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Role of Cerium Compounds in Fusarium Wilt Suppression and Growth Enhancement in Tomato (*Solanum lycopersicum*)

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Supporting Information

ABSTRACT: The use of nanoparticles in plant protection may reduce pesticide usage and contamination and increase food security. In this study, three-week-old *Solanum lycopersicum* seedlings were exposed, by root or foliar pathways, to CeO_2 nanoparticles and cerium acetate at 50 and 250 mg/L prior to transplant into sterilized soil. One week later, the soil was inoculated with the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (1 g/kg), and the plants were cultivated to maturity in a greenhouse. Disease severity, biomass/yield, and biochemical and physiological parameters were analyzed in harvested plants. Disease severity was significantly reduced by 250 mg/L of nano-CeO_2 and CeAc applied to the soil (53% and 35%, respectively) or foliage (57% and 41%, respectively), compared with non-treated infested controls. Overall, the findings show that nano-CeO_2 has potential to suppress Fusarium wilt and improve the chlorophyll content in tomato plants.

KEYWORDS: *nano-CeO_2, nanofertilizer, nanopesticide, tomato, Fusarium wilt*

INTRODUCTION

It has been estimated that the agricultural field in the United States loses hundreds of millions of dollars annually due to soil borne diseases, resulting in displacement of industries and discontinuation of product lines.[1,2] Soil borne diseases are difficult to manage and can potentially reduce crop yields by 20%.[1] Fungal pathogens alone reduce economic return on yield by approximately $200 million, in spite of the more than $600 million spent per year on control efforts.[3] Fusarium wilt is one of the most destructive fungal diseases, decreasing agricultural yield and nutritional value of crops, such as soybean, watermelon, eggplant, and tomato, resulting in billions of dollars in annual losses.[4] This scourge, coupled with increasing human population, drastic climate change, and loss of arable land for agriculture, will make the need to double food production by 2050 extremely difficult.[1] Hence, there is urgent need for novel approaches to tackle this menace.

The United States is one of the largest global producers of tomato, the second most consumed vegetable in the country, which generates over $2 billion in annual revenue.[5] Several diseases affect tomato production in the U.S., but Fusarium wilt is recognized as the most destructive soil borne disease of this plant. The disease is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*, which can affect tomato both in the field and under protected cultivation.[6]

The control of Fusarium wilt is difficult because the fungus may remain dormant in the soil in the form of chlamyspores for a long period of time.[6] The most successful control strategy for plant pathogens has been host resistance. However, this technique has been limited for tomato due to a lack of resistant genes, consumer-driven preference for susceptible heirloom cultivars, and social unease surrounding the use of genetically modified foods. Another traditional control method is the use of fungicides, but this approach is environmentally unsustainable and cost ineffective.[4] Hence, there is significant need to develop novel and more effective strategies for fungal pathogen control.

It has been reported that an improvement in a plant's nutritional status can increase defense against pathogenic diseases.[4] Nitrogen fertilization has been shown to improve plant defenses against pathogenic infection.[7] However, continuous nitrogen fertilization causes imbalances in soil microbial communities and is not sustainable.[8] Currently, there is great interest in the application of nanotechnology to enhance the growth, yield, and nutritional quality of crops.[9]

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This is because of the unique ultrasmall size and large surface area of nanoparticles (NPs), which significantly enhance biological activity and functions in biological living systems.

Little is known about the impact of NPs on the suppression of plant pathogenic diseases; recent results highlight increased crop production, pest/disease control, and plant health.[4] The antimicrobial properties of particles such as Ag, Mg, Si, TiO_2, and ZnO can directly reduce fungal pathogen activity.[4] For instance, ZnO NPs reduced *Fusarium graminearum* growth in mung bean (*Vigna radiata*) broth by 26%, as compared with the bulk oxide and controls.[10] ZnO NPs at 3−12 mmol also suppressed the growth of *Penicillium expansum* and *Botrytis cinerea* by 61−91% and 63−80%, respectively.[11] This ability to successfully reduce pathogen activity and to improve growth suggests that nanoscale nutrients such as ZnO may be a better control option than antimicrobials such as AgNPs to manage fungal infection.[10]

Foliar application of micronutrient NPs such as CuO, MnO, and ZnO reduced disease symptoms (such as yellowing and browning of older leaves and stunted growth) in tomato grown in soil infested with *F. oxysporum*.[12] Elmer and White[12] also reported that CuO NPs increased the growth and yield of both tomato and eggplants (*Solanum melongena* L.) cultivated in infested soils. Unlike Cu and Mn, Ce is not a nutritional element for plants; however, it has been reported that nano-CeO_2 enhances plant growth, although the mechanism is still unclear.[4,13] Additionally, Ce is the major component of "Changle", a rare earth element (REE) fertilizer that contains about 50% Ce and is used in rice, wheat (*Triticum aestivum* L.), and other vegetables.[14] Nano-CeO_2 was reported to stimulate soybean (*Glycine max* (L.) Merr.) growth,[15] increasing both shoot and root lengths and chlorophyll content in tomato.[16] Moreover, Ce was reported to enhance photosynthetic activity and reduce the inhibition of UV-b radiation in soybean seedlings.[17] Nonetheless, to the best of the authors' knowledge, there is no information on the role of nano-CeO_2 in the suppression of Fusarium wilt in plants. The objective of this study was to evaluate the potential of nano-CeO_2 to suppress Fusarium wilt disease and to enhance tomato production. Cerium acetate was used as an ionic control for comparison. A UV/vis spectrophotometer was used for catalase and polyphenol oxidase assays, a single photon avalanche diode (SPAD) was used for chlorophyll measurement, and inductively coupled plasma-optical emission spectroscopy (ICP-OES) instrumentation was used to quantify Ce and micro/macro element contents.

MATERIALS AND METHODS

Nanoparticle Suspension Preparation. Nano-CeO_2 (Meliorum Technologies) was obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to Keller and co-workers,[18] nano-CeO_2 have a primary size of 8 ± 1 nm, aggregate to 231 ± 16 nm in deionized (DI) water, have a surface area of 93.8 $m^2\ g^{-1}$, and are 95.14% pure. Cerium acetate (CeAc, Sigma-Aldrich) has a size of about 5 μm. Following the procedure previously described by Barrios and co-workers,[16] NP suspensions and CeAc solutions were prepared in DI water at 0, 50, and 250 mg/kg, for compound-based concentrations relative to 3 kg of soil.[16]

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

The nano-CeO_2 suspensions and CeAc solutions were applied to the roots/soil or leaves of the tomato plants. For the root application, the 3 kg soil mixture was homogeneously amended with the prepared suspensions/solutions prior to seedling transplant. For the foliar application, the shoots of 21-day-old seedlings were sprayed with 5 mL of the nano-CeO_2 and CeAc suspensions/solutions that had been amended with one drop of a non-ionic surface active agent (Lesco Spreader-Sticker) to allow retention to the leaf surface. The shoots were allowed to dry, with the suspensions/solutions kept off the roots prior to transplant into the pots containing the soil mixture.

