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Nanoscale CuO Charge and Morphology Control Fusarium Suppression and Nutrient Biofortification in Field-grown Tomato and Watermelon

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Abstract: Limited data exist on how surface charge and morphology impact the effectiveness of nanoscale copper oxide (CuO) as an agricultural amendment under field conditions. This study investigated the impact of these factors on tomatoes and watermelons following foliar

treatment with CuO nanosheets (NS-) or nanospikes (NP+ and NP-) exhibiting positive or negative surface charge. Results showed plant species-dependent benefits. Notably, tomatoes infected with *Fusarium oxysporum* had significantly reduced disease progression when treated with NS-. Watermelons benefited similarly from NP+. Although disease suppression was significant and trends indicated increased yield, the yield effects weren't statistically significant. However, several nanoscale treatments significantly enhanced the fruit's nutritional value, and this nano-enabled biofortification was a function of particle charge and morphology. Negatively charged nanospikes significantly increased the Fe content of healthy watermelon and tomato (20-28%) and Ca in healthy tomato (66%), compared to their positively charged counterpart. Negatively charged nanospikes also outperformed negatively charged nanosheets, leading to significant increases in the content of S and Mn in infected watermelon (37-38%), Fe in healthy watermelon (58%), and Ca (42%) in healthy tomato. These findings highlight the potential of tuning nanoscale CuO chemistry for disease suppression and enhanced food quality under field conditions.

Keywords: nanotechnology, agriculture, field yield, tomato, watermelon, fusarium, nutrient quality

Introduction:

By 2050, total food production needs to increase by approximately 70% to support the projected global population (Alexandratos and Bruinsma - FAO, 2012). The challenges posed by a changing climate are actively confounding efforts to maintain global food security. In addition, many conventional agricultural

strategies have proven to be unsustainable, particularly with regard to inputs of water and energy, and notably, few human activities have a more profound negative impact on the environment than does agriculture. For example, the efficiency of agrichemical delivery is quite low, often below 40% (Xu *et al.*, 2022). This often results in growers over-applying materials to ensure sufficiency. Consequently, excess pesticides and fertilizers may be subject to drift and runoff, leading directly to negative environmental consequences such as greenhouse gas emissions, air pollution, eutrophication, and damage to non-target species (Ghormade *et al.*, 2011).

One of the most significant limitations to agricultural productivity is damage from pests and pathogens, both at the pre- and post-harvest stage. Along the food chain, up to 30% of food production is lost to disease and pathogenic contamination (Savary *et al.*, 2019). Crop yield in the field can also be dramatically impacted by the presence of plant diseases, including those caused by agents such as viruses, bacteria, fungi, and nematodes. Disease pressure can also compromise crop nutritional quality, further escalating losses (Wang *et al.*, 2022a). Fungal pathogen infections are often soilborne, making management particularly challenging; these pathogens are known to significantly impact the productivity of tomatoes (*Solanum lycopersicum* L), watermelon (*Citrullus lanatus*), and a broad range of additional crops (Jones *et al.*; Hartman *et al.*, 2015; Kousik *et al.*, 2018; Lopez-Lima *et al.*, 2021; Balasubramaniam *et al.*, 2023). In some cases, soilborne fungal pathogens can cause up to 70% of the yield loss in these species (Godfray *et al.*, 2016). *Fusarium* is a common fungal genus of soil-borne pathogens, with species impacting over two dozen important crops, including vegetable and grain species (Rampersad, 2020). Common pathogenic species include *Fusarium oxysporum* *F. sp. niveum* (FON), which causes Fusarium wilt in watermelon, and *Fusarium oxysporum* *f. sp. lycopersici* (FOL), which causes Fusarium wilt in tomato. Fusarium wilt is a destructive vascular disease where the pathogen infects young roots, with subsequent fungal hyphae development that inhibits the flow of water and nutrients to the shoot tissue, resulting in symptomatic wilting, as well as reduced

photosynthesis, nutrient content, and yield (Gordon, 2017). The management of plant fungal diseases is challenging, although strategies such as fungicides, soil fumigation, and crop rotation may partially alleviate disease pressure (Hartman *et al.*, 2015). However, conventional management options have significant shortcomings and as such, there is urgent need for more effective, economical, and environmentally friendly management tools for *Fusarium* pathogens.

Nanotechnology has demonstrated exciting potential in agriculture, including improvement of use efficiency, either as nanoscale nutrients or as novel nanoscale carriers that deliver conventional or nanoscale active ingredients that can act as fertilizers, pesticides, or growth promoters. Several recent reports have shown that various nanomaterial-containing micro-nutrients can significantly improve crop yield, disease resistance, and nutrient utilization efficiency. For example, Wang *et al.* reported that foliar applied steric acid-coated nanoscale sulfur significantly improved the yield of healthy and *Fusarium* infested tomatoes in the field by 33.4% and 107%, respectively (Wang *et al.*, 2022b). Others have shown that fluorescent silica nanoparticle promoted rice (*Oryza sativa* L.) growth under hydroponic conditions, increasing shoot biomass by 33.6%, compared with conventional Si ion fertilizers (Cheng *et al.*, 2021). Graphite carbon nanoparticles at 1% (wt basis), combined with 70% of the recommended dose fertilizer, decreased nitrate leaching by 57%, and exerted no negative effects on lettuce yield compared to conventional fertilizer regimes (Pandorf *et al.*, 2020). Collectively, these studies highlight a nanoscale-specific effect that consistently outperforms conventional materials, although clearly dosing, plant species, and treatment timing are critical parameters.

Nanoscale CuO (nCuO) has attracted considerable attention due to its known antibacterial properties, influence on oxidative stress systems, and biological activity as a co-factor to several important defensive enzyme systems in plants (Dimkpa *et al.*, 2012). In a greenhouse study, lettuce (*Lactuca sativa*) infected with *Fusarium oxysporum* f. sp. *lactucae* that had been treated with a soil amendment of nCuO had significantly increased biomass (26.1%) compared with the infected control and outperformed a

commercial Cu fungicide (Shang *et al.*, 2021). Conversely, Wang *et al.* reported that soil-exposure nCuO did not significantly impact the biomass of green onion (*Allium fistulosum* L.), although the nutrient and allicin content in the bulbs were increased, compared with the control (Wang *et al.*, 2020). Ma *et al.* found that *Fusarium virguliforme* (FV) infection reduced soybean shoot mass by 58%, and that foliar applied CuO nanosheets alleviated much of the damage, compared with the infested controls (Ma *et al.*, 2020). Interestingly, the authors reported differences in plant response as a function of particle morphology and composition, both experimentally and computationally. Similarly, NPs surface charge may greatly affect particle adhesion, uptake and translocation in plants. For example, Borgatta *et al.* found that when tomato plants were treated with negatively surface charged CuO, they exhibited a significant decrease in disease progression and an increase in biomass. Conversely, positively surface charged NPs and CuSO₄ had minimal effects (Borgatta *et al.*, 2023).

Notably, only a limited number of studies have evaluated the impact of nCuO chemical and physical properties on crop yield and quality after full life cycle exposure under field conditions (Wang *et al.*, 2021). To fill this research gap, here, nCuO with different surface charge (positive and negative) and morphology (nanosheet and nanorhombus), were foliar applied on two plant species (watermelon and tomato) that were subsequently cultivated under field conditions in the presence or absence of fungal pathogens. After a full life cycle, the matured fruits were harvested, and Cu uptake, nutrient accumulation, and agronomic parameters were evaluated. The findings of this study provide important information on the design and application of nanomaterials as novel fertilizers or as crop management tools in support of sustainable agricultural production systems.

Materials and Method

Materials Properties and Application.

Powders of nanoscale (n) CuO sheets (NS-) and nCuO spikes with either positive or negative surface charge (NP+, NP-) were synthesized akin to previously reported methods, where particle morphology and surface charge were controlled by modifying solution pH (Borgatta *et al.*, 2023). The zeta potential of CuO nanoparticles in 50 mg/L suspensions was analyzed with a Malvern Zetasizer Nano ZS. Positively charged nanospikes had a zeta potential of 24.6 ± 0.3 mV. Negatively charged nanosheets and nanospikes had zeta potentials of -19.7 ± 0.3 mV and -18.1 ± 0.4 mV, respectively. Scanning electron microscope (SEM) images for each particle type are detailed below; additional characterization information can be found in the Supporting Information.

