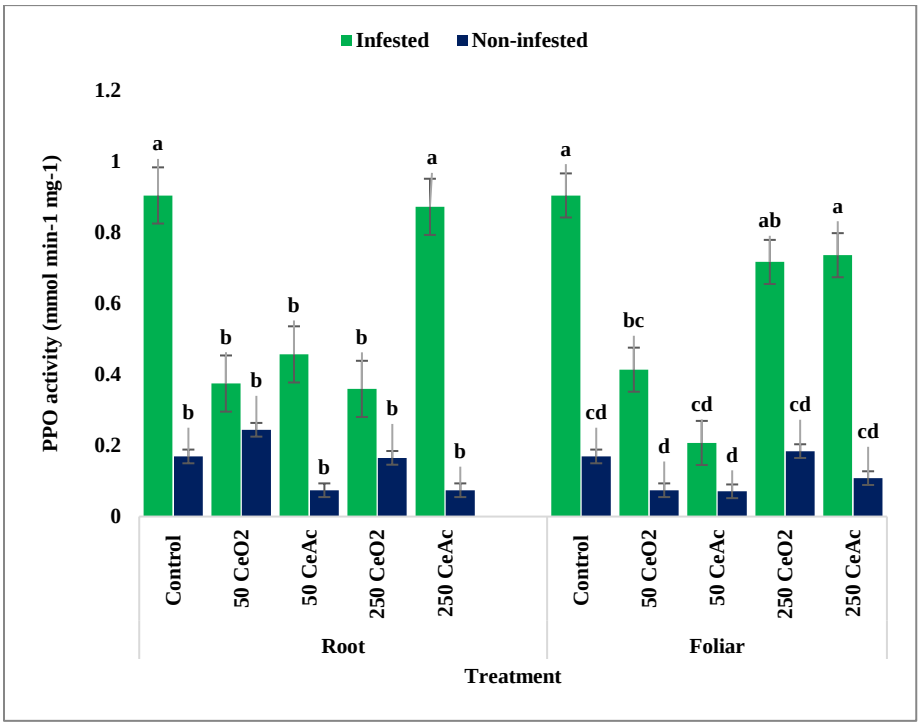


Figure 2.5 Effect on root polyphenol oxidase of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

2.3.5 Effects of cerium compounds on agronomical parameters

The number and weight of fruits are presented in Figures 2.6 and 2.7, respectively. The shoot fresh and dry weights and the shoot length are shown in Table 1. The total fruit weight was not affected by the infestation when the untreated infested control was compared with the untreated non-infested control (Figure 2.6). In addition, none of the root treatments (nano-CeO_2 and CeAc at 50 and 250 mg/kg) altered the total fruit weight in both infested and non-infested treated plants. In foliar application, infestation did not affect the total fruit weight in all treatments when treated infested plants were compared with the treated non-infested plants. However, foliarly exposed plants to CeAc at 50 mg/L reduced the total fruit weight (59 %, $p \leq 0.05$), compared with the infested control (Figure 2.6). Although the light intensity of the green house (340 µmol/m^2 s^{-2}) is good enough for plant growth, it seems it is not high enough for fruit production (Cao *et al.*, 2017). However, the significant reduction observed in fruit yield in term of total fruit weight by the CeAc can be attributed to the dynamic relationship between acetate metabolism and photosynthetic activity that involves both chloroplast and mitochondrion (Heifetz *et al*., 2000). Heifetz *et al*. (2000) reported that acetate can induce reduction in photosynthetic performance in plants, which can ultimately affect the plant yield.

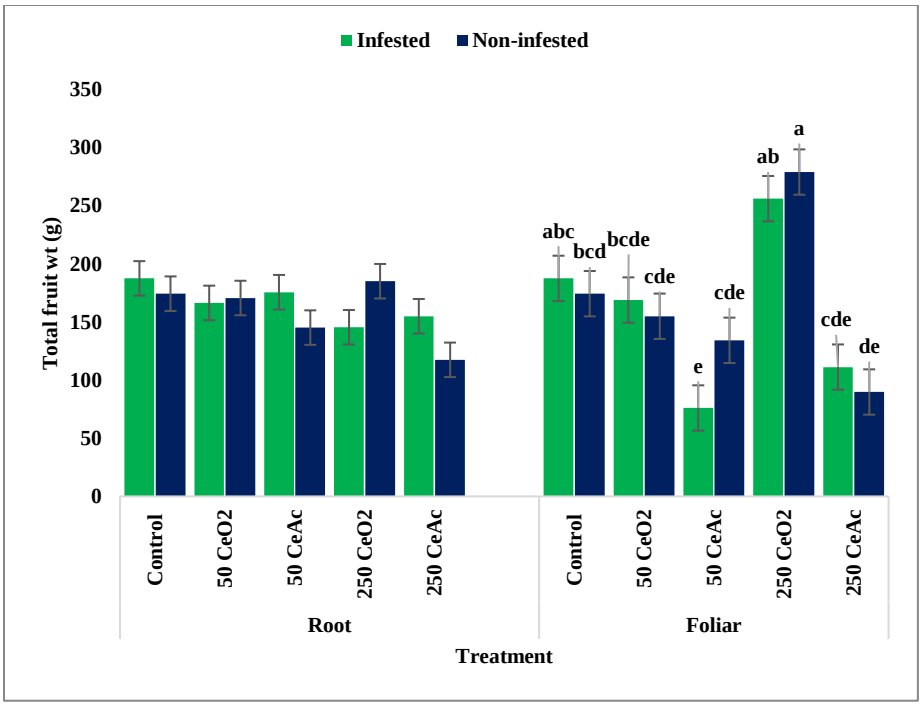


Figure 2.6. Effect on total fruit weight of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

Only non-infested plants foliarly exposed to nano-CeO_2 at 250 mg/L had significant increase in total number of fruit produced (85 %, $p \leq 0.05$), compared with non-infested control (Figure 2.7). The total number of fruits was not affected in the infested control, compared with the non-infested control (Figure 7). Similarly, root and foliarly treated infested plants indicated no changes in total number of fruits, compared with the treated non-infested plants. In addition, none of the treatments (root and foliar) affected the total number of fruits produced in infested plants, compared with the infested control. Barrios *et al*. (2017) also reported no significant changes in

the tomato fruit size and weight (fresh and dry) upon exposure to 0-500 mg/kg; however, at 125 mg/kg, the fruit water content increased by 72 %.

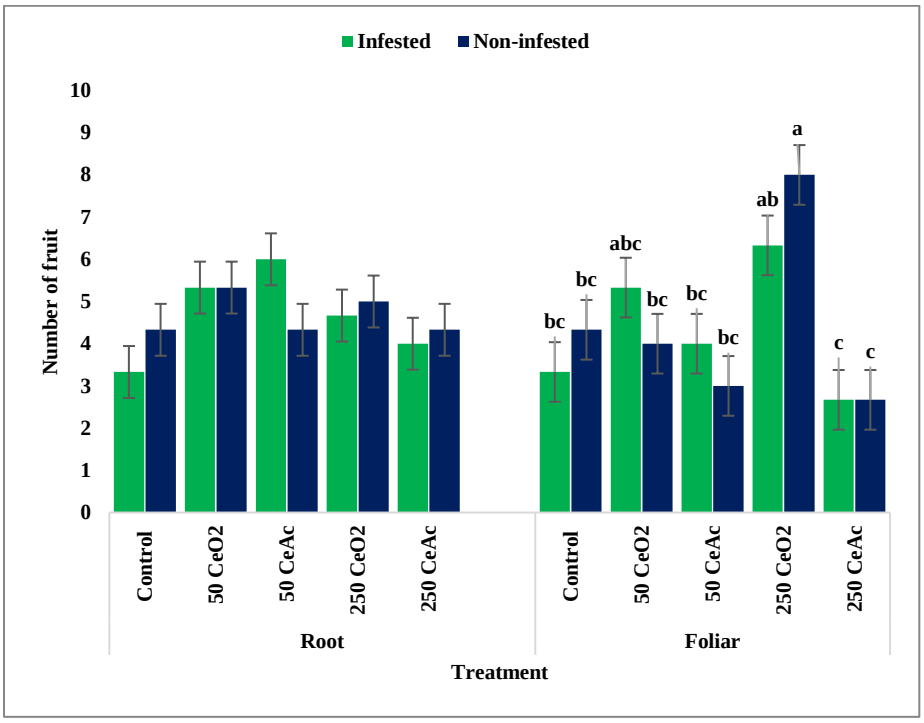


Figure 2.**7** Effect on number of fruit produced in infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

None of the treatments affected the shoot fresh weight (Table 2.1). There was no significant change in the shoot fresh weight of untreated infested controls, compared with untreated non-infested controls. This suggests that Fusarium infestation did not affect the shoot fresh weight of the tomato plants. Similar results were obtained when root or foliarly treated infested plants were

compared with the respective treated non-infested plants. In addition, none of the treatments (root or foliar) affected the shoot fresh weight of infested and non-infested plants, compared with the respective control. Wang *et al*. (2012) did not report changes in size and average weight of tomato plants exposed to 130 mg/L of nano-CeO_2. In the current study, the shoot dry weight was not affected by the Fusarium infestation, when the infested control was compared with the non-infested control (Table 2.1). None of the non-infested treatments affected the shoot dry weight. However, in root application, only infested plants exposed through the roots to nano-CeO_2 at 50 mg/kg had 75 % and 74 % reduction in shoot dry weight, compared respectively, with the non-infested counterpart and the infested control ($p \leq 0.05$). In foliar treatment, only nano-CeO_2 at 250 mg/L exposure reduced the shoot dry weight (56 %, $p \leq 0.05$) in infested plants, compared with the infested control. It has been reported that tomato plants cultivated under controlled greenhouse conditions can emit different volatile organic compounds (VOCs) such as (3E, 7E)-4, 8, 12-trimethyl-1, 3,7, 11-tridecatetraene (TMTT) and n-hexanal, 2-carene, β-caryphyllene (Takayama *et al.,* 2012). Although VOCs were not measured in this study, it is possible that the pathogen and the CeAc can increase the emission of these compounds, thereby, reducing the dry weight (Barrios *et al.,* 2017). In non-infested plants, none of the treatments significantly affected the shoot dry weight.

Table 2.2 Shoot length, fresh, and dry weights of Fusarium wilt infested and non-infested tomato plants exposed through roots or leaves to nano-CeO_2 and CeAc at 0, 50 and 250 mg/L. Measurements were performed 18 weeks (full maturity) after inoculation. Averages with different letters are statistically significant ($p \leq 0.05$), compared with the respective control; n = 3.

	Treatment	Shoot fresh wt (g)	Shoot dry wt (g)	Shoot length (cm)
Root	CTRL/INF	511.33^{ab}	154.33^{ab}	130.67^{ab}
	CTRL/NI	761.33^{a}	181^{ab}	127^{ab}
	50/INFCeO_2	163^{b}	39.67^{c}	94.67^{b}
	50/INFCeAc	397^{ab}	88^{bc}	138.67^{a}
	50/NI CeO_2	585.33^{ab}	156.33^{ab}	159.33^{a}
	50/NICeAc	592^{ab}	149^{ab}	136.33^{a}
	250/INFCeO_2	637.33^{a}	199.67^{a}	126.33^{ab}
	250/INFCeAc	347^{ab}	73.33^{bc}	126.33^{ab}
	250/NICeO_2	619.33^{ab}	170^{ab}	131.67^{ab}
	250/NICeAc	531.67^{ab}	138.33^{abc}	146.67^{a}
Foliar	CTRL/INF	511.33	154.33^{abc}	130.67^{bc}
	CTRL/NI	761.33	181^{ab}	127^{c}
	50/INFCeO_2	658.67	159.33^{abc}	131.33^{bc}
	50/INFCeAc	712	145.33^{abcd}	172^{a}
	50/NICeO_2	755	207.67^{a}	148.33^{abc}
	50/NICeAc	528	122^{bcd}	156^{abc}
	250/INFCeO_2	317	68^{d}	140^{bc}
	250/INFCeAc	485.33	100^{cd}	151.67^{abc}
	250/NICeO_2	746.33	171.33^{abc}	158.67^{ab}
	250/NICeAc	670.67	132^{abcd}	156.33^{abc}

The shoot length was not affected in the infested control, compared with the non-infested control (Table 2.1). Also, none of the root treatments affected the shoot length of the infested plants, compared with the infested control. However, only nano-CeO_2 at 50 mg/kg exposed via roots reduced the shoot length (41 %, $p \leq 0.05$) in infested plants, compared with the treated non-infested plants. This revealed that the treatment triggered the reduction in the shoot length since

the infestation did not affect the parameter in the infested control. In foliar application, only plants exposed to nano-CeO_2 at 250 mg/L increased the shoot length (25 %, $p \leq 0.05$) in non-infested plants, compared with the non-infested control. Moreover, none of the treatments affected the shoot length in infested plants except those treated with CeAc at 50 mg/L, which had 32 % increase in shoot length, relative to the infested control ($p \leq 0.05$). Under insufficient light like in the greenhouse, tomato plants are stressed but tended to grow taller (Barrios *et al.*, 2017). However, Lopez-Moreno *et al*. (2010) reported that nanocera at most concentrations used in the experiment (0-4000 mg/L) promoted shoot elongation in alfalfa and cucumber plants (20-100%). In addition, Majumdar *et al*. (2014) reported that 500 mg/L of nano-CeO_2 increased (26%) the root biomass of kidney beans. However, Trujillo-Reyes *et al.* (2013) reported that nano-CeO_2 reduced the stem length and root biomass of radish seedlings, even though the radish was not diseased at the time. Also, Barrios *et al*. (2016) reported that CeAc reduced the stem length of tomato plants at 250 and 500 mg/kg (12 and 25%, respectively). This was suggested to result from the cerium acetate's superoxide scavenging activity but not catalase activity, which enhances its toxicity (Barrios *et al*., 2016; Pirmohamed *et al.*, 2010). On the other hand, Barrios *et al*. (2017) reported that CeAc at 125 mg/kg increased the water content in tomato, which could result in an increase in shoot length. However, there is little information on the impacts of nano-CeO_2 and CeAc exposure on plant shoot length under the pathogen stress.

2.3.6 Elemental analysis

Concentration of Ce, micro, and macro elements across the tissues of infested and non-infested tomato plants are shown in Table 2.2. Among the essential elements, only those that

showed significant differences in concentration, compared with the respective controls, are discussed.

2.3.6.1 Cerium accumulation

Table 2.3 shows cerium contents across the tissues of infested and non-infested tomato plants exposed to nano-CeO_2 or CeAc, through roots or leaves. Fusarium infection did not affect the Ce accumulation in the roots of infested control, compared with non-infested control. Surprisingly, only infested plants exposed to nano-CeO_2 at 250 mg/kg exhibited significant decrease in the root Ce uptake (71 %, $p \leq 0.05$), compared with the non-infested plants exposed to the same root treatment. It is suggested that the Fusarium infection hindered the Ce element uptake in the root of the plants treated with the nanoparticles via the roots. Moreover, in the root application, only infested plants exposed to nano-CeO_2 at 50 mg/kg, had 219 % increase in root Ce uptake, relative to the infested control ($p \leq 0.05$). The altered accumulation of Ce across the tissues, as a function of disease in the tomato plants, suggests an interaction between the pathogens and Ce; in infested plants specifically, there were changes in Ce accumulation as a function of exposure. The uptake of metal elements by roots can be impacted by both the biotic and abiotic factors, including soil composition, pH, microorganisms, and metal immobilization in the root cell walls (López-Moreno *et al.*, 2010). *Fusarium oxysporum* is known to produce a mycotoxin known as fusaric acid (FA) (Eged, 2005). Fusaric acid (5-butylpiconic acid) is an organic compound capable of chelating divalent metals (Eged, 2005). It is possible that in infested plants, Ce was retained in the soil complexed with FA. In addition, similar results were found in non-infested plants treated with nano-CeO_2 at 250 mg/kg via roots (1058 % increase, $p \leq 0.05$), when compared with the non-infested control. However, none of the treatments affected the root Ce uptake in

infested and non-infested plants exposed to foliar treatment of both nano-CeO_2 or CeAc. Several factors including Ce speciation, soil chelates, and the Casparian strip in plant roots could cause poor translocation of Ce across plant tissues (Hu *et al.,* 2004). A previous study has shown that nano-CeO_2 was poorly translocated to other plant tissues when applied to either roots or foliage, although the concentration used was quite low and the exposure duration was short (Birbaum *et al.,* 2010). Other studies have shown a basipetal movement of Ce from the leaves to other plant tissues (Elmer and White, 2016). However, in the present study, Ce translocation from either application was not enough to achieve statistically significant differences. One of the reasons could be the low dose applied (1.25 mg of Ce to 21-day old plants) and the length of the growth (more than 100 days) that diluted the Ce in the new biomass.

Table 2.3 Concentration of Ce (µg/g) in roots, stems, and leaves of of Fusarium wilt infested and non-infested tomato plants exposed through roots or leaves to nano-CeO_2 and CeAc at 0, 50 and 250 mg/L. Measurements were performed 18 weeks (full maturity) after inoculation. Averages with different letters are statistically significant ($p \leq 0.05$), compared with the respective control; n = 3.

		Root		**Stem**		**Leaf**	
Ce (µg/g)	**Treatment**	**Infested**	**Non-infested**	**Infested**	**Non-infested**	**Infested**	**Non-infested**
Root	Control	1.81^{c}	0.93^{c}	0.03	0	0.38	0.001
	50 CeO_2	5.77^{b}	3.41^{bc}	0	0.02	0.373	0.241
	50 CeAc	1.08^{c}	0.82^{c}	0.06	0.03	0.285	0.233
	250 CeO_2	3.15^{bc}	10.77^{a}	0.05	0.01	0.367	0.317
	250 CeAc	3.92^{bc}	3.88^{bc}	0.06	0.01	0.271	0.126
Foliar	Control	1.8	0.93	0.03	0	0.38^{a}	0.001^{c}
	50 CeO_2	2.18	0.62	0.05	0.01	0.133^{bcd}	0.14^{bcd}
	50 CeAc	0	1.4	0	0	0.02^{cd}	0.002^{d}
	250 CeO_2	0.8	1.53	0.05	0.11	0.285^{ab}	0.186^{bc}

250 CeAc	1.41	3.06	0	0	0.174^{bcd}	0.037^{cd}

In the stem, neither the infestation nor the Ce-compound exposure affected the Ce accumulation. In addition, Ce accumulation in the leaves was not affected by root treatments significantly, regardless of the Fusarium infestation. Conversely, in foliar treatment, leaf Ce accumulation increased by 37, 900%, in infested control, compared with the non-infested control ($p \leq 0.05$). Foliar exposure of infested plants to nano-CeO_2 at 50 mg/L decreased the Ce accumulation in the leaves (65 %, $p \leq 0.05$), relative to the infested control. Moreover, infested plants exposed to CeAc at 50 and 250 mg/L through the leaves showed significant decrease (95 % and 54 %, respectively) Ce translocation to the leaves, compared with the infested control ($p \leq 0.05$). However, only non-infested plants treated with CeAc at 50 mg/L through foliage showed significant increase in the translocation of the Ce element in the leaves (100 %, $p \leq 0.05$), compared with the non-infested control. The increase of Ce in roots is not surprising since Ce was applied to the soil and, given that the roots were acid washed, one can assume much of the Ce was absorbed, although some small amount could remain adhered to surface negative charge of the root cells (Barrios *et al.,* 2016; Hong *et al.,* 2016; Wang *et al*., 2012; Trujillo-Reyes *et al.,* 2013). The increase of Ce in non-infested treated plants is in agreement with the findings of López-Moreno *et al.* (2010) and Wang *et al*. (2012), which showed that soybean and tomato plants accumulate Ce across the plant tissues. In addition, Barrios *et al.* (2016) reported that uncoated nCeO_2 at 62.5 mg/kg increased Ce accumulation in the leaves of tomato plants.