The *F. oxysporum* f. sp. *lycopersici* Race 2 inoculum, isolated from an heirloom tomato cultivar, was obtained from the Scratch Farm, Cranston, RI. Procedures for producing inoculum were as described by Elmer and White.[12] After 7 days of the NP/ionic exposure, six treatment replicates were divided into two groups. To infest the soil, triplicates of each treatment were inoculated with *F. oxysporum* by carefully removing the plants and thoroughly mixing the soil with 3 g of the inoculum per pot (1 g/kg soil ∼100,000 colonies) to ensure homogeneity; the seedlings were then retransplanted. The remaining triplicates were treated as non-infested controls. Plants were watered with 150 mL of water as needed for plant growth. Peter's soluble 20:20:20 nitrogen/phosphorus/potassium (NPK) fertilizer was applied on a weekly basis, and the plants were cultivated until full maturity (126 days).

Disease Severity. Disease severity in each triplicate pot was assessed weekly for 18 weeks, as the symptoms manifested, using a 1−6 scale, where 1 = no disease, 2 = 1−10% disease, 3 = 11−25% disease, 4 = 26−50% disease, 5 = 51−75% disease, and 6 = >75% disease or dead.[19] The disease progress was plotted against time, and the area-under-the-disease progress curve (AUDPC) was calculated using the trapezoid rule:

$$\mathrm{AUDPC} = \sum (Y_i + Y_i)/2 \times (t_{i+1} - t_i)$$

where Y_i = disease rating at time t_i.[19]

In Vitro Antifungal Activity Test. Potato dextrose agar (PDA) was used for an in vitro inhibitory test of nano-CeO_2 against *F. oxysporum,* following the method of Fraternale and co-workers[20] with some modification. Nanoparticle suspensions were prepared at 0, 50, 100, and 250 mg/L with DI water, which was then amended with 25% PDA. The mixtures were autoclaved, poured into 10 cm diameter Petri dishes, and were allowed to solidify by cooling. Mycelial plugs of 4 mm diameter in size were cut from the edge of the *Fusarium* isolates grown on PDA for 7 days and were placed at the center of triplicate Petri dishes containing the nano-CeO_2 suspensions. The inoculated dishes were then incubated at 28 °C for 7 days. The inhibitory potential of nano-CeO_2 was determined by mycelial expansion (in centimeters), with measurements of the diameter of the spore germination at 2, 4, and 6 d intervals.[20]

Chlorophyll Content. The chlorophyll content was determined by using a hand-held single photon avalanche diode (SPAD, Minolta Camera, Japan).[9] Six leaves per plant were randomly selected, and the average chlorophyll content was determined using SPAD at 5 weeks after transplant, when the symptoms of Fusarium wilt had developed, and at harvest (18th week).

Plant Harvest and Agronomical Parameters. At full maturity (126 days), the plant tissues (roots and shoots) were washed and rinsed 3 times with 5% $CaCl_2$ and Millipore water (MPW).[21] The length and weight of individual fresh plant tissues were recorded. The fresh root samples were collected for enzyme assays; the leaf, stem, and root samples were also separated for elemental analysis. The remaining plants were oven-dried for 72 h at 60 °C to determine the total biomass. The fruit from each plant was collected and weighed

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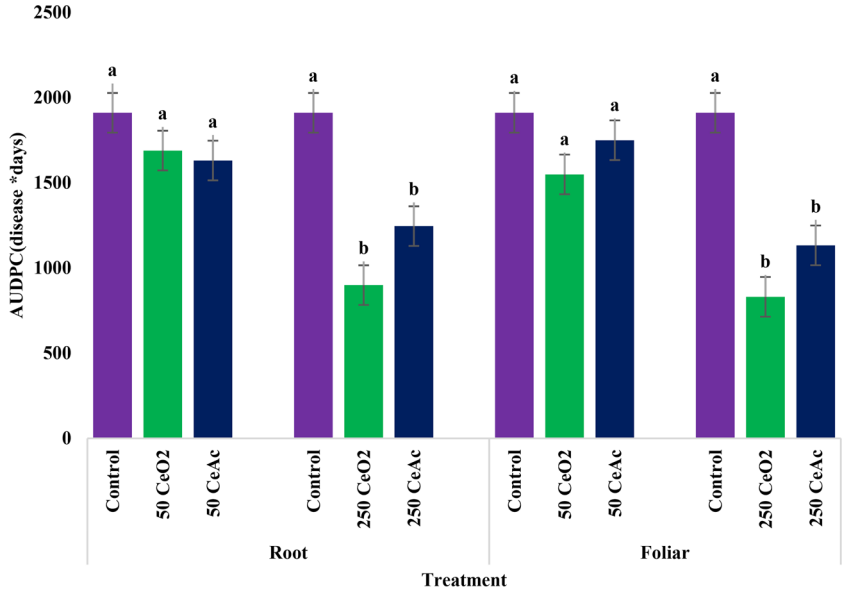

Figure 1. Effect of root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L on Fusarium wilt infested tomato plants grown for 18 weeks. The disease progression was monitored and estimated over time using AUDPC between the 5th and 18th weeks. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

upon ripening until day 126. The size, total mass, and total number of fruit produced by each plant was determined at harvest.

Enzyme Assays. Activities of a typical defense enzyme (polyphenol oxidase; EC 1.14.18.1) and stress enzyme (catalase; EC 1.11.1.6) were examined in the plant roots. Root extracts following the procedure described by Barrios and co-workers[16] were used for enzymes analysis. The extracts were centrifuged at 9600g for 10 min at −4 °C (Eppendorf AG bench centrifuge 5417R, Hamburg, Germany), and the supernatants were collected in 2 mL Eppendorf tubes for analysis.[16]

Catalase (CAT; EC 1.11.1.6) Activity. Following the method described by Gallego and co-workers,[22] a reaction mixture containing 950 μL of 10 mM H_2O_2 and 50 μL of the enzyme extract was shaken three times in a quartz cuvette. The absorbance of the mixture was read and recorded for 3 min at 240 nm using a PerkinElmer Lambda 14 UV/vis spectrophotometer (single-beam mode, PerkinElmer, Uberlingen, Germany). Catalase activity was expressed as the amount of enzyme required to degrade 1 μmol of H_2O_2 per minute.