Particle morphology was determined using a Zeiss Supra55 SEM. To prepare samples, a suspended solution of particles in isopropyl alcohol (IPA) was dropcast onto a silicon wafer and dried overnight prior to analysis. Representative nanoparticles are shown below (Figure 1). Both nanosheets and nanospikes consist of thin 2-dimensional layers but are morphologically distinct. The nanosheets are more rectangular and slightly thicker, while the nanospikes have tapered ends and are thinner, resembling a spike. The nanospikes have a higher surface area as a result of being a thinner material.

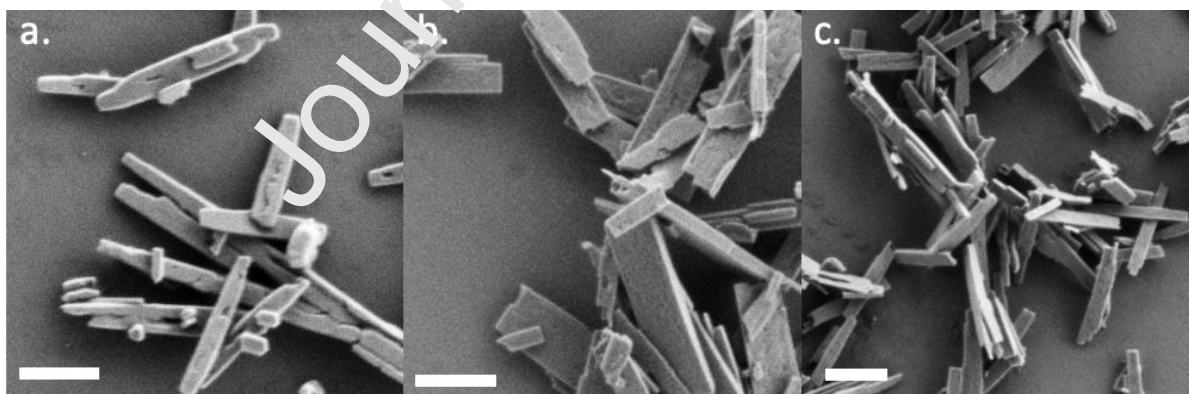


Figure 1. Representative micrographs of a. positively charged nanospikes, b. negatively charged nanosheets, and c. negatively charged nanospikes. The scalebar is 400 nanometers.

In preparation for the foliar-exposure field experiment (below), all materials were suspended in 18 M Ω Millipore water (MW) to achieve a final Cu concentration of 125 mg/L for tomato and 250 mg/L for watermelon. These concentrations were selected for its environmental relevance and were determined based on prior research (Shacklette *et al.*; Rawat *et al.*, 2018; Borgatta *et al.*, 2023). The nanoscale suspensions were freshly prepared and sonicated in a water bath (FS220 Ultrasonic Cleaner, Fisher Scientific) at 25 °C for 25 min. There were five treatments in both infected and healthy groups: 1). Control; 2). NS-; 3). NP+; 4). NP-; 5). CuSO₄ solution. The CuSO₄ was obtained commercially (C1297, Sigma-Aldrich). The concentration of CuSO₄ was adjusted to 314 and 628 mg/L to match the amount of Cu used in the nanoscale treatments. There were 10 replicates in each treatment. CuO NS- and NP- were used to investigate the role of particle morphology in biological response of the plant and pathogen; NP+ and NP- were used to compare the role of surface charge in response; CuSO₄ was used to differentiate the effects of nanoscale materials relative to conventional ionic copper. After sonication, leaves of twenty-eight-day-old seedlings (species described below) were dipped into the suspensions/solutions of Cu-based compounds for two min. Control plants were treated with MW. Treated plants were then prepared for transplanting to the field site.

Field Experiments.

Two different crops were selected to evaluate species differences in response to material exposure and pathogen infection. Tomato (*Solanum Lycopersicum* L. cv Bonnie Best; Harris Seed Co., Rochester, NY), and watermelon (*Citrullus lanatus* L. cv Sugar Baby; Harris Seed Co., Rochester, NY) are known to be susceptible to infection by *Fusarium* (Everts and Himmelstein, 2015; Zhang *et al.*, 2015; Wang *et al.*, 2022b). Seeds were germinated and grown in plastic liners (36-cell, 5.66 X 5.66 X 4.93 cm³) with potting soil substrate (Pro-Mix BX, Premier Hort Tech, Quakertown, PA) for four weeks prior to transplanting. No fertilizer was applied during this period. Four-week-old seedlings of uniform size were selected for the field study. The pathogen inoculum was prepared according to previous reports (Elmer and White, 2016;

Adisa *et al.*, 2018). Briefly, millet (*Echinochloa esculenta*) seeds were autoclaved in distilled water (1:1, wt:wt) for one hour seeded with two agar plugs colonized with FON or FOL. After incubating at 22-25 °C for three weeks, the millet was air-dried, ground, sieved to one mm, and added to the soil.

The experimental site was established at the Connecticut Agricultural Experiment Station's Lockwood farm located in northern Hamden, Connecticut. The soil was previously characterized as a Cheshire Fine Sandy Loam with a pH of 5.9 (Wang *et al.*, 2022b). Field microplots were established in the Spring of 2021 and 2022; rows were 0.9 m wide and separated by 6 m. The plots were fertilized with 112 kg/ha of 10-10-10 NPK, covered with black plastic mulch, and irrigated with drip tape. The soil nutrient content is described in SI. The rows were then divided into thirty microplots separated by 30 cm. Seedlings previously treated with the experimental test materials were transplanted into the field in a randomized block design. For the infested microplots, the planting holes were hand mixed with 0.75 g of millet inoculum immediately prior to transplanting (Borgatta *et al.*, 2023). For non-infested plots, adjacent rows were prepared using the same method but no pathogen was added. Plants were rated weekly for disease progression based on plant's shoot system, and the area-under-the-disease-progress curve (AUDPC) was calculated (Wang *et al.*, 2022b). Disease severity was determined based on a scale of 1-5, where 1 = healthy, 2 = slightly stunted, 3 = partially stunted, 4 = severely stunted, 5 = completely stunted or dead (Borgatta *et al.*, 2018). AUDPC calculations were made using the trapezoid rule:

$$\text{AUDPC} = [D_i + D_{(i+1)}]/2 \times (t_{(i+1)} - t_i)$$

where D_i = the disease rating at time t_i .

Plant Harvest and Elemental Analysis.

At harvest (tomato ninety-one days; watermelon eighty-five days), total shoot and fruit biomass were harvested at maturity. Fruit and shoot tissues were obtained, carefully cleaned with DI water to remove the surface particles and collected in prelabeled bags. Samples were then dried in an oven for seventy-

two hours at 70 °C. Approximately 0.2 g of dried sample was weighed into digestion tubes with five mL of plasma pure nitric acid (HNO₃; Fisher Scientific). Samples were digested at 115 °C for forty-five min in a hot block (DigiPREP MS, SCP SCIENCE, Quebec City, Canada). DI water was used to dilute the digests to fifty mL. Three replicate samples of each tissue were analyzed by ICP-OES (iCAP 6500, Thermo Fisher Scientific, Waltham, MA) to determine macro- (Ca, K, Mg, P, S) and micronutrient (Zn, Fe, Mn, Cu) levels. Blank (no plant tissues), Cu spike (1, 5, 10, 50 mg/kg nCuO powder), and standard reference materials (NIST-SRF 1570a and 1547, Metuchen, NJ) were digested and analyzed as part of the QA/QC protocol. Yttrium (Y) was used as an internal standard, and a continuing calibration verification (CCV) (1 ppm Cu) sample was analyzed every fifteen samples to ensure accurate analysis. The recovery of all the analyzed elements ranged between 90 and 110%.

Statistical Analyses.

Agronomic and elemental data were determined with the Statistical Package for the Social Sciences program 26 (SPSS 26, Chicago, IL, USA). A one-way ANOVA and Tukey-Kramer multiple comparison test was used to compare the mean values of the control group and treatments. A student's t-test was also used to compare the differences between the control groups and specific treatments. Results are described as mean $\pm$ standard error (SE), and significant differences were determined at $P < 0.05$.

