2019

The Effects of Zinc Nanofertilizers on Tomato Plants

Ketsira Pierre
University of Central Florida

THE EFFECTS OF ZINC NANOFERTILZERS ON TOMATO PLANTS

by

KETSIRA PIERRE

A thesis submitted in partial fulfillment of requirements
for the Honor in the Major Program in Interdisciplinary Studies
in the College of Undergraduate Studies
and in the Burnett Honors College
at the University of Central Florida
Orlando, Florida

Summer Term 2019

Thesis Chair: Swadeshmukul Santra

ABSTRACT

Farmers around the world aim to use soil with adequate nutrients to produce sufficient and quality crops to the world's ever-growing population. Unbalanced use of nutrients in the soil will lead to soil deficiency, which is usually seen in South and Southeast Asian countries. This soil deficiency is often due to loss of micronutrient(s) within the soil from farming practices. Micronutrient deficiency affects not only plant growth but human health. Plants grown in nutrient deficient soil produce food with nutrient deficiencies, which affect people dependent on these foods for nutrients (Kathmandu, 2004). Nutrient deficient diseases and disorders like malnutrition are often seen in such cases. Current farming practices often involve leaching, mineralization, and bioconversion, which result in 50-70% loss of micronutrients. Smart practices from nanotechnology can lead conventional farming to more sustainable agriculture (Chhippa, 2016). This study aims to improve the dispersibility and uptake of zinc in plants different dual combination of 'green' capping agents in zinc nanoparticles. The results of this study suggest tomato plants treated with urea coated with 3% Zn (w/w) using NAC-SAL ZnO showed a higher number of leaves and number of fruits set compared to controls.

ACKNOWLEDGEMENTS

To my thesis chair, Dr. Santra, thank you for taking a chance on me and seeing my skills as an asset in your work and research. Without you I would not have discovered my passion for or even understood all that it takes to do research. You always have a smile and willingness to help me understand.

I would also like to thank my committee member Maria Campos. Thank so much for all your patience in all my questions and in learning new laboratory techniques. You were always there when I needed help and always encouraged me to be more involved in all facets of research. Thank you for making less nervous and smile in my introduction to research. Thank you for all you have done for me throughout this process.

A special thank you to Stephen Smith, Ryan Heetai, and Zon Thwin for all their help in experiments, using lab equipment, and monitoring and watering plants when I could not.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

B – Boron

Ca – Calcium

Cl – Chloride

Cu – Copper

Cys – Cysteine

Fe – Iron

H_2O_2 – Hydrogen Peroxide

HCl – Hydrochloric Acid

HNO_3 – Nitric Acid

His – Histidine

K – Potassium

Mg – Magnesium

Mn – Manganese

Mo – Molybdenum

NAC – N-Acetyl Cysteine

NaClO – Sodium Hypochlorite

NF – Nanofertilizers

NP – Nanoparticles

N – Nitrogen

Ni – Nickel

P – Phosphorus

SAL – Sodium Salicylate

S – Sulfur

VAD – Vitamin A Deficiency

VAM – Vesicular Arbuscular mycorrhiza

Zn – Zinc

ZF – Zinc Finger

$Zn(NO_3)_2$ $6H_20$ – Zinc Nitrate Hexahydrate

CHAPTER 1: INTRODUCTION

Micronutrient deficiencies affect more than one-third of the world's population. The term 'Hidden Hunger' was coined to describe the lack of essential the lack of essential vitamins and minerals needed for human health and development (Harding, Aguayo, Webb, 2018) due to their widespread presence and association in health and developmental consequences. Deficiencies in zinc (Zn), Iodine, Iron (Fe), Vitamin A (VAD) pose the greatest global public health concern; one-third of VAD children are under the age of 5 and come from low- and middle- income countries. About 18% of children under the age of 5 suffer from Fe-deficiency anemia, 30% of people from poor iodine intake, and 17% from insufficient zinc intake.

Although these micronutrient deficiencies affect people across the globe, populations and individuals in poorer regions of south Asia and Sub-Saharan Africa have more affected and more serve cases of these deficiencies. In 2013, 95% of the 1.7% of deaths among children under the age of 5 from VAD came from South Asia and Sub-Saharan Africa (Stevens et al, 2013, as cited by Harding et al 2018). Anemia from lack of Fe among pregnant women (52%) and children under the age of 5 (58%) exceed the global presence (38% and 43% respectively). Insufficient iodine intake in South Asia (32%) is comparable to the global average (30%), while inadequate Zn intake accounts for 30% of the global 17% (Stevens et al, 2015; Andersson et al, 2012; Wessells et al, 2012, as cited by Harding et al 2018). Despite South Asia's progress, micronutrient deficiencies have seen little improvement over the past decades.

Harding *et al* consider South Asia a paradox. At one angle, it considered the fastest developing region in the world. According to the Asian Development Bank in 2016 and the

UnICEF South Asia 2016 progress report, India has strong economic growth, Bangladesh has significant poverty reduction, falling rates of undernutrition in children in Nepal illiteracy declining in Sri Lanka, and growing agricultural productivity in Pakistan. At another angle, this region is home to the largest reported malnutrition. In 2015, the International Food Policy Research Institute reported South Asia has the largest number of stunted children under the age of 5, rising rates of overweight and obesity related to diabetes and chronic heart disease and continue to suffer from a wide range of micronutrient deficiencies.

Unbalanced use of nutrients in the soil will lead to soil deficiency, which is usually seen in South and South East Asian countries. This soil deficiency is often due to loss of micronutrient(s) within the soil from farming practices. Micronutrient deficiency affects not only plant growth, but human health. Plants grown in nutrient deficient soil produce food with nutrient deficiencies, which affect people dependent on these foods for nutrients (Kathmandu, 2004). Nutrient deficient diseases and disorders like malnutrition are often seen in such cases. Current farming practices often involve leaching, mineralization, and bioconversion which result in 50-70% loss of micronutrients. Smart practices from nanotechnology can lead conventional farming to a more sustainable agriculture (Chhippa, 2016).

Micronutrients

Essential nutrients in plants are divided into two categories, micronutrients (Mn, Zn, Mo, Fe, Cu, Ni, B, Cl) and macronutrients (N, P, K, Ca, Mg or S) (Sharma, 2016). All these nutrients are crucial for optimal plant growth and quality. Although micronutrients are required in small quantities (less than 100ppm), they are just as important as macronutrients for metabolic

processes in plants. According to Sharma, micronutrients play key roles in metabolism regulation, reproduction, and protection against biotic and abiotic stresses as well as protection against pathogenic infection. They also play a role in the organic structure, enzyme activation, and osmoregulation (Sharma, 2016). Micronutrients are not localized to particular parts in plants; therefore, it is not enough for micronutrients to be present; the correct quantity is also essential for the biochemical requirement. Deficiency in micronutrients is reflected in plant structure, function, development, and adaptive response. Their deficiencies lead to severe diseases and manifestation in plants, and also the quantity and quality of food (Tripathi et al 2015). Zn and Fe are most commonly seen in short supply.

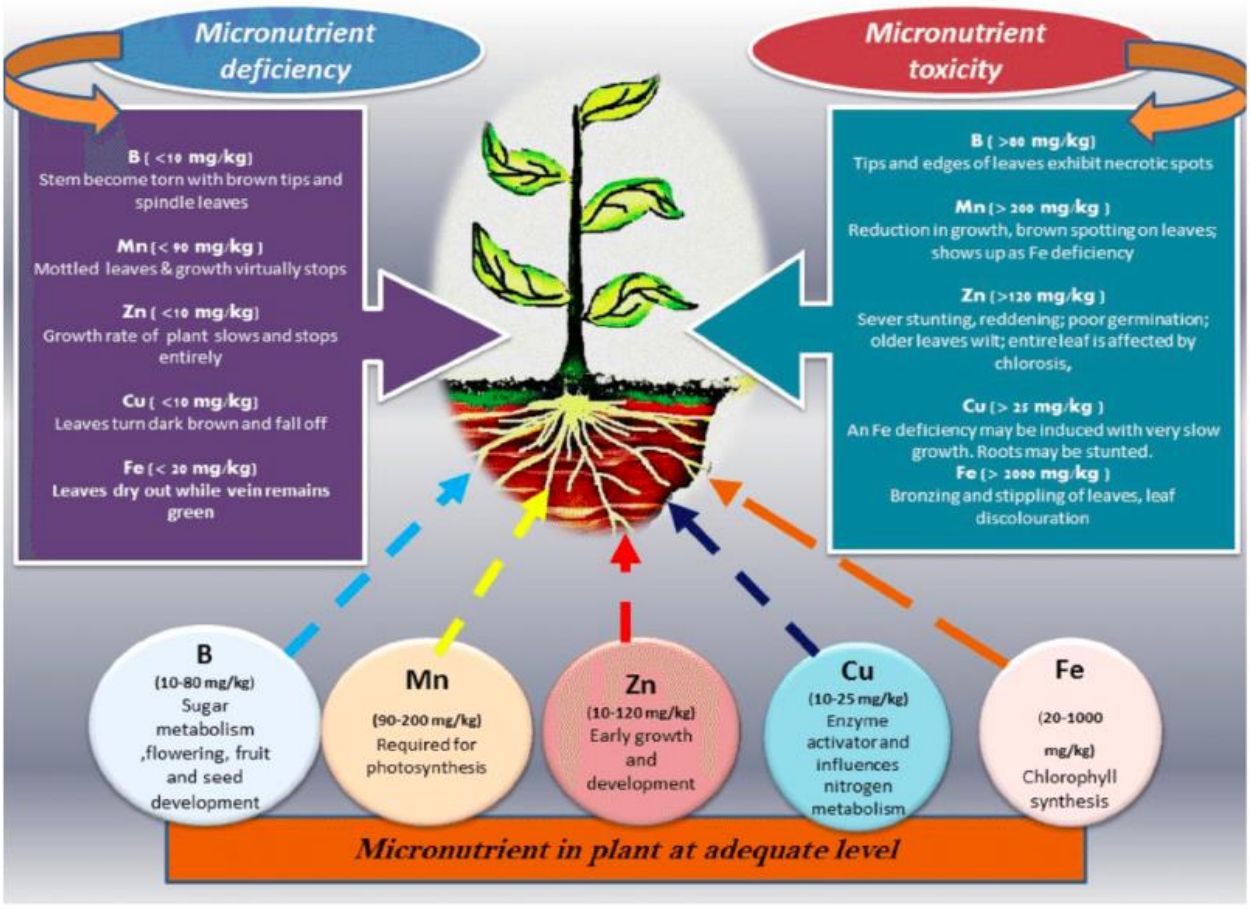

Figure 1: Appropriate micronutrient levels in soil as well as their physiologic response to deficiency and toxicity (Epstein and Bloom 2005; Marschner 2012 as cited by Tripathi et al 2015)