Polyphenol Oxidase (PPO; EC 1.14.18.1) Activity. The PPO activity was determined following a method by Mayer and co-workers[23] with slight modification, as previously reported by Anusuya and Sathiyabama.[24] The reaction mixture containing 1.5 mL of 0.1 M potassium phosphate buffer at pH 6.5 and 0.2 mL of the enzyme extract was initiated by addition of 0.2 mL of 0.01 M catechol. The absorbance was recorded at 495 nm using a PerkinElmer Lambda 14 UV/vis spectrophotometer (single-beam mode, PerkinElmer, Uberlingen, Germany) to determine the enzyme activity. The PPO activity was defined as the change in absorbance at 495 nm per minute per milligram of protein.[23]

Accumulation of Cerium, Micro, and Macro Elements in Plant. Cerium and selected micro/macro element (Ca, Fe, Zn, Cu, Mn, Al, P, and K) concentrations were determined in the plant tissues. At harvest, portions of root, stem, and leaf tissues were rinsed 3 times using a 5% $CaCl_2$ and Millipore water (MPW) and were oven-dried at 70 °C for 72 h. Plant tissues were acid digested for elemental analysis following an EPA method as described by Ebbs and co-workers.[25] The Ce and micro/macro element content was quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES, PerkinElmer, Optima 4300 DV, Shelton, CT). To validate the digestion and the analytical methods employed, blanks, spikes, and a standard reference material (NIST 1547, Gaithersburg, MD, peach leaves) were used. To ensure quality control and quality assurance, ICP readings of the blank and the standard were repeated after every 15 samples (95% recovery).

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

RESULTS AND DISCUSSION

Disease Severity. The symptoms of Fusarium wilt became evident on the infested plants at the fourth week after soil inoculation; disease progression was monitored until harvest and was estimated using AUDPC (Figure 1). The root or foliar application of nano-CeO_2 at 50 mg/L had no impact on disease suppression, compared with the non-treated infested control (Figure 1). However, at 250 mg/L both root and foliar applications significantly decreased the disease severity by 53% and 57%, respectively, compared to the control ($p \leq 0.05$). Similar results were also observed with CeAc. There was no effect at 50 mg/L, whereas 250 mg/L of foliar or root application reduced the disease progression by 41% and 35%, respectively ($p \leq 0.05$), compared to the infested control (Figure 1). The potential of Ce compounds to enhance plant growth and improve resistance against infection could be attributed to the characteristics of the lanthanide group of elements (such as antioxidant and photosynthetic enhancement), to which cerium belongs.[17] Microfertilizers containing rare elements have been extensively used in China since the 1970s to promote plant growth, productivity, and improve resistance against stress.[17,26] A rare earth nitrate fertilizer known as "Changle", which is more than 50% CeO_2 in composition, is commonly used in China to fertilize rice, wheat, soybean, and peanut crops.[14] However, since a similar effect was observed in infested plants treated with CeAc, the antifungal activity could be attributed to the antioxidant property of Ce in general. Cerium coexists in Ce^{3+} and Ce^{4+} oxidation states,[27] which enhances its antioxidant properties. Liang and co-workers[17] reported that Ce improves photo-

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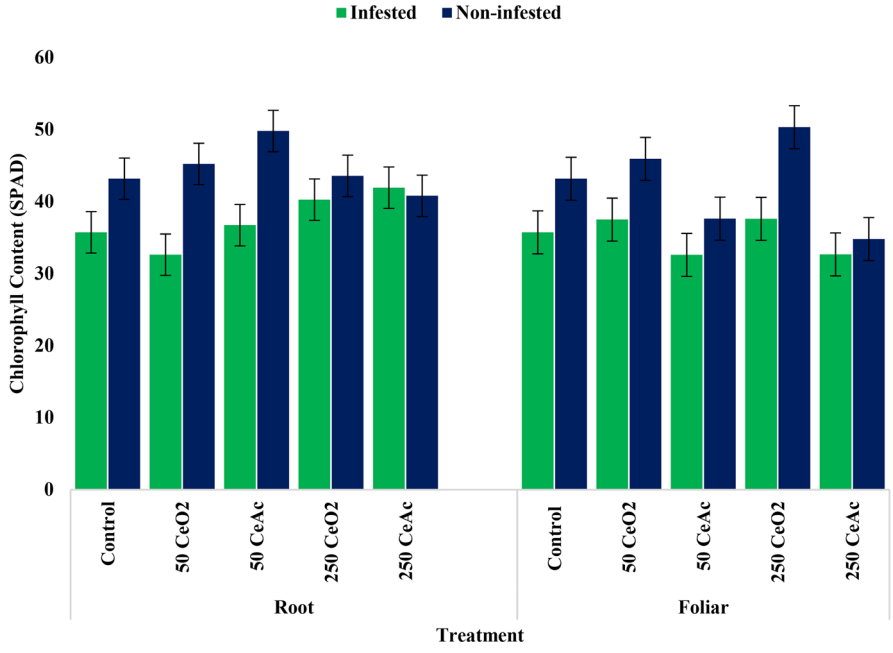

Figure 2. Effect on the leaf chlorophyll content of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L at the 5th week. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

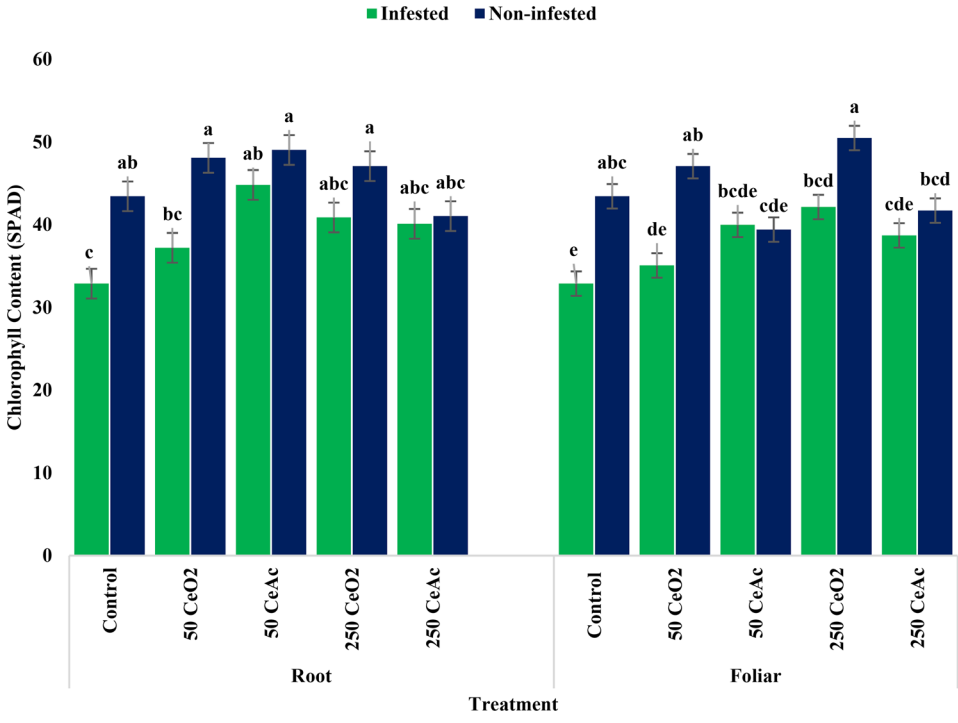

Figure 3. Effect on the leaf chlorophyll content of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L at the 18th week. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) is indicated by the letters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

synthetic parameters, reducing the inhibition of UV-b radiation in soybean seedlings. The mechanism by which the cerium compounds suppress disease is unknown; however, previous reports have indicated that CeO_2 NPs inhibit the growth of *Escherichia coli* and *Bacillus subtilis*.[28] Yan and co-workers[29] revealed the protective potential of rare earth elements on the growth and physiological metabolism of wheat under acid rain stress. Huang and co-workers[26] also reported that Ce can reduce the inhibitory effects of acid rain on the growth and germination of barley by quenching excessive free radicals generated by the acid stress and by promoting chlorophyll synthesis and root growth. It is possible that reactive oxygen species (ROS) generated by pathogen infection can be mitigated by the cerium compounds.[32]