Results and Discussion:

Effect of NPs on Disease Severity

Control tomato plants grown in infested soil and not treated with nanoparticulate or ionic Cu developed disease symptoms seven days after planting, with significant wilt symptoms evident by day thirteen ($p < 0.05$). Starting from day sixteen, the AUDPC continued to increase and reached a value of 57.8 ± 13.2 after twenty-eight days (Figure 2 and S2). However, by day twenty-eight exposure of these plants to both of the negatively charged Cu-based treatments significantly slowed disease progression, compared

to the infected control, with a 31.7% and 14.6% suppression for NS- ($p < 0.05$) and NP- treated crops, respectively. Conversely, the other Cu-based treatments did not inhibit disease progression. Notably, NS- reduced disease by 37% and 40%, as compared to NP+ and CuSO_4 , respectively ($p < 0.05$). Figure 2 and S2 depict the progression of disease in *Fusarium*-infected watermelon. Similar to tomato, watermelon grown in infected soil without Cu treatment developed disease symptoms four days after planting, with significant wilt symptoms evident by day eight ($p < 0.01$). All Cu-based treatments, including NPs and ionic treatments, decreased disease in *Fusarium*-infected watermelon starting from day four. By day eight, the NP+ treatment exhibited strong *Fusarium* symptom-suppression effects, decreasing disease progression by 37.5% and 34.8% at day eight and twelve, respectively ($p < 0.05$). After sixteen days, the AUDPC of the infected control watermelon reached 63.1 ± 18.3 (Figure 2). Unlike tomato, by harvest none of the negatively charged nanoparticle treatments showed significant wilt-suppression as compared to the infected controls. However, the NP+ treatment significantly reduced disease by 30.9% compared with infected controls ($p < 0.05$).

Shang et al. reported similar findings with *Gibberella fujikuroi* (*Fusarium fujikuroi*) infected rice (*Oryza sativa* L.); upon foliar exposure to 10 mg/L Cu-based nanopesticide, disease impacts were significantly decreased by approximately 30% compared to untreated plants. Interestingly, roughly equivalent disease suppression has also been reported for other nanoscale elements, such as S, Ce, and Zn (Shang et al., 2020). Wang et al. conducted a time-dependent field study and demonstrated that disease progress was significantly diminished by soil and foliar applied S-based nanomaterials in field-grown tomato, with the greatest effect being with foliar applied S (32% reduction) (Wang et al., 2022b). Adisa et al. reported that foliar application of nanoscale CeO_2 at 250 mg/L reduced *Fusarium* wilt damage by 41% in greenhouse grown tomato plants (Adisa et al., 2018). In a field experiment, Graham et al investigated nanoscale control of citrus canker in grapefruit trees and reported that cuprous oxide/zinc oxide applied at 0.56 kg of Cu per ha and 0.56 kg of Zn per ha reduced *Xanthomonas citri* incidence

between 25 and 63% (Graham *et al.*, 2016). The potential mechanisms underlying these results could involve the nanomaterials directly supplementing nutritional Cu that is required by the plants, resulting in enhanced defense response pathways that may involve ROS signaling and lead to improved overall health (Zhao *et al.*, 2022). It is also possible that the excess Cu has a direct antimicrobial impact on the pathogen in the root system or if exuded, perhaps in the rhizosphere itself.

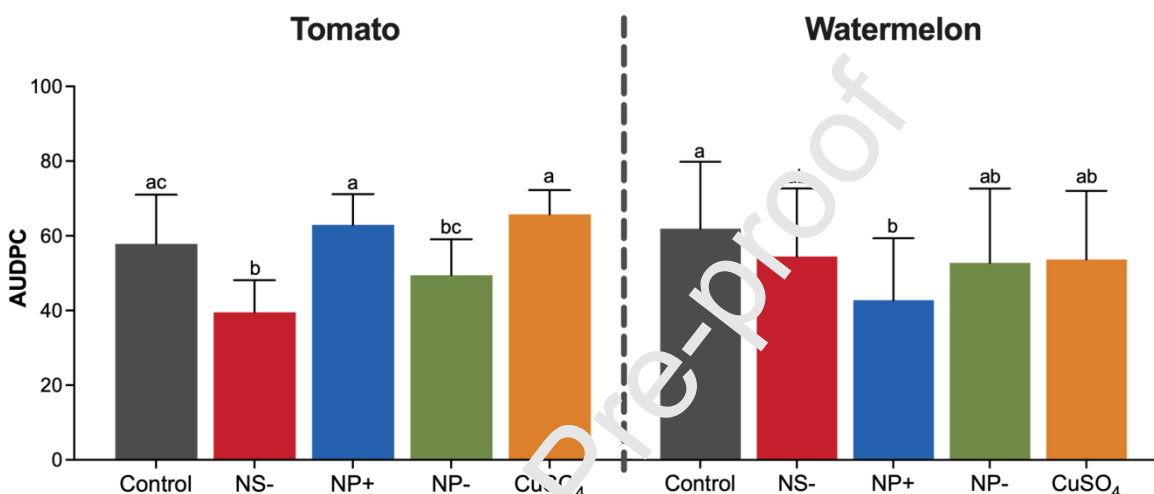


Figure 2. Disease progress (AUDPC) of *Fusarium*-infected (left) tomato and (right) watermelon upon exposure to nCuO sheet (NS-), nCuO spike with positive and negative surface charge (NP+, NP-), and CuSO₄ prior to harvesting. Different lowercase letters within the same plant species represent significant differences among different treatment groups ($p < 0.05$) (Student t-test). Error bars correspond to the SE of the mean ($n = 6$ and 10 for tomato and watermelon, respectively).

Effect of NPs on Plant Biomass

The above ground (shoot and fruit) biomass of both healthy and *Fusarium*-infected tomato under different treatments are shown in Figure S3 A and B, respectively. The presence of disease reduced shoot biomass by 39.3% ($p < 0.1$) but importantly had no impact on the harvested fruit yield, suggesting modest disease pressure. For the plant shoot biomass, although significant difference was not observed

among the different treatment groups due to high replicate variability common in field studies, the trends in the data align with disease progress findings, with negatively charged treatments, particularly the NP-, seeming to increase shoot biomass (65.2%; non-significant) as compared with the healthy controls. Similarly, there was also a trend for NS- to increase fruit and shoot weight of the infested plants, as compared to other NPs treatments. For the infected tomatoes, exposure to NP+ seemed to reduce fruit weight, although significant replicate variability precluded statistical significance. As noted, these trends agree with previously described AUDPC results. Compared to the positively charged NP treatment, the application of negatively charged NPs, including NS- and NP-, seemed to improve fruit production for tomatoes. Had disease pressure been higher, it is likely that nanoscale treatment-based benefits would have become significant as has been noted in the literature. The mechanisms underlying why the reduced disease damage from the positively charged nanomaterials did not lead to increased fruit yield as observed with the negatively charged treatments are unknown. High replicate variability indicative of field trials was certainly a factor with the positively charged treatment. In addition, charge-based differences in the kinetics and magnitude of Cu accumulation and transport may underlie the differences in yield. Consequently, field studies with time dependent harvest and molecular analysis are currently being planned so as to explore the kinetics of plant response to nanoscale Cu as a function of charge and pathogen presence. For example, Elmer et al. found that foliar exposure of CuO nanoparticles to *Fusarium* infected tomato improved yield by 33% as compared with untreated controls (Elmer and White, 2016). Similarly, Ashraf et al reported that 300mg/L nanoscale CuO treatments improved tomato plant fresh biomass under diseased conditions, increasing growth by more than 65%, compared with control (Ashraf *et al.*, 2021).