2.3.6.2 Micro and macro element concentrations

The concentration of essential elements (Ca, Fe, Zn, Cu, Mn, P and K) and Al, a non-essential element, is shown in Table 2.4. Three micronutrients (Cu, Mn, and Fe), Al, and the macronutrients Ca and K were altered by the Ce treatments. In the soil application, the root uptake of elements was different in infested and non-infested plants. In infested plants, none of the treatments affected Ca and Mn accumulation. However, nano-CeO_2 at 50 mg/kg increased Cu in roots by 108 %, compared with infested control ($p \leq 0.05$). On the other hand, in non-infested plants, none of the treatments affected Mn and K uptake. In contrast, nano-CeO_2 at 50 and 250 mg/kg, increased Ca by 76 % and 72 %, respectively, compared with the non-infested control. In addition, nano-CeO_2 at 50 mg/kg increased Cu in the roots by 318 %, compared to non-infested control ($p \leq 0.05$). None of the soil treatments affected the uptake of Fe and Al.

Calcium can be translocated to the xylem as Ca^{2+} solely through the root apoplast (White, 2001). It has been reported that rare earth elements (REEs) possess relatively similar characteristics as Ca (Hu *et al.,* 2004). Their ionic radii are within the range of 9.6-11.5 nm, compared to that of Ca, which is 9.9 nm (Hu *et al.,* 2004). Thus, REEs can displace Ca^{2+} at root level, and ultimately, can affect its transportation and physiological function in plants. Surprisingly, in this study nano-CeO_2 increased root uptake of Ca in non-infested plants. Calcium is a messenger that is involved in many physiological responses such as plant growth and development (White, 2001), hormone production, enzymatic activity, nodulation, biotic, and abiotic environmental stressors. Calcium can also be taken up either as Ca^{2+} or can be complexed with organic acids (White, 2001).

Copper is accumulated as Cu^{2+} through the cell membranes by ATPase Cu-transporters (Ma *et al.,* 2016). However, it can also be taken up as Cu^{+} by high-affinity copper transporter

proteins; these proteins are up regulated in the roots by Cu deficiency (Ma *et al.,* 2016). Important enzymes such as polyphenol oxidase (PPO) require Cu as a co-factor for metabolic activity. However, significant reduction in the activity of PPO observed in the infected plants exposed to nano-CeO_2 at 50 mg/kg indicated a reverse response relative to Cu accumulation in the roots. It is hypothesized that the disease was reduced because Cu was used in other defensive enzymes and PPO was not needed. Root exposure of infested plants to CeAc at 250 mg/kg increased K uptake in roots by 444% compared with the infested control. Plant-microbe communication and interactions can be beneficial to both the host plant and the microbes. It has been reported that fungi could act as bioinoculants, altering the membrane permeability of the root cells and subsequently changing plant metabolic activity (Ma *et al.,* 2016). This could facilitate the availability of mineral elements such as K, as observed in the infested plants (Ma *et al.,* 2016). In addition, La and Ca have been reported to inhibit K uptake during short exposures, but enhance its uptake in under longer time periods (Ma *et al.,* 2017). Importantly, the data suggest that CeAc acted similarly to La in accelerating K uptake by tomato roots.

The translocation of elements from roots to stems and leaves was varied as a function of disease/infection. None of the root treatments affected the translocation of Fe, Al and K from roots to the above plant parts in regardless of infestation status. In addition, the translocation of Ca and Cu to the shoots was not affected in infested plants. However, Ca increased by 53 % and 70 % in stems of non-infested plants exposed to 50 or 250 mg/kg of nano-CeO_2, respectively, as compared with non-infested control. Moreover, at such concentrations, nano-CeO_2 increased Ca in the leaves by 39 % and 55 %, respectively. This study revealed a consistent trend with Ca accumulation in tissues of non-infested tomato plants. The data suggest that Ce favored the translocation of Ca from the roots to the shoots. The data also suggests that pathogen presence impacted Ca through

the secretion of fusaric acid. Fusaric acid can bind divalent metals and other organic matter to form chelating complexes in soil. This could reduce the amount of Ca in the tissues of infested plants. Non-infested plants exposed to 50 mg/kg of nano-CeO_2 exhibited 287 % increase in Cu accumulation in the stem as compared with the non-infested control. There is the possibility that the positively charged nano-CeO_2 associated with the fusaric acid, enabling the positively charged Cu particles to be bound by the negative charge of the root surface in the diseased plants (Wang *et al.*, 2014).

Only CeAc affected the translocation of Mn to the aboveground tissues. In infested plants, CeAc at 250 mg/kg increased Mn in stems by 135% compared to infested controls, while at 50 mg/kg, Mn increased in the leaves of non-infested plants by 216%). It is thought that Mn is accumulated by plants mostly in form of Mn^{2+}, depending on environmental factors such as soil pH, plant species, and concentration. The ionic form can move freely in the xylem sap with the transpiration stream (White *et al.*, 1981). However, it has been suggested that Mn could form a complex with other biomolecules, such as carbohydrates or amino acids (White *et al.*, 2009). White *et al.* (1981) reported that most Mn is found freely in the xylem sap of tomato and soybean plants but about 40 % formed complexes with malate and citrate (White *et al.*, 1981). The data from this study suggests that complexation with CeAc may be responsible for the high Mn content observed in the above tissues of infested and non-infested tomato plants. The CeAc may serve as chelating agent for cations and increase their absorption (Barrios *et al.*, 2016).

In foliar applications, both infested and non-infested plants exhibited relatively similar response on the root uptake of some elements. None of the treatments altered root Cu, Mn, Fe, and K concentrations regardless of infestation status. On the other hand, nano-CeO_2 at 250 mg/L increased the concentration of Ca in roots of infested plants by 60 % but reduced Al by 82 %

compared with infested control. However, none of the treatments altered Ca and Al in roots of non-infested plants. A previous study mentioned that Ce can be transported via phloem from the leaves to the rest of the plant (Hong *et al.*, 2016). It is possible that the enzyme mimetic activity of Ce reduced ROS, and favored the uptake of cations that could ultimately increase accumulation of select elements in the root (Yan, 1999). However, this phenomenon requires additional study.

Table 2.4 Concentrations of micro and macro elements (µg/g) in the roots, stems and leaves of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50 and 250 mg/L, and cultivated till full maturity (126 days weeks). Data represent mean (n=3) at confidence level $p \leq 0.05$. Only elements within detection limit and statistically significant from respective controls are presented.

			Root		Stem		Leaf	
Element	Exposure Route	Treatment	Infested	Non-Infested	Infested	Non-Infested	Infested	Non-Infested
Ca		Control	19319±2279^{ab}	13822±1757^{cd}	21271±1954^{a}	11835±1250de	36594±3167^{a}	20508±1478^{bc}
	Root	50 CeO_2	26690±2279^{a}	24334±1757^{a}	21210±1954^{a}	18077±1250^{abc}	42013±3167^{a}	28519±1478^{a}
		50 CeAc	12344±2279^{bc}	10929±1757^{cd}	22746±1954^{a}	14384±1250^{bcd}	36994±3167^{a}	27445±1478^{ab}
		250 CeO_2	16227±2279^{abc}	23771±1757^{ab}	18434±1954^{ab}	20151±1250^{ab}	33088±3167^{ab}	31848±1478^{a}
		250 CeAc	18215±2279^{abc}	15250±1757^{bcd}	21899±1954^{a}	11667±1250de	30246±3167^{abc}	19613±1478^{cd}
	Foliar	50 CeO_2	13437±2279^{bc}	10420±1757^{cd}	21387±1954^{a}	13415±1250^{cde}	29291±3167^{abc}	27174±1478^{ab}
		50 CeAc	15388±2279^{bc}	14761±1757^{cd}	6616±1954^{c}	8576±1250de	15105±3167^{c}	12705±1478^{d}
		250 CeO_2	7748±2279^{c}	8038±1757^{d}	22312±1954^{a}	21168±1250^{a}	27200±3167^{abc}	26316±1478^{abc}
		250 CeAc	15400±2279^{bc}	17069±1757^{abc}	9908±1954^{bc}	7359±1250^{e}	18406±3167^{bc}	13149±1478^{d}
Fe		Control	527±103^{ab}	261±67^{ab}	35±6	23±3	120±12	72±5.45^{b}
	Root	50 CeO_2	660±103^{a}	401±67^{ab}	32±6	18±3	101±12	78±5.45^{ab}
		50 CeAc	145±103^{b}	128±67^{b}	32±6	22±3	77±12	61.82±5.45^{b}

		250 CeO_2	155±103^{ab}	484±67^{a}	33±6	16.95±3	115±12	85±5^{ab}
		250 CeAc	222±103^{ab}	153±67^{ab}	35±6	24.56±3	85±12	79±5^{ab}
	Foliar	50 CeO_2	122±103^{b}	109±67^{b}	28±6	19.17±3	77±12	68±5^{b}
		50 CeAc	174±103^{ab}	190±67^{ab}	23±6	25.28±3	65±12	61±5^{b}
		250 CeO_2	117±103^{b}	86±67^{b}	37±6	22.35±3	121±12	99±5^{a}
		250 CeAc	151±103^{ab}	376±67^{ab}	27±6	25.21±3	105±12	77±5^{ab}
Zn		Control	45±15	42±5^{ab}	55±11^{ab}	24±3.81	40±5^{ab}	23±^{ab}
	Root	50 CeO_2	33±15	47±5^{ab}	88±11^{a}	34±3.81	37±5^{ab}	34±3^{a}
		50 CeAc	31±15	33±5^{b}	46±11^{ab}	23±3.81	24±5^{b}	24±3^{ab}
		250 CeO_2	52±15	35±5^{ab}	61±11^{ab}	31±3.81	45±5^{ab}	30±3^{ab}
		250 CeAc	70±15	50±5^{ab}	41±11^{ab}	27±3.81	35±5^{ab}	28±3^{ab}
	Foliar	50 CeO_2	71±15	27±5^{b}	53±11^{ab}	20±3.81	23±5^{b}	25±3^{ab}
		50 CeAc	44±15	58±5^{a}	30±11^{b}	28±3.81	25±5^{b}	18±3^{b}
		250 CeO_2	24±15	26±5^{b}	46±11^{ab}	27±3.81	54±5^{a}	34±3^{a}
		250 CeAc	84±15	45±5^{ab}	41±11^{ab}	31±3.81	42±5^{ab}	26±3^{ab}
Cu		Control	66±12^{b}	51±16^{b}	10.20±2	3±1^{b}	22±64	5±6
	Root	50 CeO_2	137±12^{a}	215±16^{a}	12.74±2	11±1^{a}	36±64	29±6
		50 CeAc	65±12^{b}	54±16^{b}	9.73±2	6±1^{ab}	103±64	22±6
		250 CeO_2	70±12^{b}	104±16^{b}	12.49±2	7±1^{ab}	251±64	20±6

		250 CeAc	40±12^{b}	63±16^{b}	8.99±2	4±1^{b}	13±64	14±6
	Foliar	50 CeO_2	35±12^{b}	46±16^{b}	9.7±2	4±1^{ab}	27±64	11±6
		50 CeAc	43±12^{b}	57±16^{b}	4.50±2	2±1^{b}	8±64	5±6
		250 CeO_2	34±12^{b}	26±16^{b}	9.49±2	5±1^{ab}	16±64	12±6
		250 CeAc	53±12^{b}	92±16^{b}	8.42±2	4±1^{ab}	16±64	7±6
Mn		Control	62±37	118±35	47±10^{bcd}	30±12^{ab}	149±23^{abcd}	76±29^{c}
	Root	50 CeO_2	80±37	144±35	62±10^{abcd}	33±12^{b}	140±23^{bcd}	153±29^{abc}
		50 CeAc	60±37	148±35	67±10^{abc}	86±12^{a}	157±23^{abc}	234±29^{ab}
		250 CeO_2	124±37	67±35	59±10^{bcd}	37±12^{ab}	195±23^{ab}	96±29^{bc}
		250 CeAc	148±37	70±35	111±10^{a}	35±12^{ab}	223±23^{ab}	90±29^{c}
	Foliar	50 CeO_2	32±37	37±35	26±10^{cd}	35±12^{ab}	74±23^{cd}	100±29^{bc}
		50 CeAc	38±37	58±35	17±10^{d}	26±12^{ab}	41±23^{d}	48±29^{c}
		250 CeO_2	133±37	24±35	91±10^{ab}	76±12^{ab}	254±23^{a}	254±29^{a}
		250 CeAc	60±37	50±35	27±10^{cd}	22±12^{b}	61±23^{cd}	35±29^{c}
Al		Control	609±100^{a}	287±59^{abcd}	6.68±3	4±0.76	58±10	24±4^{bc}
	Root	50 CeO_2	527±100^{ab}	409±59^{ab}	7.62±3	1±0.76	66±10	19±4^{c}
		50 CeAc	148±100^{ab}	129±59^{bcd}	3.47±3	3±0.76	33±10	19±4^{c}
		250 CeO_2	162±100^{ab}	447±59^{a}	2.64±3	3±0.76	54±10	34±4^{abc}
		250 CeAc	248±100^{ab}	157±59^{abcd}	3.11±3	3±0.76	40±10	34±4^{abc}

	Foliar	50 CeO_2	117±100^{ab}	114±59^{cd}	7.90±3	3±0.76	29±10	20±4^{bc}
		50 CeAc	175±100^{ab}	100±59^{abcd}	2.05±3	4±0.76	20±10	28±4^{abc}
		250 CeO_2	109±100^{b}	96±59^{d}	6.46±3	5±0.76	67±10	48±4^{a}
		250 CeAc	153±100^{ab}	392±59^{abc}	8.50±3	3±0.76	68±10	40±4^{ab}
P		Control	6305±1210	5708±1034	8662±1254	5559±540^{ab}	9351±1245	5052±581
	Root	50 CeO_2	7468±1210	7891±1034	9517±1254	4902±540^{b}	8211±1245	7133±581
		50 CeAc	3890±1210	5568±1034	9486±1254	6659±540^{ab}	7214±1245	6781±581
		250 CeO_2	7707±1210	6936±1034	10560±1254	6235±540^{ab}	9736±1245	5870±581
		250 CeAc	9505±1210	6892±1034	9094±1254	6329±540^{ab}	7871±1245	7399±581
	Foliar	50 CeO_2	6947±1210	4070±1034	8647±1254	6017±540^{ab}	8047±1245	6379±581
		50 CeAc	5613±1210	7311±1034	6383±1254	7758±540^{a}	6864±1245	5476±581
		250 CeO_2	3705±1210	3832±1034	9630±1254	7275±540^{ab}	9337±1245	7487±581
		250 CeAc	6475±1210	6382±1034	10563±1254	7704±540^{a}	9301±1245	6106±581
K		Control	8410±3195^{b}	4267±1870	57380±6772	46613±2635^{ab}	56374±4741	42316±3838^{ab}
	Root	50 CeO_2	6532±3195^{b}	4199±1870	60682±6772	53038±2635^{a}	49730±4741	45556±3838^{ab}
		50 CeAc	2916±3195^{b}	1330±1870	50500±6772	35574±2635^{b}	37732±4741	33172±3838^{b}
		250 CeO_2	8457±3195^{b}	7361±1870	65571±6772	53053±2635^{a}	55953±4741	59830±3838^{a}
		250 CeAc	29790±3195^{a}	8010±1870	55076±6772	43950±2635^{ab}	51493±4741	38027±3838^{b}
	Foliar	50 CeO_2	9381±3195^{b}	2706±1870	59143±6772	45589±2635^{ab}	46503±4741	47059±3838^{ab}

	50 CeAc	6454±3195[b]	4255±1870	37573±6772	38819±2635[b]	35497±4741	32807±3838[b]
	250 CeO_2	3322±3195[b]	6662±1870	65220±6772	47244±2635[ab]	42944±4741	32130±3838[b]
	250 CeAc	1611±3195[b]	10007±1870	45510±6772	38379±2635[b]	39740±4741	35623±3838[b]

The translocation and accumulation of most elements in the stems was the similar in both infested and non-infested plants. None of the treatments affected the translocation of Cu, Mn, Fe, Al, and K to the stems and leaves of infested and stems of non-infested plants. Moreover, none of the treatments altered Cu and K accumulation in the leaves of non-infested plants. Divergent effects were observed on Ca accumulation in stems and leaves of infested and non-infested plants exposed to CeAc and nano-CeO_2. In infested plants, CeAc at 50 and 250 mg/L reduced Ca in stems by 69 % and 53 %, and leaves by 59 % and 50 %, respectively, as compared with infested control ($p \leq 0.05$). In addition, in non-infested plants CeAc at 50 at 250 mg/L also decreased Ca in leaves by 38 % and 36 %, respectively, compared with non-infested control. However, nano-CeO_2 at 250 mg/L increased Ca in stem by 79 % in non-infested plants.

Contrary to what was observed in soil application, foliar application of the Ce compounds generally decreased the Ca accumulation in the plant tissues, the exception being in non-infested plants exposed to nano-CeO_2 at 250 mg/L, which showed a significant increase of Ca in stems. However, no effects were observed in roots, which suggest that Ce was retained at the stem level. The consistent decrease in the Ca uptake and accumulation across the plant tissues could be correlated with the positive zeta potential of Ce (Barrios *et al.*, 2016), which could repel other positive elements. Foliar exposure to CeAc at 250 mg/L increased the leaf Mn by 234 % in non-infested plants, compared with non-infested control ($p \leq 0.05$) (Barrios *et al.*, 2016). Additionally, nano-CeO_2 at 250 mg/L increased Fe and Al in the leaves of non-infested plant by 38 % and 102 %, respectively, relative to the non-infested control. The possibility of nano-CeO_2 binding with Fe and Al oxides, which are widespread soil colloids, may explain the increase in their concentration in the roots and leaves of the exposed plants (Pullagurala *et al.*, 2018; Zhao *et al.*, 2014).

2.4 Conclusion

In summary, this work revealed that at 250 mg/L, nano-CeO_2 and CeAc reduced Fusarium wilt and improved the chlorophyll content and the nutritional value of the tomato. The level of Ce exposure across the plant tissues is critical to optimizing both food safety and security concerns. In this study, Ce compounds suppressed diseases, increased yield, and enhanced nutrient utilization, all without accumulating in plant tissues, except in roots. However, more research work needs to be done to examine the effect of Ce on fruit quality and to optimize the disease suppressing effects. It has been reported that the antifungal potential of NPs may be enhanced by surface coating with agents that can improve their bio-interactions and, consequently, have positive physiological effects in plants (Medina-Velo *et al.*, 2017). For example, Barrios *et al.* (2016) revealed that citric acid coated CeO_2 NPs at 250 mg/kg significantly increased the chlorophyll content in tomato plants. However, no studies have been performed with coated nano-CeO_2 in diseased plants. Clearly, additional research is necessary to understand the mechanism by which nutrient and non-nutrient nanoparticles in suppress disease and increase agricultural productivity.

Chapter 3

Cerium Oxide Nanoparticles alter the Nutritional Status of Tomato (*Solanum lycopersicum*) Fruit Grown in *Fusarium* Infested Soil

3.1 Introduction

Interest in the use of engineered nanomaterials in agriculture has increased significantly. Current projections indicate that by 2050, the global food production will need to double to ensure food security (Kegan, 2016). Importantly, efforts to increase production will be confounded by a changing climate, loss of arable soil and increased pest/pathogen activity (Deutsch *et al.*, 2018); FAO report (2015). Globally, there is an increase in utilization of pesticides and fertilizers, respectively, to control pests and diseases, and to replenish nutrients in soils that are continuously used for crop production. Approximately 386 and 1,806 million kilograms of pesticides are utilized annually in the United States and China, respectively (Dimkpa and Bindraban, 2017; Kah and Hofmann, 2014). In addition, nearly 200 hundred thousand kilograms of fertilizers are used yearly since 2013 (Dimkpa and Bindraban, 2017; Chhipa, 2017). In United States alone, more than $600 million annually are spent to combat plant fungal pathogens (FAO report (2015); Servin *et al*., 2015). The use of nanoscale platforms such as nanofertilizers, nanopesticides and nanosensors has shown significant potential for enhancing agricultural efficiency (Elmer and White, 2018; Kim *et al.*, 2018; Dimkpa and Bindraban, 2017; Chhipa, 2017; Servin *et al*., 2015). Several recent reports have shown that a number of nanoparticles can be used to increase crop yield (Dimkpa and Bindraban, 2017), control plant diseases and pests, and enhance nutrient use efficiency (Kah *et al*., 2018; White and Gardea-Torresdey, 2018; Kim *et al.*, 2018).