Antifungal Activity Test. There were no significant changes in the diameter of spore germination at two, four,

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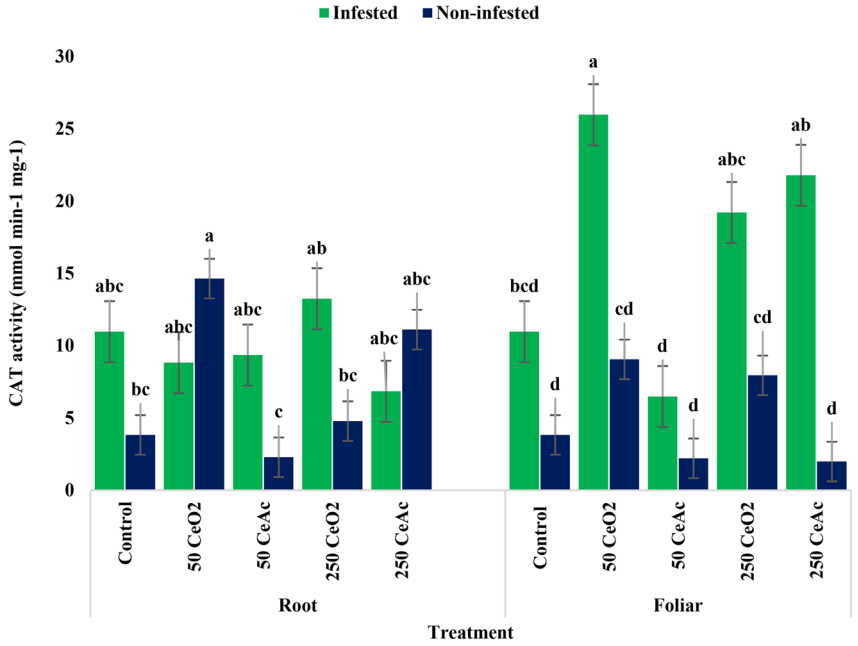

Figure 4. Effect on root catalase activity of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

and six days upon exposure to 50, 100, and 250 mg/L as compared with the control ($p \leq 0.05$). This demonstrates that nano-CeO_2 is not acting as a direct inhibitor on the pathogen, at least under in vitro conditions. Previous studies have demonstrated the antimicrobial properties of nano-CeO_2. Pelletier and co-workers[28] revealed that CeO_2 NPs (at 0.5% wt/vol) can inhibit bacteria and reduce overall viability. The reasons for this discrepancy are not known but could be related to differences in the nature of the exposure or the pathogen (bacteria vs fungi).

Effect of Cerium Compounds on Chlorophyll Content. Figures 2 and 3 display the chlorophyll content in leaves of tomato plants exposed to nano-CeO_2 and CeAc with or without *F. oxysporum* infestation at weeks 5 and 18 after transplant, respectively. At week 5, the relative chlorophyll content of the plants was not affected by the root and foliar applications of nano-CeO_2 and CeAc, regardless of the concentration or infestation (Figure 2). This could be a result of the early stage of infection and plant growth. Cao and co-workers[15] reported that uncoated nano-CeO_2 at 10, 100, and 500 mg/kg soil had no significant impact on total chlorophyll in soybean. At week 18, the chlorophyll content of Ce treated, non-infested plants, was similar to that of the non-infested control (Figure 3). However, the chlorophyll content of infested control reduced by 32% ($p \leq 0.05$), compared with the non-infested control. This is an indication that the Fusarium infestation affected the photosynthetic system of the infested plants. Similarly, the chlorophyll content of infested plants exposed to nano-CeO_2 at 50 mg/kg via the roots decreased by 29% ($p \leq 0.05$) compared with the non-infested plants treated with nano-CeO_2 at 50 mg/kg via the roots (Figure 3). However, none of the treatments in the non-infested plants affected the chlorophyll content at week 18, compared with the non-infested control. Plants grown in infested soil treated with CeAc at 50 mg/kg exhibited a 36% increase in chlorophyll content, compared with the infested control ($p \leq 0.05$) (Figure 3). Infested plants foliarly exposed to 250 mg/L of nano-CeO_2 also exhibited significant increases in chlorophyll content (28%, $p \leq 0.05$), compared with the infested control (Figure 3). Conversely, exposure of infested plants with 250 mg/L of nano-CeO_2 or CeAc via the roots, and CeAc at 250 mg/L via the leaves, did not affect the chlorophyll content. Leaf pigments, including chlorophyll, are known to change in response to stress.[30] It has been previously reported that nano-CeO_2 and other NPs alter chlorophyll content in plants.[30,15] Cao and co-workers[15] reported that PVC-coated CeO_2 NPs at 10 mg/kg increased the total chlorophyll content in soybeans. However, Du and co-workers[30] found that CeO_2 NPs at 400 mg/kg decreased total chlorophyll content in wheat. The significant increase in chlorophyll content, and likely photosynthetic output at week 18, could be an indication that, relative to infested controls, the treated plants had enhanced tolerance to infection. The stress generated from infection could inhibit the movement of water and nutrients required for photosynthetic activities through the xylem. The data suggest that Ce mitigates the negative impacts of infection, perhaps due to its antioxidant activity. This is in agreement with Rossi and co-workers,[31] who reported a significant increase in the chlorophyll content in *Brassica napus* exposed to CeO_2 NPs when grown under stress conditions. Conversely, Rico and co-workers[32] reported that in non-stressed rice plants, nano-CeO_2 at 125 mg/L reduced the chlorophyll content. Clearly, additional investigations are needed to determine the conditions under which Ce (NP or otherwise) impact photosynthesis under a range of stressed and non-stressed conditions.