The watermelon shoot and fruit biomass are presented in Figure S4 A and B. Similar to tomato, the presence of disease reduced the biomass of watermelon's shoot tissue by 69.0% ($p < 0.05$) but did not influence the fruit yield. For the healthy plants, similar trends of fruit and shoot weight were noticed,

although the results were not of statistical significance. Specifically, Cu NP amendment regardless of the surface charge tended to increase both fruit and shoot tissues, compared to the controls. A similar effect was also evident in the *Fusarium*-infected plants, which showed a slight but statistically insignificant increase in biomass upon exposure to both NP+ and NP- by 18.0% and 27.5% for fruit biomass, and 7.5% and 18.3% for shoot biomass, compared with infected controls. Similar to tomato, the biomass results align with the AUDPC data, although with watermelon the positively charged particles also seem to convey some benefit. These results indicate that both particle morphology and charge influence plant growth under *Fusarium*-infection; these findings align with previous work on watermelon (Borgatta *et al.*, 2018; Lopez-Lima *et al.*, 2021). For example, Borgatta *et al.* observed that Cu-based nanosheets ($\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$) alleviated the growth suppression effect induced by *F. oxysporum*. This effect was achieved at a significantly lower mass concentration (10 mg/L) of particles when compared to commercially available CuO NPs (used at 1000 mg/L). The improved efficacy of $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ nanosheets was attributed to various factors, such as the reduced overall particle size, the unique sheet-like structure of the particles, and the faster initial release of ions from nanosheets in comparison to CuO NPs. These characteristics collectively contribute to the enhanced performance of nanosheets in mitigating the disease (Borgatta *et al.*, 2018). Similarly, El-Abeid demonstrated that reduced graphene oxide nanosheet-coated CuO nanoparticles could exhibit remarkable enhancement of their antifungal activity against wild strains of *Fusarium oxysporum*, which commonly affect tomato and pepper plants. This enhanced antifungal activity was also found to be significantly superior to that of commercial Cu-based fungicides (El-Abeid *et al.*, 2020). Additionally, observations have been made in other plant species regarding the suppressive effects of CuO NPs with different surface-coatings and morphology on plant diseases, such as musk melon (Shah *et al.*, 2022), eggplant (Elmer and White, 2016), wheat (Zakharova *et al.*, 2019), tea (Ponmurugan *et al.*, 2016), and soybean (Ma *et al.*, 2020).

Nutrient accumulation

Cu accumulation in Fruit

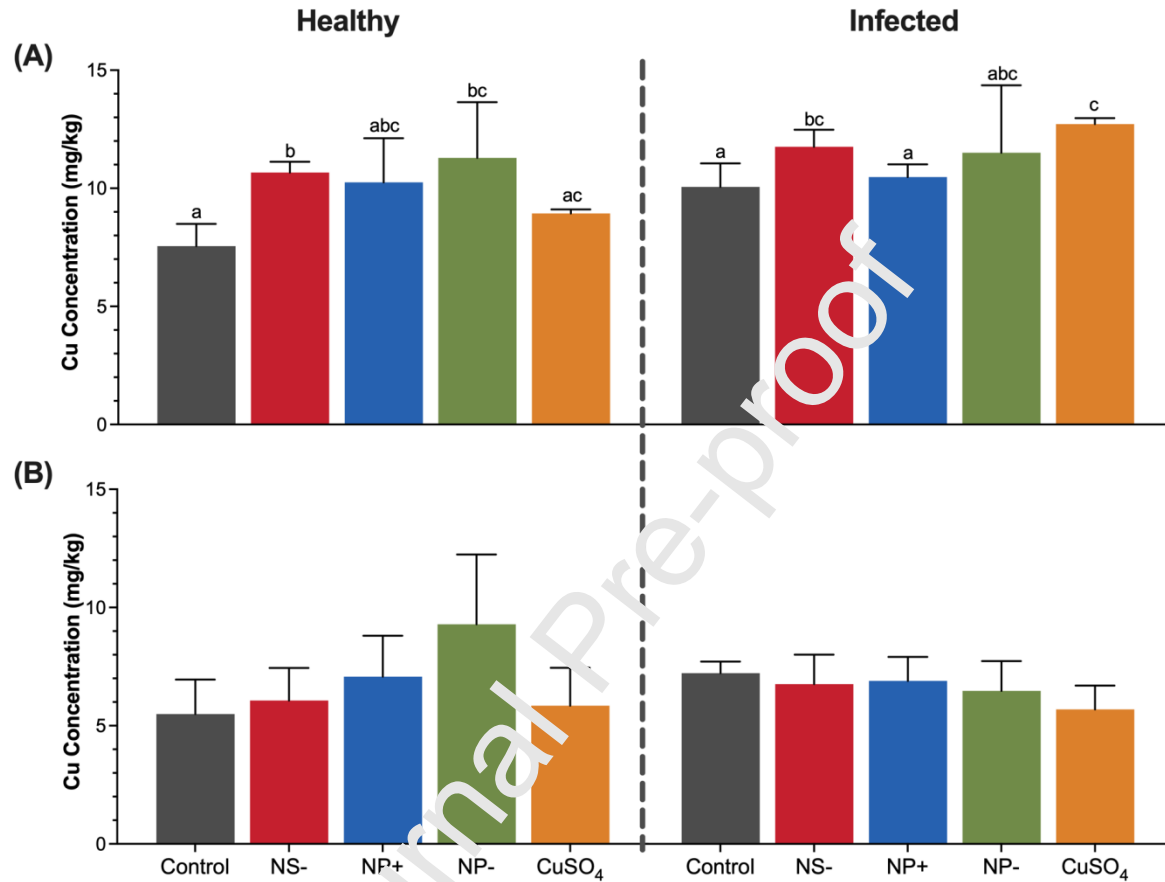


Figure 3. Cu concentration (mg of Cu/kg dry sample) in (A) tomato and (B) watermelon fruit of healthy and *Fusarium*-infected plants treated with NS-, NP+, NP-, and CuSO₄. Different lowercase letters within the same plant species represent significant differences between the control and specific treatment ($p < 0.1$) (Student t-test). Error bars correspond to the SE of the mean ($n = 3$).

The Cu concentrations in the tomato and watermelon fruits are shown in Figure 3. For the tomato, nearly all Cu treatments of the seedlings resulted in greater Cu content in the fruit. Specifically, NS- significantly increased Cu content by 41% and 17% in healthy and *Fusarium*-infected fruit, respectively,

compared with the control ($p < 0.05$). Similarly, treatment with NP- significantly improved the Cu concentration in healthy tomato fruit by 50%, compared with the control ($p < 0.05$). Last, CuSO_4 significantly increased the Cu content by 26% in infected tomato fruit compared with control. The fact that a foliar amendment of a very low amount (1-2 mg) of Cu to twenty-eight-day-old seedlings would yield increased Cu content in fruit formed months later is quite interesting and important and suggests that the Cu is translocated from the vegetative tissue into the reproductive organ. Conversely, the Cu content of watermelon fruit was not significantly impacted by treatment, although the trend was for increased Cu content in fruit of seedlings treated with NP-. This species-specific effect is not necessarily surprising and could be the result of any number of physiological differences between the two crops. For instance, after exposure to Ag, CuO, and TiO_2 nanoparticles, the corresponding metal uptake in the wheat and rice was greater than that in amaranth and maize plants (Bai *et al.*). In this study, the Cu-based treatment (125 mg/L) was only foliar applied once at the beginning of the experiment. Moreover, surfactants may contribute to the penetration of nanoparticles into leaf tissues. Similar results were observed in previous studies; the application of CuO nanoparticles in soil at levels below 150 mg/kg did not significantly influence Cu concentration in bok choy leaves (Deng *et al.*, 2020) and rice grain (Deng *et al.*, 2022b). Conversely, higher concentrations of Cu-based exposure in soil at 300 and 600 mg/kg significantly improved the Cu content in bok choy (Deng *et al.*, 2020).

Effect of surface charge and morphology on fruit nutrient accumulation

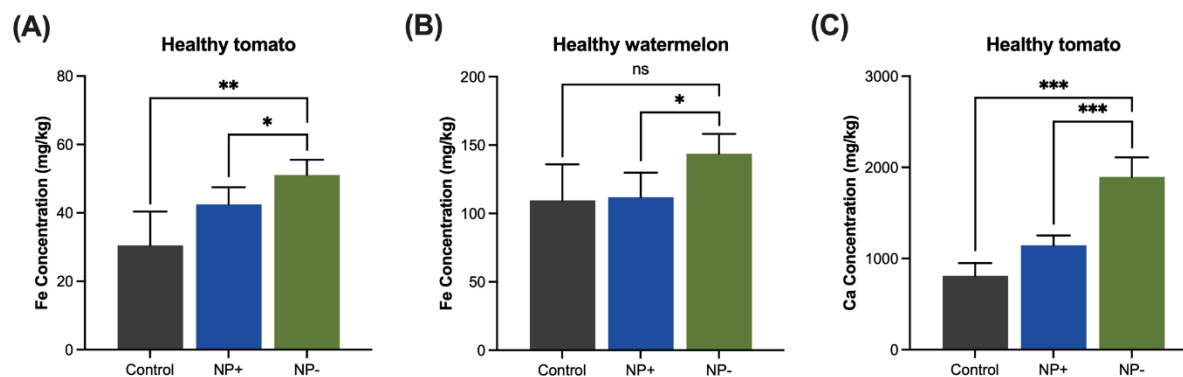


Figure 4. Effect of nanoparticle charge on nutrient concentration (mg/kg dry sample) in tomato and watermelon fruits of healthy and *Fusarium*-infected plants treated with NP+ and NP-. Asterisk represents significant differences between treatments (* and *** represent $p < 0.1$ and 0.01 , respectively, ns represent $p > 0.1$). Error bars correspond to the SE of the mean ($n = 3$).