Tomato is an economically important vegetable worldwide, generating over $2 billion in annual revenue (Minor and Bond, 2018). In United States, tomato is negatively impacted by a

number of diseases, including bacterial spot, bacterial wilt, early and late blight, septoria leaf spot, leaf mold, tomato pith necrosis, tomato spotted wilt virus, anthracnose and Fusarium wilt. Fusarium wilt is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. l*ycopersici* (Bawa, 2016). This wilt is difficult to control and quite destructive, causing millions of dollars annually in economic loss (Bawa, 2016). In addition, there is a growing concern about the accumulation of Cu in soil due to excessive use of non-nano Cu-containing pesticides to control plant diseases (Zhou *et al.,* 2011). Apart from being an excellent source of fiber, sugars, proteins, vitamins, lipids and carbohydrates (USDA, 2018), tomato also contains high amounts of important phytochemicals such as lycopene (Minor and Bond, 2018). Lycopene is an antioxidant carotenoid present in the chromoplasts and it accumulates during fruit ripening (Hornero-Méndez and Britton, 2002; Stahl *et al*., 1997). Additionally, tomato fruit also contains several essential elements, which include calcium (Ca), chlorine (Cl), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), phosphorus (P), potassium (K), silicon (Si), sodium (Na), sulfur (S), and zinc (Zn) (USDA, 2018). This high nutritional value and general ease of cultivation have led to it being the second most consumed fruit in the world.

Nanoparticles such as Ag, CeO_2, CuO, MnO and ZnO have been shown to improve plant productivity and suppress plant pathogenic diseases (Dimkpa *et al.,* 2019; Pullagurala, *et al*., 2018; Elmer *et al.,* 2018; Dimkpa *et al.,* 2018; Adisa *et al*., 2018; Dimkpa *et al.* 2017; Elmer and White, 2016; Lamsal *et al.,* 2011). Lamsal *et al.*(2011) demonstrated the efficacy of Ag nanoparticles (NP) against powdery mildew in cucumber and pumpkin leaves. Powdery mildew is caused by the fungal pathogens *Golovinomyces cichoracearum* or *Sphaerotheca fusca* on cucumber and pumpkin; relative to the commercial fungicide, Ag NP at 100 mg/L suppressed powdery mildew more effectively in cucumber (14 and 9%) and pumpkin (7 and 5%), before and after disease

outbreak, respectively (Lamsal *et al.* 2011). Elmer *et al.* (2018) demonstrated in both greenhouse and field experiments that foliar exposure to NP CuO (500-1000 mg/L) reduced Fusarium wilt in watermelon by 29% and increased fruit yield in the two field experiments by 39 and 53% as compare with the untreated control. Elmer and White (2016) also showed that foliar exposure to NP CuO, MnO, and ZnO at 1000 mg/L significantly reduced the severity of wilt diseases and increased fruit yield in both tomato and eggplant infested with *Verticillium dahliae* and *F. oxysporum*, respectively. In a previous study, we found that root and foliar exposure to NP CeO_2 at 250 mg/L significantly suppressed Fusarium wilt in tomato by 57 and 53%, respectively, compared with controls (Adisa *et al.,* 2018). Interesting, when compared to controls the chlorophyll content in plants affected by Fusarium wilt was 28% higher following foliar treatment with NP CeO_2 (Adisa *et al.,* 2018). The known antimicrobial activity of Ce-containing compounds is thought to be due to mimetic catalase activity at either +3 or +4 oxidation state in plant cells, coupled with its superoxide scavenging function (Pirmohamed *et al.,* 2010). A rare earth element (REE) fertilizer known as "Changle" is commonly used in China and has been reported to contain 41.4% of Ce (Hu *et al.,* 2004). "Changle" is often used as fertilizer for rice, wheat and other vegetables (Hu *et al.,* 2004). Barrios *et al*. (2017) reported that citric acid coated NP CeO_2 significantly altered the nutritional value of tomato fruit, although the plants were not exposed to any pathogens. Specifically, root treatment with NP CeO_2 at 62.5, 125 and 500 mg/kg decreased the reducing sugar content by 63, 75, and 52%, respectively, and at 125 mg/kg, the starch content was decreased by 78% compared with untreated control. Apart from no exposure of the plants to pathogens, the CeO_2 NPs are coated with citric acid.

There is very limited information available on the impact of NP CeO_2 on the nutritional values of tomato harvested from plants infested with *F. oxysporum*. The current study builds upon

a previous investigation where we showed that root and foliar exposure to NP CeO_2 and cerium acetate (CeAc) at 250 mg/L suppressed Fusarium wilt disease and improved the chlorophyll content in tomato plants (Adisa *et al.,* 2018). The current study evaluates the impact of NP CeO_2 on the fruit physiological parameters, lycopene content, non-structural carbohydrates (reducing and total sugars) and nutritional elements of tomato cultivated in *Fusarium* infested and non-infested soils. CeAc was used as an ionic control for comparison. Lycopene content and non-structural carbohydrates were determined using UV-Vis spectrophotometry and Ce and micro/macro element content was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

3.2 Materials and Methods

3.2.1 Nanoparticle suspension preparation and experimental design

Cerium oxide nanoparticles (Meliorum Technologies) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). Characterization of these NPs has been previously published by Keller *et al.* (2010). Briefly, the Nano-CeO_2 have a primary size of 8 ± 1 nm, with a surface area of 93.8 m^2 g^{-1} and 95.14% purity; the particles aggregate in deionized (DI) water to 231 ± 16 nm.[21] The suspension/solution of NP CeO_2 and cerium acetate at 0, 50 and 250 mg/L were prepared, compound-based concentrations, in DI water. Tomato seeds (*Solanum lycopersicum* cv Bonny Best) were procured from Totally Tomato, Randolph, WI. The seeds were germinated in vermiculite for 21 days before transplant into plastic pots containing 3 kg of soil mixture (1:2, natural to commercial potting mix) as described in the supplementary information (Adisa *et al.,* 2018). For root/soil treatment, the NP CeO_2 suspensions and CeAc solutions at 50 and 250 mg/kg of soil were thoroughly mixed with 3

kg of the soil to ensure homogeneity. For the foliar treatment, the tomato plants shoots were sprayed with suspension/solution of the Ce-compounds at 0, 50, and 250 mg/L (Adisa *et al.,* 2018).

Inoculation with *F. oxysporum* f. sp. *lycopersici*

The inoculum of isolate of *F. oxysporum* f. sp. *lycopersici* Race 2 was obtained from the Scratch Farm, Cranston, RI. The inoculum was prepared following the procedure described by Elmer and White (2016). Briefly, the inoculum was prepared by growing the colonies of virulent isolates of *F. oxysporum f. sp. lycopersici* on autoclaved millet seed. Dried inoculum was milled and sieved through a 1 mm sieve (Elmer and White, 2016). One week after NP CeO_2 or CeAc exposure, six treatment replicates were divided into two groups; infested and non-infested treatments. The infested group were inoculated with *F. oxysporum* by thoroughly mixing the inoculum with the soil mixture (1 g per kg of soil, 1 g of inoculum ~100,000 colonies), as described by Adisa *et al.* (2018). Plants were watered as needed and Peter's soluble 20:20:20 (nitrogen: phosphorous: potassium (NPK)) fertilizer was applied to individual pots weekly for plant growth. The plants were cultivated until full maturity (126 days), in a greenhouse with photoperiod of 14 h, under light intensity of 340 μmol m^{-2} s^{-1}, day and night temperature of 25 and 20 °C, and relative humidity of 70%.

3.2.2 Plant harvest and nutritional assessment

Fruit were collected as they ripened and were washed and rinsed 3 times with a 5% $CaCl_2$ and Millipore water (MPW) (Hong *et al.,* 2016). The fruit dimensions (height and width) and mass were recorded. Selected fruit were oven dried at 60 ºC for 72 h; the dried samples were ground to a homogenized powder prior to analysis for carbohydrates and elements. Additional fruit from

each treatment were flash frozen in liquid nitrogen and stored at -80 ºC for further analysis (Barrios *et al*., 2017).

3.2.3 Determination of lycopene content

The lycopene content was determined following the method of Barrett and Anthon (2000). The frozen fruit were homogenized using a mortar and pestle, and centrifuged at -4 ºC 9600 rpm for 10 min using Eppendorf AG bench centrifuge 5417 R (Hamburg, Germany). In 15 ml test tubes, 100 µL of the supernatant and 8 mL of acetone: ethanol: hexane (1:1:2) were added, capped, shaken lightly and incubated in the dark for 1 h. Millipore water (1 mL) was then added and briefly shaken, and the samples were allowed to stand for 10 min release any air and ensure phase separation. The absorbance of the upper layer was read at 503 nm using PerkinElmer Lambda 14 UV-Vis spectrometer (single-beam mode, PerkinElmer, Uberlingen, Germany), and the lycopene content was estimated following Barrett *et al.* (2007) using the following equation:

mg lycopene/kg fresh wt.= $A_{503} \times 537 \times 8 \times 0.55/(0.10 \times 172) = A_{503} \times 137.4$;

where A is the absorbance, 537 g/mol is the molecular weight of lycopene, 8 mL is the total volume of the solvent mixture, 0.55 is the volume ratio of the upper layer, 0.10 g is the weight of the sample added, and 172 mM^{-1} is the extinction coefficient for lycopene in hexane.

3.2.4 Determination of non-structural carbohydrates: total and reducing sugars

The total and reducing sugar content were determined following Dubois *et al.* (1965) and Nelson-Somogyi (1952) methods, respectively, with slight modifications. Ten mL of 80% ethanol was added onto 100 mg of dried powder of tomato fruit; the samples were boiled at 80 ºC for 30 min in a water bath, and were centrifuged at 22,000 rpm for 20 min. The supernatants were

collected and the extraction procedure was repeated three times for each sample. The supernatants were evaporated to 3 mL and were diluted with MPW to a final volume of 25 mL.

For total sugar determination, 100 µL of the extracts, a glucose standard (Sigma-Aldrich, 99.9% pure) and water (blank) were diluted to 1 mL with MPW. One mL 5% phenol and 5 mL 96% H_2SO_4 were added, mixed and allowed to cool at room temperature for 30 min. A calibration curve was obtained using the glucose standard at 0.0005, 0.001, 0.002, 0.004, 0.006 g/mL. The sample absorbance was read at 490 nm using a Perkin-Elmer Lambda 14 UV-Vis spectrophotometer.

For reducing sugar determination, 100 µL of the extracts was diluted to 2 mL with MPW and 1 mL of alkaline copper tartrate was added prior to boiling in a water bath for 30 min. The mixture was allowed to cool, 1 mL of arsenomolybdolic acid reagent was added, and the mixture was diluted with MPW to a final volume of 10 mL. The mixture was allowed to stand for 10 min and the absorbance was read at 620 nm in a Perkin-Elmer Lambda 14 UV-Vis spectrophotometer. The same calibration curve for total sugar was used to determine reducing sugar content.

3.2.5 Quantification of cerium, micro and macro elements in tomato fruits

At harvest, the concentration of Ce, as well as a range of micro and macro elements, were determined in the fruit by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer, Optima 4300 DV, Shelton, CT) as described by Ebbs *et al.* (2016). To validate the digestion and the analytical methods employed, blanks, spikes, and a standard reference material (NIST 1547, Gaithersburg, MD, peach leaves) were used. The blank and the standards were injected after every 15 samples to ensure quality control and quality assurance with 95% recovery.

The limit of detection for cerium was determined by reading eight replicas of the blank. The mean, plus three standard deviations (μ± 3SD) was in the range of 50 μg/L.

3.2.6 Statistical analysis

Triplicate samples were used for all experiments. All data sets were subjected to one-way ANOVA and Fisher LSD tests ($p \leq 0.05$) using IBM SPSS 25 software package (Chicago, IL) to determine the variance of the experiment and the differences between treatments. Data are presented as mean ± standard errors (SE).

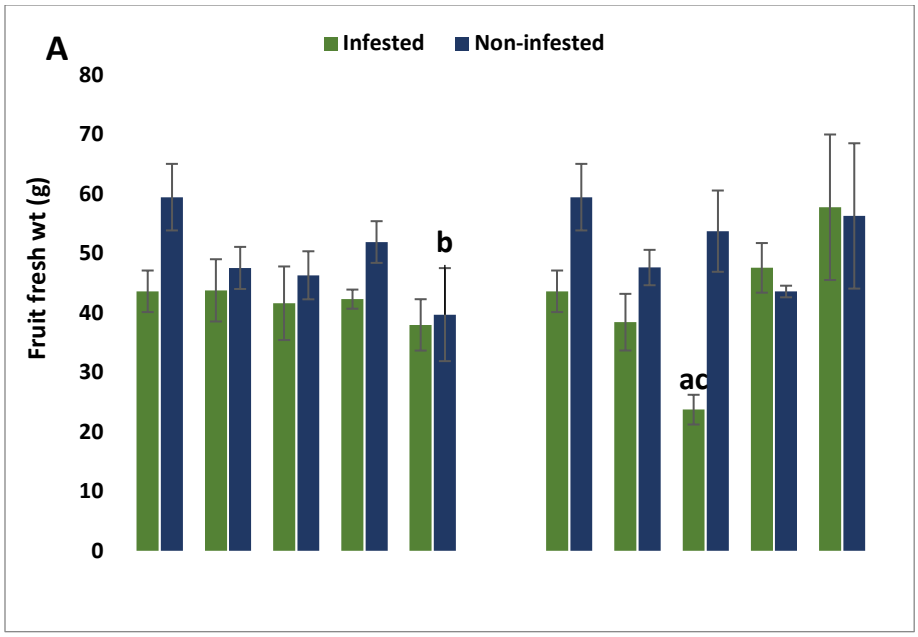
3.3 Result and Discussion

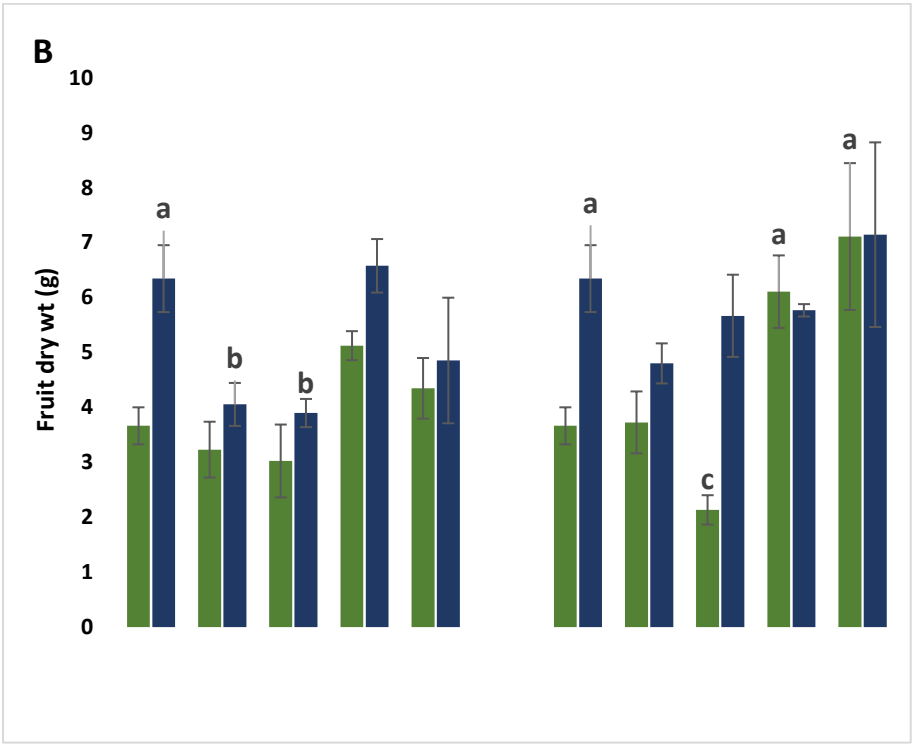
3.3.1 Effect on fruit agronomical parameters

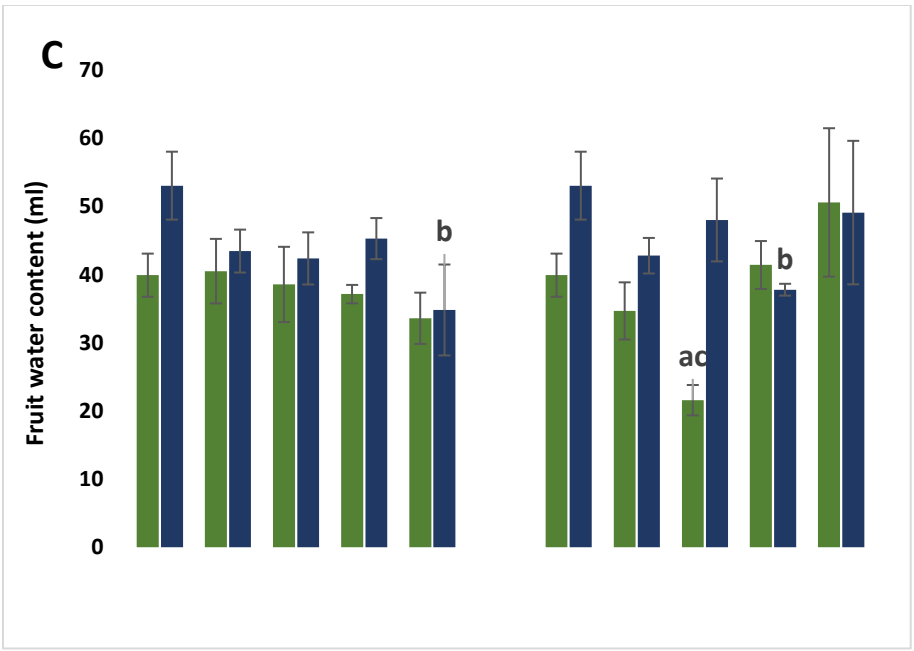
The effect of application mode of the cerium compounds on the fruit phenotypic parameters are presented in Figures 3.1 (A-C) and Table 1. As seen in Figure 1A, none of the NP CeO_2 treatments significantly affected the fruit fresh weight ($p \leq 0.05$) when infested treated plants are compared with respective controls. However, foliar exposure to CeAc at 50 mg/L significantly decreased the fruit fresh weight by 46% ($p \leq 0.05$), relative to infested control. Moreover, CeAc at 50 mg/L, applied through the leaves, decreased fruit fresh weight by 56% in Fusarium infested treated plant, compared with non-infested treated plants ($p \leq 0.05$). This is an indication that the Fusarium pathogen significantly reduced the fruit productivity even in the presence of CeAc. Interestingly, the fruit fresh weight of non-infested plants exposed via the roots to CeAc at 250 mg/L also decreased by 33% ($p \leq 0.05$), compared with non-infested untreated control. Overall, CeAc treatments negatively impacted the fruit fresh weight regardless of the exposure routes. On the other hand, the fruit dry weight reduced significantly by 42% ($p \leq 0.05$) in infested untreated