Effects of Cerium Compounds on Enzyme Activity. *Catalase (CAT) Activity in the Roots.* Root catalase activity was not affected when the infested control was compared with the non-infested control (Figure 4). Root exposure to both nano-CeO_2 and CeAc at 50 and 250 mg/kg did not alter the root CAT activity in infested plants, compared with the non-infested treaments. Also, none of the treatments affected the CAT activity, compared with the infested control. This indicated that the infestation has no effect on CAT activity in the root

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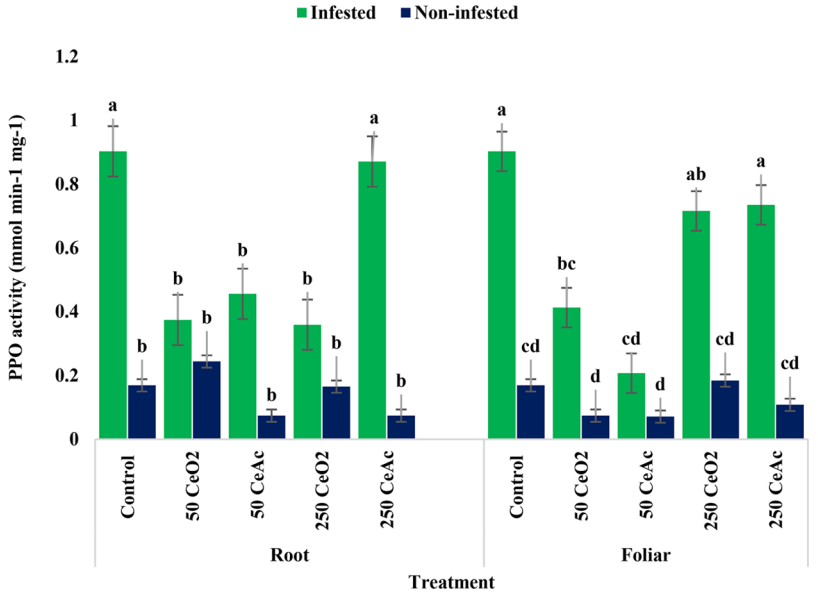

Figure 5. Effect on root polyphenol oxidase of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the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

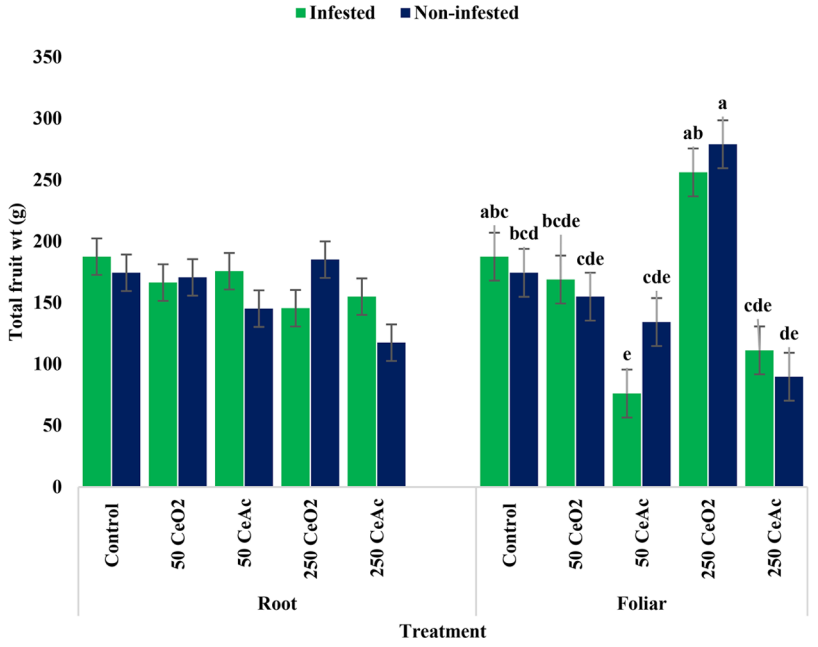

Figure 6. Effect on total fruit weight of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by the letters using one-way ANOVA follow by Tukey's test. The treatments are reported only when the differences in means are significant statistically.

treaments. Similar results were found in foliar exposure to CeAc at 50 mg/L and CeO_2 at 250 mg/L in infested treated plants, compared with non-infested treated plants. However, foliarly treated infested plants with nano-CeO_2 at 50 mg/L and CeAc at 250 mg/L significantly increased the catalase activities by 65% and 91% ($p \leq 0.05$), respectively, compared with the relative treated non-infested plants. However, the root catalase activity significantly increased (137%, $p \leq 0.05$) after foliar exposure to nano-CeO_2 at 50 mg/L, compared with the untreated infested control (Figure 4). Nano-CeO_2 is considered an excellent antioxidant because of its role in scavenging free radicals.[27,32] Plants have evolved complex defensive systems against pathogens and oxidative stress, which include the production of antioxidant enzymes such as catalase.[27] The antioxidant potential of nano-CeO_2 is due to the presence of Ce^{3+} and Ce^{4+} oxidation stages.[27,32] Though disease severity was not significantly reduced by foliar exposure to 50 mg/L nano-CeO_2, an increase in catalase activity for this treatment can likely be attributed to the antioxidant properties of nano-CeO_2 in response to oxidative stress resulting from infection. It is thought that the stress imposed by the pathogens can trigger the generation of H_2O_2, which could possibly be mitigated by the presence of Ce. However, additional investigation is needed to understand the potential antioxidant behavior of foliarly

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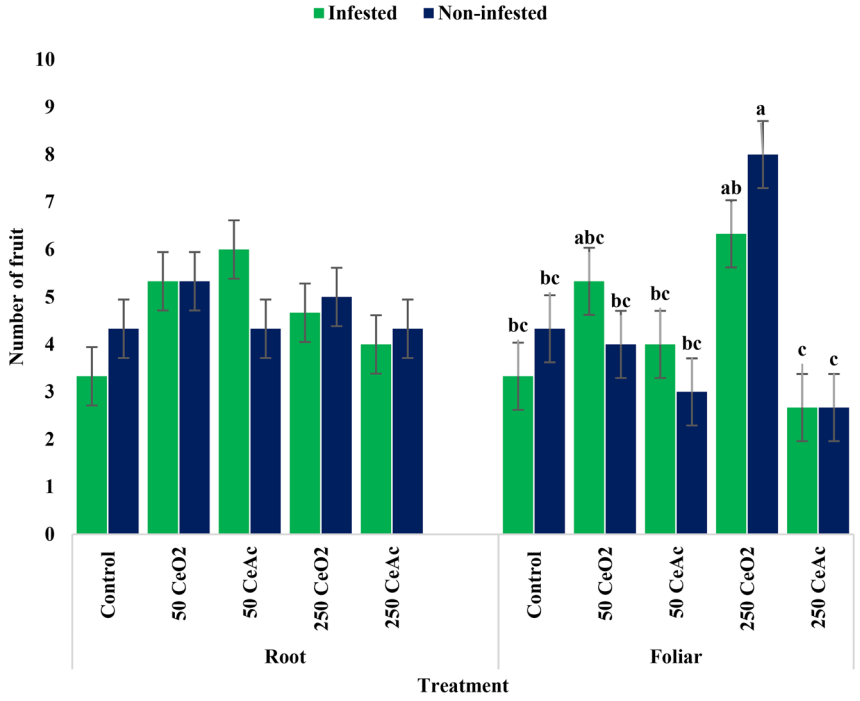

Figure 7. Effect on the number of fruit produced by infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L. Values represent the mean ± SE (n = 3). The significant difference ($p \leq 0.05$) relative to the controls is indicated by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

applied nano-CeO_2. Previous studies have shown contradictory roles of CeO_2 NPs as either a potential scavenger of free radicals[29] or an inducer of oxidative stress.[27] These roles depend on the size and surface charge of the NPs, exposure duration, plant species, and age.[27] However, surprisingly, the CAT activity did not increase in plants exposed to 250 mg/L of nano-CeO_2 or CeAc. Perhaps at this concentration, Ce controlled the excess ROS and the plant cells did not need to increase CAT activity since no additional stress was evident.