(1) Surface charge effect (comparison between NP+ and NP-)

Significant differences in nutrient accumulation in tomato and watermelon fruits were observed as a function of NP charge (NP- vs NP+) (Figure 4). Specifically, for the healthy tomato, NP- seedling exposure resulted in significantly higher (20%) fruit Fe concentration than NP+ (Figure 4A). A similar effect was observed in healthy watermelon, with NP- increasing Fe accumulation by 28%, compared to NP+ (Figure 4B). Previous studies have reported that the accumulation of Fe in rice and green onion (*Allium fistulosum* L.) can increase with Cu-based NPs exposure (Wang *et al.*, 2020; Deng *et al.*, 2022a). More specifically, NP exposure can increase the production of ferritin in plant tissues, an intracellular protein with a high affinity to bind and store Fe (Deák *et al.*, 1999). Interestingly, there is no significant difference between NP+ and NP- treatments in infected tomato and watermelon. The mechanism by which negatively charged Cu NPs increases Fe content in healthy but not diseased fruit is unknown, although disease presence clearly results in the activation of significantly different metabolic pathways (Ma *et al.*, 2020; Wang *et al.*, 2022a). In addition, NP- treatment also increased Ca accumulation in

healthy tomato by 66%, compared with the NP+ (Figure 4C). It is possible that negatively charged nanoparticles increase the Fe and Ca (both positively charged) content in plants because of electrostatic interactions [40]. However, the positively charged Cu did not influence the accumulation of anionic nutrients; the reason for this difference is not known. Additionally, these charge-based effects also varied with plant species and disease presence. For example, the Ca content in infected tomato and watermelon treated with any form of Cu regardless of charge was significantly higher (119% - 159%) than the content in healthy fruits ($p < 0.05$). Similarly, Fe content was increased by 11-41% in the infected fruits compared with healthy controls.

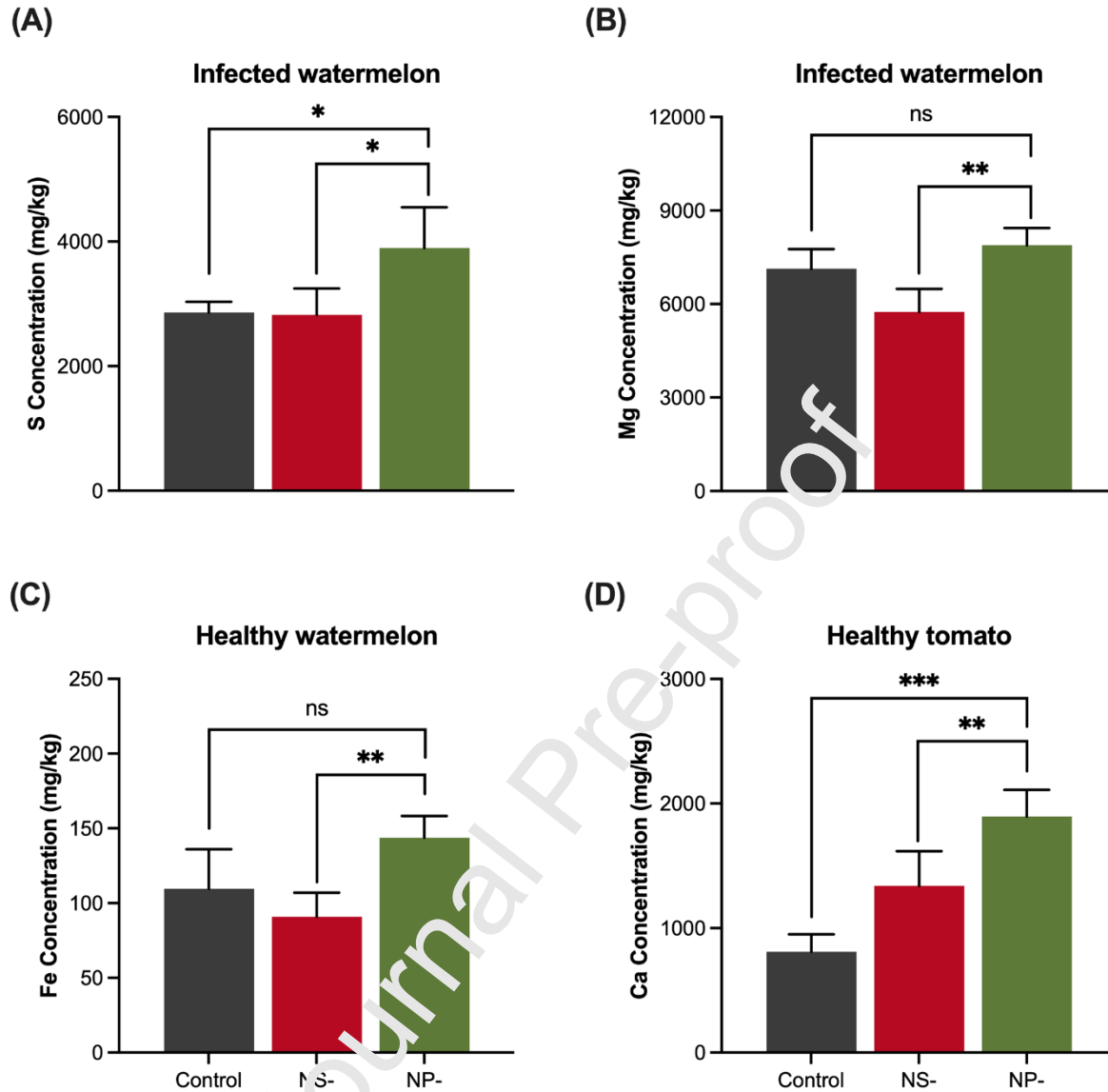


Figure 5. Effect of NP morphology on nutrient concentration (mg/kg dry sample) in tomato and watermelon fruits of healthy and Fusarium-infected plants treated with NS- and NP-. Asterisks represent significant differences between treatments (*, **, and *** represent $p < 0.1$, 0.05, and 0.01, respectively, ns represent $p > 0.1$). Error bars correspond to the SE of the mean ($n = 3$).

(2) NP morphology effect (comparison between NS- and NP-)

In addition to charge, particle morphology also impacted fruit nutrient content. Specifically, sheet- and spike- shaped negatively charged CuO NPs were compared. Previous work from our group with different copper nanomaterials demonstrated differences in uptake as a function of morphology (Borgatta *et al.*, 2018, 2023). In the current study, CuO nanospikes led to higher Cu content in the fruit than with the nanosheets; this may have been a function of greater dissolution and more facile physical movement of the particle across the leaf biointerface. Previous studies have also shown that the nanospike structured CuO exhibited notably greater release of Cu ions into the simulated plant xylem fluid, as compared to the nanosheets structure (Borgatta *et al.*, 2023). In watermelon, S (Figure 5A), Mg (Figure 5B), and Fe (Figure 5C) content were significantly increased (38%, 37%, and 58%, respectively) in the fruit of plants treated with the nanospike form, as compared with nanosheets. In healthy tomato, the Ca content was also significantly higher (42%) after nanospike treatment (Figure 5D). Given this difference in plant species, it appears that watermelon may be more sensitive or responsive to differences in nanoparticle morphology. The mechanisms for this difference are unknown but could be related to physiological differences in or on the leaf surface. Similar to the observations for surface charge, the Ca content in infected tomato and watermelon treated with both nanoparticle morphologies (NP- and NS-) was significantly ($p < 0.05$) higher (94% - 159%) than in healthy fruit. In addition, regarding the two nanoparticle morphologies (NP- and NS-), the Fe content in fruits treated with both materials showed an increasing trend (1% - 41%) in infected plants, but statistical significance was only evident in healthy tomato (41%, $p < 0.05$). This further indicates the beneficial effect of nanomaterials on plant health, as well as the potential to tune nanomaterial chemistry for edible tissue biofortification which is critical for human nutrition security.