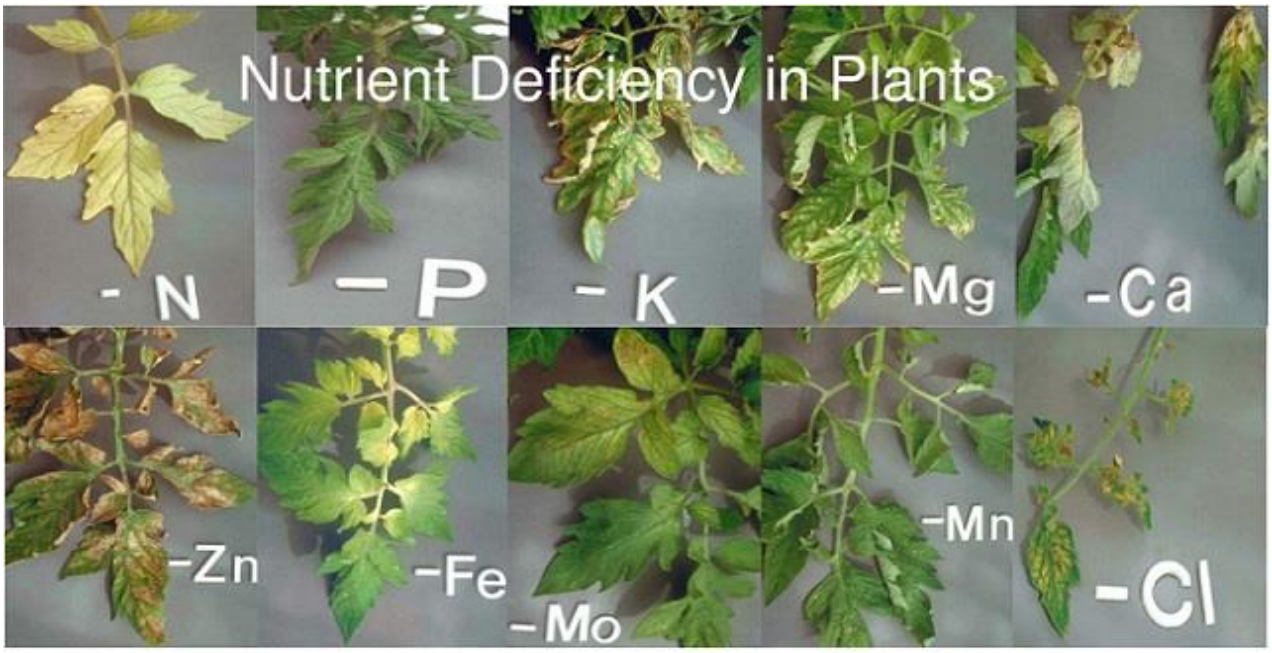

Figure 2: Tomato plant response to deficiency in different micronutrient and macronutrients ("Deficiency Symptoms in Tomato Crops," 2012)

Zinc

A transition metal bearing the atomic number 30, Zn plays an essential role in the structure and function of plants. As a structural component to hundreds of proteins, Zn provides stability to many regulatory proteins and enzymes. Zn is a divalent cation (Zn^{++}) with completely filled d-shell orbitals, making the element redox-stable thus forming more stable complexes (meaning no redox activity in plants) unlike other metal ions such as Mn, Fe, and Cu (Brown et al., 1993; Lohry, 2007). High concentrations of other divalent metals such as P inhibit Zn uptake (Lohry, 2007). Zn acts as a structural, regulatory, and functional cofactor to hundreds of proteins and enzymes with catalytic and regulatory functions (Sharma, 2006). Zn (II) ions also facilitate folding of protein subunits (Kluska et al., 2018).

According to Takatsuji (1998) at the National Institute of Agrobiological Resources, plants have evolved new functional motifs unique to plants alone that have modified their protein structures for regulatory processes. These structural motifs are known as 'zinc finger' (ZF). ZF structural role in Zn (II) ion was first proposed when found in *xenopus laevis* transcription factor IIIA (Kluskak et al., 2018). ZF utilize sequence motifs, CCHH, in which two cysteines (cys) and/or two histidines (his) coordinate Zn atoms to form local peptide structures necessary for their specific function. The CCHH sequence allows for the Zn (II) ion to adopt a $\beta\beta\alpha$ fold with hydrophobic structures that provide additional stabilization to the ZF domain (Kluskak et al., 2018; Takatsuji, 1998). Classified by Zn-binding amino acid sequence arrangement, ZF motifs regulate transcription factor and play crucial roles in interaction with other molecules (Takasuji, 1998; Kluska et al., 2018). ZF domain in Zn(II) binding adopt tetrahedral coordination which adds to providing stabilization to protein domains (Kluska et al., 2018). Kluska et al. also state, entropically driven binding of Zn(II) to cys residues in ZF make the stability of the core more sensitive to factors such as ionic strength, pH, or temperature enhancing reactivity with other molecules and metal ions. Any displacement of Zn(II) ions by other metals will lead to structural and/or functional defects of ZN (2018).

Zinc role in plant metabolism is essential to activates like carbonic anhydrase which is critical in the photosynthesis of C4 and C3 plants. Without zinc, RNA polymerase cannot occur, and the enzyme inactivated. Zinc is also involved in ribosomal fraction stabilization, cytochrome synthesis, and carbohydrate metabolism (Hafeez et al 2013). Zinc activates enzymes involved in protein synthesis, pollen formation, and regulation of auxin. It is also involved in gene expression which is necessary for the tolerance of environmental stresses in plants. Zinc plays a

critical role in plant reproduction, water stress prevention, and protects against toxic effects of reactive oxygen species (Sharma, 2006). Presence of Zn as a cofactor, protects plants from oxidative stress. Zinc also provides defense mechanisms against harmful pathogens (Tripathi et al, 2015).

Studies have shown that zinc uptake is concentration-dependent and saturable, which implies it is carrier-mediated transport. The cherra corellina model shows a linear dependence on the concentration of at least 50ppm for uptake. The average concentration is 80ppm and as high as 100ppm. Uptake is known to be inhibited at low temperatures — zinc aids in the maintenance of structural integrity of biomembranes. Zinc decreases the permeability of plant plasma membranes by decreasing leakage from structural loss. A study conducted by Welch et al. (1982) depicted deficiency of zinc in the soil led to the leakage of P and Cl in roots of wheat plants. By adding zinc, the leakage effects were reversed due to structural integrity (Sharma, 2006).

Zinc Deficiency in Plants

Common in South Asian countries, zinc deficiency results in a decrease yield and nutrient quality in plants. Deficiency can lead to a yield decrease up to 40% in plants (Cullen et al., 2008). Micronutrient Zn deficiency can also decrease the quality of products harvested (Lohry, 2007). Zinc deficiency is often seen with the use of phosphorus fertilizers, heavy use of such fertilizers increases plant yield, but decrease Zn in food (Hafeez et al., 2013). Raun Lohry (2007) describes the induced deficiencies as a result of diluted zinc from increased growth from added P, inhibited uptake from other divalent cations, and enhanced Zn absorption to hydroxides and oxides of Fe, Al, and CaCO3. The additional P-Zn interactions inhibit Zn' translocation from the

roots to the shoots. A series of studies conducted by Camak and Marschner (1986) on phosphorus-induced Zn deficiency in cotton plants found evidence of enhanced P uptake rates, impaired shoot control of P uptake and translocation, and changes in physiological availability of Zn. Results concluded that P-induced Zn deficiency is caused by increase P uptake (increased from $5x10^{-5}$ to $1.25x10^{-3}$ M). Phosphorus toxicity characterized by leaf puckering and grayish-brown marginal necrosis as well as Zn deficiency symptoms such as interveinal chlorosis were seen in P induced Zn deficient cotton plants (Carmark and Marschner, 1986).

A complication to Zn is the infection of roots with Vesicular Arbuscular mycorrhiza (VAM) which take up more Zn than uninfected roots (Lohry, 2007). VAM is formed from a symbiotic relationship between phycomycetous fungi and angiosperm roots (Sullia, 1991). VAM infections increase P uptake and improve plant resistance to soil-born pathogens, especially wilt and rot pathogens (Sullia, 1991; Sharma et al., 1992). Unfortunately, high nutrient concentrations from VAM-infections in mycorrhizal plants make such plants more susceptible to foliar pathogens (Sharma, 1992). Foliar disease includes: Septoria tritici (leaf blotch), Puccinia striiformis (yellow rust), Erysiphe graminis (powdery mildew) and Puccinia triticina (brown rust) (Audsley, 2005).

Plant pH is significant for all plant nutrients, especially zinc. Deviation in pH range 5.5 to 7.0, can decrease Zn2+, which is the zinc concentration readily available to plants7. As pH increases, zinc available will decrease. Zinc concentration decreases from 10-4 M to 10-10 M when pH increases from 5 to 8. The availability of Zn is reduced more in alkaline soils than in acidic soils due to the lower solubility of soil Zn. The higher carbonate contents in alkaline soils absorb zinc and hold it in a form that is not bioavailable. Liming acidic soils reduce Zn uptake causing Zn deficiency (Hafeez et al., 2013). Zinc available declines in flooding and submerged

soil conditions because of the changes in pH and insoluble zinc compounds8. Deficiency is also affected by climate, weather, high HCO_3^- presence in soil, highly leached soils, sandy soils, calcareous granite, and acidic soils.

According to Sharma (2006), Zinc deficiency symptoms appear on the subterminal leaves after early stages of healthy growth. Development of bronze or reddish-brown tints or blotches associated with fading lamina and interveinal chlorosis, reduced leaf size, and condensation of shoot growth are common symptoms of zinc deficiency (Sharma, 2006). Necrosis at root apex and inward curling of leaf lamina are also seen (Tripathi et al., 2015). Plants are seen to have stunted growth noticeable 2 to 3 weeks after transplantation and may die in severe cases. Symptoms initiate in tips of young leaves as fading lamina and appearance of light brown necrotic lesions that spread and connect as deficiency continues. Leaves of tomato plants also show inward curling of lamina and epinasty in response to zinc deficiency4. Although zinc deficiency is harmful to plants, excess Zn is toxic. Therefore, an adequate supply of Zn is essential for proper growth and development.

Zinc Deficiency in Human Health

Zinc deficiency affects not only plants but human health as well. Developing countries get the majority of nutrients from plant-based diets. Reliance on plant-based diets may be due to the economic, traditional, religious, and or cultural reasons (Solomons, 2000). Therefore, plants low in nutrients will transfer to people. Due to this phenomenon, high prevalence of deficiency in vitamin A, Fe, Zn, riboflavin, and vitamin B12 are often seen. Many of these nutrients are available in meat.

Zn is required for catalyst activity of enzymes, plays a role in protein synthesis, wound healing, immune function, DNA synthesis, and cell division. Zinc is also required for proper taste and smell. It is also necessary for proper growth and development during childhood, adolescence, and pregnancy ("Zinc: Fact Sheet for Health Professionals," n.d.).

According to the National Institute of Health Office of Dietary Supplements, recommended dietary supplements, recommended dietary allowance for Zn are: 2-3mg in infants, 5mg in children, 8-11mg in adolescents and adults, and 11-13mg for pregnant and lactating women. Insufficient intake or absorption of Zn will lead to Zn deficiency. Consequences of Zn deficiency include growth retardation (more severe in children, hypogonadism in males, changes in neuro-sensory (abnormal taste sensation) and delayed wound healing (Prasad, 1998). Hair loss and alteration in hair color from black to a reddish-brown have also been observed (Maret and Sandstead, 2006). Other significant adverse clinical effect of Zn deficiency is impairment of cognitive function (memory loss), increased oxidative stress, and upregulation of inflammatory cytokines (Prasad, 2017). Lymphocyte proliferation is also affected due to Zn involvement in DNA synthesis and cell division. Tymulin, a hormone involved in T-lymphocyte maturation is Zn-dependent, therefore adversely affected by low Zn presence (Prasad, 1995). Effects on the nervous system included impaired brain function, never decreased conduction, ataxia, disorientation, and impaired neuropsychological performance (Maret and Sandstead, 2006).

