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Research article

Effects of foliar application of zinc sulfate and zinc nanoparticles in coffee (*Coffea arabica* L.) plants

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ABSTRACT

A greenhouse study comparing the physiological responses and uptake of coffee (*Coffea arabica* L.) plants to foliar applications of zinc sulfate ($ZnSO_4$) and zinc nano-fertilizer (ZnO NPs) was conducted with the aim to understand their effects on plant physiology. One-year old coffee plants were grown in greenhouse conditions and treated with two foliar applications of 10 mg/L of Zn as either zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) or zinc oxide nanoparticle (ZnO NPs 20% w/t) and compared to untreated control plants over the course of 45 days. ZnO NPs positively affected the fresh weight and dry weight (FW and DW) of roots and leaves, increasing the FW by 37% (root) and 95% (leaves) when compared to control. The DW increase was 28%, 85%, and 20% in roots, stems, and leaves, respectively. The net photosynthetic rate increased 55% in response to ZnO NPs treatment at the end of experiment when compared to control. ZnO NPs-treated leaves contained significantly higher amounts of Zn (1267.1 ± 367.2 mg/kg DW) when compared to $ZnSO_4$-treated plants (344.1 ± 106.2 mg/kg DW), while control plants had the lowest Zn content in the leaf tissue (53.6 ± 18.9 mg/kg DW). X-ray micro-analyses maps demonstrated the increased penetrance of ZnO NPs in coffee leaf tissue. Overall, ZnO NPs had a more positive impact on coffee growth and physiology than conventional Zn salts, which was most likely due to their increased ability to be absorbed by the leaf. These results indicate that the application of ZnO NPs could be considered for coffee systems to improve fruit set and quality, especially in areas where Zn deficiency is high.

1. Introduction

The addition of fertilizers to supplement natural soil fertility is a routine practice in modern agriculture, although temperate and tropical soils commonly remain deficient in micronutrients, particularly zinc (Zn) (Kaya and Higgs, 2001; Barker and Pilbeam, 2015). Zn is necessary for the activity of enzymes such as dehydrogenases, aldolases, isomerases, transphosphorylases, and RNA and DNA polymerases (Lacerda et al., 2018). It is also involved in the synthesis of tryptophan, cell division, maintenance of membrane structure and photosynthesis, and acts as a regulatory cofactor in protein synthesis (Lacerda et al., 2018; Marschner, 2011). Coffee (*Coffea* spp.) is one of the most significant tropical crops in developing countries and historically understudied in topics of crop nutrition and management. Microelements have important roles in fruit set and retention, as well as in fruit yield and quality of coffee plants. Particularly, Zn is an essential microelement in coffee trees and it is required for macromolecule synthesis and serves as a regulatory cofactor in protein synthesis. Although Zn is required for optimal metabolism, yet deficiency is prevalent in part due to the plant's inefficiency at absorbing and translocating the micronutrient (Martinez et al., 2011; Wintgens, 2009). Further, it has been demonstrated that Zn fertilization improves production and quality of coffee beans by positively impacting polyphenol oxidase activity, color index, contents of sucrose, caffeine, trigonelline (Lacerda et al., 2018), and chlorogenic acid (Perrone et al., 2009). With an increasing demand for specialty high-quality coffee, further investigation on the utilization, technical application, and uptake of Zn is warranted to meet the objectives of both producers and consumers (Rice, 2001).

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Coffee is grown in some of the most biodiverse and environmentally sensitive regions on the planet, thus a vigilant fertilization method is essential to protect these environments while allowing farms to prosper (Somarriba et al., 2004). A growing interest in foliar fertilization for sustainable crop management has taken place to address issues such as soil conditions with limited availability of nutrients, high loss rates of soil applied fertilizers, and limitations brought forth when the environmental conditions constrain nutrient delivery to plant organs during critical stages of growth (Fernández and Brown, 2013). Foliar fertilization has proven to mitigate micronutrient deficiencies, avoid toxicity symptoms, and reduce fertilizer-related pollution (Alexander and Schroeder, 1987; Fageria et al., 2009; Kuepper, 2003; Kannan, 2010).