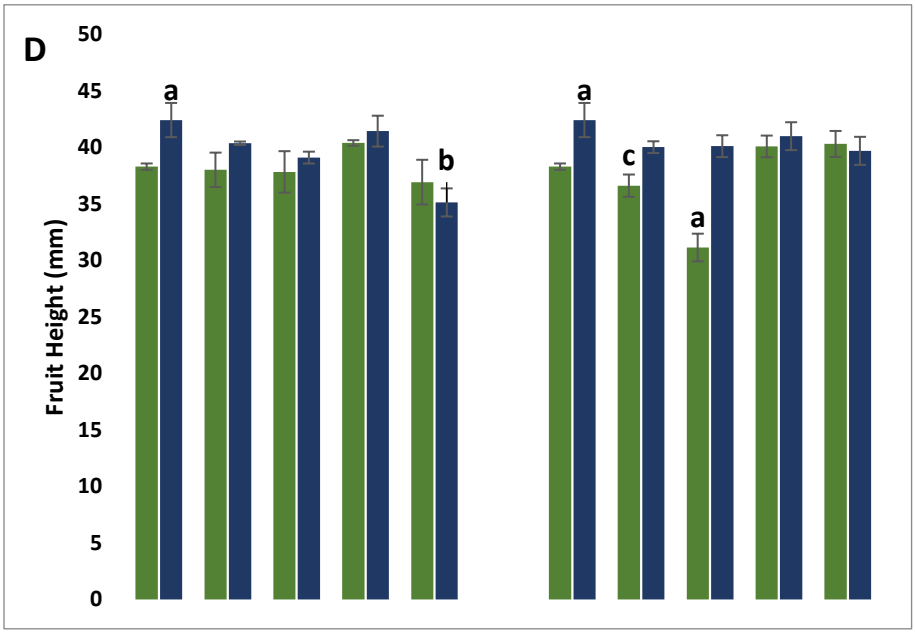
control, compared with non-infested untreated control (Figure 3.1B). However, foliar exposure to NP CeO_2 and CeAc at 250 mg/L significantly increased fruit dry weight of infested plants by 67 and 94% ($p \leq 0.05$), respectively, compared with infested untreated control (Figure 3.1B). Root exposure to NP CeO_2 and CeAc at 50 mg/kg reduced the fruit dry weight by 36 and 38%, respectively compared with non-infested untreated control. Moreover, foliar exposure to CeAc at 50 mg/L reduced the fruit dry weight (62%) in infested plants, compared with non-infested treated plants (Figure 3.1B). Figure 3.1C indicated none of the root treatments affected the fruit water content in infested plants. However, root exposure to CeAc at 250 mg/kg reduced the fruit water content by 34%, compared with non-infested untreated control. On the other hand, foliar exposure to CeAc at 50 mg/L decreased the fruit water content (46%) in infested plants, compared with infested untreated control ($p \leq 0.05$). Moreover, the fruit water content of infested plants foliarly exposed to CeAc at 50 mg/L reduced (55%) significantly, compared with non-infested treated plants ($p \leq 0.05$). It is suggests that the CeAc treatment could not ameliorated the reduction of fruit water content caused by the Fusarium pathogen infection. Similarly, foliar exposure to NP CeO_2 at 250 mg/L reduced fruit water content (29%) in non-infested plants, compared with non-infested untreated control. Similar to the impact on other fruit yield, the fruit height of infested untreated control reduced by 10%, compared with non-infested untreated control ($p \leq 0.05$) (Figure 3.1D). Generally, the fruit dimension (Figure 3.1D and E); height and width are not significantly affected by any of the exposure to NP CeO_2 and CeAc at all concentrations in infested plants, except foliar exposure to CeAc at 50 mg/L, which significantly reduced the height of fruits of infested tomato by 19%, compared with infested untreated control ($p \leq 0.05$). As previously reported, CeAc impact on fruit yield can be attributed to the systemic relationship between photosynthetic activities and acetate metabolism which involves two organelles (mitochondrion and chloroplast) (Adisa *et al*.,

2018; Heifetz *et al*., 2000). This can also be correlated with the impact on chlorophyll content in the plant as reported in our previous study, in which the impact of Fusarium infestation cannot be overemphasized (Adisa *et al*., 2018). In addition, infested plants exposed through leaves to NP CeO_2 at 50 mg/L had 9% reduction in fruit height, compared with non-infested treated plant. Overall, results were somewhat similar to that reported by Barrios *et al.* (2017). The authors revealed that NP CeO_2 root treatments had no significant impact on the fruit dimensions, fresh and dry weights, and water content. Conversely, CeAc at 125 mg/kg increased the fruit water content by 72% relative to the control (Barrios *et al.*, 2017). However, the growing conditions are relatively similar but primarily, the current study include Fusarium infestation with different tomato cultivar (Bonny Best) as against Roma tomato cultivar used by Barrios *et al*. (2017). Wang *et al*. (2012) also revealed no significant impact of periodic exposure of NP CeO_2 on the size and average weight of tomato fruit. Conversely, there are reports of other particle types positively impacting tomato fruit yield. For example, Raliya *et al*. (2015) demonstrated that 250 mg/kg of TiO_2 nanoparticles significantly increased the fruit biomass of tomato plant fruit by 70%, compared with untreated control. In addition, Elmer and White (2016) also reported significant increase tomato yield when exposed to foliar treatment with NP CuO in both greenhouse and field experiments. Overall, our study revealed no significant impact of NP CeO_2 on the tomato fruit biomass or physical properties, although Fusarium infestation clearly negatively impacted plant health and productivity.


A
Infested
Non-infested
Fruit fresh wt (g)
80
70
60
50
40
30
20
10
0
b
ac


B
Fruit dry wt (g)
10
9
8
7
6
5
4
3
2
1
0
a
b
b
a
c
a
a


C
Fruit water content (ml)
70
60
50
40
30
20
10
0
b
ac
b


D
Fruit Height (mm)
50
45
40
35
30
25
20
15
10
5
0
a
b
a
c
a

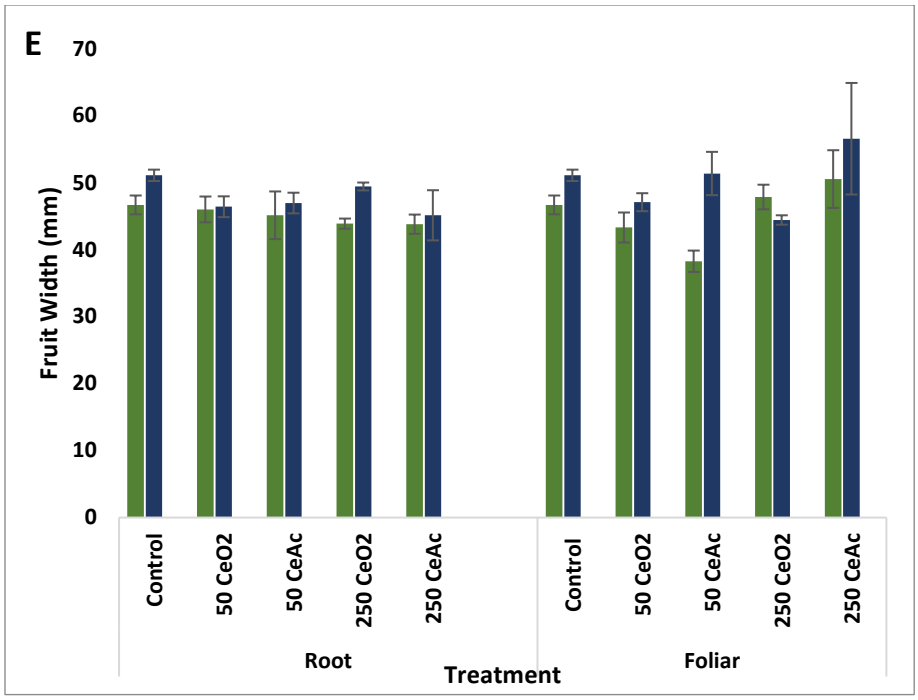


Figure 3.1 Effect on fresh (A), and dry (B) weight, water content (C), height (D) and width (E) of tomato fruit from plants exposed to root and foliar applications of NP CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

3.3.2 Effect on lycopene content

Figure 3.2 shows the lycopene content of tomato fruit exposed to root and foliar treatments with NP CeO_2 and CeAc with or without infestation. The lycopene content in untreated diseased fruit was significantly reduced by 17% compared with fruits of non-infested untreated control ($p \leq 0.05$). However, none of the root treatments affected fruit lycopene concentration as compared to the relevant controls, except those exposed to NP CeO_2 and CeAc at 50 mg/kg, which significantly increased fruit lycopene content by 9 and 11%, respectively, in infested plants, compared with infested untreated control ($p \leq 0.05$). Conversely, in non-infested plants, fruit

lycopene content reduced significantly on exposure via root to NP CeO_2 at 50 mg/kg (13%), CeAc at 50 mg/kg (17%), and CeAc at 250 mg/kg (13%), compared with non-infested untreated control ($p \leq 0.05$). Similarly, foliar exposure to NP CeO_2 at 250 mg/L, and CeAc at 50 and 250 mg/L significantly reduced the lycopene content by 18, 16, and 20% in the fruits of non-infested treated plant, compared with non-infested untreated control ($p \leq 0.05$). Although there is no known information on the impact of nanoparticles on the lycopene content in Fusarium-infested tomato plants, there are some reports available in a non-infested treated tomato plants and other fruit with different types of nanoparticles. Contrary to our findings, Barrios *et al.* (2017) found no significant impact of both bare and citric acid coated NP CeO_2 on the lycopene concentration in fruit from healthy treated plants. Interestingly, and relatively similar, the authors did report a significant reduction in lycopene content in tomato fruit after root treatment with bulk CeO_2 at 62.5 (92%), 250 (61%), and 500 mg/kg (72%); CeAc at 62.5, 125 and 500 mg/kg reduced lycopene content by 69, 79, and 81%, respectively (Barrios *et al.*, 2017). Alternatively, Kole *et al.* (2013) showed that fullerols (carbon-based nanoparticles) at 47.2 nM significantly increased lycopene content in bitter melon by 82% compared with untreated control. However, our findings are in partial agreement with results of Raliya *et al*. (2015), although the tomato plants in that study were not infested with *Fusarium oxysporum*, different tomato cultivar (tomato cherry super sweet 100) and different nanoparticles (TiO_2 and ZnO) were used in the experiment, which could play a role in plant response to the treatments. Specifically, the authors reported that all plants treated with TiO_2 and ZnO nanoparticles at 100 mg/L had 80 and 113%, respectively, significantly elevated levels of fruit lycopene, compared with untreated control (Raliya *et al*., 2015).

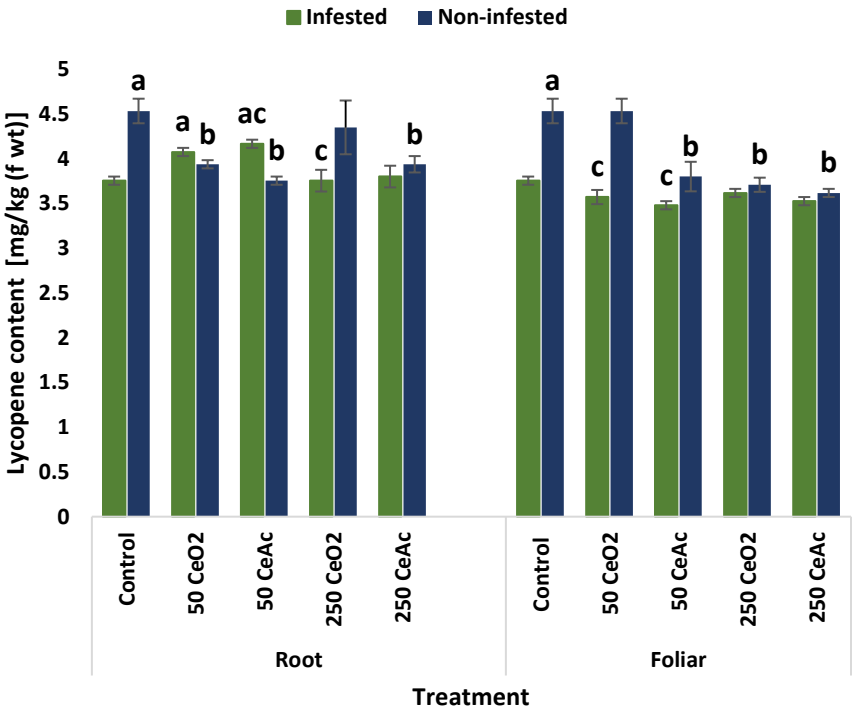


Figure 3.2 The lycopene content of tomato fruit from plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters using one-way ANOVA follow by LSD test. The mean differences of treatments were compared with the respective controls.

Lycopene is an important natural antioxidant that scavenges free radicals and protects human cells against oxidative damage and chronic diseases such as cancer (Palozza *et al*., 2013). It is important that any treatment to improve crop productivity or control disease and pests not alter its lycopene content. In this study, treatment of Fusarium wilt disease in tomato plant with NP CeO_2 by root or foliar exposure had no negative effect on the lycopene concentration but slightly improve the fruit concentration. It is clear that depending on conditions, pathogen infestation and plant species may change the dynamics of the lycopene concentration in the treated

plant. Additional study is needed to ensure that the integrity of overall fruit quality and safety is not compromised by nanoscale treatment.

3.3.3 Effect on fruit total and reducing sugar content

Figures 3.3 and 3.4 show the effect of root and foliar applications of NP CeO_2 and CeAc at 0, 50 and 250 mg/L on the total and reducing sugar concentration, respectively, in tomato fruit from plants grown in infested and non-infested soils ($p \leq 0.05$). Infested untreated plants (control) indicated significant increase (60%) in total sugar, compared with non-infested untreated control. However, root exposure to NP CeO_2 at 50 and 250 mg/kg decreased the total sugar concentration by 63 and 54% in infested plants, respectively, compared with infested untreated control ($p \leq 0.05$). In addition, infested plants treated with CeAc at 50 mg/kg via root had 46% reduction in fruit total sugar, compared with the control ($p \leq 0.05$). Similarly, foliar exposure of infested to NP CeO_2 at 50 and 250 mg/L decreased the fruit total sugar by 50% each, compare with the control ($p \leq 0.05$). Conversely, significant increase in fruit total sugar was observed in non-infested plants exposed via root to CeAc at 250 mg/kg (93%), and via leaves to NP CeO_2 at 50 (56%) and CeAc at 250 mg/kg (77%), compared with non-infested untreated control. Overall, the impacts of the Ce-compound treatments on non-structural carbohydrates are in line with the findings of Barrios *et al.* (2017) although that study involved only root exposure in non-infested soil and the use of surface coating agent (citric acid) on the NPs. The authors reported that NP CeO_2 had no impact on the fruit total sugar content of treated plants compared with untreated controls. However, in the same experiment citric acid coated NP CeO_2 decreased the total fruit sugar concentration at 62.5 (84%), 250 (78%) and 500 mg/kg (81%), as compared with the untreated control. Moghaddam and Ende (2012) reported that sucrose, one of the most common non-reducing sugars in plants, plays vital

role in plant response to stresses, including biotic stress from pathogens. In addition, upregulation of sucrose synthesis in cucumber treated with NP CeO_2 was reported to be a possible stress response in the plant (Zhao *et al*., 2014). However, in the current study, in spite of the biotic stress generated by the pathogen infection and exposure to cerium compounds, the total fruit sugar content was not significantly altered.

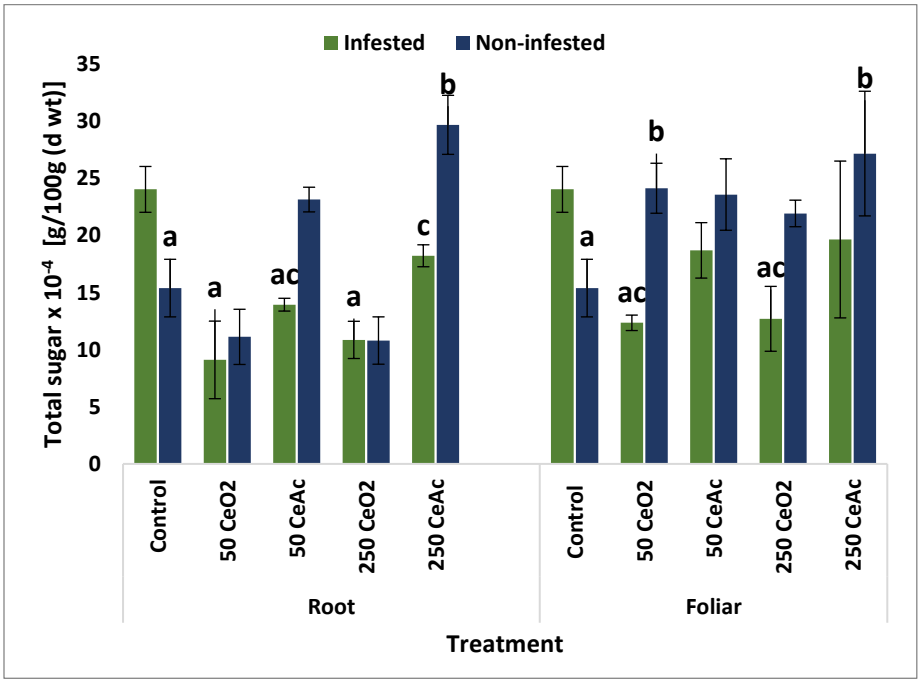


Figure 3.3 The total sugar concentration in tomato fruit from plants exposed to root and foliar applications of NP CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls

As evident in Figure 3.4, none of the treatments had a significant effect on the reducing sugar content in the fruit of infested and non-infested plants as compared to relevant controls. As shown in Figures S1 and 2, in a previous study, Adisa *et al.*(2018), found that cerium compounds

have the potential to suppress of Fusarium wilt disease in tomato, with no significant impact on the reducing sugar content of the fruits. In a similar manner, Barrios *et al*. (2017) reported no significant impact of root exposure of tomato plants to NP CeO_2 at 62.6-500 mg/kg. Conversely, the same study reported that citric acid coated NP CeO_2 at 62.5, 125 and 500 mg/kg decreased the fruit reducing sugar content by 56, 63 and 75%, respectively, relative to untreated controls (Barrios *et al*. 2017). In addition, CeAc was also shown to decrease the reducing sugar content at 62.5 mg/kg (58%), compared with the untreated control. However, the authors also reported that bulk CeO_2 increased the reducing sugar by 67% and 58% at 250 and 500 mg/kg (Barrios *et al*. 2017). It was suggested that the sweetness of exposed tomato fruit was modified by citric acid coated NP CeO_2 and CeAc (Barrios *et al*. 2017). Notably, carbohydrates are produced in plant leaves by photosynthesis. The rate of photosynthesis correlates directly with the amount of sugars produced in the plants; therefore, reductions in photosynthesis result in the reduction of sugars in the plant (Goodman *et al.*, 1986). A previous study demonstrated non-significant impact of the Ce-compounds treatments on the chlorophyll content (Adisa *et al*., 2018), which can be correlated with non-significant impacts on the non-structural carbohydrates in tomato fruits. Findings from this work are in contrast with the findings of Barros *et al.*(2017), which showed no significant impact of $nCeO_2$ on the total sugar content. Rico *et al.* (2013) also reported non-significant changes to the sugar content of rice exposed to 500 mg/L $nCeO_2$ when compared with the untreated control. Generally, carbohydrates are the most abundant organic macromolecules in plants, being both the major source of chemical energy and of structural support for plants (Boysen, 2007). Therefore, they are critical to the estimation of the nutritional value of plants (Loewus and Tanner, 2012). Overall, the findings from our current work and the literature suggest that further study is needed

to elucidate the underlying mechanisms and role of different nanoscale compounds on the synthesis of different plant sugars.