(3) Nanoscale effects

Figure 6 shows heat maps depicting the macro (S, Ca, Mg, P) and micro (Fe) nutrient profile of the tomato and watermelon fruit from plants exposed to NS-, NP+, NP-, and ionic CuSO₄ at the seedling stage. It is clear that the average value of S, Ca, Mg, P and Fe content in both watermelon and tomato fruits increased with certain Cu exposures as compared to corresponding controls. The related statistical analysis is shown in Table S1. Overall, these findings demonstrate the potential of controlling nanoscale chemistry as a biofortification strategy.

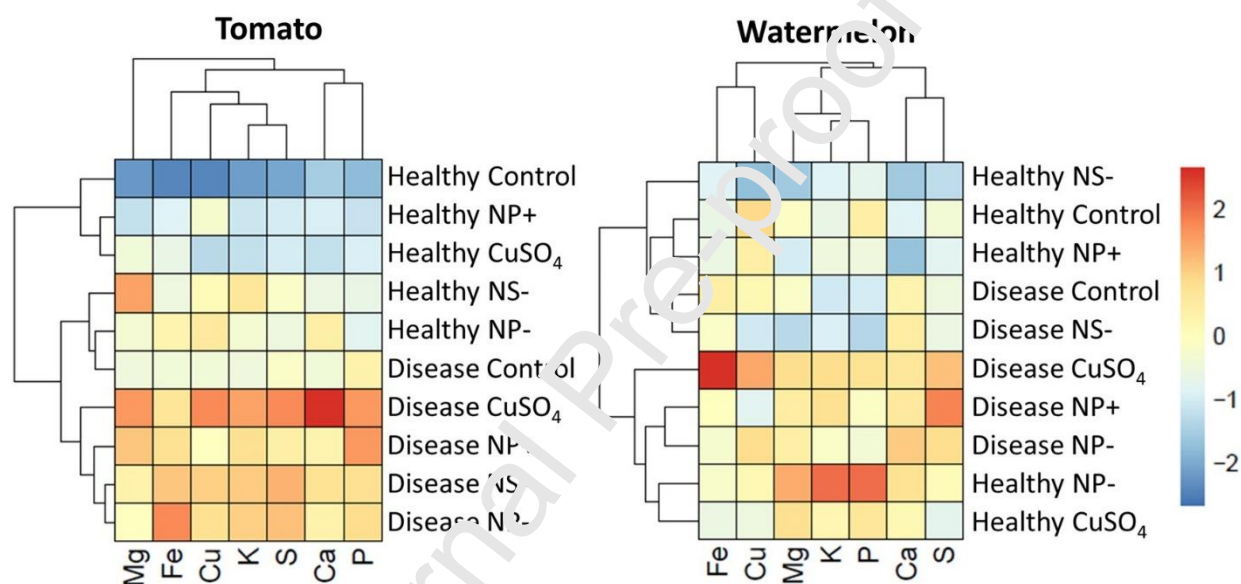


Figure 6. Macro- and Micro-nutrients in tomato and watermelon fruits of healthy and *Fusarium*-infected plants treated with NS-, NP+, NP-, and CuSO₄.

For healthy and infected tomato fruits, treatment with NS-, NP+, and CuSO₄ significantly increased the accumulation of S by 23% - 45%, 9% - 25%, and 24% - 31%, respectively, as compared to the corresponding controls. In addition, NP- increased the S content in healthy tomato by 35%. For watermelon, the S content was increased by 36%, 61%, and 44% upon exposure to NP+, NP-, and ionic treatments in disease fruits, respectively. Sulfur is involved in the synthesis of several metal-binding

polypeptides and in plant antioxidant capacity (Deng *et al.*, 2022b). A previous study has reported that Cu NPs increased sulfur assimilation and enhanced the content of glutathione in tomato leaves (Hernández-Hernández *et al.*, 2019). Moreover, sulfate transporters Sultr 1;1 and Sultr 1;2 were upregulated in plant roots with ionic Cu treatment (Takahashi *et al.*, 2000; Gigolashvili and Kopriva, 2014).

All seedling Cu exposures increased Ca accumulation in healthy tomato healthy fruit by 41% -134%. In addition, NS- and CuSO₄ increased the Ca content in diseased tomato fruits by 45% and 11%, respectively. The increase in Ca content with foliar application of Cu NPs has been reported in tomato leaves by Perez-Labrade *et al.* (Pérez-Labrada *et al.*, 2019). In addition, Deng *et al.* reported that CuO NPs treated soybean leaves and roots accumulated more Ca than control plants (Deng *et al.*, 2022a). No significant difference in Ca content was evident with Cu-based treatments in healthy or infected watermelon.

In tomato, NS-, NP-, and CuSO₄ significantly increased the Mg content in healthy and infected fruit by 11% - 65%, 18% - 21%, and 28% - 32%, respectively. NP- treatment also increased the Mg concentration by 34% in healthy tomato plants. For watermelon, NP+ was the only treatment that increased nutrient levels in infected plants; Mg accumulation was increased by 11%. In a previous study, CuO NPs increased the Mg content in soybean leaves (Deng *et al.*, 2022a). CuSO₄ also increased the concentration of Mg in both healthy and infected tomato fruits. These findings are consistent with Rawat *et al.*, who reported that ionic Cu treated bell pepper leaves had higher Mg content (26%) than the controls (Rawat *et al.*, 2018). For the watermelon, only NP+ treatment had a significant effect on Mg accumulation, relative to the controls.

NP+ increased the P content in both healthy and infected tomato fruit by 29% compared to controls. The NS- and CuSO₄ treatments also improved the P content in healthy tomato by 53% and 39%, respectively. In infected watermelon, NP+ and ionic CuSO₄ treatments caused significant increases in

the P content by 21% to 41%. Similarly, Hong et al reported that a high level of ionic Cu treated alfalfa shoot contained higher P content than the control (Hong *et al.*, 2015). For infected tomato, NP- increased Fe accumulation (37% - 68%) compared to controls; similarly, CuSO₄ treatments also increased the Fe level (19%) in infected tomato fruits. However, the application of CuO NPs did not show any significant change on Fe content in either healthy or infected watermelon crops.

Collectively, the data demonstrate that foliar nanoscale Cu amendment to seedlings can be an effective strategy to increase the concentration of important plant macro and micro nutrients in fruit tissues and perhaps more importantly, that nanomaterial properties such as charge and morphology can be tuned to optimize those effects. Given the global problem of nutrition insecurity or hidden hunger, we believe these findings to be quite significant. The mechanisms controlling these impacts, as well as the occurrence of species-specific responses to treatment are topics of ongoing investigation.

Conclusion:

This study describes the physiological response of field grown tomato and watermelon plants after foliar seedling treatment with nCuO of different surface charges and morphologies. Disease progression in treated plants was significantly impacted as a function of both charge and morphology. For tomato, NS- and NP- significantly slowed disease progression by 32% and 15%, compared to the infected control, respectively. Conversely, the other Cu-based treatments did not inhibit disease progression. Notably, NS- reduced disease by 37% and 40% as compared to NP+ and CuSO₄, respectively. For watermelon, the negatively charged NPs had no effect on wilt-suppression as compared to the infected controls, but NP+ reduced disease by 30.9%. Both NS- and NP- significantly increased Cu content in healthy tomato fruit. In addition, the accumulation of other nutrients varied in the fruit as a function of plant species, disease presence and Cu type. Specifically, changes in the accumulation of nutrients in the edible parts were observed to vary with the morphology (S, Ca, Fe, and Mg) and surface charge (Fe and Ca) of the

nanomaterials. This study demonstrates the importance of developing synthesis methods tailored to plant species and specific nutrients. This work also suggests that the complex process through which nanoparticles enter the plant after foliar exposure is important to its eventual impact on the plant. The elucidation of this entry may be accelerated through computational molecular scale simulations at the leaf surface which are sensitive to the local charge and morphology of the specific plants. Overall, the data indicates that a one-time foliar treatment during the seedling stage with nCuO improves plant health and disease resistance and also offers a novel and tunable strategy for the biofortification of important micronutrients in food crops.