A further advance in foliar fertilization is the use of nano-technologies (Solanki et al., 2015). Materials that are smaller than 100 nm, at least in one dimension, are defined as nano-materials. Applications of this new technology are found in agriculture and nano-technologies are already applied to production, processing, storage, packing and transportation of agricultural products (Khot et al., 2012; Nair et al., 2010). Nano-fertilizer foliar sprays have proven to be convenient for field use because they can feed plants gradually and in a more controlled manner than salt fertilizers (Kah et al., 2018; Subramanian et al., 2015) thus reducing toxicity symptoms that may occur after soil application of the same microelements.

Oversupplying Zn can create phytotoxic symptoms by directly reducing photosynthesis (Andrade et al., 2010) or by creating nutritional imbalance by interactions with other nutrients (Kabata-Pendias, 2010). Nano-fertilizers show potential to avoid the induction of phytotoxicity in plants via slower and more tailored delivery of micronutrients while decreasing potential soil pollution and other environmental risks that may occur when using chemical fertilizers directly applied to the soil (Solanki et al., 2015). Another advantage of using nano-fertilizers is that application can be done in smaller amounts than common fertilizers (Davarpanah et al., 2016). A recent study proved that Fe_2O_3 NPs can replace traditional Fe fertilizers in the cultivation of peanut (*Arachis hypogaea* L.) plants in sandy soil (Rui et al., 2016). A study in apple (*Malus pumila* Miller) showed that plant growth characteristics (such as plant height, diameter, leaf number and leaf area) increased with Fe and Zn nano-fertilizer treatment (Mohasedat et al., 2018). Positive effects of ZnO NPs were reported on seed germination, seedling vigor, leaf chlorophyll content, stem and root growth in peanut (Prasad et al., 2012) and pomegranate (Davarpanah et al., 2016). Although these studies have demonstrated the positive physiological impacts nanoparticle fertilizer has on crop growth, the unique properties of nanoparticles (NPs) can also induce oxidative stress and toxicity in plants (Nhan et al., 2015) and other organisms in the ecosystem (Heinlaan et al., 2008; Baek and An, 2011; Hajipour et al., 2012). A recent study concluded that nanotoxicity depends on both the nanoparticle composition and the plant species exposed (Nhan et al., 2015). Particularly, a concentration of 200 mg/L of ZnO NPs (zinc, 35 nm) and ZnO (zinc oxide, 20 nm) inhibited germination in ryegrass and corn, respectively. The root growth of radish, rape canola, ryegrass, lettuce, corn, and cucumber species was inhibited upon exposure to 2000 mg/L nano-sized Zn and ZnO (Lin and Xing, 1987).

The possible physiological interactions between coffee and the new application of nano-fertilizers has not been extensively explored and, to date, only a few studies have been published on foliar fertilization (Wang and Nguyen, 2018). To understand the interactions between nanoparticles and coffee plant physiology, the aims of this investigation were (i) to compare plant growth responses of coffee plants to zinc sulfate ($ZnSO_4$) and zinc nano-fertilizer (ZnO NPs) and (ii) to analyze the Zn uptake and the physiological changes in coffee leaves treated with $ZnSO_4$ and ZnO NPs.