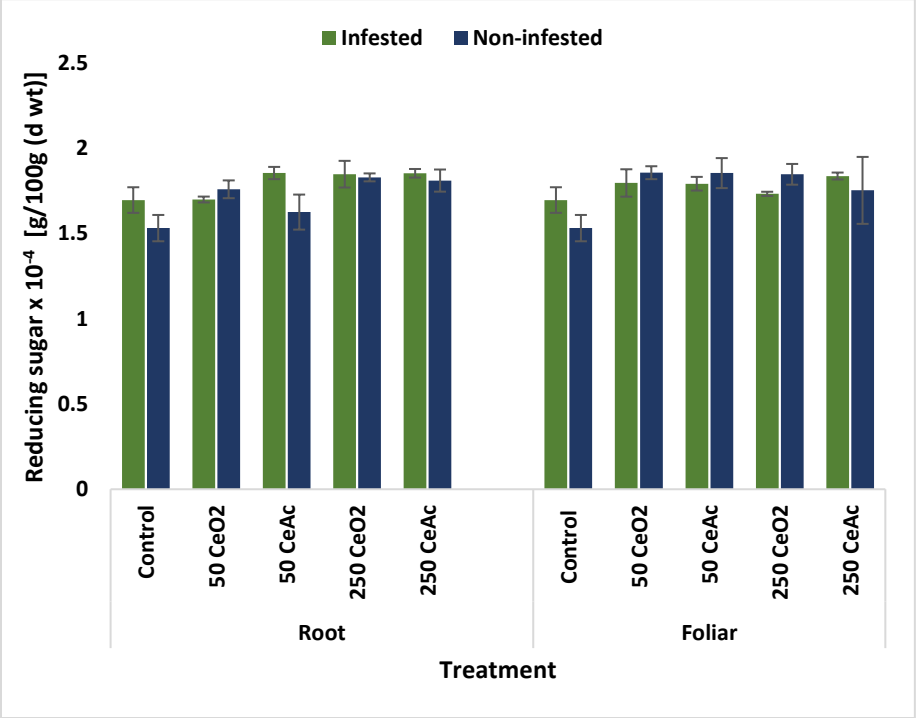


Figure 3.4 The reducing sugar concentration in tomato fruit from plants exposed to root and foliar applications of nano-CeO_2 and CeAc, at 0, 50 and 250 mg/L. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls

3.3.4 Effect on the fruit micro- and macronutrient content

Table 3.1 shows the impact of root and foliar exposure of NP CeO_2 and CeAc, at 0, 50 and 250 mg/L on macro- and micronutrient (Ca, Cu, Fe, K, Zn, Mn and P) content in the fruit of infested and non-infested tomato plants. Notably, the cerium concentration in the fruit of all treated infested and non-infested plants was below the detection limit ($p \leq 0.05$). These findings are in line with Barrios *et al.* (2017) where cerium accumulation in the fruit was below the detection limit

even at a higher exposure concentration (500 mg/kg). Similarly, Birbaum *et al.* (2010) reported that root exposure of corn to NP CeO_2 did not translocate significant Ce accumulation in the other plant tissues. Conversely, some studies have demonstrated concentration-dependent translocation and accumulation of Ce across plant tissues. Wang *et al.* (2012) reported a significant dose dependent increase in cerium accumulation in tomato fruit from plants treated with NP CeO_2 twice per week at concentrations from 0.1 to 10 mg/L, with the highest fruit Ce accumulation (0.5 mg-Ce/g-tissue) in plant treated 10 mg/L. The authors did observe that the Ce concentration decreased significant from root to shoot to edible tissue (fruits); ~5 to ~4.8 to ~0.5 mg-Ce/g-tissue (Wang *et al.*, 2012). Moreover, other studies have also demonstrated a similar trend of Ce accumulation in the above ground tissues, including pumpkin leaves (Zhang *et al*., 2011), soybean (López-Moreno *et al*., 2010), rice (Rico, *et al*., 2013), and wheat (Schwabe *et al.*, 2015). Schwabe *et al.* (2015) demonstrated Ce can not only be taken as NP CeO_2 into the plant but also as released Ce(III) ions, which can then re-precipitate as NP CeO_2 in the above ground tissues of the plants (pumpkin, sunflower and wheat). Specie dependent translocation of Ce from NP CeO_2 to the above ground tissues was reported to be size dependent, with the largest accumulation from smaller sized NP CeO_2 (10 nm). Importantly, 17.2 μg g^{-1} Ce was reportedly found in wheat leaves using ICP-OES/MS (with 0.2 μg L^{-1} LOD for ICP-MS and 0.3 mg L^{-1} for ICP-OES), when 0.1 mM of Ce (III)-citrate solution was applied the plant. In addition, Ce (III)-citrate treated pumpkin and sunflower accumulated more Ce in leaves than those treated with NP CeO_2. The current discrepancy in the literature with regard to plant response to Ce exposure can likely be attributed differences in plant species and exposure details such as concentration, media, and growth conditions (Schwabe *et al.*, 2015). Although suppression of diseases and enhanced productivity is highly desirable, concerns over food safety with regard to nanomaterial use in agriculture is still

important; therefore, the lack of detectable Ce in the fruit of treatment plants is a significant finding.

Table 3.1 Concentration of macro- and micro element (µg/g) in tomato fruit from Fusarium wilt infested and non-infested plants exposed to root or foliar application of nano-CeO_2 and CeAc at 0, 50 and 250 mg/L. Averages with different letters are statistically significant ($p \leq 0.05$), compared with the respective control; $n = 3$ using one-way ANOVA follow by LSD test.

	Element	Exposure route	Treatment	Infested	Non-Infested
Macro	**Ca**		Control	0.84± 0.24	0.35±0.02[a]
		Root	50 CeO_2	0.73± 0.17	0.56± 0.16
			50 CeAc	0.70±0.04	0.43±0.04
			250 CeO_2	0.59±0.11	0.45±0.08
			250 CeAc	0.50±0.03	0.25±0.02
		Foliar	50 CeO_2	1.30±0.50[c]	0.36±0.04
			50 CeAc	0.49±0.10	0.33±0.02
			250 CeO_2	0.40±0.07	0.33±0.05
			250 CeAc	0.48±0.09	0.52±0.15
	Mg		Control	2.20±0.22	2.07±0.21
		Root	50 CeO_2	2.63±0.29[c]	2.03±0.09
			50 CeAc	2.55±0.04[c]	1.87±0.02
			250 CeO_2	2.74±0.11	2.49±0.08
			250 CeAc	2.27±0.03	1.95±0.03
		Foliar	50 CeO_2	2.73±0.16	2.31±0.13
			50 CeAc	2.24±0.11	1.91±0.06
			250 CeO_2	2.46±0.00	2.07±0.06

		250 CeAc	2.44±0.56	2.01±0.31
K		Control	39.76±4.13	32.70±2.50
	Root	50 CeO_2	43.65±3.66	37.80±1.90
		50 CeAc	43.57±1.09[c]	31.69±1.02
		250 CeO_2	44.53±3.37	41.38±1.29[b]
		250 CeAc	37.03±0.51	33.00±1.11
	Foliar	50 CeO_2	46.69±1.21	38.62±2.39
		50 CeAc	37.57±0.73	31.28±1.84
		250 CeO_2	41.74±0.89[c]	32.39±1.31
		250 CeAc	45.18±8.73[c]	30.48±1.88
P		Control	9.31±0.68	7.59±0.36
	Root	50 CeO_2	9.73±0.90	8.37±0.52
		50 CeAc	11.21±0.36[c]	8.20±0.38
		250 CeO_2	11.00±1.14	9.94±0.56
		250 CeAc	9.77±0.22	8.03±0.37
	Foliar	50 CeO_2	11.57±0.68[c]	9.15±0.29
		50 CeAc	10.24±0.36	8.06±0.16
		250 CeO_2	10.42±0.47	8.34±0.34
		250 CeAc	11.92±2.54[ac]	7.96±0.41
S		Control	3.01± 0.33	2.64± 0.22
	Root	50 CeO_2	3.21± 0.28	2.54± 0.10
		50 CeAc	3.32±0.04[c]	2.51±0.06
		250 CeO_2	3.58±0.18	3.05±0.19
		250 CeAc	3.14±0.07	2.49±0.13
	Foliar	50 CeO_2	3.59±0.36[c]	2.83±0.24
		50 CeAc	3.06±0.12	2.71±0.06
		250 CeO_2	2.96±0.13	2.73±0.13

			250 CeAc	3.39±0.73[c]	2.56±0.18
Micro	**Cu**		Control	0.010±0.001	0.008±0.001
		Root	50 CeO_2	0.012±0.001	0.012±0.002[b]
			50 CeAc	0.013±0.001[c]	0.008±0.001
			250 CeO_2	0.016±0.002[ac]	0.012±0.001[b]
			250 CeAc	0.013±0.000	0.010±0.001
		Foliar	50 CeO_2	0.014±0.001	0.016±0.002[b]
			50 CeAc	0.013±0.001[c]	0.008±0.000
			250 CeO_2	0.010±0.000	0.008±0.000
			250 CeAc	0.011±0.002	0.010±0.001
	Fe		Control	0.045±0.010	0.050±0.009
		Root	50 CeO_2	0.046±0.005	0.050±0.008
			50 CeAc	0.057±0.001	0.042±0.004
			250 CeO_2	0.056±0.008	0.047±0.006
			250 CeAc	0.049±0.002	0.046±0.004
		Foliar	50 CeO_2	0.049±0.004	0.048±0.002
			50 CeAc	0.049±0.000	0.033±0.001
			250 CeO_2	0.046±0.003	0.041±0.003
			250 CeAc	0.051±0.012	0.040±0.000
	Mn		Control	0.019±0.006	0.016±0.001
		Root	50 CeO_2	0.018±0.003	0.018±0.003
			50 CeAc	0.024±0.000	0.016±0.001
			250 CeO_2	0.031±0.005[a]	0.023±0.004
			250 CeAc	0.022±0.001	0.014±0.001
		Foliar	50 CeO_2	0.019±0.003	0.019±0.000
			50 CeAc	0.019±0.002	0.013±0.000
			250 CeO_2	0.022±0.001	0.019±0.004

		250 CeAc	0.023±0.007	0.016±0.005
Zn		Control	0.032±0.005	0.028±0.003
	Root	50 CeO_2	0.035±0.003	0.030±0.002
		50 CeAc	0.037±0.001[c]	0.030±0.002
		250 CeO_2	0.035±0.002	0.034±0.001
		250 CeAc	0.034±0.000	0.031±0.001
	Foliar	50 CeO_2	0.038±0.001	0.034±0.001
		50 CeAc	0.036±0.001[c]	0.028±0.000
		250 CeO_2	0.029±0.003	0.028±0.001
		250 CeAc	0.031±0.005	0.027±0.001

As a function of infestation, of all the macro- and micronutrients analyzed, only Ca increased by 140% in the fruit of infested untreated control, compared with non-infested untreated control ($p \leq 0.05$). This suggests the infestation impact the nutrient accumulation in the tomato fruits of untreated plants. None of the treatments affected any of the analyzed macronutrients in the fruit samples, except P concentrations that increased 28% by foliar exposure to CeAc at 250 mg/L, compared with infested untreated control. Although some of the changes were statistically non-significant, when compared with the infested control. However, definite trends of significant increase in the amount of fruit macronutrients (Ca, K, Mg, P and S) were evident, as regards infestation within the same treatment. For example, root exposure of infested tomato plants to CeAc at 50 mg/kg significantly increased the concentration of fruit K, P and S by 37, 37, and 32%, compared with non-infested treated plants ($p \leq 0.05$). Moreover, root exposure of non-infested plants to NP CeO_2 at 250 mg/kg increased fruit K content by 27%, compared with non-infested control ($p \leq 0.05$). With regard to the foliar treatments, similar trends were observed in plants exposed via leaves to NP CeO_2 at 50 mg/kg, which had significant increase in Ca, P and S fruit

content (261, 26 and 27%, respectively) in the infested plants relative to non-infested treated plants ($p \leq 0.05$). Moreover, foliar exposure to NP CeO_2 at 250 mg/kg in infested plants also increased the fruit K content by 29%, compared to non-infested treated plants. In addition, infested plants treated foliarly with CeAc at 250 mg/L had significant increase in fruit concentration of K (48%), P (50) and S (32%), relative to non-infested treated plants. It can be suggested that apart from other factors, Fusarium pathogen plays role in the increase of the macronutrients. Importantly, there is no information available on the impact of Ce-compounds on Ca, K, Mg, P and S accumulation in tomato fruits as a function of fungal disease. However, alluded to previous study (Adisa *et al*., 2018), significant increase in Ca translocation to the shoot was observed in plants treated via the root with NP CeO_2 at 50 (53%) and 250 mg/L (70%) in non-infested tomato plants, while no significant changes were observed in infested treated plants. This can be correlated with the findings of the current study, where no significant changes in the fruit Ca content was observed in tomato plant treated with NP CeO_2 via root. Conversely, Barrios *et al* (2017) reported a 59% decrease in Ca concentration of fruit of plant exposed to NP CeO_2 at 125 mg/kg as compared to the untreated controls. The dynamics in the Ca accumulation can be attributed to differences in tomato cultivar and the concentration of NP CeO_2 used in the experiments. However, in the same study, CeAc at 62.5 and 500 mg/kg increased fruit Ca concentration by 157% (Barrios *et al* (2017). In our study, the changes in Ca may be attributed to the similar characteristics of Ca to rare earth elements such as Ce as previously discussed by Hu *et al*. (2004), and the role of Fusarium pathogen cannot be overemphasized. However, further study is needed to elucidate the mechanism behind the discrepancy in the uptake, translocation and accumulation of macronutrients across the plant tissues.

Among the analyzed micronutrients (Cu, Fe, Mn and Zn), only Fe was not affected significantly by any treatments, regardless of the infestation. Root exposure to NP CeO_2 at 250 mg/kg increased the fruit accumulation of Cu (51%) and Mn (59%) concentrations in infested plants, compared with infested untreated control. In addition, Cu concentration increased in the fruit of non-infested tomato plants exposed via root to NP CeO_2 at 50 (50%) and 250 mg/kg (50%), and via leaves to NP CeO_2 at 50 mg/L (100%), compared with non-infested untreated control ($p \leq 0.05$). In addition, infested plant exposed to NP CeO_2 at 250 mg/L via had 33% increase in fruit Cu content, compared with non-infested treated plants. Moreover, fruit Cu content increased by 63% in Fusarium infested plants exposed via root and foliar to CeAc at 50 mg/L, compared with non-infested treated plants. Similar to Cu accumulation in the fruits of infested treated plants, Zn concentration also increased in the fruits of infested plants exposed to CeAc at 50 mg/L via root (33%) or foliar (29%), compared with non-infested treated plants. Considering the results obtained from the previous study (Adisa *et al*., 2018), the uptake and translocation of the micronutrients may influence low accumulation observed in the fruits in this current study. For instance, our previous study indicated significant increase in Cu uptake in fungal infected plants treated via root with 50 mg/kg $nCeO_2$ (108%) but the stem concentration was not affected, compared with the infested untreated control. Importantly, the stage of plant growth, size, and other environmental factors can also impact the concentration of these important elements in the aboveground tissues. Overall, there is no known information to explain these subtle findings, however, the uptake and translocation of the nutrients, as well as the role of the pathogen in the plants could not be left out (Adisa *et al*., 2018). Notably, in this study some of the treatments increased the concentrations of some essential nutrients (Ca, Cu, Mn, and P). This can give further research insight into nutrient enhancement or biofortification in plants, to improve the nutritional values of crops.

3.4 Conclusion

This study revealed that the fusarium wilt decreases crop yield and possibly the nutritional content of the tomato fruits. However, no evidence of phytoxicity or overall negative impacts in the fruit of plants treated with nanoscale Ce as part of a novel disease management strategy. Although the impact of treatment on lycopene and carbohydrates are subtle, it is noteworthy relative to respective control; none of the treatments on a large scale negatively altered the nutritional values of the fruits. Notably, NP CeO_2 at 250 mg/kg via root exposure significantly increased the Cu and Mn concentration in the fruit from infested plants. In our previous study, root exposure to nCeO_2 at 250 mg/kg significantly suppressed Fusarium wilt disease in infected tomato plants; collectively, the two studies suggest NP CeO_2 has potential as a novel disease management strategy with negatively impacting the nutritional quality of the fruit. These findings are significant for future determination of the sustainability of nano-enabled disease suppression platforms in agriculture, although additional molecular-level mechanistic evaluation is recommended to fully understand and guarantee the safety of these approaches.

Chapter 4

Physiological and Biochemical Impact of Copper Oxide Nanoparticles on *Fusarium* Infested Tomato (*Solanum lycopersicum*) plant

4.1 Introduction

Diseases and pests caused by soil pathogens threaten the goal to double global food production in order to meet the supply by 2050 (Kegan, 2016). These exacerbate existing pressure from increasing global human population, drastic climate change, lack of arable land for farming, and shortage of water supply. Economic losses caused by soil borne diseases in crops can never be overemphasized. In the United States alone, agricultural losses, caused by soil pathogenic diseases, run into several millions of dollars annually. More than 20 % reduction in crop yield stems from plant infections; over $600 million is spent annually to control diseases and pests (Pandey *et al.*, 2018; Tuite and Lacey, 2013; FAO report, 2015; Servin *et al*., 2015). This has resulted in distortion of the production line of farm produce and displacement of food and agricultural industries (Dehne and Oerke, 2004). One of the most destructive plant pathogenic diseases is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersicum* (Bawa, 2016). This disease affects tomato plants and reduces its productivity.. Management of soil pathogenic diseases is a difficult task. In addition, the conventional methods are shadowed with environmental concerns, low nutrient bioavailability, and high cost; hence, aa better alternative strategy is necessary (Servin *et al*., 2015).

Recently, there is an increasing effort to explore nanoscale elements as pesticides and fertilizers to control diseases and pests and improve plant productivity (Kah *et al*., 2018; White and Gardea-Torresdey, 2018; Kim *et al.,* 2018). For a very long time, copper or coper-based compounds (such as copper hydroxide ($Cu(OH)_2$), cuprous oxide (Cu_2O), and copper oxychloride

($Cu(OH)_2 \cdot CuCl_2$) have been used as a major component of commercially available pesticides such as Kocide 2000® and Kocide 3000® (Giannousi *et al.*, 2013; Zuverza-Mena *et al*., 2015). However, taking the advantage of the larger surface/volume ratio, nanoparticles are more reactive and efficient as pesticides and fertilizers, compared to the bulk counterparts. On the other hand, the excessive use of these non-nano Cu-based pesticides could lead to an accumulation of Cu in the environment, which could pose a threat to the agri-ecosystem (Zhou *et al.*, 2011). Numerous studies have evaluated the antimicrobial function of nano-Cu against plant pathogens (Kanhed *et al.* 2014; Zabrieski *et al*. 2015; Strayer-Scherer *et al.* 2018) and its potential to improve plant productivity (Zuverza-Mena *et al*., 2015). The ability of nano-Cu to basipetally translocate to plant roots was well documented by Elmer and White (2016) and Wang *et al.* (2013). When foliarly applied, nCuO was able to fertilize maize (Wang et al., 2013), eggplant and tomato (Elmer and White, 2016), with increased Cu content in the unexposed region and significant reduction in wilt disease incidences caused by *F. oxysporum f. sp. lycopersici* and *Verticillium dahliae* in tomato and eggplant, respectively (Elmer and White 2016; Wang *et al.*, 2013). In addition, Elmer *et al.* (2018) reported that foliar exposure to nCuO (500-1000 mg/L) did not only suppress the Fusarium wilt disease (29%) in watermelon, in both greenhouse and field experiments, but also increased the fruit yield in the two field experiments by 39 and 53%, relative to untreated control. The mechanism behind antimicrobial function of nCuO is still unclear, however, Cu is known as a cofactor, which involves in the activation of many important proteins including: oxidases, peroxidases, plastocyanins. It has also been suggested that Cu damages the respiratory chain and affect proteins and enzymes in microbes. Many of these enzymes play significant role in plant defense mechanisms. More importantly, it has been documented that increased Cu ion from nCuO, enhanced polyphenol peroxidase activity in the plant systems under pathogen infection. Moreover,

other nanoparticles such as Ag, CeO_2, MnO, and ZnO have been shown to improve plant productivity and suppress plant pathogenic diseases (Dimkpa *et al.,* 2019; Pullagurala, *et al*., 2018; Dimkpa *et al.,* 2018; Adisa *et al*., 2018; Dimkpa *et al.* 2017; Lamsal *et al.,* 2011).

The primary goal of this study was to evaluate the physiological and biochemical impact of nCuO on tomato plant grown on Fusarium infested soil at flowering stage. Commercial fungicide, Kocide 3000 and ionic counterpart, $CuSO_4$ were used to compared the relative impact of nCuO on the plant. The root and shoot biomass, chlorophyll content, enzyme activities (polyphenol oxidases and catalase), total proteins, micro and macro elements were evaluated. UV-Vis spectrophotometry, inductively coupled plasma-optical emission spectroscopy (ICP-OES), and single photon avalanche diode (SPAD) were used in this study to elucidate the response of the plant to the treatments. To the best of author's knowledge, this is the first study that evaluated the impact of nCuO on defense and stress enzymes in tomato plants infested with *Fusarium oxysporum*.

4.2 Materials and Methods

4.2.1 Copper nanoparticle and other chemicals

Copper oxide nanoparticles (nCuO) (US Research Nanomaterials Inc. Houston, TX), $Cu(OH)_2$, the commercial fungicide, (Kocide® 3000 ($Cu(OH_2)$), Dupont, Wilmington, DE), and ionic $CuSO_4$ (Spectrum Chemical,® Sigma) were used in this study. Characterization of these NPs and the commercial fungicides have been previously published by Hong *et al.* (2015).