Severe dermatitis manifests later as Zn deficiency increases. Mild deficiency in pregnant women results in increased maternal morbidity, abnormal taste sensation, prolonged gestation, inefficient labor, and atonic bleeding (Prasad, 1998). Further insufficient intake can lead to

stunted brain development in fetus and infertility in men (Hafeez, 2013). High morbidity and death from diarrhea, pneumonia, and other infections in children can also occur from foodborne illnesses have been reported in developing counties. Zinc deficiency is often associated with Fe deficiency (both are inhibited by P increase) and affects cognitive and reproductive performance, provoke pregnancy complications leading to low birth weight and defects (Akhtar et al., 2013).

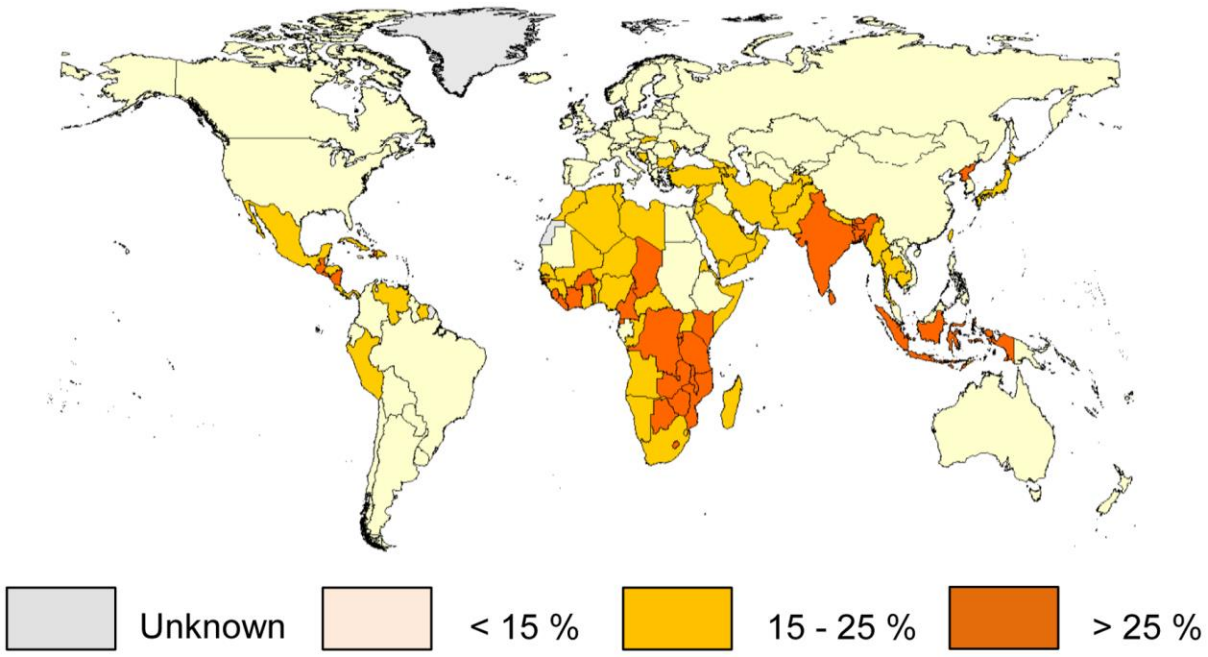

Figure 3: Estimated prevalence of inadequate zinc intake across the globe (Wessells and Brown, 2012).Figure 3: Estimated prevalence of inadequate zinc intake across the globe (Wessells and Brown, 2012).

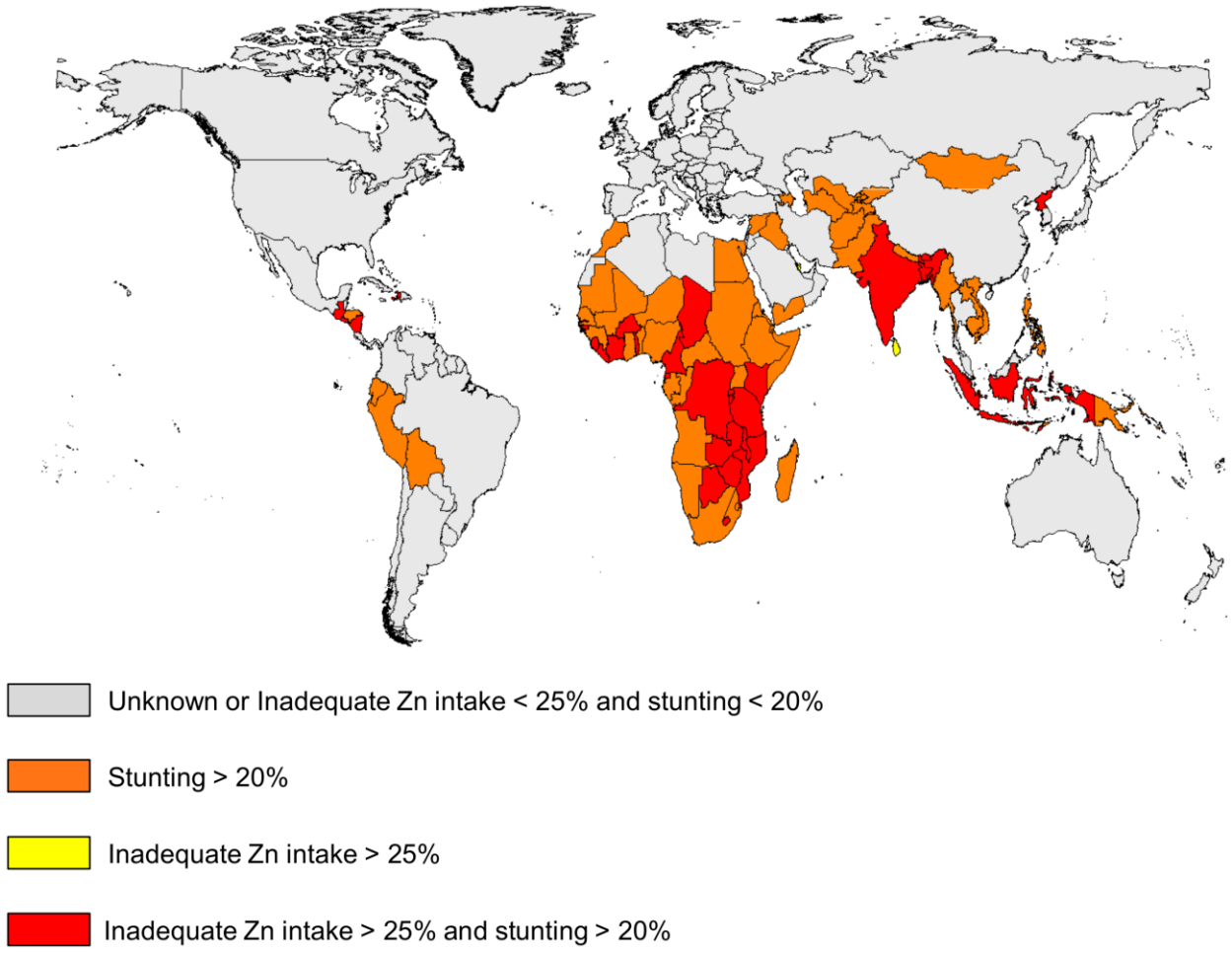

Figure 4: Risk of zinc deficiency based on prevalence of childhood shunting and prevalence of inadequate zinc intake (Wessells and Brown, 2012).

Application of Nanoparticles to Soil

Current farming practices often involve leaching, mineralization, and bioconversion, which result in 50-70% loss of micronutrients. Smart practices from nanotechnology can lead conventional farming to more sustainable agriculture (CChipa, 2016). Nanotechnology involves technology at a nanoscale (1-100nm) (Nasrollahzadeh et al., 2019). It is a "field that consists of

creating and utilizing chemical, physical, and biological systems from single atoms and molecules to form functional structures at a nanoscale. Nanotechnology allows a wide range of diverse application amongst disciples like chemistry, agriculture, physics, medicine, and various fields of engineering. Nanotechnology can improve sustainable agriculture with Nano-fertilizers (NF) and Nano-pesticides in the form of precision farming by converting essential plant nutrients into Nano-coated or Nano-encapsulated particles and delivering to plants. NF improve nutrient use efficiencies through slowly releasing nutrients. Nanoparticles provide several advantages to promoting agricultural productivity, including enhancement of seed germination and growth against stress. Zinc-nanoparticles are widely used due to their high reactivity; they enhance Zn availability as well as other nutrients in the soil (Aziz et al., 2019). The potential that this smart and controlled method has can lead to more sustainable environmental solutions (CChipa, 2016).

According to Kharissova et al. (2013), due to problems associated with environmental contamination from synthesis practices of nanomaterials, the use of 'greener 'environmentally friendly methods are on the rise. The synthesis of using such materials can lower the toxicity of resulting materials and byproducts. More environmentally friendly routes to NP and nanomaterials involve extracts from plants and other natural products; such products include tea, coffee, banana, pure amino acids, wine, table sugar, and glucose, have been used as capping and reducing agents during synthesis (Kharissova et al., 2013).

Capping agents are often used to inhibit NP overgrowth and aggregation as well as control structural NP characteristics in a precise manner (Niu and Li, 2013). In this study, to improve ZnO solubility, urea, sodium salicylate (SAL), and n-acetyl cysteine (NAC) were used as capping agents. Urea, SAL, and NAC all have a natural presence in plants. Urea is a known N

macronutrient metabolite in plants (Merigout et al., 2008). SAL is a plant hormone that induces plant defense against abiotic stresses (War et al., 2011). NAC is a natural antioxidant. It contributes to the beneficial effects of onion and garlic in warding off illness and cardiovascular protection (Souza et al., 2011).

This study aims to investigate the effects of NP synthesized with a combination of different capping agents (urea, SAL, NAC) to improve ZnO dispersibility and uptake by plants. Due to the difficulty of monitoring NP in soil, a greenhouse experiment was developed by growing tomato plants in different NF treatments and comparing phenotypic developments such as height, number of leaves, fruit, and flowers. Weekly measurements were taken and averaged to analyze the comparable benefits of NF treatments to controls.

CHAPTER 2: MATERIALS AND METHODS

Materials

All reagents and chemicals were purchased and provided by the Santra Research Group at the Nanoscience Technology Center, University of Central Florida. The Santra Research Group also prepared and provided the Zn, NPs, urea granules and capping agents used for this study.

Soil Preparation and Digestion for Characterization

For the atomic absorption spectroscopy machine to perform elemental analysis of a sample, the sample must be in a form the instrument can process which is usually liquid. For soil preparation, the soil was first was dried at 60 C for 48 hours and sieved through a 2mm mesh strainer. Then 1g of the sieved soil was applied to each centrifuge tube (3 tubes were used).