2. Materials and methods

2.1. Plant material

Approximately 100 seeds of two coffee species (*Coffea arabica* L. var. 'Anacafe 14' and *C. canephora* Pierre ex A. Froehner var. 'Nemaya') were imbibed on March 11, 2016 for 24 h in reverse osmosis water and then sown in seed trays filled with Sungro® Sunshine LC1 medium (sphagnum peat moss, bark, perlite, vermiculite, and clay; Sun Gro Horticulture, Bellevue, WA, USA). Trays were placed in greenhouse (27 °C day/22 °C night) at the Institute for Plant Biology and Biotechnology, Norman Borlaug Southern Crop Improvement Greenhouse complex, Texas A&M University, in College Station, Texas, USA (30.6280° N, 96.3344° W, 103 m a.s.l.) in complete darkness for approximately three weeks. Seed emergence started between the third and the fourth week after sowing and continued for approximately 6 weeks. On May 19, 2016, *C. arabica* and *C. canephora* seedling uniform in size were carefully removed from the seed trays and the top portion of each *C. arabica* seedling was cleft-grafted onto the bottom portion of a *C. canephora* seedling. Grafting was performed to mimic a common practice in countries where root nematodes represent a serious threat to coffee production. Immediately after grafting, seedlings were transferred to 12-L pots (one seedling per pot) containing Sungro® Sunshine LC1 medium and placed under a mesh shade cloth receiving an average midday photosynthetic photon flux of about 700 $\mu mol\, m^{-2} s^{-1}$ to mimic shade grown conditions, which are ideal for *C. arabica* cultivation. Plants were hand-watered bi-weekly using reverse osmosis water.

2.2. Experimental design

In April 2017, 18 plants were randomly divided into three groups each assigned to a different foliar fertilization treatment: (i) zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$; 10 mg Zn/L; Alpha chemicals, Cape Girardeau, MO, USA), (ii) zinc oxide nanoparticle (ZnO NPs 20% w/t; 10 mg Zn/L, and (iii) control (no fertilization). The Zn concentration (*i.e.,* 10 mg/L) was chosen following the guidelines that can be found in Wintgens (2009). Foliar applications were applied mid-morning twice during the course of the experiment, at the start of experiment (D0) and 14 days after the initial spray (D14) using a ½ gallon handheld polyethylene tank sprayer. Approximately 0.25 L was applied to each plant. Applications continued until leaves were thoroughly wet and stopped before dripping point. To avoid any contact with the soil, each plant was sprayed separately, and a plastic film was used to cover the top of each pot before the spraying. A sample of the growing media was collected before the first application to determine the background Zn content in soil. The experiment was concluded 45 days after D0 (D45).

2.3. ZnO NPs

The dispersion of ZnO NPs was obtained from the US Research Nanomaterials, Inc. (Houston, TX). The ZnO NPs size was obtained by measuring more than 270 individual NPs with an image processing software ImageJ (ver. 1.49, National Institutes of Health, Bethesda, MD, USA). The Transmission Electron Microscopy (TEM) images of ZnO NPs used in this study are shown in Fig. 1A and B. The ZnO NPs were predominantly spherical, but other polygonal shapes were also found. The TEM images indicated most ZnO NPs fell in the size range of 15–137 nm, with an average size of 68.14 nm, consistent with our previous characterization results of ZnO NPs from the same batch (Wang et al., 2018). ZnO NPs aggregated in liquid solution and the average hydrodynamic size of ZnO NPs in 100 mg/L of solution was measured as 621 nm by dynamic light scattering (DLS) method. The zeta potential of the nanoparticles was -28.80 ± 2.04 mV.