4.2.2 Experimental design, plant cultivation, and inoculation with *F. oxysporum* f. sp. *lycopersici*

The CuO nanoparticle suspension (nCuO) was prepared at 250 and 500 mg/ concentrations (Cu-based concentration), in DI water with the help of sonication (Crest Ultrasonics, Trenton, NJ Model 275 DA; 120 V, 3 A, 59/60 Hz), in water bath to ensure homogeneity. The solutions of $CuSO_4$ (at 25 and 50 mg/L, Cu-based concentration) and Kocide 3000 (500 mg/L Cu), were also prepared in DI water. Tomato (*Solanum lycopersicum* cv Bonny Best, Totally Tomato, Randolph, WI) seeds rinsed with 1% NaClO and DI water were germinated in vermiculite for three weeks. The seedlings were treated foliarly with the nanoparticles (0, 250 and 500 mg/L), the ionic form (0, 25 and 50 mg/L) and Kocide 3000 (~500 mg/L of Cu) before transplant into plastic pots containing 1 kg of soil mixture (1:2, natural to commercial potting mix. Separately, a set of seedlings were also treated with via the root by thoroughly mixing only the nanoparticles at 0, 250 or 500 mg/kg of nCuO with the autoclaved soil mixture. Root exposures are denoted by R250 and R500, while foliar exposures are denoted by F25, F50, F250 and F500, in respect of the concentrations of the nanoparticles and ionic counterpart.

One week after exposure to the Cu-compounds, the treatment replicates were divided into infested and non-infested. The infested group was inoculated with *F. oxysporum* f. sp. *lycopersici* Race 2 (Scratch Farm, Cranston, RI). The inoculum was prepared by growing colonies of virulent isolates of *F. oxysporum f. sp. lycopersici* on autoclaved millet. The millet seed were then dried, milled, and sieved through a 1 mm sieve (Elmer and White, 2016). The infested group were inoculated with *F. oxysporum* by thoroughly mixing the inoculum with the soil mixture (1 g per kg of soil, 1 g of inoculum ~100,000 colonies), as described by Adisa *et al.*

(2018). Plants were watered as needed and with Peter's soluble 20:20:20 (nitrogen: phosphorous: potassium (NPK)) fertilizer weekly for plant growth.

4.2.3 Plant harvest and sample collection

Six weeks after transplanting, the plant tissues (root and shoot) were harvested, washed, and rinsed with a 5% $CaCl_2$ and Millipore water (MPW) (Hong *et al.*, 2016). The root and shoot biomass, as well as their lengths were recorded. Part of the fresh samples were flash frozen with liquid nitrogen and stored at -80 ºC for further analysis, and the other parts were oven dried for 72 h at 60 ºC and ground to a homogenized powder for elemental analysis.

4.2.4 Chlorophyll content

To determine the chlorophyll content, hand held single photon avalanche diode (SPAD, Minolta Camera, Japan) was used randomly on selected six plant leaves to measure the chlorophyll content and average was determined and recorded (Adisa *et al.*, 2018).

4.2.5 Extraction of biomolecules

Root extract of each sample was made by grinding about 0.2 g of the stored fresh root tissues using mortar and pestle with 1800 µL of a phosphate buffer solution (25 mM KH_2PO_4 at pH 7.4). The extracts were centrifuged at 9600 x g (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany), for 10 min at -4 ºC and the supernatants were collected in 2 mL Eppendorf tubes for analysis.

4.2.6 Protein quantification and enzyme assay

Protein content was determined using Bradford method by adding 980 μL of Bradford reagent to 20 μL of sample extract. The absorbance for each sample was read at 595 nm in microplate using UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA). A standard bovine serum albumin (0.02–0.1 mg/mL) was used to create a calibration curve (Table S1).

Stress enzyme, catalase (CAT, EC 1.11.1.6) and the defense enzyme, polyphenol oxidase (PPO, E.C.1.14.18.1) activities were determined using the plant root extract of each sample. To determine the CAT activity, 50 μL of the root extract was added to 950 μL of 10 mM H_2O_2 and was shaken three times in a quartz cuvette following the procedure described by Gallego *et al.* (1996). The absorbance of the reaction mixture was read and recorded at 240 nm for three using a Perkin Elmer Lambda 14 UV/Vis Spectrophotometer (single-beam mode, Perkin Elmer, Uberlingen, Germany). CAT activity was expressed as the amount of enzyme required to degrade 1 μmol of H_2O_2 per minutes.

To determine PPO activity, a method described by Mayer *et al.* (1965), with slight modification, as previously reported by Anusuya and Sathiyabama (2015), was used. The root extract (50 μL) was added to 0.1 M potassium phosphate buffer at pH 6.5 (138 μL), and the reaction was initiated in 96-well microplate by adding 0.01 M catechol (25 μL). The absorbance was recorded at 495 nm using a Perkin Elmer Lambda 14 UV/Vis Spectrophotometer (single-beam mode, Perkin Elmer, Uberlingen, Germany) to determine the enzyme activity. The PPO activity was defined as change in absorbance at 495 nm per minute per milligram protein.

4.2.7 Elemental analysis

About 0.2 g of oven dried tissue samples (root and shoot) were acid digested with 4 mL of plasma pure HNO_3 for 45 min at 115 ºC in DigiPREP MS digestion hot block (SCP Science, NY). Each

digest was adjusted with Millipore water (MPW) to 50 mL and analyzed for mineral elements (micro and macronutrients) using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer, Optima 4300 DV, Shelton, CT). For quality control and assurance (QC/QA), blanks, spikes, and a standard reference material (spinach leaves, NIST-SRM1570a, Gaithersburg, MD) were used; the ICP readings of the blank and the standard were repeated after every 15 samples with 95% recovery. The limit of detection for copper was determined by reading eight replicas of the blank. The mean, plus three standard deviations (μ± 3SD), was in the range of 50 μg/L.

4.2.8 Statistical analysis

All data sets in triplicates were subjected to one-way ANOVA to determine the level of significance of means differences and a LSD test at confidence level ($p \leq 0.05$) was used to determine average differences (IBM SPSS 25 software package, Chicago, IL). Data are presented as mean ± standard errors (SE). Averages with letters a and b are statistically significant to untreated infested control and commercial fungicide, respectively ($p \leq 0.05$).

4.3 Result and Discussion

4.3.1 Disease symptoms

It is noteworthy to mention that at harvest, the disease symptoms were relatively absent in all treated infested plants, including the untreated infested control. Plants were harvested at 6th week after transplant. At this growing stage (anthesis), pathogen (Verticillium) infestation symptoms is expected to manifest physically on the plants (Elmer & Ferrandino, 1994). However, the delay in the disease incidence is unclear. Generally, Cu have been reported to suppress plant diseases and increase crop yield (Malandrakis *et al*., 2019; Elmer *et al*., 2018; Elmer and White,

2016; Evans *et al.* 2007; Romheld and Yruela 2009). This study further analyzed other parameters to study the impact of copper-based nanoparticle on Fusarium infested tomato plants.

4.3.2 Copper uptake and translocation

Figure 4.1 displays the root Cu content in Fusarium infested and non-infested tomato plants exposed to foliar treatment with nCuO and $CuSO_4$, commercial fungicide and root exposure to nCuO. Surprisingly, none of the treatments significantly affected the root Cu content, relative to untreated infested control. However, root exposure to nCuO at 250 and 500 mg/kg increased the root Cu accumulation by 217 and 550%, respectively, in infested plants, compared with the commercial fungicide. Notably, the ICP-OES analysis indicated that the shoot Cu content was below the detection limit (LOD; 50 ug/L) in all treatments in infested and non-infested plants, including the control. This finding is very strange and is not consistent with previous studies. Elmer *et al.* (2018) and Elmer and White (2016) reported an increase in root and leaf Cu content in plants infested with Fusarium (watermelon and tomato) and Verticillium (eggplant) pathogens, when treated foliarly with nCuO. However, the rinsing of the plant tissue $CaCl_2$ in this study may affect tissue Cu concentrations. In addition, a number of studies have reported increased concentration of Cu in the root of exposed plants (Tamez *et al.,* 2019; Zuverza-Mena *et al.,* 2015; Peng *et al.,* 2015; Trujillo-Reyes *et al.*, 2014), although most of the studies are conducted without infestation with pathogens. Copper as an important plant nutrient is known to play critical role in the activation of some important proteins (many act in plant defense) and has been implicated in suppression of plant diseases in previous studies. However, the role and mechanism of Cu in this regards is yet to be fully elucidated.

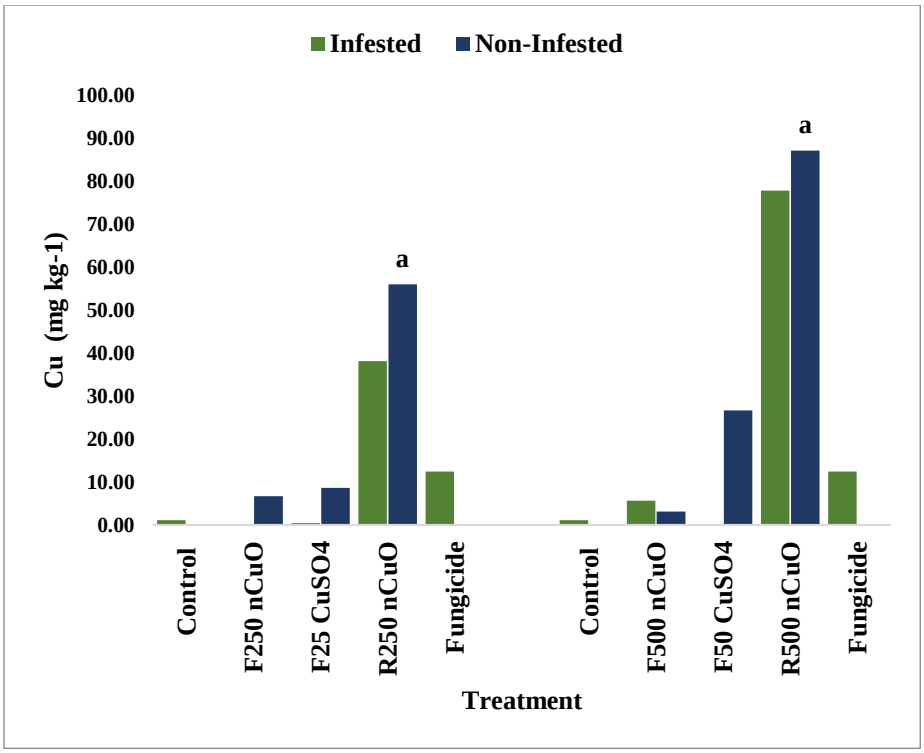


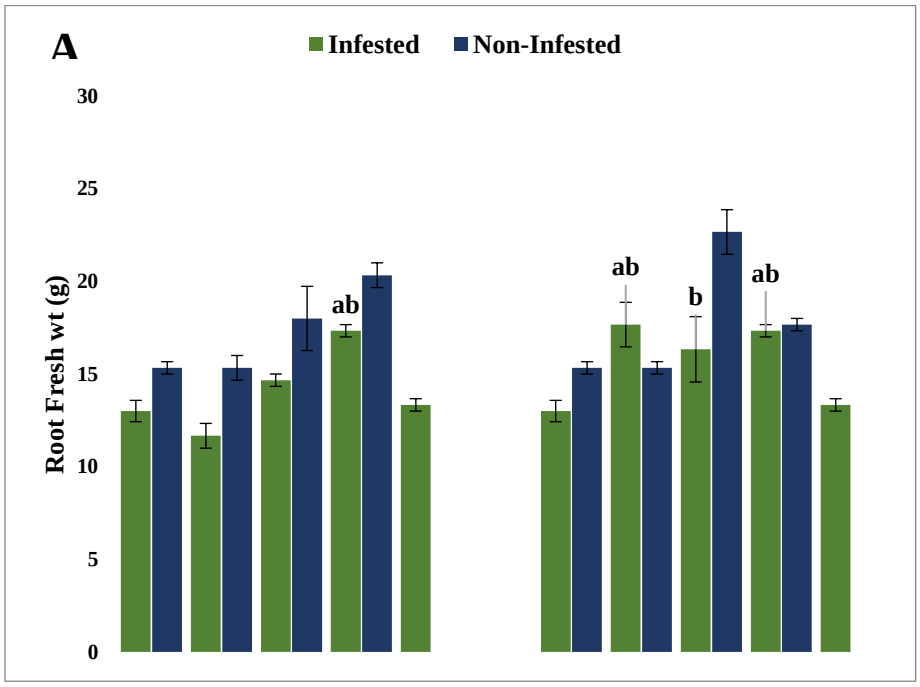
Figure 4.1 Root Cu concentration in infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, compared with untreated infested control and the commercial fungicide, respectively., using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.3 Effect on plant agronomical parameters

The root and shoot fresh biomass are displayed in Figure 4.2. The root biomass of infested tomato plants exposed to nCuO at 250 and 500 mg/kg, via root increased significantly by 33%, compared with untreated infested control ($p \leq 0.05$) (Figure 4.2A). However, none of the foliar treatments in infested plants impacted the root fresh weight, except those exposed to 500 mg/kg, with 36% increase, compared with infested control. Interestingly, root fresh weight was

significantly increased in those infested plants treated foliarly with nCuO (33%) and $CuSO_4$ (23%) at 50 mg/L and, and via root, nCuO at 250 and 500 mg/kg (30% each), relative to the commercial fungicides, Kocide 3000 ($p \leq 0.05$). On the other hand, none of the foliar treatments affected the shoot fresh biomass, when compared with untreated infested control (Figure 4.2B). However, root exposure to nCuO at 500 mg/kg increased shoot fresh weight by 18%, compared with infested control ($p \leq 0.05$). In contrast to the trend observed in plant root fresh weight, reduction in shoot fresh weight was observed in plants exposed foliarly to nCuO at 250 (15%) and 500 mg/L (14%), and $CuSO_4$ at 25 mg/L (17%), relative to commercial fungicide (Kocide 3000). However, root exposure to nCuO at 500 mg/kg increased the shoot biomass by 6%, compared with commercial fungicide ($p \leq 0.05$). None of the treatment affected the plant root length, regardless of infestation (Figure 4.3A). However, some treatments exhibited significant reduction in shoot length, when compared with the commercial fungicide (Figure 4.3B). Shoot length reduced on foliar exposure to nCuO at 250 (14%) and 500 mg/L (15%), and $CuSO_4$ at 25 mg/L (19%), as well as root exposure to nCuO at 500 mg/kg (14%) in infested tomato plants, relative to the commercial fungicide ($p \leq 0.05$) (Figure 4.3B). Overall, increased root biomass can be attributed to the role of Cu in plant nutrition as an essential micronutrient. Unique physicochemical properties of nCuO also improved the plant biomass, compared with the commercial fungicides. Our findings are partially in agreement with what was reported by Tamez *et al*. (2019). The authors reported no significant changes in the effect of nCuO (at 50 and 200 mg/kg) treatments on the root and shoot lengths of Zucchini plants (*Cucurbita pepo*), compared with the control. However, in our case, a significant reduction in root length was observed in nCuO treated infested plants, relative to the commercial fungicide. Overall, the agronomical parameter suggest that nCuO with its antimicrobial properties appears to be more effective in terms of plant yield, when compared with the commercial

fungicide. Our findings are in consistent with the previous studies that reported increased yield in watermelon, tomato, and eggplant grown on pathogen infested soil, when exposed to nCuO foliarly (Elmer *et al.*, 2018; Elmer and White, 2016). Moreover, Cu-based nanoparticles have also been reported to suppress diseases and increase yield in tea plant, finger millet (*Eleusine coracana*) and maize (*Zea mays*) (Sathiyabama and Manikandan, 2018; Choudhary *et al.*, 2017; Ponmurugan *et al.*, 2016). In addition, a number of studies have also reported growth potential of other nanoparticles in tomato plants. Silver containing nanoparticles have been reported to increase yield of tomato plant in *Alternaria solani* or *Phytophthora infestans* infested soil (Kumari *et al.*, 2017; Zakharova, 2017). Variation in plant response to nanoparticle treatment may be attributed to differences in experimental design, plant species, exposure route and time.



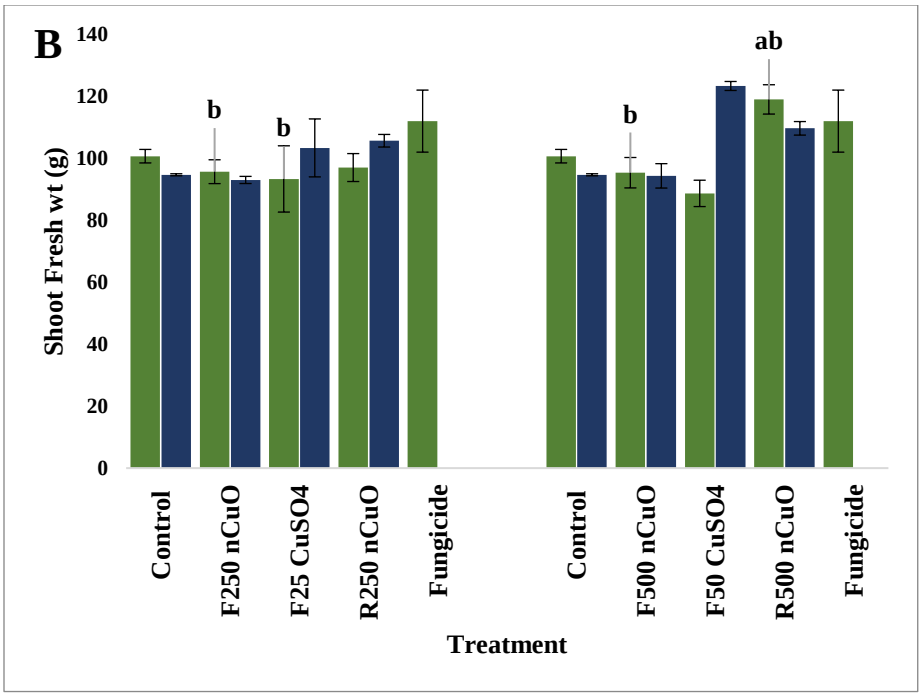


Figure 4.2 Effect on root (A), and shoot (B) fresh weight of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to untreated infested control and the commercial fungicide, respectively, using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

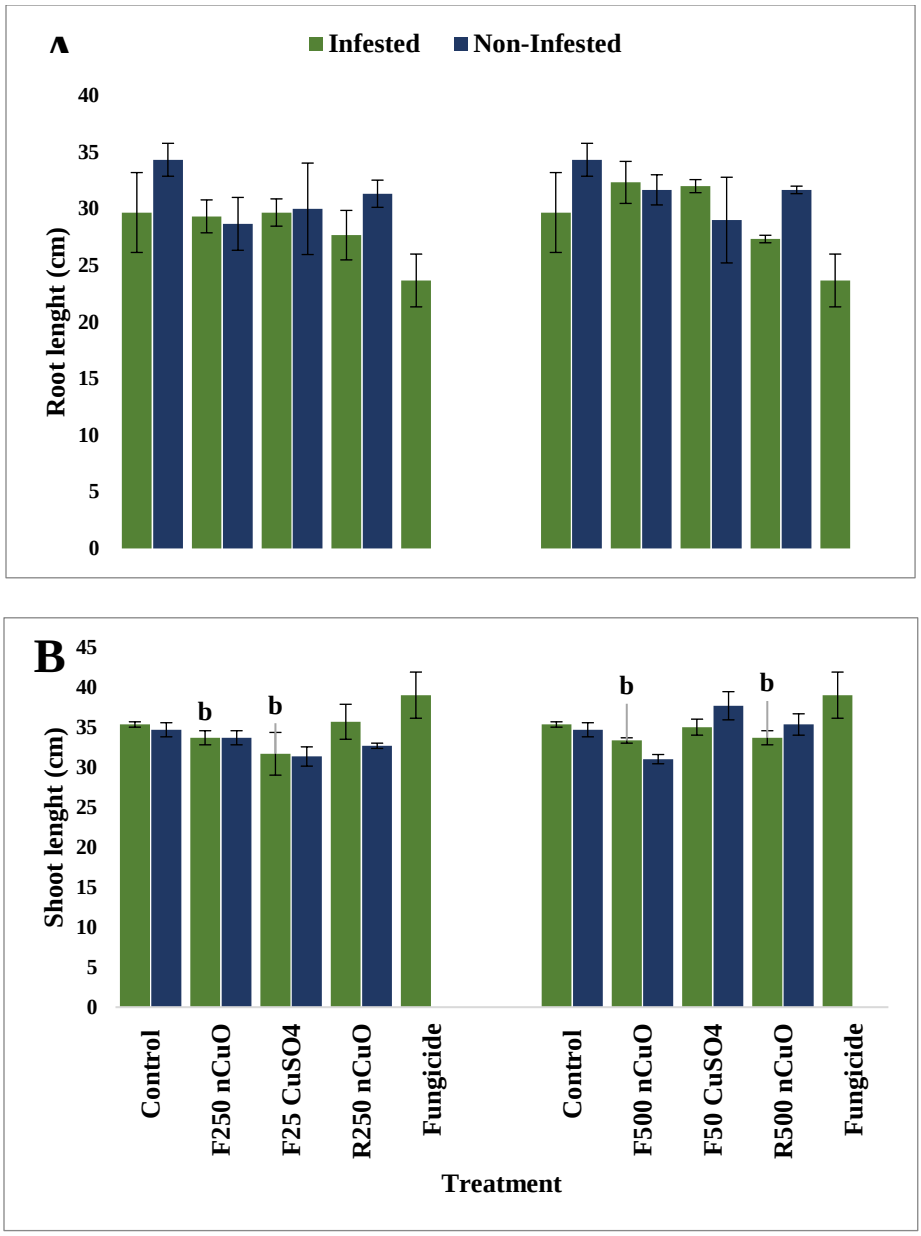


Figure 4.3 The root (A) and shoot (B) length of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to untreated infested control and the commercial fungicide, respectively, using one-

way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.4 Chlorophyll content

As shown in Figure 4.4, the leaf chlorophyll content of untreated infested control reduced significantly by 11%, compared with untreated non-infested control ($p \leq 0.05$). This is an indication that the Fusarium infestation impacted the leaf chlorophyll content. Notably, none of the nano treatments in infested plants affected the leaf chlorophyll content, compared with untreated infested control. However, infested plants exposed foliarly to $CuSO_4$ at 25 and 50 mg/L exhibited 8 and 9% increase in chlorophyll content, respectively, compared with untreated infested control ($p \leq 0.05$). Remarkably, the chlorophyll content of infested plants treated with the commercial fungicide decreased significantly (9%), compared with the untreated infested control. Moreover, significant increase in chlorophyll content was observed in infested plants exposed to foliar treatment with nCuO at 250 (10%) and 500 mg/L (14%), and $CuSO_4$ at 25 mg/L (15%), compared with the commercial fungicide ($p \leq 0.05$). In addition, infested plant exposed to nCuO at 500 mg/kg exhibited 14% increase in chlorophyll content, compared with the commercial fungicide. Copper is essentially required in photosynthetic activity in plant, and it assists in the metabolism of carbohydrates and proteins. The positive impact on the leaf chlorophyll content observed in Cu-treated infested plants can be attributed to these critical functions in plant. Our findings are consistent with many studies that have reported a significant increase in chlorophyll content in plants on exposure to Cu-based nanoparticles. Choudhary et al. (2017) revealed that foliar exposure Cu-chitosan nanocomposite reduced disease leaf spot disease in maize plant and increased the chlorophyll content in both greenhouse and field experiment.