For sample digestion, a water bath was set between 95-98°C in a fume hood. In the fume hood, 5 mL of DI water and 15 mL of concentrated HNO_3 were added to each tube via pipette. The slurry was then mix and added to the water bath and covered. The samples were heated for a 1 hour then refluxed without boiling. If brown fumes generated, the heating steps were repeated until there were no more brown fumes (about 4 hours). Then 5 mL of HNO_3 was added to samples and put back into the water bath for 30 more minutes to confirm soil was entirely digested. Samples were allowed to cool down to room temperature. Then 5 mL of H_2O_2 was added to each tube and put back into the bath for another hour. Samples were then removed from the bath, and 5 mL of HCl was added to each sample. Samples were then filtered and diluted for

AAS characterization (Robinson et al., 2005 as cited by JoVe Science Education Database, 2019).

ZnO Nanoparticles and Soil Characterization for Zn Content

Atomic absorption spectroscopy (AAS) is an elemental analysis used to determine ppm levels of trace metals. For this experiment, AAS was used to confirm zinc presence and determine average percent of Zn content for each treatment. A___lamp was used to test for Zn concentration. Three samples of each treatment were prepared by Santra Research Group. The samples were tested against Zn standards at 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, and 2.5 ppm. Samples with high concentrations were diluted. The absorbance and calibration curve equation were used to determine the concentration of Zn for each sample. The concentration of Zn was multiplied by dilution to find the concentrations in ppm of Zn. Percent Zn was calculated using concentrations in ppm of Zn over the total weight of NP per 1mL. To find the average percent Zn content, percent Zn concentration of all three samples per treatment was added and divided by 3. The average percent of Zn content was used to prepare 3ppm NP solutions for coating urea and seed germination experiment.

Urea Coating with ZnO Nanoparticles

Nanoparticles were dispersed in a saturated urea solution in absolute ethanol. Then 3% (w/w) of metallic Zn was incorporated to 2 mm urea granules. Mineral oil and PVP were used to improve the coating process. Food coloring was added to help differentiate the different

treatments. Samples were rotated until the urea granules were completely coated. Once coated, samples were set to dry before being added to the soil for the greenhouse experiment.

Greenhouse Experiment

The effects of the different combinations of capping agent on Zn NP was evaluated using tomato plants, BHN 602 VFFF Hybrid, purchased from Amazon and organic potting soil, Nature's Care Organic Potting Soil Mix, purchased from Home Depot. The organic potting soil was analyzed for Zinc using AAS. The soil was dried to provide a constant weight when measured. The tomato plants were grown in a temperature-regulated greenhouse. There were 27 tomato plants, 5 for each treatment (UREA only, NAC-Urea, Urea-SAL, NAC-SAL, and Bulk ZnO), and 2 with only the commercial soil. The seeds were planted in trays about 1/8 inch deep. The potted seeds were watered in and then germinated in an elevated plastic dome to promote soil-moisture retention (trap moisture and keep the heat high) for about 7-10 days until seedlings. Once the true leaf emerged, the plants then transplanted into 3-inch pots containing the 1lbs dry soil and different treatments, coated NP with varying combinations of capping agents (fertilizer). Of the 1lbs dried soil, 3% was the created fertilizer which contains 3% Zn. This treatment only occurred once. Tomato cages were added to each plant to support the stems and decrease susceptibility to rot or damage from insects. The tomato plants were observed and watered at the same time with the same volume of water. Plant phenotype such as height, quality, number of leaves, number of flowers, and yield was measured and recorded every week.

Seed Germination Experiment

Seed germination experiment was conducted to test the effects of NF treatments on seed germination rate and root length. For this experiment, the NPs were tested in solution (3ppm metallic Zn) . Zinc is a micronutrient, therefore, amount added is very small, with urea (nitrogen macronutrient) coating we would not be able to see the effects of the treatment. 35 mL of each test solution was prepared (DI water, NAC-Urea, NAC-SAL, Urea-SAL, Bulk ZnO, and Zn $(NO_3)_2$ $6H_2O$) into centrifuge tubes. Filter paper was labeled Petri dishes with 5 mL of each test solution. Ferry-Morse beefsteak tomato seeds purchased from Home Depot were sterilized with 10% NaClO (or 10% H_2O_2) for 10 minutes. Seeds were then washed with DI water three times to wash off sterilizing solution. Sterilized seeds were soaking in test solutions for 2 hours (20 mL each). Then seeds were drained and transferred onto filter paper. Each Petri dish contained ten seeds 1 cm apart. Six replicates total for each test solution: 3 each for the 7th and 10th day for germination and root length measurements. Petri dishes were wrapped in aluminum foil and left in a growth chamber (no light, 25°C, 80% humidity). Germination was checked on the 7th and 10th day (Lin et al., 2007).

CHAPTER 3: RESULTS AND DISCUSSION

Atomic Absorption Spectroscopy

AAS results indicate a 17.98 ± 0.66 ppm Zn concentration in the digested solution, that was calculated to 0.54 mg of Zn content per gram of soil. **Figure 1** provides an example of Zn concentration standards and absorbances obtained from AAS was used to create a calibration curve equation applied to determine Zn concentration in the samples. The Zn content in the NPs samples are presented in **Table 1**.

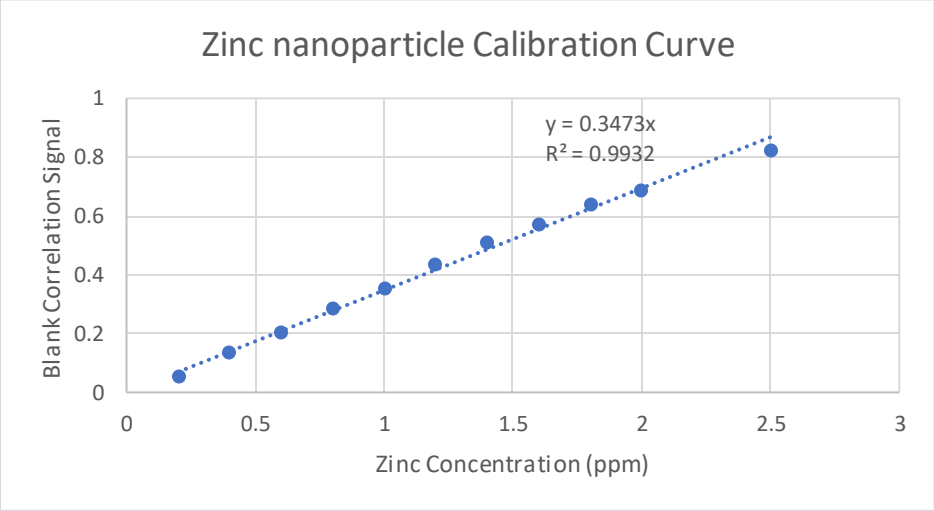

Figure 5: Generated calibration curve equation from correlation between standard Zn concentration and absorbance

Table 1: Average Percent Zn for each Treatment

Treatment	**% Zn**
Urea-SAL ZnO	13.6%
NAC-SAL ZnO	17.8%
NAC-Urea ZnO	13.5%
Bulk ZnO	80%

Greenhouse Experiment

Tomato plants were grown in a temperature-controlled greenhouse for 12 weeks to allow fruits to mature. Of the 6 treatments, 3 were controls: the UREA Only treatment did not contain any Zn to test the compare the effect of the treatments containing capping agents, UREA/Bulk ZnO contained no NP only Zn salts to compare the impact of NP; Soil Only had no Zn NP nor did it contain urea granules this was done to test the phenotypic development of the tomato plants without the boost from the fertilizer treatments compared to tomato plants that did receive the fertilizer treatment **(Table 2)**. The phenotypic events throughout the experiment were recorded and analyzed **(Table 3-9)**. A steady increase in the number of leaves and plant height were observed with comparable data between each treatment. On average, a higher number of leaves were seen in plants with NAC as part of the capping agent. NAC-SAL had the highest number of leaves **(Figure 5)**. For height, Urea Only and Urea-SAL had the tallest measurements **(Figure 6)**. Soil Only had the most elevated height measurements near the start of the experiment, then Urea only near the end. This may be because the soil used in Soil Only was not dried, but the soil was for all other treatments **(Table 4-11)**. NAC-SAL had the most flowers **(Figure 7)**. NAC-Urea and Bulk ZnO were the first to have fruit forming (week 6). In week 11, NAC-SAL had the most fruits, although NAC-Urea had the most substantial yield **(Figure 8 and 9)**. Urea-SAL had the greatest biomass at 3.87 lbs and averaged was 3.22 lbs. Second to Urea-SAL was NAC-SAL with average biomass of 2.89 lbs **(Table 11 and Figure 10)**. On average, for every phenotypic measurement, Soil Only had the lowest measurements. While drying soil allowed for an actual measurement on soil weight, it did take time for the moisture to return to the soil, which in part lead to nutrient washing. The leaching of nutrients from the soil occurred

in a combination of dried soil, and the high volume of water needed for successful transplanting, therefore, leaves took on a yellow-green color (a sign of nutrient deficiency). Fortunately, color and plant condition improved as moisture returned **(Table 4-11)**. Results suggest NAC-SAL has the greatest potential overall in showed higher number of leaves and number of fruits set compared to controls.

Table 2: Summary of Tomato Plant Distribution for Nano-Zinc Treatments

Treatment	**Number of Plants**
UREA Only	5
UREA/Nano-ZnO-NAC-Urea	5
UREA/Nano-ZnO-Urea- SAL	5
UREA/Nano-ZnO-NAC-SAL	5
UREA/Bulk ZnO	5
Soil Only	2