Polyphenol Oxidase (PPO) Activity in the Roots. As shown in Figure 5, the root polyphenol oxidase activity increased significantly (81%, $p \leq 0.05$) in the untreated infested control, compared with the untreated non-infested control. In root applications, only CeAc at 250 mg/kg increased the polyphenol oxidase activity (92%, $p \leq 0.05$) in treated infested plants, compared with treated non-infested plants. Other root treatments did not alter the polyphenol oxidase activity in treated infested plants, compared with treated non-infested plants (Figure 5). However, polyphenol oxidase activity decreased significantly in infested plants exposed through root to nano-CeO_2 at 50 and 250 mg/kg (59% and 60%, respectively; $p \leq 0.05$) or CeAc at 50 mg/kg (49%, $p \leq 0.05$), compared with the infested control. Polyphenol oxidase activity in non-infested plants was unaffected by root or foliar exposure to nano-CeO_2 or CeAc at both concentrations. Polyphenol oxidases are copper-containing enzymes that catalyze the oxidation of phenolic compounds to highly reactive quinones. Quinones may confer resistance to the host plant against pathogenic invasion.[33] Several studies have demonstrated that PPO plays a vital role in the defense response against pathogens, although there is no clear mechanistic evidence for this role.[23,33] In this study, PPO in roots of all infested Ce treated adult plants showed no increased activity, which contrasts with the possible defense response by the enzymatic activity. It is possible that the antioxidant properties of the Ce compounds minimized the plants' PPO response.

Effects of Cerium Compounds on Agronomical Parameters. The number and weight of fruits are presented in Figures 6 and 7, respectively. The shoot fresh and dry weights and the shoot length are shown in Table 1. The total fruit weight was not affected by the infestation when the untreated infested control was compared with the untreated non-infested control (Figure 6). In addition, none of the root treatments (nano-CeO_2 and CeAc at 50 and 250 mg/kg) altered the total fruit weight in either the infested or the non-infested treated plants. In foliar application, infestation did not affect the total fruit weight in all treatments when treated infested plants were compared with the treated non-infested plants. However, foliarly exposed plants to CeAc at 50 mg/L reduced the total fruit weight (59%, $p \leq 0.05$), compared with the infested control (Figure 6). Although the light intensity of the greenhouse (340 μmol/m^2 s^{-2}) is good enough for plant growth, it seems it is not high enough for fruit production.[15] However, the significant reduction observed in fruit yield in terms of total fruit weight by CeAc can be attributed to the dynamic relationship between acetate metabolism and photosynthetic activity that involves both chloroplast and mitochondrion.[34] Heifetz and co-workers[34] reported that acetate can induce a reduction in the photosynthetic performance in plants, which can ultimatetly affect the plant yield.

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

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Table 1. Shoot Length, Fresh, and Dry Weights of Fusarium Wilt Infested and Non-Infested Tomato Plants Exposed through Roots or Leaves to Nano-CeO_2 and CeAc at 0, 50, and 250 mg/L[a]

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

[a]Measurements were performed 18 weeks (full maturity) after inoculation. Averages with different letters are statistically significant ($p \leq 0.05$), compared with the respective control; $n = 3$.

treatments (root and foliar) affected the total number of fruits produced in infested plants, compared with the infested control. Barrios and co-workers[36] also reported no significant changes in the tomato fruit size and weight (fresh and dry) upon exposure to 0−500 mg/kg; however, at 125 mg/kg, the fruit water content increased by 72%.

None of the treatments affected the shoot fresh weight (Table 1). There was no significant change in the shoot fresh weight of untreated infested control, compared with untreated non-infested control. This suggets that Fusarium infestation did not affect the shoot fresh weight of the tomato plants. Similar results were obtained when root or foliarly treated infested plants were compared with the respective treated non-infested plants. In addition, none of the treatments (root or foliar) affected the shoot fresh weight of infested and non-infested plants, compared with the respective control. Wang and co-workers[37] did not report changes in the size and average weight of tomato plants exposed to 130 mg/L of nano-CeO_2. In the current study, the shoot dry weight was not affected by the Fusarium infestation, when the infested control was compared with the non-infested control (Table 1). None of the non-infested treatments affected the shoot dry weight. However, in root application, only infested plants exposed through the roots to nano-CeO_2 at 50 mg/kg had a 75% and 74% reduction in shoot dry weight, compared respectively, with the non-infested counterpart and the infested control ($p \leq 0.05$). In foliar treatment, only nano-CeO_2 at 250 mg/L exposure reduced the shoot dry weight (56%, $p \leq 0.05$) in infested plants, compared with the infested control. It has been reported that tomato plants cultivated under controlled greenhouse conditions can emit different volatile organic compounds (VOCs), such as (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and *n*-hexanal, 2-carene, and *β*-caryphyllene.[38] Although VOCs were not measured in this study, it is possible that the pathogen and the CeAc could increase the emission of these compounds, thereby reducing the dry weight.[36] In non-infested plants, none of the treatments significantly affected the shoot dry weight.

The shoot length was not affected in the infested control, compared with the non-infested control (Table 1). Also, none of the root treatments affected the shoot length of the infested plants, compared with the infested control. However, only nano-CeO_2 at 50 mg/kg exposed via the roots reduced the shoot length (41%, $p \leq 0.05$) in infested plants, compared with the treated non-infested plants. This revealed that the treatment triggered the reduction in the shoot length since the infestation did not affect the parameter in the infested control. In foliar application, only plants exposed to nano-CeO_2 at 250 mg/L increased the shoot length (25%, $p \leq 0.05$) in non-infested plants, compared with the non-infested control. Moreover, none of the treatments affected the shoot length in infested plants except those treated with CeAc at 50 mg/L, which had a 32% increase in shoot length relative to the infested control ($p \leq 0.05$). Under insufficient light like in the greenhouse, tomato plants are stressed but tend to grow taller.[36] However, Lopez-Moreno and co-workers[39] reported that nanocera at most concentrations used in the experiment (0−4000 mg/L) promoted shoot elongation in alfalfa and cucumber plants (20−100%). In addition, Majumdar and co-