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References:

- Adisa IO, Reddy Pullagurala VL, Sowat S, Hernandez-Viezcas JA, Dimkpa CO, Elmer WH, White JC, Peralta-Videa JR, Gardea-Torresdey JL.** 2018. Role of Cerium Compounds in Fusarium Wilt Suppression and Growth Enhancement in Tomato (*Solanum lycopersicum*). *Journal of Agricultural and Food Chemistry* **66**, 5959–5970.
- Alexandratos N, Bruinsma - FAO J.** 2012. World agriculture towards 2030/2050: the 2012 revision. doi: 10.22004/AG.ECON.288998.
- Ashraf H, Anjum T, Riaz S, Ahmad IS, Irudayaraj J, Javed S, Qaiser U, Naseem S.** 2021. Inhibition mechanism of green-synthesized copper oxide nanoparticles from *Cassia fistula* towards *Fusarium oxysporum* by boosting growth and defense response in tomatoes. *Environmental Science: Nano* **8**, 1729–1748.
- Bai T, Zhang P, Guo Z, et al.** Different physiological responses of C3 and C4 plants to nanomaterials. doi: 10.1007/s11356-021-12507-7/Published.

Balasubramaniam J, Goh KS, Sani SF, Alam MW, Ismail NA, Gleason ML, Rosli H. 2023. *Fusarium falciforme* and *F. oxysporum* causing postharvest fruit rot of watermelon (*Citrullus lanatus*) in Malaysia: A first report. *Crop Protection* **163**, 106115.

Borgatta J, Ma C, Hudson-Smith N, Elmer W, Pérez CDP, Torre-Roche RD La, Zuverza-Mena N, Haynes CL, White JC, Hamers RJ. 2018. Copper Based Nanomaterials Suppress Root Fungal Disease in Watermelon (*Citrullus lanatus*): Role of Particle Morphology, Composition and Dissolution Behavior. *ACS Sustainable Chemistry & Engineering* **6**, 14847–14856.

Borgatta J, Shen Y, Tamez C, et al. 2023. Influence of CuO Nanoparticle Aspect Ratio and Surface Charge on Disease Suppression in Tomato (*Solanum lycopersicum*). *Journal of Agricultural and Food Chemistry* doi: 10.1021/ACS.JAFC.2C09153.

Cheng B, Chen F, Wang C, Liu X, Yue L, Cao X, Wang Z, Xing B. 2021. The molecular mechanisms of silica nanomaterials enhancing the rice (*Oryza sativa* L.) resistance to plant hoppers (*Nilaparvata lugens* Stal). *Science of The Total Environment* **767**, 144967.

Deák M, Horváth G V., Davletova S, Török K, Sass L, Vass I, Baraňa B, Király Z, Dudits D. 1999. Plants ectopically expressing the ironbinding protein, ferritin, are tolerant to oxidative damage and pathogens. *Nature Biotechnology* 1999 17:2 **17**, 192–196.

Deng C, Wang Y, Cantu JM, et al. 2022a. Soil and foliar exposure of soybean (*Glycine max*) to Cu: Nanoparticle coating-dependent plant responses. *NanoImpact* **26**, 100406.

Deng C, Wang Y, Cota-Ruiz K, et al. 2020. Bok choy (*Brassica rapa*) grown in copper oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation, biodistribution and nutritional disturbance. *Journal of Hazardous Materials* **398**, 122978.

Deng C, Wang Y, Navarro G, Sun Y, Cota-Ruiz K, Hernandez-Viezcas JA, Niu G, Li C, White JC, Gardea-Torresdey J. 2022b. Copper oxide (CuO) nanoparticles affect yield, nutritional quality, and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains. *Science of The Total Environment* **810**, 152260.

Dimkpa CO, McLean JE, Latta DJ, Manangón E, Britt DW, Johnson WP, Boyanov MI, Anderson AJ. 2012. CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *Journal of Nanoparticle Research* **14**, 1–15.

El-Abeid SE, Ahmed Y, Daròs JA, Mohamed MA. 2020. Reduced graphene oxide nanosheet-decorated copper oxide nanoparticles: A potent antifungal nanocomposite against fusarium root rot and wilt diseases of tomato and pepper plants. *Nanomaterials* **10**.

Elmer WH, White JC. 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. *Environmental Science: Nano* **3**, 1072–1079.

Everts KL, Himmelstein JC. 2015. *Fusarium* wilt of watermelon: Towards sustainable management of a re-emerging plant disease. *Crop Protection* **73**, 93–99.

Ghormade V, Deshpande M V., Paknikar KM. 2011. Perspectives for nano-biotechnology enabled protection and nutrition of plants. *Biotechnology Advances* **29**, 792–803.

- Gigolashvili T, Kopriva S.** 2014. Transporters in plant sulfur metabolism. *Frontiers in Plant Science* **0**, 442.
- Godfray HCJ, Mason-D'Croz D, Robinson S.** 2016. Food system consequences of a fungal disease epidemic in a major crop. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**.
- Gordon TR.** 2017. *Fusarium oxysporum* and the Fusarium Wilt Syndrome. <https://doi.org/10.1146/annurev-phyto-080615-095919> **55**, 23–39.
- Graham JH, Johnson EG, Myers ME, Young M, Rajasekaran P, Das S, Santra S.** 2016. Potential of nano-formulated zinc oxide for control of citrus canker on grapefruit trees. *Plant Disease* **100**, 2442–2447.
- Hartman GL, Chang HX, Leandro LF.** 2015. Research advances and management of soybean sudden death syndrome. *Crop Protection* **73**, 60–66.
- Hernández-Hernández H, Quiterio-Gutiérrez T, Cadenas-Pliego G, Ortega-Ortiz H, Hernández-Fuentes AD, De La Fuente MC, Valdés-Reyna J, Juárez-Maldonado A.** 2019. Impact of Selenium and Copper Nanoparticles on Yield, Antioxidant System, and Fruit Quality of Tomato Plants. *Plants* 2019, Vol. 8, Page 355 **8**, 355.
- Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea-Torresdey JL.** 2015. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environmental Science: Processes & Impacts* **17**, 177–185.
- Jones JB (Jeffrey B, Zitter TA (Thomas A, Momeni M, Miller SA, American Phytopathological Society.** *Compendium of tomato diseases and pests*.
- Kousik CS, Brusca J, Turechek WW.** 2018. Diseases and Disease Management Strategies Take Top Research Priority in the Watermelon Research and Development Group Members Survey (2014 to 2015). <https://doi.org/10.1094/PHP-S-15-0047> **17**, 53–58.
- Lopez-Lima D, Mtz-Enriquez AI, Carrón G, Basurto-Cereceda S, Pariona N.** 2021. The bifunctional role of copper nanoparticles in tomato: Effective treatment for Fusarium wilt and plant growth promoter. *Scientia Horticulturae* **277**, 105810.
- Ma C, Borgatta J, Hudson EG, et al.** 2020. Advanced material modulation of nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. *Nature Nanotechnology* 2020 15:12 **15**, 1033–1042.
- Pandorf M, Pourzahedi L, Gilbertson L, Lowry G V., Herckes P, Westerhoff P.** 2020. Graphite nanoparticle addition to fertilizers reduces nitrate leaching in growth of lettuce (*Lactuca sativa*). *Environmental Science: Nano* **7**, 127–138.
- Pérez-Labrada F, López-Vargas ER, Ortega-Ortiz H, Cadenas-Pliego G, Benavides-Mendoza A, Juárez-Maldonado A.** 2019. Responses of Tomato Plants under Saline Stress to Foliar Application of Copper Nanoparticles. *Plants* 2019, Vol. 8, Page 151 **8**, 151.
- Ponmurugan P, Manjukurambika K, Elango V, Gnanamangai BM.** 2016. Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. *Journal of Experimental Nanoscience* **11**, 1019–1031.

Rampersad SN. 2020. Pathogenomics and Management of Fusarium Diseases in Plants. *Pathogens* 2020, Vol. 9, Page 340 **9**, 340.