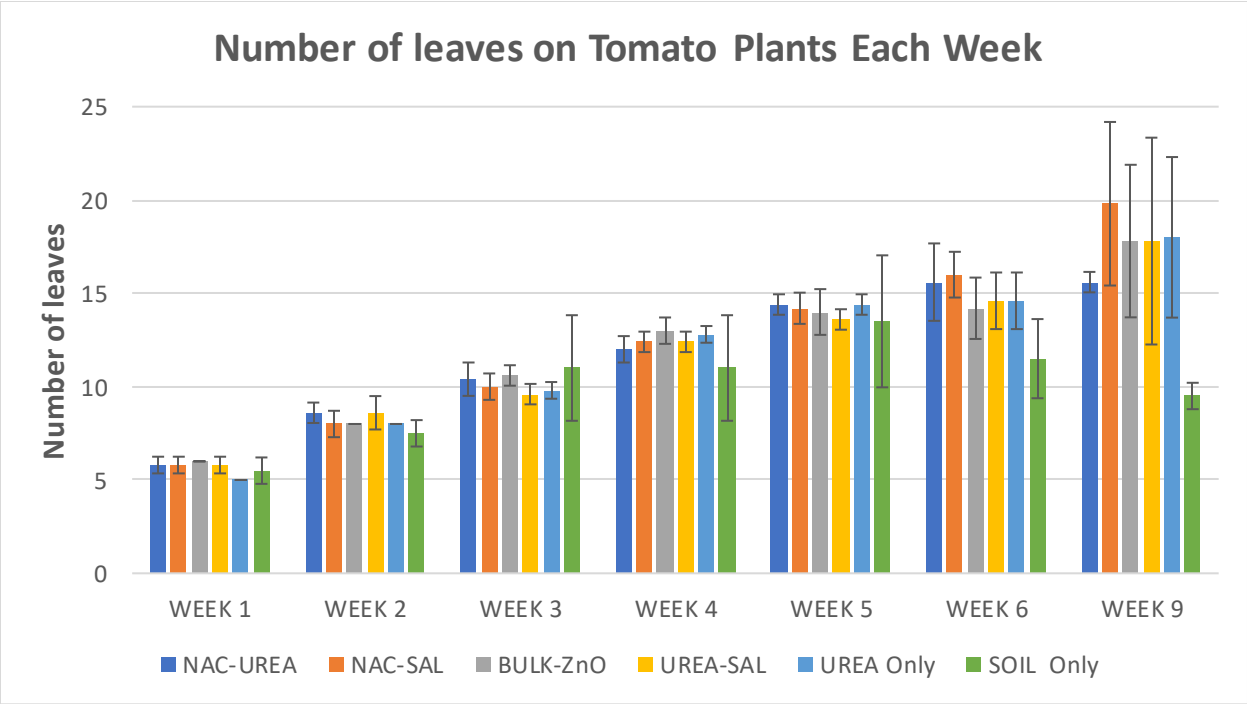

Figure 6: Average number of tomato plant leaves over time per treatment

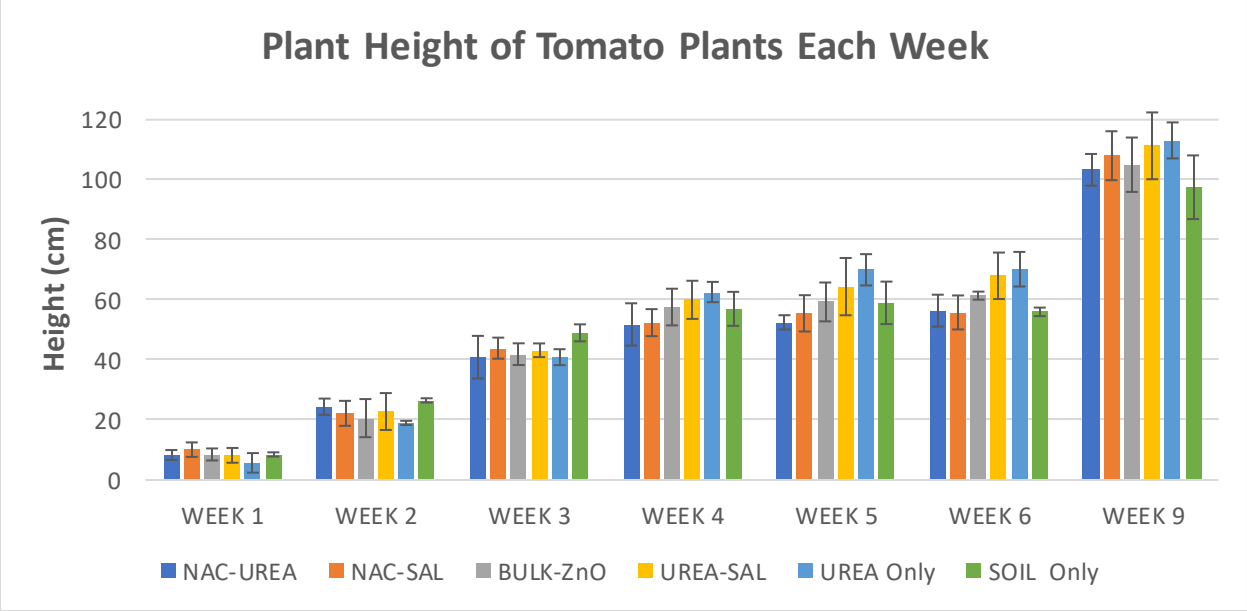

Figure 7: Average height of tomato plants over time per treatment

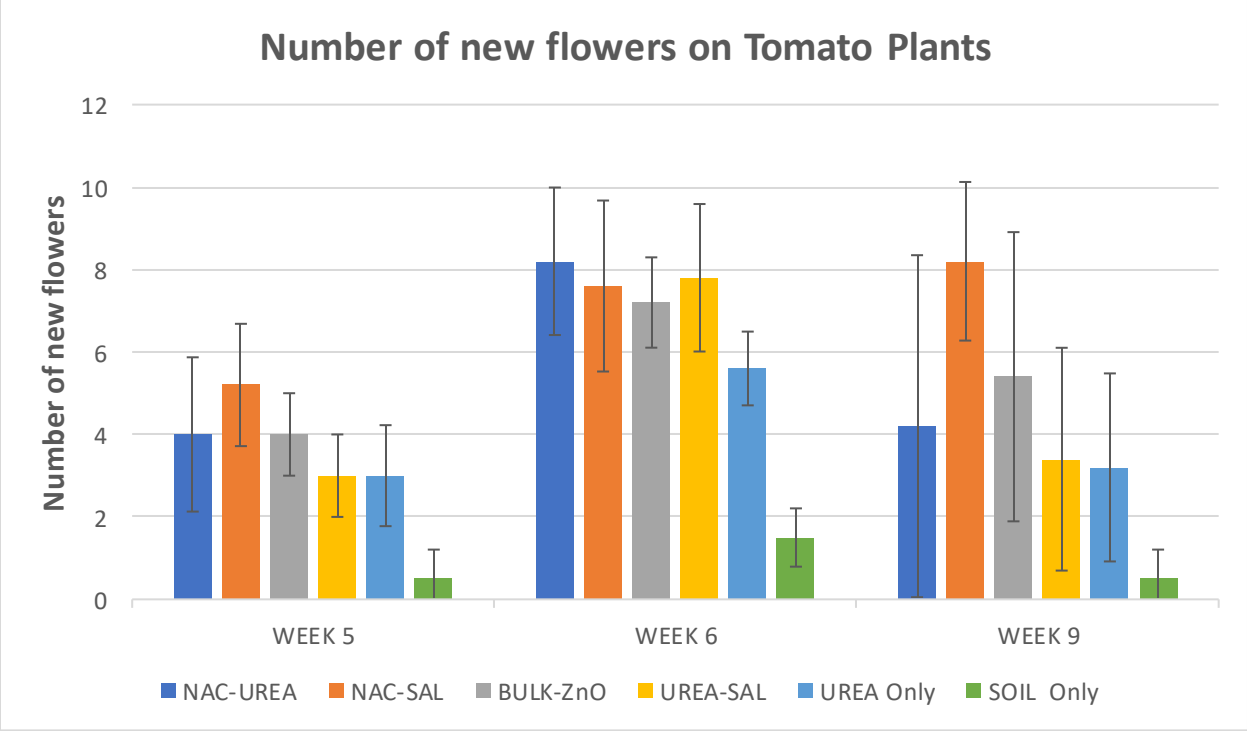

Figure 8: Average number of flowers per treatment

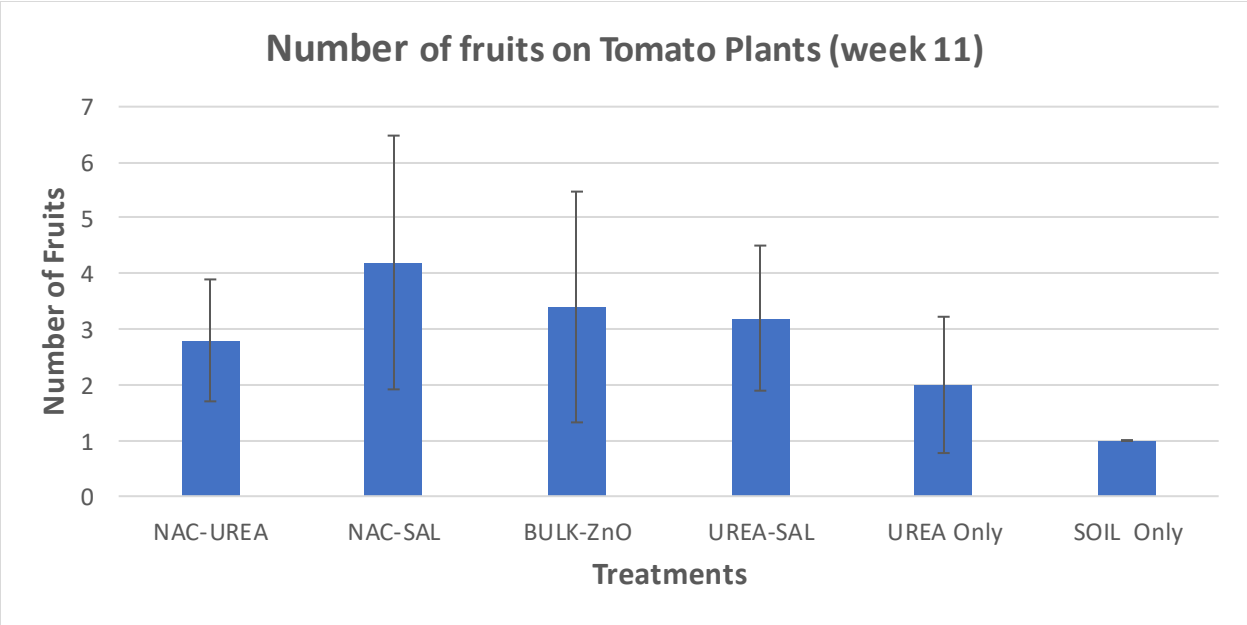

Figure 9: Average number of fruits per plant treatment

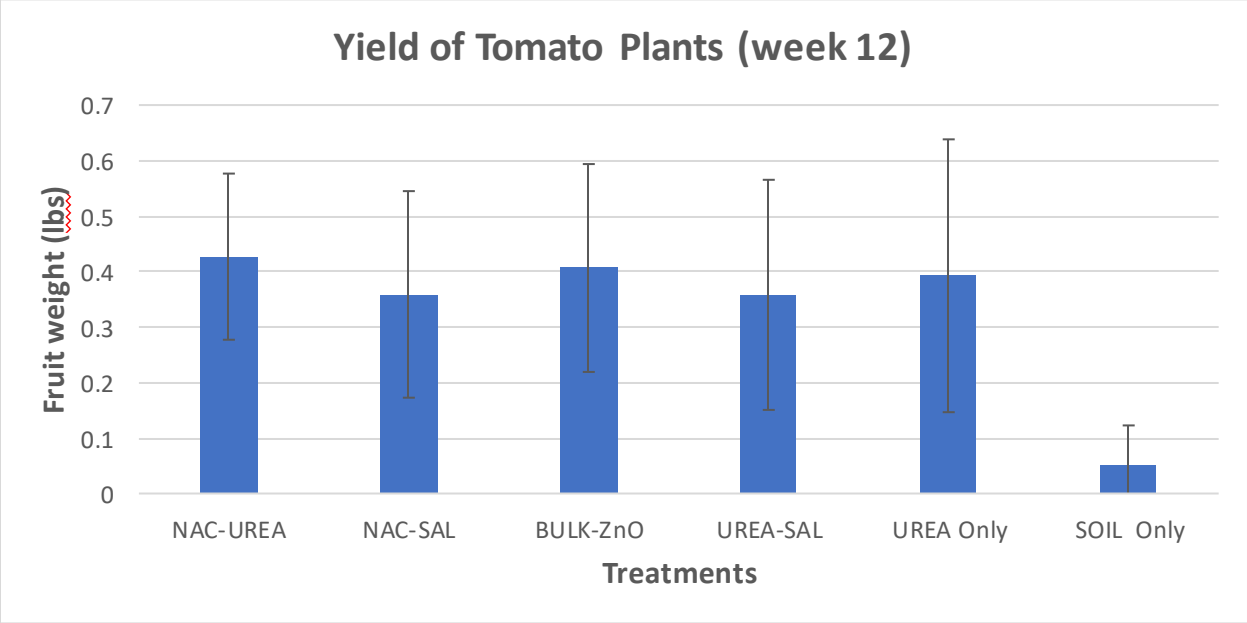

Figure 10: Average weight of fruits per treatment

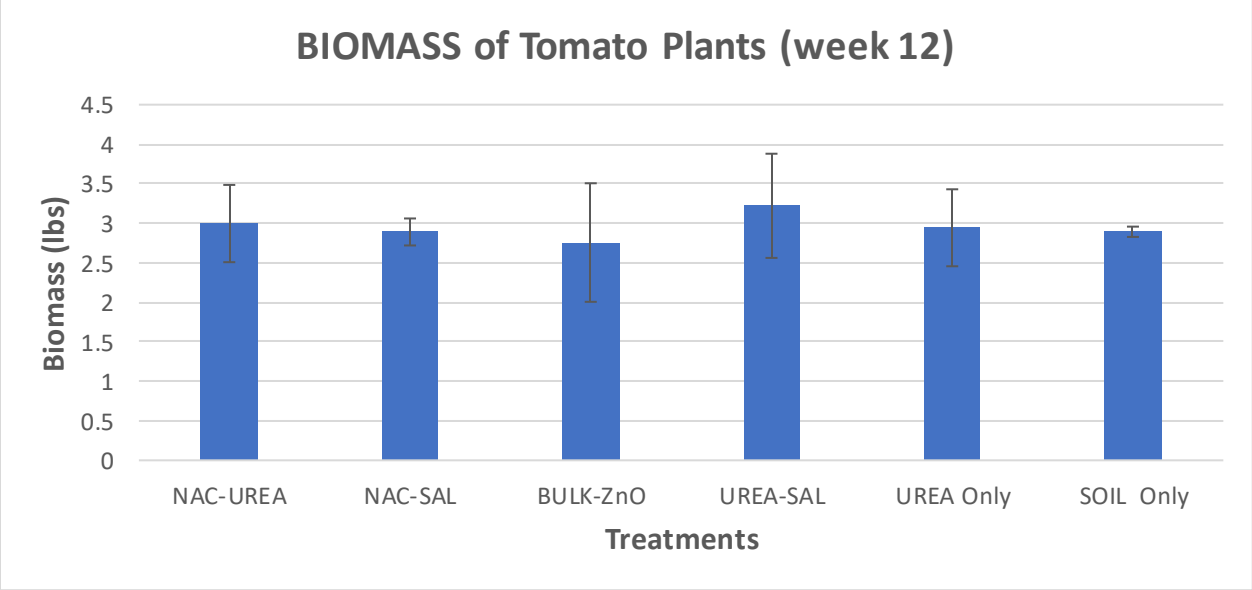

Figure 11: Average biomass per treatment

Seed Germination Experiment

The benefits of NF with dual capping agents were further tested with tomato seed germination and root length (Lin et al., 2007). **Figure 11** shows the germination percentage of each treatment after 7 and 10 days of incubation in zero light, 26°C, and 80% humidity in the growth chamber. Results showed all NF had a germination rate above 85%. On the 7th day, NAC-Urea had the highest germination success at 100% and DI water the lowest at 87%. For the 10th day, NAC-SAL and Zn $(NO_3)_2$ $6H_2O$ had 100% germination success. On the 10th day, DI water Petri dishes were dried. Nanofertilzer solutions have a higher boiling point than DI water; therefore, DI water Petri dishes dried quicker. **Figure 12** shows the root length in each treatment after 7 and 10 days. Results show root length was longest in Urea-SAL at 13.62 cm on the 7th day and NAC-SAL at 15.71 cm on the 10th day. Urea- SAL had comparable results with DI