Table 2. Concentration of Ce (*μ*g/g) in the Roots, Stems, and Leaves of Fusarium Wilt Infested and Non-Infested Tomato Plants Exposed through Roots or Leaves to Nano-CeO_2 and CeAc at 0, 50, and 250 mg/L[a]

		root		stem		leaf	
Ce (*μ*g/g)	treatment	infested	non-infested	infested	non-infested	infested	non-infested
root	control	1.81c	0.93c	0.03	0	0.38	0.001
	50 CeO_2	5.77b	3.41bc	0	0.02	0.373	0.241
	50 CeAc	1.08c	0.82c	0.06	0.03	0.285	0.233
	250 CeO_2	3.15bc	10.77a	0.05	0.01	0.367	0.317
	250 CeAc	3.92bc	3.88bc	0.06	0.01	0.271	0.126
foliar	control	1.8	0.93	0.03	0	0.38a	0.001c
	50 CeO_2	2.18	0.62	0.05	0.01	0.133bcd	0.14bcd
	50 CeAc	0	1.4	0	0	0.02cd	0.002d
	250 CeO_2	0.8	1.53	0.05	0.11	0.285ab	0.186bc
	250 CeAc	1.41	3.06	0	0	0.174bcd	0.037cd

[a]Measurements were performed 18 weeks (full maturity) after inoculation. Averages with different letters are statistically significant ($p \leq 0.05$), compared with the respective control; $n = 3$.

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workers[40] reported that 500 mg/L of nano-CeO_2 increased (26%) the root biomass of kidney beans. However, Trujillo-Reyes and co-workers[41] reported that nano-CeO_2 reduced the stem length and root biomass of radish seedlings, even though the radish was not diseased at the time. Also, Barrios and co-workers[16] reported that CeAc reduced the stem length of tomato plants at 250 and 500 mg/kg (12 and 25%, respectively). This was suggested to result from the cerium acetate's superoxide scavenging activity but not its catalase activity, which enhances its toxicity.[16,35] On the other hand, Barrios and co-workers[36] reported that CeAc at 125 mg/kg increased the water content in the tomato plants, which could result in an increase in shoot length. However, there is little information on the impacts of nano-CeO_2 and CeAc exposure on plant shoot length under the pathogen stress.

Elemental Analysis. The concentration of Ce is shown in Table 2, while the concentrations of micro and macroelements are shown in Table S1. Among the essential elements, only those that showed significant differences in concentration, compared with the respective controls, are discussed.

Cerium Accumulation. Table 2 shows cerium contents across the tissues of infested and non-infested tomato plants exposed to nano-CeO_2 or CeAc through roots or leaves. Fusarium infection did not affect the Ce accumulation in the roots of infested control, compared with non-infested control. Surprisingly, only infested plants exposed to nano-CeO_2 at 250 mg/kg exhibited a significant decrease in the root Ce uptake (71%, $p \leq 0.05$), compared with the non-infested plants exposed to the same root treatment. It is suggested that the Fusarium infection hindered the Ce element uptake in the root of the plants treated with the nanoparticles via the roots. Moreover, in the root application, only infested plants exposed to nano-CeO_2 at 50 mg/kg had a 219% increase in root Ce uptake, relative to the infested control ($p \leq 0.05$). The altered accumulation of Ce across the tissues, as a function of disease in the tomato plants, suggests an interaction between the pathogens and Ce; in infested plants specifically, there were changes in Ce accumulation as a function of exposure. The uptake of metal elements by roots can be impacted by both biotic and abiotic factors, including soil composition, pH, microorganisms, and metal immobilization in the root cell walls.[39] *F. oxysporum* is known to produce a mycotoxin known as fusaric acid (FA).[42] Fusaric acid (5-butylpiconic acid) is an organic compound capable of chelating divalent metals.[42] It is possible that in infested plants Ce was retained in the soil complexed with FA. In addition, similar results were found in non-infested plants treated with nano-CeO_2 at 250 mg/kg via roots (1058% increase, $p \leq 0.05$), when compared with the non-infested control. However, none of the treatments affected the root Ce uptake in infested and non-infested plants exposed to foliar treatement of both nano-CeO_2 or CeAc. Several factors including Ce speciation, soil chelates, and the Casparian strip in plant roots could cause poor translocation of Ce across plant tissues.[14] A previous study has shown that nano-CeO_2 was poorly translocated to other plant tissues when applied to either roots or foliage, although the concentration used was quite low and the exposure duration was short.[43] Other studies have shown a basipetal movement of Ce from the leaves to other plant tissues.[12,21] However, in the present study, Ce translocation from either application was not enough to achieve statistically significant differences. One of the reasons could be the low dose applied (1.25 mg of Ce to 21-day-old plants) and the length of the growth (more than 100 days) that diluted the Ce in the new biomass.

In the stem, neither the infestation nor the Ce compound exposure affected the Ce accumulation. In addition, Ce accumulation in the leaves was not affected by root treatments significantly, regardless of the Fusarium infestation. Conversely, in foliar treatment, leaf Ce accumulation increased by 37900% in the infested control, compared with the non-infested control ($p \leq 0.05$). Foliar exposure of infested plants to nano-CeO_2 at 50 mg/L decreased the Ce accumulation in the leaves (65%, $p \leq 0.05$), relative to the infested control. Moreover, infested plants exposed to CeAc at 50 and 250 mg/L through the leaves showed a significant decrease (95% and 54%, respectively) in Ce translocation to the leaves, compared with the infested control ($p \leq 0.05$). However, only non-infested plants treated with CeAc at 50 mg/L through foliage showed significant increase in the translocation of the Ce element in the leaves (100%, $p \leq 0.05$), compared with the non-infested control. The increase of Ce in roots is not surprising since Ce was applied to the soil, and given that the roots were acid washed, one can assume much of the Ce was absorbed, although some small amount could remain adhered to surface of negative charge of the root cells.[16,21,37,41] The increase of Ce in non-infested treated plants is in agreement with the findings of López-Moreno and co-workers[44] and Wang and co-workers,[37] which showed that soybean and tomato plants accumulate Ce across the plant tissues. In addition, Barrios and co-workers[16] reported that uncoated nano-CeO_2 at 62.5 mg/kg increased Ce accumulation in the leaves of tomato plants.

Micro and Macro Element Concentrations. The concentrations of essential elements (Ca, Fe, Zn, Cu, Mn, P, and K) and Al, a non-essential element, are shown in Table S1. Three micronutrients (Cu, Mn, and Fe), Al, and the macronutrients Ca and K were altered by the Ce treatments. In the soil application, the root uptake of elements was different in infested and non-infested plants. In infested plants, none of the treatments affected Ca and Mn accumualtion. However, nano-CeO_2 at 50 mg/kg increased Cu in roots by 108%, compared with the infested control ($p \leq 0.05$). On the other hand, in non-infested plants, none of the treatments affected Mn and K uptake. In contrast, nano-CeO_2 at 50 and 250 mg/kg increased Ca by 76% and 72%, respectively, compared with the non-infested control. In addition, nano-CeO_2 at 50 mg/kg increased Cu in the roots by 318%, compared to the non-infested control ($p \leq 0.05$). None of the soil treatments affected the uptake of Fe and Al.