Rawat S, Pullagurala VLR, Hernandez-Molina M, Sun Y, Niu G, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL. 2018. Impacts of copper oxide nanoparticles on bell pepper (*Capsicum annuum* L.) plants: a full life cycle study. *Environmental Science: Nano* **5**, 83–95.

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. 2019. The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution* 2019 3:3 **3**, 430–439.

Shacklette HT, Boerngen JG, Clark WP, Peck DL. Element Concentrations in Soils and Other Surficial Materials of the Conterminous United States UNITED STATES DEPARTMENT OF THE INTERIOR. *in press*.

Shah IH, Ashraf M, Khan AR, Manzoor MA, Hayat K, Arif S, Sabir IA, Abd-Allah M, Niu Q, Zhang Y. 2022. Controllable synthesis and stabilization of Tamarix aphylla-mediated copper oxide nanoparticles for the management of Fusarium wilt on musk melon. *3 Biotech* **12**, 1–15.

Shang H, Ma C, Li C, White JC, Polubesova T, Chefetz B, Xing B. 2020. Copper sulfide nanoparticles suppress *Gibberella fujikuroi* infection in rice (*Oryza sativa* L.) by multiple mechanisms: contact-mortality, nutritional modulation and phytohormone regulation. *Environmental Science: Nano* **7**, 2632–2643.

Shang H, Ma C, Li C, Zhao J, Elmer W, White JC, Xing B. 2021. Copper Oxide Nanoparticle-Embedded Hydrogels Enhance Nutrient Supply and Growth of Lettuce (*Lactuca sativa*) Infected with *Fusarium oxysporum* f. sp. *lactucae*. *Environmental Science and Technology* **55**, 13432–13442.

Takahashi H, Watanabe-Takahashi A, Smith FW, Blake-Kalff M, Hawkesford MJ, Saito K. 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. *The Plant Journal* **23**, 171–182.

Wang Y, Deng C, Cota-Ruiz K, et al. 2020. Improvement of nutrient elements and allicin content in green onion (*Allium fistulosum*) plants exposed to CuO nanoparticles. *Science of The Total Environment* **725**, 138387.

Wang Y, Deng C, Elmer WH, et al. 2022a. Therapeutic Delivery of Nanoscale Sulfur to Suppress Disease in Tomatoes: In Vitro Imaging and Orthogonal Mechanistic Investigation. *ACS Nano* **16**, 11204–11217.

Wang Y, Deng C, Rawat S, Cota-Ruiz K, Medina-Velo I, Gardea-Torresdey JL. 2021. Evaluation of the Effects of Nanomaterials on Rice (*Oryza sativa* L.) Responses: Underlining the Benefits of Nanotechnology for Agricultural Applications. *ACS Agricultural Science and Technology* **1**, 44–54.

Wang Y, Deng C, Shen Y, Borgatta J, Dimkpa CO, Xing B, Dhankher OP, Wang Z, White JC, Elmer WH. 2022b. Surface Coated Sulfur Nanoparticles Suppress Fusarium Disease in Field Grown Tomato: Increased Yield and Nutrient Biofortification. *Journal of Agricultural and Food Chemistry* **2022**, 14377–14385.

Xu T, Wang Y, Aytac Z, Zuverza-Mena N, Zhao Z, Hu X, Ng KW, White JC, Demokritou P. 2022. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core-Shell Nanostructures. *ACS Nano* **16**, 6034–6048.

Zakharova O, Kolesnikov E, Shatrova N, Gusev A. 2019. The effects of CuO nanoparticles on wheat seeds and seedlings and *Alternaria solani* fungi: In vitro study. IOP Conference Series: Earth and Environmental Science. Institute of Physics Publishing.

Zhang M, Xu JH, Liu G, Yao XF, Li PF, Yang XP. 2015. Characterization of the watermelon seedling infection process by *Fusarium oxysporum* f. sp. *niveum*. *Plant Pathology* **64**, 1076–1084.

Zhao, L., Bai, T., Wei, H., Gardea-Torresdey, J. L., Keller, A., White, J. C. 2022. Nanobiotechnology-based strategies for enhanced crop stress resilience. *Nature Food*, **3**(10), 829-836.

Journal Pre-proof

Author Contribution

JCW, and WHE: conceptualization; CD, CRP, YW, JB, COD, and EHW: methodology; CD, CRP, YW, JB, HJB, and KR-O: investigation; CD, CRP, and YW: writing - original draft; CD, CRP, YW, JZ, PW, VG, COD, and JCW: writing - review & editing; CD, CRP, YW, JZ, PW, and JCW: visualization; COD, RH, RJH, JCW, and WHE: supervision; RH, RJH, and JCW: funding acquisition

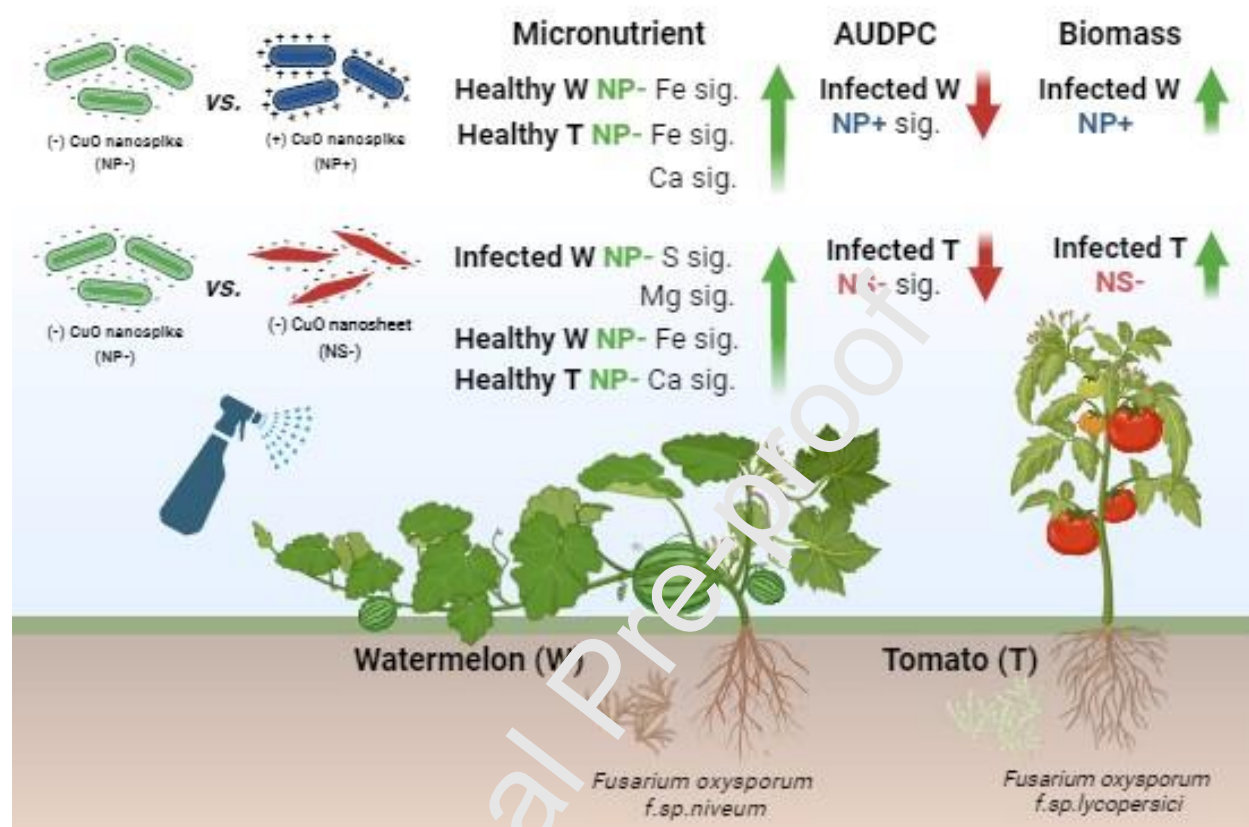
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Graphical abstract



Highlights

- Nanoscale CuO surface charge and morphology influence efficacy as a foliar treatment under field conditions.
- Beneficial impacts on plants were species and nanomaterial specific.
- All nanoscale treatments increased the fruit nutritional quality (i.e., nano-enabled biofortification) of both species.

Journal Pre-proof