water and Zn $(NO_3)_2$ $6H_2O$ for 7th-day root length. On average, root length and percent germinated was more significant on the 10th day. These results suggest NAC-SAL NF has more significant potential for seed germination and root elongation.

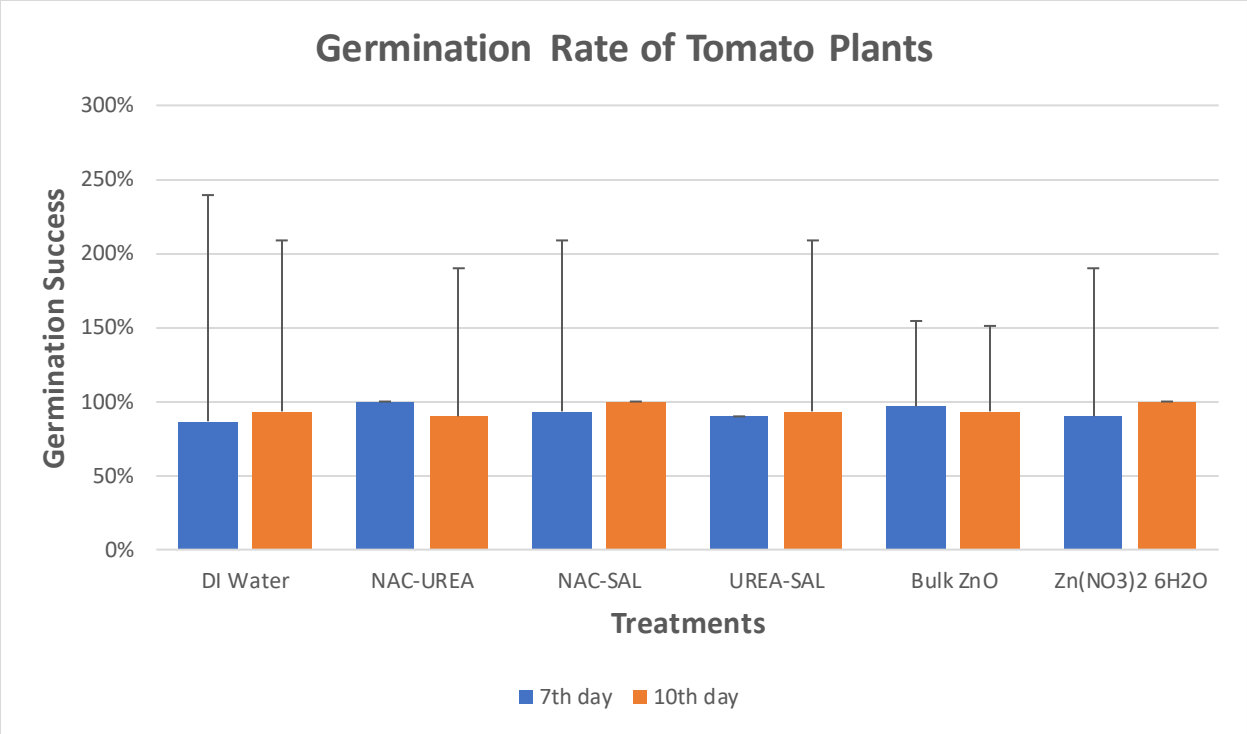

Figure 12: Average germination on the 7th and 10th day

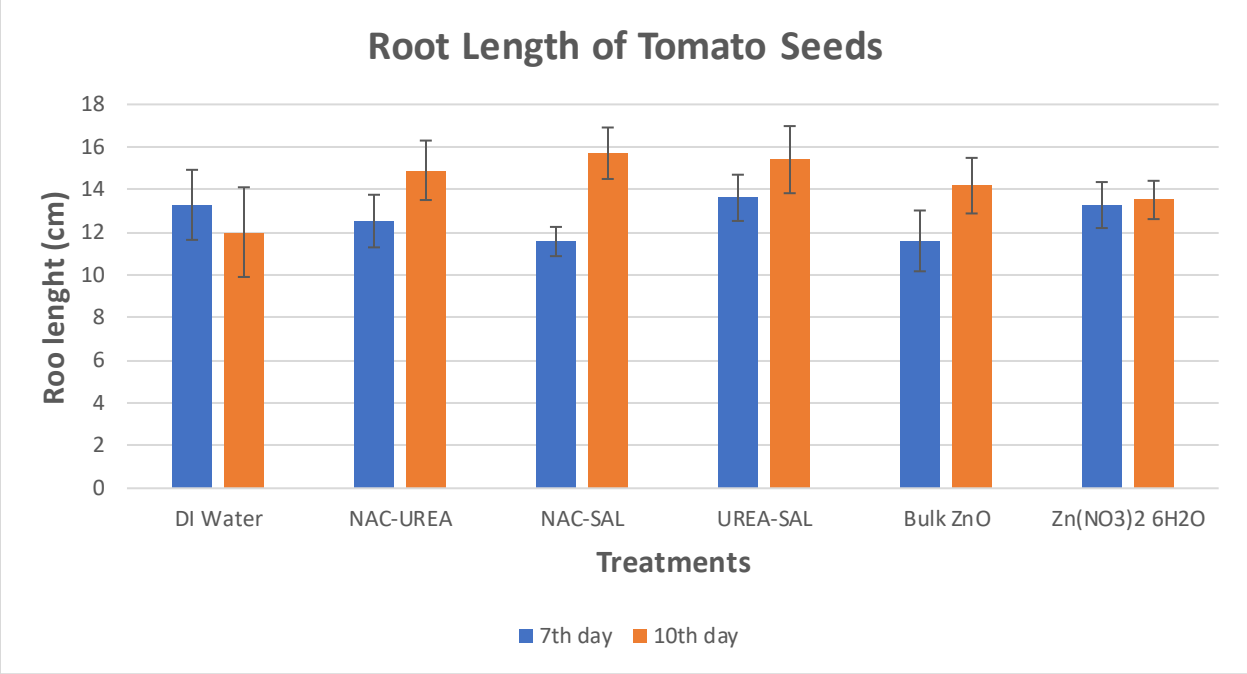

Figure 13: Average root length on 7th and 10th day

CHAPTER 4: CONCLUSIONS

Zinc deficiency is characterized by bronze or reddish-brown tints or blotches associated with fading lamina and interveinal chlorosis, reduced leaf size, and condensation of shoot growth are common symptoms of zinc deficiency (Sharma, 2006). Zinc deficiency can manifest in people dependent on such crops. Tomato plants treated with urea coated with 3% Zn (w/w) using NAC-SAL ZnO showed a higher number of leaves and number of fruits set compared to controls. It is concluded ZnO NP with dual capping agents had a successful impact and can be used as a source of Zn micronutrient. For the seed germination experiment, NAC-SAL NF has more significant potential for seed germination and root elongation.