Calcium can be translocated to the xylem as Ca^{2+} solely through the root apoplast.[45] It has been reported that rare earth elements (REEs) possess relatively similar characteristics as Ca.[14] Their ionic radii are within the range of 9.6−11.5 nm, compared to that of Ca, which is 9.9 nm.[14] Thus, REEs can displace Ca^{2+} at root level and, ultimately, can affect its transportation and physiological function in plants. Surprisingly, in this study nano-CeO_2 increased root uptake of Ca in non-infested plants. Calcium is a messenger that is involved in many physiological responses, such as plant growth and development,[45] hormone production, enzymatic activity, nodulation, and biotic and abiotic environmental stressors. Calcium can also be taken up either as Ca^{2+} or can be complexed with organic acids.[45]

Copper is accumulated as Cu^{2+} through the cell membranes by ATPase Cu-transporters.[46] However, it can also be taken up as Cu^{+} by high-affinity copper transporter proteins; these

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proteins are up-regulated in the roots by Cu deficiency.[46] Important enzymes such as polyphenol oxidase (PPO) require Cu as a cofactor for metabolic activity. However, significant reduction in the activity of PPO observed in the infected plants exposed to nano-CeO_2 at 50 mg/kg indicated a reverse response relative to Cu accumulation in the roots. It is hypothesized that the disease was redcued because Cu was used in other defensive enzymes and PPO was not needed. Root exposure of infested plants to CeAc at 250 mg/kg increased K uptake in roots by 444%, compared with the infested control. Plant−microbe communication and interactions can be beneficial to both the host plant and the microbes. It has been reported that fungi could act as bioinoculants, altering the membrane permeability of the root cells and subsequently changing plant metabolic activity.[47] This could facilitate the phytoavailability of mineral elements such as K, as observed in the infested plants.[47] In addition, La and Ca have been reported to inhibit K uptake during short exposures but enhance its uptake under longer time periods.[27] Importantly, the data suggest that CeAc acted similarly to La in accelerating K uptake by tomato roots.

The translocation of elements from roots to stems and leaves was varied as a function of disease/infection. None of the root treatments affected the translocation of Fe, Al, and K from roots to the above plant parts regardless of infestation status. In addition, the translocation of Ca and Cu to the shoots was not affected in infested plants. However, Ca increased by 53% and 70% in stems of non-infested plants exposed to 50 or 250 mg/kg of nano-CeO_2, respectively, compared with the non-infested control. Moreover, at such concentrations, nano-CeO_2 increased Ca in the leaves by 39% and 55%, respectively. This study revealed a consistent trend with Ca accumulation in tissues of non-infested tomato plants. The data suggest that Ce favored the translocation of Ca from the roots to the shoots. The data also suggest that pathogen presence impacted Ca through the secretion of fusaric acid. Fusaric acid can bind divalent metals and other organic matter to form chelating complexes in soil. This could reduce the amount of Ca in the tissues of infested plants. Non-infested plants exposed to 50 mg/kg of nano-CeO_2 exhibited a 287% increase in Cu accumulation in the stem as compared with the non-infested control. There is the possibility that the positively charged nano-CeO_2 associated with the fusaric acid, enabling the positively charged Cu particles to be bound by the negative charge of the root surface in the diseased plants.[48]

Only CeAc affected the translocation of Mn to the aboveground tissues. In infested plants, CeAc at 250 mg/kg increased Mn in stems by 135% compared to infested controls, while at 50 mg/kg, Mn increased in the leaves of non-infested plants by 216%). It is thought that Mn is accumulated by plants mostly in the form of Mn^{2+}, depending on environmental factors such as soil pH, plant species, and concentration. The ionic form can move freely in the xylem sap with the transpiration stream.[49] However, it has been suggested that Mn could form a complex with other biomolecules, such as carbohydrates or amino acids.[46] White and co-workers[49] reported that most Mn is found freely in the xylem sap of tomato and soybean plants but about 40% formed complexes malate and citrate.[49] The data from this study suggest that complexation with CeAc was responsible for the high Mn content observed in the above tissues of infested and non-infested tomato plants. The CeAc may serve as a chelating agent for cations and increase their absorption.[16]

In foliar applications, both infested and non-infested plants exhibited relatively a similar response on the root uptake of some elements. None of the treatments altered root Cu, Mn, Fe, and K concentrations regardless of infestation status. On the other hand, nano-CeO_2 at 250 mg/L increased the concentration of Ca in roots of infested plants by 60% but reduced Al by 82%, compared with the infested control. However, none of the treatments altered Ca and Al in roots of non-infested plants. A previous study mentioned that Ce can be transported via phloem from the leaves to the rest of the plant.[21] It is possible that the enzyme mimetic activity of Ce reduced ROS and favored the uptake of cations that could ultimately increase accumulation of select elements in the root.[29] However, this phenomenon requires additional study.

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

Contrary to what was observed in the soil application, foliar application of the Ce compounds generally decreased the Ca accumulation in the plant tissues, with the exception being in non-infested plants exposed to nano-CeO_2 at 250 mg/L, which showed a significant increase of Ca in stems. However, no effects were observed in roots, which suggest that Ce was retained at the stem level. The consistent decrease in the Ca uptake and accumulation across the plant tissues could be correlated with the positive zeta potential of Ce,[16] which could repel other positive elements. Foliar exposure to CeAc at 250 mg/L increased the leaf Mn by 234% in non-infested plants, compared with the non-infested control ($p \leq 0.05$).[16] Additionally, nano-CeO_2 at 250 mg/L increased Fe and Al in the leaves of non-infested plant by 38% and 102%, respectively, relative to the non-infested control. The possibility of nano-CeO_2 binding with Fe and Al oxides, which are widespread soil colloids, may explain the increase in their concentration in the roots and leaves of the exposed plants.[50,51]

In summary, this work revealed that at 250 mg/L, nano-CeO_2 and CeAc reduced Fusarium wilt and improved the chlorophyll content. The level of Ce exposure across plant tissues is critical to optimizing both food safety and security concerns. In this study, Ce compounds suppressed diseases, increased yield, and enhanced nutrient utilization, all without accumulating in plant tissues, except in roots. However, more research work needs to be done to examine the effect of Ce on fruit quality and to optimize the disease suppressing effects. It has been reported that the antifungal potential of NPs may be enhanced by surface coating with agents that can improve their biointeractions and, consequently, have positive physiological effects in plants.[52] For example, Barrios and co-workers[16] revealed that citric acid coated CeO_2 NPs at 250 mg/kg significantly increased the chlorophyll content in tomato plants.

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However, no studies have been performed with coated nano-CeO_2 in diseased plants. Clearly, additional research is necessary to understand the mechanism by which nutrient and non-nutrient nanoparticles can suppress disease and increase agricultural productivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b01345.

Concentrations of micro and macro elements (μg/g) in the roots, stems, and leaves of infested and non-infested tomato plants exposed to root and foliar applications of nano-CeO_2 and CeAc at 0, 50, and 250 mg/L, and cultivated until full maturity (126 days weeks) (Table S1) and abbreviation table (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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