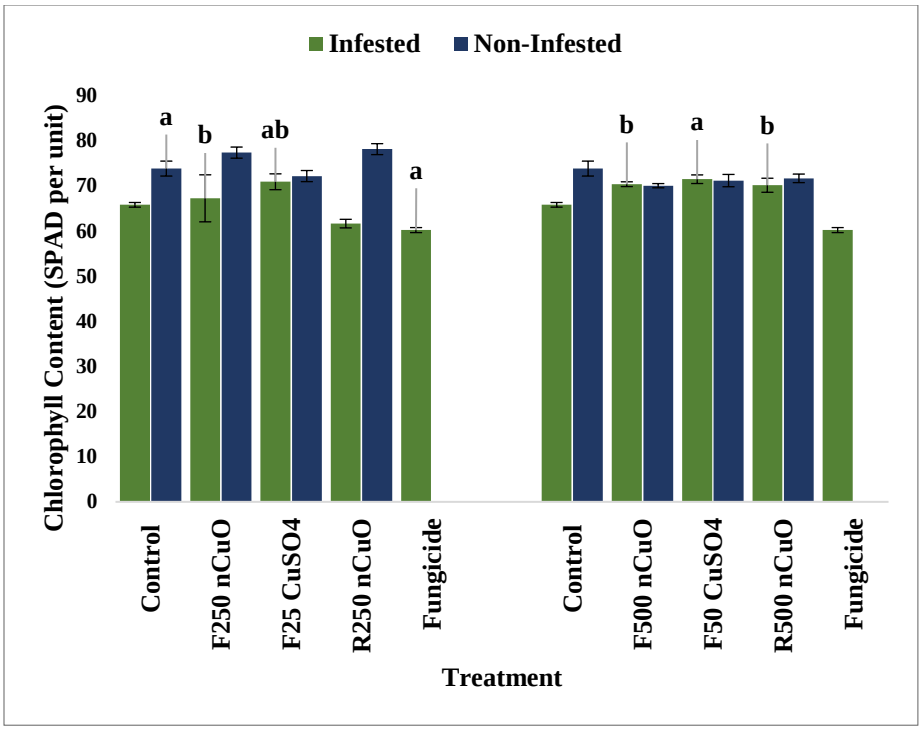


Figure 4.4. Effect on the leaf chlorophyll content of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to untreated infested control and the commercial fungicide, respectively, using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.5 Effect on total protein

The amount of root total protein estimated in both infested and non-infested tomato plants exposed to nCuO, $CuSO_4$ and Kocide 3000 treatments are shown in Figure 4.5. None of the nCuO and $CuSO_4$ treatments affected the total protein of infested plants, compared with untreated infested control. However, the commercial fungicide exhibited an elevated total protein (219%),

compared with untreated infested control ($p \leq 0.05$). Interestingly, significant reduction in total protein was observed in infested plant exposed foliarly to nCuO at 250 (45%) and 500 mg/L (49%), $CuSO_4$ at 25 (64%) and 50 mg/L (57%), and via root to nCuO at 250 (56%) and 500 mg/kg (80%), compared with the commercial fungicide ($p \leq 0.05$). These results indicated diverse response of the infected tomato plants to Cu-based exposure. There is no available information on why the commercial fungicide exhibited significant elevated total protein than other Cu-based compounds used in the study. However, regulation of specific enzyme activities could clarify the disparity in total protein level in treated plants.

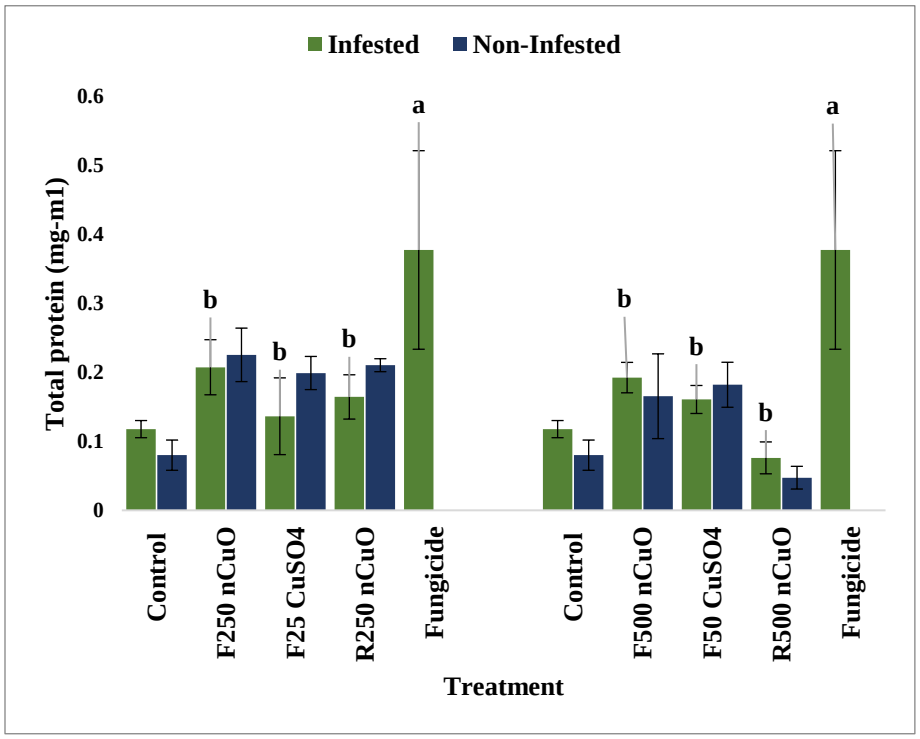


Figure 4.5 Effect on total protein of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to

untreated infested control and the commercial fungicide, respectively, using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.6 Catalase (CAT) activity

Figure 4.6 depicts the root catalase activities in infested and non-infested tomato plants exposed to nCuO, $CuSO_4$ and Kocide 3000. Significant increase in CAT activities were observed in plants treated foliarly with 500 mg/L of nCuO (138%) and Kocide 3000 (178%), compared with untreated infested control ($p \leq 0.05$). Root catalase activity was not altered by $CuSO_4$ treatments. Similarly, none of the root exposure of the nCuO affected the catalase activities, regardless of infestation (Figure 4.6). Notably, the catalase activities in infested plants treated with nCuO foliarly at 250 mg/L, and via roots at 500 mg/kg, decreased by 58 and 61%, respectively, compared with the commercial fungicide ($p \leq 0.05$). Overall, the findings revealed that both nCuO and commercial fungicides had elevated catalase activities in the infested tomato plants. Cu is known to act as cofactor in the activation several proteins in plants. The Cu ions from both nCuO and $Cu(OH)_2$ could enhance the activity of CAT in infested treated plants, in response to pathogen invasion. Increased CAT activity in plant is an indication of plant response to stress. Plant responds to stress such as pathogen infection by scavenging the reactive oxygen species generated by the pathogens. CAT is known to convert H_2O_2 to H_2O and O_2. Increase CAT activity signals Cu-based compounds enhance the enzyme activity in the plant systems to fight the stress generated by the pathogen. Choudhary *et al*. (2017) reported an enhanced level of activity of antioxidant enzyme (SOD) in *Culvularia lunata* infested maize treated Cu-chitosan nanomaterial.

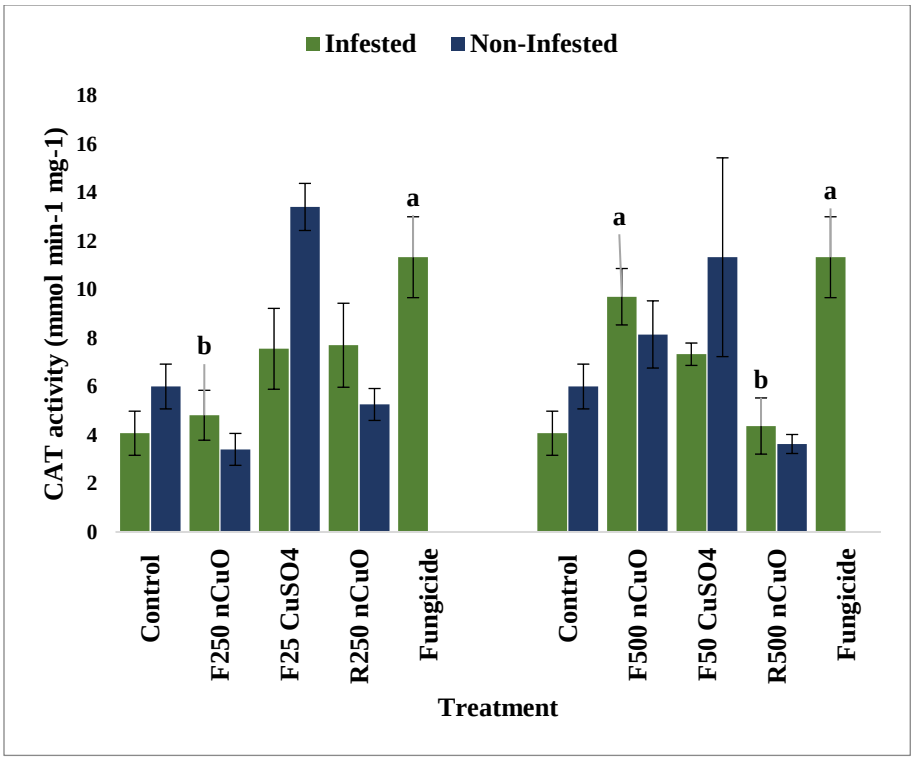


Figure 4.6 Root catalase (CAT) activities of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to untreated infested control and the commercial fungicide, respectively, using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.7 Polyphenol oxidase (PPO) activity

Polyphenol oxidase activities in plant roots are shown in Figure 4.7. Relative to untreated infested control, none of the treatments in infested plants significantly affected the PPO activities, except root exposure to CuO at 500 mg/kg, which had 175% increase in PPO activities ($p \leq 0.05$). Interestingly, root exposure to CuO at 500 mg/kg also increased the PPO activities in infested plants

by 497%, compared with the commercial fungicide. Similarly, foliar exposure to $CuSO_4$ at 50 mg/L had 342% increase in the PPO activity, compared with commercial fungicide ($p \leq 0.05$). Polyphenol oxidases are copper containing enzymes that catalyze the oxidation of phenolic compounds to highly reactive quinones. Plant resistance against pathogen invasion may be enhanced by quinones (Isaac, 1991). Several studies have demonstrated that PPO plays a vital role in the defense response against pathogens, although full mechanistic evidence is still missing (Isaac, 1991; Mayer, 1965). In this study, elevated PPO activities in the root is an indication that the Cu treatment boosted the defense mechanism of the plants by enhancing the activity of the enzyme in the presence of Fusarium pathogens. Our results are in agreement with the findings of Choudhary *et al.* (2017), who reported an elevated activity of PPO on exposure of *Curvularia* infested maize to Cu-chitosan nanoparticles. The authors suggested that the increased activity of PPO and other stress enzymes (peroxidases, POD) can be attributed to biosynthesis of suberin, melanin and lignin (Fugate *et al*., 2016; Gómez-Vásquez *et al.,* 2004). These proteins may further strengthen the plant cell wall against pathogen invasion (Choudhary *et al.,* 2017). Relatively similar to our findings, Elmer *et al.* (2018) also reported an upregulated expression of PPO in nCuO treated watermelon infested with Fusarium. In addition, Anusuya and Sathiyabama (2015) also reported an elevated activity of PPO in *Pythium aphanidermatum* infested turmeric plant treated with β-d-glucan nanoparticles (GNPs). Overall, antifungal strength of nCuO is suggested to rely on its capacity to enhance activities of defense and antioxidant enzymes.

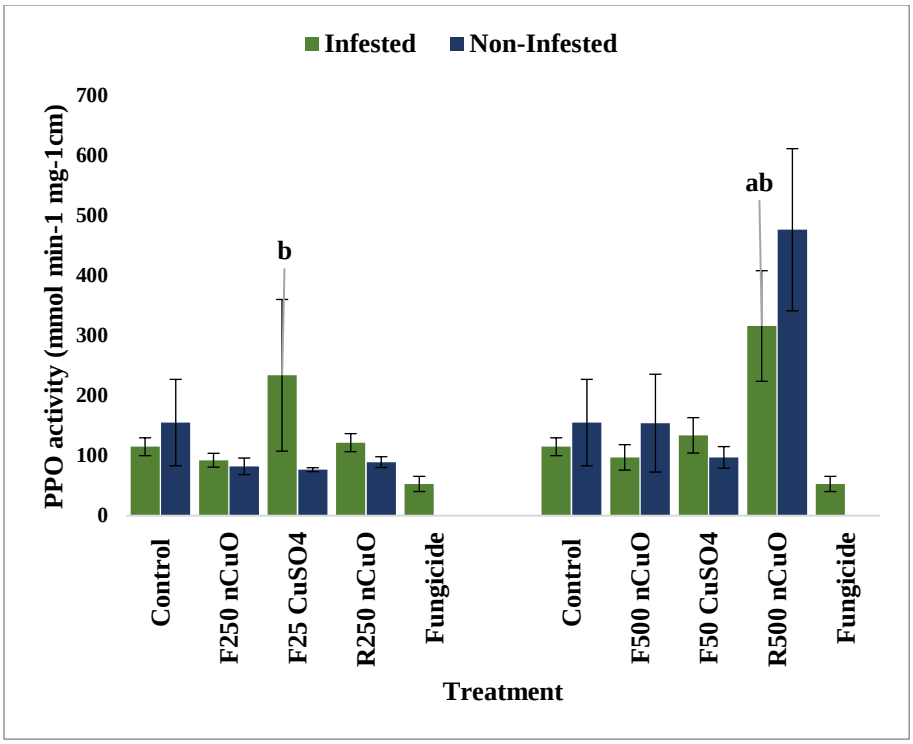


Figure 4.7 Root polyphenol oxidase (PPO) activities of infested and non-infested tomato plants exposed to foliar applications of nCuO at 250 and 500 mg/L, $CuSO_4$ at 25 and 50 mg/L and commercial fungicide, Kocide 3000 (500 mg/L Cu), and root application of nCuO at 250 and 500 mg/kg. Values represent mean ± SE (n=3). Significant differences ($p \leq 0.05$) are indicated by letters a and b, in respect to untreated infested control and the commercial fungicide, respectively, using one-way ANOVA follow by LSD test. The mean difference of treatments were compared with the respective controls.

4.3.8 Uptake and translocation of micro- and macronutrient content

Table 4.1 shows the quantified elements in the roots of infested and non-infested tomato plants. Overall, none of the treatments affected the root uptake of P, Mg, Mn and Na in both infested and non-infested tomato plants ($p \leq 0.05$). However, relative to untreated infested control, amount of S in the roots of infested plants increased in foliar exposed infested plants; $CuSO_4$ at 25

(26%) and 50 mg/L (32%). Similarly, root S uptake increased in infested plants on root exposure to nCuO at 250 (55%) and 500 mg/kg (39%), as well as commercial fungicide (55%), compared with untreated infested control ($p \leq 0.05$). It can be suggested that ionic Cu from these Cu-based compounds and the pathogen presence in the soil can form organic complexes that can influenced the root uptake of S. Organic protein complexes such as metallothionein and phytochelatins contain S as a major component in plant systems (Abrol and Ahmed, 2003). These protein complexes play crucial role in metabolic processes and cellular detoxification. However, decreasing trend in root S uptake was also observed in the plants treated foliarly with nCuO at 250 (32%) and 500 mg/L (33%), and $CuSO_4$ at 25 (19%) and 50 mg/L (15%), compared with the commercial fungicide. There is no known information to support this strange behavior of nCuO and $CuSO_4$ in infested plants, relative to the commercial fungicides.

Root Ca uptake reduced significantly by 18% in infested control, compared non-infested control. None of the root treatments significantly affected root Ca uptake. However, foliar exposure of infested plant to nCuO at 250 mg/L decrease the root Ca uptake by 27%, compared with untreated infested control ($p \leq 0.05$). Similarly, commercial fungicide treatment also decrease the Ca content by 28%, compared with infested control. In addition, foliar exposure to nCuO at 250 mg/L and $CuSO_4$ at 25 and 50 mg/L, decreased by 24, 27 and 32%, respectively, compared with the commercial fungicide ($p \leq 0.05$). Calcium is involved in several physiological processes in plant systems, where it can act as a messenger involving in plant growth and development, hormone production, enzymatic activity, nodulation, biotic, and abiotic environmental stressors (White, 2001). Calcium can also be taken up either as Ca^{2+} or can be complexed with organic acids (White, 2001). The reduced Ca content observed virtually in all treatments can be attributed to many factors including over utilization of Ca content in other physiological processes which maybe aggravated by pathogen

invasion. In addition, Fusarium secretes fusaric acid, which can bind divalent metals like Ca, thereby limiting their uptake in the plant system. Moreover, Ca can also form chelating complexes with other organic compounds in the soil and Cu has been implicated to interact with Ca channel, which interfere with Ca uptake in the plant.

Root Fe and Al (non-essential element) contents in the roots of infested plants significantly reduced by all treatments, respectively; foliar exposure to nCuO at 250 (75, 63%) and 500 mg/L (71, 58%), and $CuSO_4$ at 25 (91, 66%) and 50 mg/L (80, 63%), via root, nCuO at 250 (86, 71%) and 500 mg/L (63, 53%), and Kocide 3000 (83, 71%), compared with untreated infested control ($p \leq 0.05$). In addition, only foliar exposure to $CuSO_4$ at 50 mg/L had 26 % increase in root K uptake, compare with infested control. Similarly, foliar exposure to nCuO at 250 mg/L and root exposure to 500 mg/kg, significantly increased the Mo content in the roots of infested plants by 4 and 5 %, respectively, compared with infested control ($p \leq 0.05$). Moreover, root Si content decreased on foliar exposure to nCuO at 250 (40%) and 500 mg/L (36%), and $CuSO_4$ at 25 (54%); via root, nCuO at 250 (41%), and Kocide 3000 (33%), compared with untreated infested control ($p \leq 0.05$).