Although favorable results were collected depicting the benefits of capped NPs, increasing nutrient deficiency was seen in later weeks; Zn was replaced, but because other essential nutrients were not; deficiency in micronutrients and macronutrients lead to a decline in plants resulting in the experiment being shortened. Increasing the number of plants in each treatment will also provide more accurate results — further studies adding NP a week after transplant is suggested. Bigger plant pots and NPs on top of the soil rather than mixed in may reduce nutrient deficiency. For the seed germination experiment, adding more solution to Petri dishes is recommended to avoid them to dry at long periods of incubation.

APPENDIX: Weekly Data Collection and Observations During Greenhouse Experiment

Table 3: Key of leaf condition over 12 weeks

Score	Leaf Condition
1	Healthy
2	Slight green discoloration
3	lighter green
4	Yellow-green

Table 4: Week 1- Phenotype data collection and observation

Week 1			
Treatment	**Pant Condition**	**Leaf Number**	**Height**
NAC-UREA 1	4	6	7 cm
NAC-UREA 2	4	6	10 com
NAC-UREA 3	1	6	10.05 cm
NAC-UREA 4	2	6	8 cm
NAC-UREA 5	2	5	6.5 cm
NAC-SAL 1	2	6	7 cm
NAC-SAL 2	2	6	9.5 cm
NAC-SAL 3	2	6	12 cm
NAC-SAL 4	1	6	9 cm
NAC-SAL 5	2	5	13 cm
BULK-ZnO 1	4	6	7.5 cm
BULK-ZnO 2	2	6	10 cm
BULK-ZnO 3	4	6	8 cm
BULK-ZnO 4	4	6	6 cm
BULK-ZnO 5	1	6	11 cm
UREA-SAL 1	2	6	10 cm
UREA-SAL 2	2	6	9.5 cm
UREA-SAL 3	2	6	9.5 cm
UREA-SAL 4	2	6	8 cm
UREA-SAL 5	4	5	4 cm
UREA ONLY 1	3	5	3.5 cm
UREA ONLY 2	4	5	9.5 cm
UREA ONLY 3	4	5	3 cm
UREA ONLY 4	4	5	9 cm
UREA ONLY 5	4	5	3.5 cm
SOIL ONLY 1	1	5	8 cm
SOIL ONLY 2	1	6	9 cm

Table 5: Week 2 - Phenotype data collection and observation

Week 2			
Treatment	**Pant Condition**	**Leaf Number**	**Height**
NAC-UREA 1	2	9	20 cm
NAC-UREA 2	2	9	27 cm
NAC-UREA 3	2	9	24 cm
NAC-UREA 4	2	8	26 cm
NAC-UREA 5	2	8	25 cm
NAC-SAL 1	4	8	22 cm
NAC-SAL 2	4	8	21 cm
NAC-SAL 3	4	8	25.5 cm
NAC-SAL 4	4	7	16 cm
NAC-SAL 5	4	9	26.5 cm
BULK-ZnO 1	4	8	18 cm
BULK-ZnO 2	4	8	22 cm
BULK-ZnO 3	4	8	15 cm
BULK-ZnO 4	4	8	17 cm
BULK-ZnO 5	4	8	31 cm
UREA-SAL 1	2	9	25 cm
UREA-SAL 2	2	9	27 cm
UREA-SAL 3	2	9	26 cm
UREA-SAL 4	2	9	24 cm
UREA-SAL 5	4	7	12 cm
UREA ONLY 1	4	8	19 cm
UREA ONLY 2	4	8	20 cm
UREA ONLY 3	4	8	19 cm
UREA ONLY 4	4	8	19 cm
UREA ONLY 5	4	8	18 cm
SOIL ONLY 1	1	7	26 cm
SOIL ONLY 2	1	8	27 cm

Observations: The changes in leaf color could be due to the dry soil (took some time for moisture to return to soil after drying) and or nutrient washing after transplanting.

Table 6: Week 3 - Phenotype data collection and observation

Week 3			
Treatment	**Pant Condition**	**Leaf Number**	**Height**
NAC-UREA 1	2	11	40 cm
NAC-UREA 2	2	11	50 cm
NAC-UREA 3	2	11	35.5 cm
NAC-UREA 4	2	10	46 cm
NAC-UREA 5	2	9	33 cm
NAC-SAL 1	4	11	38 cm
NAC-SAL 2	4	10	47 cm
NAC-SAL 3	4	10	44 cm
NAC-SAL 4	4	9	44.5 cm
NAC-SAL 5	4	10	46 cm
BULK-ZnO 1	4	11	42 cm
BULK-ZnO 2	4	11	42 cm
BULK-ZnO 3	4	10	36 cm
BULK-ZnO 4	4	10	44 cm
BULK-ZnO 5	4	11	45.5 cm
UREA-SAL 1	2	10	44 cm
UREA-SAL 2	2	10	42 cm
UREA-SAL 3	2	9	46 cm
UREA-SAL 4	2	10	44 cm
UREA-SAL 5	4	9	40 cm
UREA ONLY 1	4	10	44 cm
UREA ONLY 2	4	9	41 cm
UREA ONLY 3	4	10	42.5 cm
UREA ONLY 4	4	10	40 cm
UREA ONLY 5	4	10	37 cm
SOIL ONLY 1	1	9	51 cm
SOIL ONLY 2	1	13	47 cm

Table 7: Week 4 - Phenotype data collection and observation

Week 4				
Treatment	**Pant Condition**	**Leaf Number**	**Height**	**Flower Number**
NAC-UREA 1	2	13	49 cm	2
NAC-UREA 2	2	12	57 cm	0
NAC-UREA 3	1	12	42 cm	1
NAC-UREA 4	1	12	60 cm	1
NAC-UREA 5	1	11	51 cm	0
NAC-SAL 1	2	12	45.5 cm	1
NAC-SAL 2	3	12	50.5 cm	2
NAC-SAL 3	1	12	57 cm	1
NAC-SAL 4	1	13	54.5 cm	2
NAC-SAL 5	3	13	54.5 cm	2
BULK-ZnO 1	2	13	56 cm	0
BULK-ZnO 2	2	14	61 cm	0
BULK-ZnO 3	3	13	64 cm	0
BULK-ZnO 4	2	13	59 cm	1
BULK-ZnO 5	1	12	48 cm	0
UREA-SAL 1	4	12	61 cm	0
UREA-SAL 2	1	13	53 cm	2
UREA-SAL 3	2	12	55.5 cm	2
UREA-SAL 4	2	12	61 cm	0
UREA-SAL 5	3	13	69.5 cm	0
UREA ONLY 1	1	13	60.5 cm	0
UREA ONLY 2	1	12	60 cm	0
UREA ONLY 3	4	13	65.5 cm	0
UREA ONLY 4	1	13	60 cm	0
UREA ONLY 5	3	13	67 cm	0
SOIL ONLY 1	4, new growth 1	9	61 cm	0
Observation: Stems of soil only plants are not as thick as stems of the others.				

Table 8: Week 5 - Phenotype data collection and observation

Week 5				
Treatment	**Pant Condition**	**Leaf Number**	**Height**	**Flower Number**
NAC-UREA 1	2	15	52 cm	5
NAC-UREA 2	2	14	65 cm	4
NAC-UREA 3	2	14	56 cm	4
NAC-UREA 4	2	14	51 cm	6
NAC-UREA 5	1	15	51 cm	1
NAC-SAL 1	2	13	46 cm	3
NAC-SAL 2	2	14	61 cm	7
NAC-SAL 3	2	14	60.5 cm	6
NAC-SAL 4	2	15	55.5 cm	5
NAC-SAL 5	2	15	54.5 cm	5
BULK-ZnO 1	3	13	62 cm	5
BULK-ZnO 2	2	16	61 cm	3
BULK-ZnO 3	2	13	64.5 cm	3
BULK-ZnO 4	2	14	61 cm	5
BULK-ZnO 5	1	14	48 cm	4
UREA-SAL 1	3	13	66 cm	2
UREA-SAL 2	2	14	53.5 cm	4
UREA-SAL 3	2	13	56.5 cm	4
UREA-SAL 4	2	14	69 cm	3
UREA-SAL 5	3	14	77 cm	2
UREA ONLY 1	1	15	72.5 cm	3
UREA ONLY 2	2	14	68 cm	2
UREA ONLY 3	2	14	77 cm	2
UREA ONLY 4	2	15	69.5 cm	3
UREA ONLY 5	1	14	63 cm	5
SOIL ONLY 1	4	11	64 cm	1
SOIL ONLY 2	4	16	54 cm	0

Observations: Not much change in height but increase in number of flowers. This is due to the amount of energy needed to produce flowers.

Table 9: Week 6 - Phenotype data collection and observation

Week 6					
Treatment	**Pant Condition**	**Leaf Number**	**Height**	**Flower Number**	**Fruit Number**
NAC-UREA 1	4	17	53 cm	7	0
NAC-UREA 2	2	16	59.5 cm	11	1 fruit forming
NAC-UREA 3	4	14	49.5 cm	9	0
NAC-UREA 4	2	18	63 cm	7	0
NAC-UREA 5	1	13	57 cm	7	0
NAC-SAL 1	4	15	47 cm	6	0
NAC-SAL 2	4	15	61.5 cm	9	0
NAC-SAL 3	2	16	60 cm	10	0
NAC-SAL 4	3	16	55 cm	8	0
NAC-SAL 5	1	18	55.5 cm	5	0
BULK-ZnO 1	2	15	63 cm	6	0
BULK-ZnO 2	2	15	60 cm	8	1 fruit forming
BULK-ZnO 3	3	12	62 cm	6	0
BULK-ZnO 4	2	13	62 cm	8	0
BULK-ZnO 5	4, new growth 1	16	60 cm	8	0
UREA-SAL 1	2	16	67.5 cm	6	0
UREA-SAL 2	1	16	69 cm	9	0
UREA-SAL 3	4	15	56 cm	10	0
UREA-SAL 4	4	13	70 cm	6	0
UREA-SAL 5	4	13	77.5 cm	8	0
UREA ONLY 1	1	13	72 cm	5	0
UREA ONLY 2	1	14	69 cm	5	0
UREA ONLY 3	4	14	78 cm	5	0
UREA ONLY 4	1	15	70 cm	7	0
UREA ONLY 5	1	17	62 cm	6	0
SOIL ONLY 1	4	10	55 cm	2	0
SOIL ONLY 2	4	13	57 cm	1	0

Observation: Whit flies and their eggs were noticed on the tomato plants – mostly on the Bulk ZnO and NAC-UREA plants. Flies and their eggs were manually removed with water. If bad enough, leaf or leaflet was removed. White flies may be a factor in leaf discoloration.

Table 10: Week 9 - Phenotype data collection and observation

Week 9						
Treatment	**Pant Condition**	**Leaf Number**	**Height**	**Flower Number**	**Fruit Number**	**Fruit Condition**
NAC-UREA 1	1	16	110 cm	11	1	Healthy
NAC-UREA 2	2	16	100 cm	0	2	Healthy
NAC-UREA 3	2	15	98.5 cm	2	4	3 w/ BER
NAC-UREA 4	2	15	108 cm	4	4	Healthy
NAC-UREA 5	1	16	100 cm	4	2	Healthy
NAC-SAL 1	2	20	99 cm	7	4	1 w/ BER
NAC-SAL 2	2	18	109 cm	8	2	Healthy
NAC-SAL 3	2	21	110 cm	9	2	Healthy
NAC-SAL 4	2	14	102 cm	6	0	
NAC-SAL 5	1	26	120 cm	11	2	Healthy
BULK-ZnO 1	2	22	118 cm	11	3	Healthy
BULK-ZnO 2	1	21	103 cm	3	3	Healthy
BULK-ZnO 3	2	13	98 cm	2	2	Healthy
BULK-ZnO 4	2	19	11 cm	5	1	Healthy
BULK-ZnO 5	2	14	96 cm	6	5	2 w/ BER
UREA-SAL 1	4	11	109 cm	0	3	Healthy
UREA-SAL 2	2	24	102 cm	7	3	1 w/ BER
UREA-SAL 3	4	16	43 cm	3	5	3 w/ BER
UREA-SAL 4	4	23	113 cm	5	0	
UREA-SAL 5	4	15	129.5 cm	2	3	1 w/ BER
UREA ONLY 1	2	21	118 cm	2	1	1 w/ BER
UREA ONLY 2	2	18	103.5 cm	6	3	1 w/ BER
UREA ONLY 3	2	11	117 cm	4	2	Healthy
UREA ONLY 4	2	22	111 cm	4	3	1 w/ BER
UREA ONLY 5	2	18	116 cm	0	2	1 w/ Damage
SOIL ONLY 1	4	9	105 cm	1	1	Healthy
SOIL ONLY 2	4	10	90 cm	0	0	

Observation: Blossom-End Rot (BER) is a brown water soaking end spot on the bottom of tomato plants. BER is a result of calcium deficiency or imbalance. All plants have taken on a rusty brown color on older leaves – this may be from Zn deficiency

color on older leaves. Some of the older leaves have been removed.

Table 11: Week 11 - Phenotype data collection and observation

Week 11 - Data collection					
Treatment	**Biomass**	**Fruit Number**	**Fruit Condition**	**Fruit weight**	**Root Condition**
NAC-UREA 1	2.764 lbs	2	Healthy	0.192 lbs	Healthy
NAC-UREA 2	3.574 lbs	2	Healthy	0.388 lbs	Healthy
NAC-UREA 3	3.238 lbs	4	2 w/ BER	0.502 lbs	Healthy
NAC-UREA 4	3.112 lbs	4	2 w/ BER	0.588 lbs	Healthy
NAC-UREA 5	2.292 lbs	2	Healthy	0.464 lbs	Healthy
NAC-SAL 1	3.074 lbs	4	1 w/ BER	0.454 lbs	Healthy
NAC-SAL 2	2.808 lbs	2	1 w/ BER	0.318 lbs	Healthy
NAC-SAL 3	2.780 lbs	3	Healthy	0.364 lbs	Healthy
NAC-SAL 4	2.714 lbs	8	Healthy	0.078 lbs	Healthy
NAC-SAL 5	3.072 lbs	4	1 w/ BER	0.580 lbs	Healthy
BULK-ZnO 1	1.862 lbs	3	1 w/ BER	0.236 lbs	Healthy
BULK-ZnO 2	2.470 lbs	2	Healthy	0.504 lbs	Healthy
BULK-ZnO 3	3.574 lbs	5	Healthy	0.336 lbs	Healthy
BULK-ZnO 4	2.372 lbs	1	Healthy	0.270 lbs	Healthy
BULK-ZnO 5	3.500 lbs	6	2 w/ BER, 1 Rotten	0.686 lbs	Healthy
UREA-SAL 1	3.462 lbs	4	1 w/ BER	0.526 lbs	Healthy
UREA-SAL 2	3.544 lbs	4	2 w/ BER	0.410 lbs	Healthy
UREA-SAL 3	3.868 lbs	4	2 w/ BER	0.494 lbs	Healthy
UREA-SAL 4	2.156 lbs	1	Healthy	0.008 lbs	Healthy
UREA-SAL 5	3.072 lbs	3	2 w/ BER	0.352 lbs	Healthy
UREA ONLY 1	2.966 lbs	2	1 w/ BER	0.310 lbs	Healthy
UREA ONLY 2	2.412 lbs	3	2 w/ BER	0.602 lbs	Healthy
UREA ONLY 3	3.728 lbs	0		0 lbs	Healthy
UREA ONLY 4	2.876 lbs	3	1 w/ BER	0.556 lbs	Healthy
UREA ONLY 5	2.73 lbs	2	Healthy	0.494 lbs	Healthy
SOIL ONLY 1	2.938 lbs	1	Healthy	0.102 lbs	Healthy
SOIL ONLY 2	2.846 lbs	1	Healthy	0.002 lbs	Healthy

Notes: Biomass includes fruits. Plant pot soil still wet from watering the day before. Weight of empty pot: 0.170 lbs – biomass was taken with pot.

REFERENCES

1. Harding, K., Aguayo, V., & Webb, P. (2018). Hidden hunger in South Asia: A review of recent trends and persistent challenges. *Public Health Nutrition, 21*(4), 785-795. doi:10.1017/S1368980017003202
2. Andersen, P. (2005). *Micronutrients in South and South East Asia: Proceedings of an International Workshop Held 8-11 September, 2004, Kathmandu, Nepal*. International Centre for Integrated Mountain Development.
3. Chhipa, H. (2017). Nanofertilizers and nanopesticides for agriculture. *Environmental Chemistry Letters, 15*(1), 15-22.
4. Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., & Issaabadi, Z. (2019). Chapter 1 - An Introduction to Nanotechnology. *Interface Science and Technology, 28*, 1–27.
5. Tripathi, Durgesh & Singh, Shweta & Singh, Swati & Mishra, Sanjay & Chauhan, Devendra & K. Dubey, N. (2015). Micronutrients and their diverse role in agricultural crops: advances and future prospective. Acta Physiologiae Plantarum. 37. 1-14. 10.1007/s11738-015-1870-3.
6. Brown P.H., Cakmak I., Zhang Q. (1993) Form and Function of Zinc Plants. In: Robson A.D. (eds) Zinc in Soils and Plants. Developments in Plant and Soil Sciences, vol 55. Springer, Dordrecht
7. HazeraUSA. (2012). Deficiency Symptoms in Tomatoes Crops
8. Cullen, A., Clark, C., & Yorgey, G. (2008). The Links between Fertilizer and Nutrition in South Asia and Africa.

9. Sharma, C. (2006). *Plant micronutrients : Roles, responses and amelioration of deficiencies*.
10. Kluska, K., Adamczyk, J., & Krężel, A. (2018). Metal binding properties, stability and reactivity of zinc fingers. *Coordination Chemistry Reviews*, *367*, 18-64.
11. Takatsuji, H. (1998). Zinc-finger transcription factors in plants. *Cellular and Molecular Life Sciences CMLS*, *54*(6), 582-596.
12. Cakmak, I., & Marschner, H. (1986). Mechanism of phosphorus-induced zinc deficiency in cotton. I. Zinc deficiency-enhanced uptake rate of phosphorus. *Physiologia Plantarum*, *68*(3), 483-490.
13. Hafeez, B., Khanif, Y. M., & Saleem, M. (2013). Role of zinc in plant nutrition-a review. *American journal of experimental Agriculture*, *3*(2), 374.
14. Sharma, A. K., Johri, B. N., & Gianinazzi, S. (1992). Vesicular-arbuscular mycorrhizae in relation to plant disease. *World journal of microbiology and biotechnology*, *8*(6), 559-563.
15. Sullia, S. B. (1991). Use of vesicular-arbuscular mycorrhiza (VAM) as biofertilizer for horticultural plants in developing countries. In *Horticulture—New Technologies and Applications*(pp. 49-53). Springer, Dordrecht.
16. Audsley, E., Milne, A., & Paveley, N. (2005). A foliar disease model for use in wheat disease management decision support systems. *Annals of Applied Biology*, *147*(2), 161-172.

17. Solomons, N. W. (2000). Plant-based diets are traditional in developing countries: 21st century challenges for better nutrition and health. *Asia Pacific journal of clinical nutrition*, *9*(S1), S41-S54.
18. Zinc, Fact sheet for Health Professionals. (n.d.). Retrieved from https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/
19. Prasad, A. S. (1998). Zinc deficiency in humans: a neglected problem.
20. Maret, W., & Sandstead, H. H. (2006). Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medicine and Biology*, *20*(1), 3-18.
21. Prasad, A. S. (2017). Discovery of Zinc for Human Health and Biomarkers of Zinc Deficiency. In *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals* (pp. 241-260). Academic Press.
22. Prasad, A. S. (1995). Zinc: an overview. *Nutrition (Burbank, Los Angeles County, Calif.)*, *11*(1 Suppl), 93-99.
23. Akhtar, S., Ismail, T., Atukorala, S., & Arlappa, N. (2013). Micronutrient deficiencies in South Asia–Current status and strategies. *Trends in food science & technology*, *31*(1), 55-62.
24. Mérigout, P., Lelandais, M., Bitton, F., Renou, J. P., Briand, X., Meyer, C., & Daniel-Vedele, F. (2008). Physiological and transcriptomic aspects of urea uptake and assimilation in Arabidopsis plants. *Plant Physiology*, *147*(3), 1225-1238.
25. War, A. R., Paulraj, M. G., War, M. Y., & Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant defense system in chickpea (Cicer arietinum L.). *Plant signaling & behavior*, *6*(11), 1787-1792.

26. Souza, G. A., Ebaid, G. X., Seiva, F. R., Rocha, K. H., Galhardi, C. M., Mani, F., & Novelli, E. L. (2011). N-acetylcysteine an allium plant compound improves high-sucrose diet-induced obesity and related effects. *Evidence-Based Complementary and Alternative Medicine*, *2011*.

27. Niu, Z., & Li, Y. (2013). Removal and utilization of capping agents in nanocatalysis. *Chemistry of Materials*, *26*(1), 72-83.

28. Kharissova, O. V., Dias, H. R., Kharisov, B. I., Pérez, B. O., & Pérez, V. M. J. (2013). The greener synthesis of nanoparticles. *Trends in biotechnology*, *31*(4), 240-248.

29. El-Ramady, H., Abdalla, N., Alshaal, T., El-Henawy, A., Elmahrouk, M., Bayoumi, Y., ... & Domokos-Szabolcsy, É. (2018). Plant nano-nutrition: perspectives and challenges. In *Nanotechnology, Food Security and Water Treatment* (pp. 129-161). Springer, Cham.

30. Aziz, Y., Shah, G. A., & Rashid, M. I. (2019). ZnO nanoparticles and zeolite influence soil nutrient availability but do not affect herbage nitrogen uptake from biogas slurry. *Chemosphere*, *216*, 564-575.

31. Wessells, K. R., & Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PloS one*, *7*(11), e50568.

32. Lin, D., & Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environmental Pollution*, *150*(2), 243-250.

33. Das, S., Wolfson, B. P., Tetard, L., Tharkur, J., Bazata, J., & Santra, S. (2015). Effect of N-acetyl cysteine coated CdS: Mn/ZnS quantum dots on seed germination and seedling

growth of snow pea (Pisum sativum L.): imaging and spectroscopic studies. *Environmental Science: Nano*, *2*(2), 203-212.

34. JoVE Science Education Database V (2019). *Environmental Science*. Lead Analysis of Soil Using Atomic Absorption Spectroscopy. JoVE, Cambridge, MA.