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Table 4.1. Root concentration of nutrient elements (µg/g) in Fusarium wilt infested and non-infested tomato plants exposed to root application of nano-CuO (0, 250 and 500 mg/kg) or foliar application of nano-CuO (0, 250 and 500 mg/L) and $CuSO_4$ (0, 25, and 50 mg/L), and a commercial fungicide, Kocide 3000 (500 mg/L Cu). Averages with superscripts a and b are statistically significant to untreated infested control and commercial fungicide, respectively ($p \leq 0.05$), compared with the respective control; n = 3 using one-way ANOVA follow by LSD test.

Nutrient	Treatment	Infested	Non-Infested
S	Control	4784.87	5279.54
	Foliar 250 CuO	5050.83[b]	4553.74
	Foliar 25 $CuSO_4$	6007.96[ab]	5664.18
	Root 250 CuO	7412.76[a]	5759.09
	Foliar 500 CuO	4952.18[b]	5037.24
	Foliar 50 $CuSO_4$	6333.92[ab]	5622.35
	Root 500 CuO	6643.23[a]	5627.27
	Fungicide, Kocide 3000	7419.90[a]	
Mg	Control	7433.45	8230.14
	Foliar 250 CuO	7668.90	7349.94
	Foliar 25 $CuSO_4$	7748.63	7380.77
	Root 250 CuO	9180.74	8121.60
	Foliar 500 CuO	7550.98	7513.42
	Foliar 50 $CuSO_4$	8508.74	8802.85
	Root 500 CuO	8567.38	7843.88
	Fungicide, Kocide 3000	8562.11	
Ca	Control	20351.60	24934.49[a]
	Foliar 250 CuO	14949.00[a]	18352.93

	Foliar 25 $CuSO_4$	20071.57[b]	18974.28
	Root 250 CuO	16777.25	25236.28
	Foliar 500 CuO	19469.17[b]	20650.74
	Foliar 50 $CuSO_4$	19497.55[b]	18381.08
	Root 500 CuO	17593.84	25494.69
	Fungicide, Kocide 3000	14727.18[a]	
Fe	Control	339.31	306.48
	Foliar 250 CuO	82.41[a]	185.24
	Foliar 25 $CuSO_4$	32.01[a]	59.09
	Root 250 CuO	49.25[a]	81.94
	Foliar 500 CuO	100.15[a]	66.28
	Foliar 50 $CuSO_4$	67.93[a]	114.61
	Root 500 CuO	125.97[a]	72.66
	Fungicide, Kocide 3000	58.06[a]	
Al	Control	339.48	323.30
	Foliar 250 CuO	123.52[a]	182.75
	Foliar 25 $CuSO_4$	115.67[a]	96.40
	Root 250 CuO	98.97[a]	124.96
	Foliar 500 CuO	141.02[a]	118.46
	Foliar 50 $CuSO_4$	125.79[a]	144.35
	Root 500 CuO	161.26[a]	145.34
	Fungicide, Kocide 3000	99.96[a]	
P	Control	13608.81	18222.40[a]
	Foliar 250 CuO	12104.35	15069.44
	Foliar 25 $CuSO_4$	13760.63	16037.79
	Root 250 CuO	15994.39	22457.79

	Foliar 500 CuO	15390.68	17574.50
	Foliar 50 $CuSO_4$	14928.87	13257.41
	Root 500 CuO	17707.61	24807.57
	Fungicide, Kocide 3000	16089.75	
K	Control	29921.97	27609.79
	Foliar 250 CuO	25851.95	26889.50
	Foliar 25 $CuSO_4$	29416.86	24329.68
	Root 250 CuO	33776.39	26707.96
	Foliar 500 CuO	28373.41	29061.67
	Foliar 50 $CuSO_4$	37584.93[a]	22305.47
	Root 500 CuO	29390.32	29755.90
	Fungicide, Kocide 3000	31740.35	
Mo	Control	10.00	9.84
	Foliar 250 CuO	10.37[a]	10.28
	Foliar 25 $CuSO_4$	9.85[b]	10.11
	Root 250 CuO	10.10	9.96
	Foliar 500 CuO	10.12	10.09
	Foliar 50 $CuSO_4$	10.06	10.10
	Root 500 CuO	10.54[a]	9.83
	Fungicide, Kocide 3000	10.27	
Si	Control	153.30	194.14[a]
	Foliar 250 CuO	92.42[a]	130.60
	Foliar 25 $CuSO_4$	70.94[a]	72.17
	Root 250 CuO	89.77[a]	95.00
	Foliar 500 CuO	97.65[a]	71.64
	Foliar 50 $CuSO_4$	118.81	116.57

	Root 500 CuO	126.87	88.30
	Fungicide, Kocide 3000	102.63[a]	
Na	Control	4349.84	3766.99
	Foliar 250 CuO	5505.97	3589.40
	Foliar 25 $CuSO_4$	3957.36	3277.93
	Root 250 CuO	4850.94	3986.92
	Foliar 500 CuO	4155.74	3445.28
	Foliar 50 $CuSO_4$	4277.94	4452.00
	Root 500 CuO	5157.09	4266.05
	Fungicide, Kocide 3000	3984.69	

The translocation of mineral elements to the aerial parts (shoot) of infested tomato plants indicated no significant changes in the amount of Ca, K, and Na, compared with untreated infested control. Infested tomato plants on exposure to the commercial fungicide had 21% significant reduction in shoot S content, compared with the infested control ($p \leq 0.05$). However, shoot S content increased in infested plant exposed foliarly to nCuO at 250 mg/L (34%), compared with the commercial fungicide. In addition, foliar exposure to $CuSO_4$ at 25 and 50 mg/L increased the shoot Mg content by 22%, compared with infested control. Similarly, root exposure to nCuO at 250 and 500 mg/kg increased the Mg content by 28 and 33%, respectively, compared with the infested control. In contrast, shoot Mg content decreased in infested plants on foliar exposure to nCuO at 250 (16%) and 500 mg/L (15%), relative to commercial fungicide. However, infested plants treated with nCuO at 250 and 500 mg/kg had 14 and 19% increase in shoot Mg content, compared with the commercial fungicide ($p \leq 0.05$).

Root and foliar exposure to nCuO at 500 mg/L decreased the shoot Zn content by 77 and 80%, respectively, compared with untreated infested control ($p \leq 0.05$). However, Zn content in the shoot

increased significantly by foliar exposure to $CuSO_4$ at 25 (93%) and 50 mg/L (116%), compared with untreated infested control. Notably, relative to the commercial fungicide, foliar exposure to $CuSO_4$ at 25 and 50 mg/L increased the Zn content by 276 and 675%, respectively. In addition, root exposure to nCuO at 250 mg/kg increased the Zn content by 149%, compared with the commercial fungicide ($p \leq 0.05$).

Non-essential element, Al, increased in the shoot of infested tomato plants on foliar exposure to $CuSO_4$ at 25 (27%), and 50 mg/L (16%), compared with the infested control. Moreover, foliar exposure to nCuO at 500 mg/L also increased shoot Al content by 16%, relative to the commercial fungicide. Similarly, nCuO at 250 mg/L, when applied through the leaves increased the shoot P content by 13%, compared with the infested control ($p \leq 0.05$). The amount of Si accumulated in the shoot increased some of the treatment in the infested tomato plants. Foliar exposure to $CuSO_4$ at 50 mg/L, the Kocide 3000, and root exposure to nCuO at 250 mg/kg increased the Si content by 41, 42, and 47% respectively, compared with the infested control. In addition, shoot Si content decreased significantly by 29% in infested plant treated foliarly with nCuO at 250 mg/L, compared with the commercial fungicide.

Table 4.2 Shoot concentration of nutrient elements (µg/g) in Fusarium wilt infested and non-infested tomato plants exposed to root application of nano-CuO (0, 250 and 500 mg/kg) or foliar application of nano-CuO (0, 250 and 500 mg/L) and $CuSO_4$ (0, 25, and 50 mg/L), and a commercial fungicide, Kocide 3000. Averages with superscripts a and b are statistically significant to untreated infested control and commercial fungicide, respectively ($p \leq 0.05$), compared with the respective control; n = 3 using one-way ANOVA follow by LSD test.

Nutrient	Treatment	Infested	Non-Infested
S	Control	6387.12	5072.25
	Foliar 250 CuO	6804.08	4605.06[a]
	Foliar 25 $CuSO_4$	5937.58[b]	5264.64
	Root 250 CuO	6152.47	4298.01
	Foliar 500 CuO	5397.36	4765.79
	Foliar 50 $CuSO_4$	6124.37	5453.31
	Root 500 CuO	5859.31	3972.20
	Fungicide, Kocide 3000	5074.74[a]	
Mg	Control	6094.84	6602.59
	Foliar 250 CuO	5768.40[b]	6215.42
	Foliar 25 $CuSO_4$	7419.01[a]	6988.93
	Root 250 CuO	7814.88[ab]	7078.01
	Foliar 500 CuO	5789.47[b]	6353.08
	Foliar 50 $CuSO_4$	7440.45[a]	7615.42
	Root 500 CuO	8131.70[ab]	6815.28
	Fungicide, Kocide 3000	6843.07	
Ca	Control	22607.31	24767.63
	Foliar 250 CuO	22126.38	19426.52

	Foliar 25 $CuSO_4$	25799.36	22577.44
	Root 250 CuO	25484.19	22532.82
	Foliar 500 CuO	19805.00	20705.40
	Foliar 50 $CuSO_4$	25364.23	24216.51
	Root 500 CuO	27038.09	20211.51
	Fungicide, Kocide 3000	22044.20	
Zn	Control	11.14	5.97
	Foliar 250 CuO	9.16	1.13
	Foliar 25 $CuSO_4$	21.48[ab]	9.45
	Root 250 CuO	14.23[b]	7.37
	Foliar 500 CuO	2.59[a]	1.98
	Foliar 50 $CuSO_4$	24.07[ab]	3.90
	Root 500 CuO	2.17[a]	0.00
	Fungicide, Kocide 3000	5.72	
Al	Control	8.03	8.87
	Foliar 250 CuO	8.43	8.90
	Foliar 25 $CuSO_4$	9.34[a]	8.16
	Root 250 CuO	8.21	8.31
	Foliar 500 CuO	10.21[ab]	10.78
	Foliar 50 $CuSO_4$	8.83	8.42
	Root 500 CuO	9.29	8.35
	Fungicide, Kocide 3000	8.83	
P	Control	15039.52	13903.18
	Foliar 250 CuO	16935.2[ab]	14061.69
	Foliar 25 $CuSO_4$	14054.04	14250.82
	Root 250 CuO	15408.45	14193.22

	Foliar 500 CuO	15014.99	13795.25
	Foliar 50 $CuSO_4$	14847.14	14278
	Root 500 CuO	14542.48	12853.83
	Fungicide, Kocide 3000	14525.91	
K	Control	68575.11	56827.87
	Foliar 250 CuO	64198.17	63780.78[a]
	Foliar 25 $CuSO_4$	63126.13	58580.14
	Root 250 CuO	65391.9	58936.71
	Foliar 500 CuO	66407.58	60468.38
	Foliar 50 $CuSO_4$	67662.58	66813.75
	Root 500 CuO	64923.73	59465.77
	Fungicide, Kocide 3000	63320.71	
Mo	Control	9.90	9.87
	Foliar 250 CuO	10.22	10.06
	Foliar 25 $CuSO_4$	9.74	9.80
	Root 250 CuO	10.08	10.21
	Foliar 500 CuO	10.13	10.00
	Foliar 50 $CuSO_4$	9.91	10.15
	Root 500 CuO	10.29	10.05
	Fungicide, Kocide 3000	10.19	
Si	Control	47.57	68.69
	Foliar 250 CuO	48.26	44.51
	Foliar 25 $CuSO_4$	61.37	65.19
	Root 250 CuO	69.97	74.57
	Foliar 500 CuO	53.76	56.02
	Foliar 50 $CuSO_4$	67.02	68.35

	Root 500 CuO	60.98	62.25
	Fungicide, Kocide 3000	67.71	
Na	Control	1634.80	1872.31
	Foliar 250 CuO	2008.51[b]	2143.28[a]
	Foliar 25 $CuSO_4$	1934.79	2008.44
	Root 250 CuO	2160.92[a]	2145.81
	Foliar 500 CuO	2151.49	1966.15
	Foliar 50 $CuSO_4$	1885.83[a]	2214.12
	Root 500 CuO	2209.08	1972.48
	Fungicide, Kocide 3000	2138.08[a]	

Applied Cu treatments altered the uptake and translocation of nutrient elements in the 6 weeks old tomato plants. Uptake of elements such as S, K and Mo, was enhanced in the root by Cu treatments. However, Ca, Fe, Si and Al (non-essential element to plant) were greatly reduced in the tomato root. In addition, generally, subtle effect was observed on nutrient elements accumulation in the tomato shoot as a result of Cu-based treatment. This mixed responses in nutrient elements accumulation can be attributed to inhibition and high affinity of copper to other mineral elements. The interaction of the pathogen with the mineral nutrients may alter the uptake and accumulation in the plant tissues. Previous studies have reported similar plant response to nCuO with altered nutrient composition, although most of these studies are conducted without pathogen infection (Tamez *et al.*, 2019; Hong *et al*, 2015). The changes in mineral composition may have underlying biomolecular effects but no measurable negative effect was observed at this stage of the tomato plants.

4.4 Conclusion

Overall, this study revealed the impacts of root and foliar exposure of infested tomato plants to nCuO at both tested concentrations (250 and 500 mg/L) at the flowering stage. These treatments show that nCuO, with antifungal activities (Elmer and White, 2016; Elmer et al., 2018;) can also mitigate significant effects of Fusarium infestation on tomato plants, improve the chlorophyll content, and enhance the activities of antioxidant/stress (CAT) and defense (PPO) enzymes. Our findings also revealed that nCuO treatments appear to be more effective in infested tomato plants, compared with the commercial fungicide, Kocide 3000. This is an indication that nCuO with reduced environmental impact can replace conventional fungicides which can release large amount of metal/agrichemicals into the environment. However, further study is required to fully understand the mechanism of nCuO in the suppression of diseases in plants.

Chapter 5

Conclusion

This doctoral research was aimed at exploring the potential of nanoscale elements, (CeO_2 and CuO), in suppression of Fusarium wilt disease caused by *F. oxysporum f. sp. lycopersici* in tomato (*Solanum lycopersicum* cv Bonny Best). The study provides useful information on the role of the nanoparticles in the disease suppression, their respective impact on crop yield, nutritional value of the tomato fruit and overall plant health upon exposure to the nanoparticles in the presence of the pathogen.

The objective of the first phase of this study was to evaluate the potential of nano-CeO_2 to suppress Fusarium wilt disease and to enhance tomato production. Cerium acetate was used as ionic control for comparison. Our findings revealed that Fusarium wilt incidence reduced significantly in infested plants by root or foliar exposure to 250 mg/L of nano-CeO_2 and CeAc, compared with untreated infested controls. In addition, the Ce-compounds enhanced the chlorophyll content of tomato plant, increased yield, and enhanced nutrient utilization, all without accumulating in plant tissues, except in roots. The potential of Ce compounds to enhance plant growth and improve resistance against infection could be attributed to characteristics of lanthanide group of elements (such as antioxidant and photosynthetic enhancement), which cerium belongs to. Micro-fertilizers containing rare elements have been extensively used in China since the 1970s to promote plant growth, productivity, and improve resistance against stress. Overall, the findings from this phase of the research show that nano-CeO_2 has the potential to suppress Fusarium wilt and improve the chlorophyll content in tomato plants, with no significant negative impact on the total plant.

The second phase of this doctoral research was aimed at filling the knowledge gap of the previous study. At this stage, we evaluated the nutritional impact of nano-CeO_2 on tomato fruit physiological parameters, lycopene content, non-structural carbohydrates (reducing and total sugars) and nutritional elements of tomato cultivated in *Fusarium* infested and non-infested soils. The ionic counterpart (cerium acetate) was used positive control to correlate the impact of the nanoparticle in the study. Our findings revealed that the Fusarium infestation negatively affected the tomato fruit quality by reducing fruit height, dry weight, and lycopene content. These fruits quality were improved in infested plants exposed to foliar treatment with nano-CeO_2 at 250 mg/L. Notably, Ce accumulation in treated tomato fruits was below the detection limit. This significantly indicated that, at the tested concentrations, the Ce-compounds do not accumulate in the fruits. While the disease suppression and productivity enhancement is highly desirable, concerns over food safety with regard to nanomaterial use in agriculture is still important. Therefore, the lack of detectable Ce in the tomato fruit of treated plants is very significant. Overall, this follow up study suggests that NP CeO_2 subtle positive effect on the fruit quality with has no severe impact on the nutritional value of tomato fruit, while suppressing Fusarium wilt disease in the tomato plant.

The last phase of this research further the exploration of the nanopesticide potential of CuO nanoparticles. It has been documented extensively that Cu-based nanoparticles have antimicrobial potency against plant pathogens, and copper is commonly used as an active ingredients of commercially available fungicides. This study aimed to evaluate the physiological and biochemical impact of nCuO on tomato plants grown in Fusarium infested soil at flowering stage. Disease incidences manifest physically on plants mostly at anthesis. We target this stage to evaluate how the nanoparticle treatment could mitigate stress resulting from pathogen invasion.

Commercial fungicide, Kocide 3000 and ionic counterpart, $CuSO_4$ were used to compared the relative impact of nCuO on the plant. The result from this study revealed that infestation reduced chlorophyll content in plants leaves but increased in plants exposed to root or foliar treatment with nCuO or $CuSO_4$, compared with plant treated with Kocide 3000. Root or foliar exposure to nCuO also increased the plant biomass, and enhanced the root polyphenol oxidase and catalase activities, compared with untreated infested control. Overall, this study showed that nCuO improved the chlorophyll content, increased plant biomass, and improve defense mechanism against the pathogen.

Collectively, the outcomes of this doctoral research suggest CeO_2 and CuO nanoparticles have potential as a novel disease management strategy with no negative impact on fruit nutritional quality, with reduced environmental impact. These findings are significant for future determination of the sustainability of nano-enabled disease suppression platforms in agriculture, although additional molecular-level mechanistic evaluation is recommended to fully understand and guarantee the safety of these approaches.

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Curriculum Vitae

Ishaq Olarewaju Adisa was born on January 13, 1979 in Ilorin, Kwara State, Nigeria. Ishaq O. Adisa earned his Bachelor of Science degree in Biochemistry from University of Ilorin, Nigeria in 2003. He had his Masters' degree in Environmental Biochemistry in 2014 from University of Port Harcourt, Nigeria. He received Nigeria Federal Government Scholarship (TETFUND) to pursue his Doctoral program in the United States in 2014/15. He joined Ph.D. program in Environmental Science and Engineering at UTEP in Fall 2015.

Dr. Adisa is a recipient of a State of Texas Public Education Grant for International Students in 2017/2018. Dr. Adisa has presented his research at several meetings including the 2017 Annual Conference Sustainable Nanotechnology Organization (SNO) Los Angeles, California, and 2018 Chemistry Research Day Seminar, at UTEP. He has authored and co-authored 14 research articles in various reputable academic journals.

While pursuing his degree, Dr. Adisa worked as a research assistant in a USDA funded research project (Nanoscale Elements Suppress Plant Disease, Enhance Macronutrient Use Efficiency, and Increase Crop Yield) under the supervision of Dr. Gardea. He was also a teaching assistant in Departments of Biology, and Chemistry and Biochemistry. In 2017 and 2018, he volunteered as a Judge 2018 UTEP Annual COURI Spring Undergraduate Symposium.

Dr. Adisa's dissertation, "Potential of nanoscale elements to control fusarium wilt disease in tomato (Solanum lycopersicum), enhance macronutrient use efficiency, and increase its yield," was supervised by Dr. Jorge Gardea-Torresdey. Dr. Adisa has authored and co-authored more than 10 research articles published in reputable academic journals.