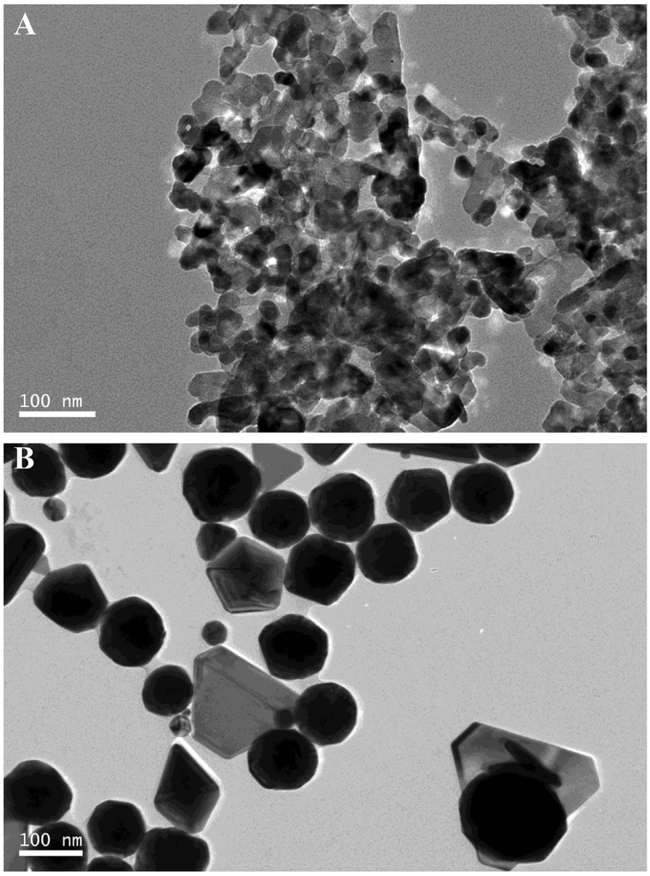

Fig. 1. Transmission Electron Microscopy (TEM) imaging showing the size variability of ZnO NPs. Primary NPs are roughly spherical (A), but other shapes are also observed (B).

2.4. Photosynthesis parameters

The net carbon assimilation rate (A) and stomatal conductance to water vapor (g_s), were measured at 0900–1200 H under artificial, saturating photosynthetic photon flux density (PPFD) of 900 μmol $m^{-2} s^{-1}$ with an infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE, USA) equipped with a standard LI-6400, 2 × 3 cm leaf chamber and a red/blue light source (6400–02B, LI-COR). Fully expanded leaves on the second branch from the top of the plant were measured on a bi-weekly basis to observe plant health and productivity. All measurements were taken at a CO_2 concentration of 400 mol $m^{-2} s^{-1}$ and with the analyzer's leaf chamber temperature set at 23 ± 2 °C. Measurements were repeated every fifteen days both for A and g_s. Leaf chlorophyll fluorescence measurements were carried out using a continuous excitation chlorophyll fluorescence analyzer (OS1p, Opti-Sciences, Hudson, NH, USA). Leaves were acclimated to the dark using lightweight leaf clips for at least 30 min before measurements were taken (Maxwell and Johnson, 2000). Baseline (F_0) and maximum (F_m) fluorescence were measured and variable ($F_v = F_m - F_0$) fluorescence and the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) ratio were calculated from these data. F_v/F_m measurements were repeated every ten days.

2.5. Chlorophyll content

To further understand whether foliar applications of $ZnSO_4$ and ZnO NPs affect plant leaf physiology, relative chlorophyll content was determined at D45 using a portable chlorophyll meter (SPAD-502, Minolta, Japan). Data were collected from three fully expanded leaves on the second branch from the top of each plant.

2.6. Tissue preparation

Plants were harvested at D45 and were separated into root, shoot, and leaf tissue. Tissues were rinsed with deionized water and blotted dry with paper towel. Fresh weight (FW) was measured for all tissue types, which were then dried in an oven at 70 °C for 48 h to measure the final dry biomass (DW).

2.7. Zinc assimilation analysis

From each plant tissue, 0.5 g of dry biomass was placed in a DigiPREP MS hot block digester (SCP science, Clark Graham, Canada). Dry shoots and roots of three replicates were ground and mixed with 4 mL of 70% (v/v) nitric acid. The mixture was predigested at room temperature overnight, and then was digested in the hot block at 95 °C for 4 h. After cooling down to room temperature, 2 mL of 30% (w/v) H_2O_2 was added to the mixture, and re-heated on the hot block at 95 °C for 2 h. Finally, the Zn in the digestate was quantified by an inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer mod. DRCII, Waltham, MA, USA). An internal standard containing 5 μg/L of Rh was used for all measurements, and instrumental fluctuations were corrected according to the internal standard density variation. Calibration curves were acquired with six concentrations of analytical-grade ICP standards of Zn and a blank and one standard solution was run for every 15 samples to ensure consistency. The plasma Ar flow was 19 L/min. The sample uptake rate is 1 mL/min and the dwell time is set as 50 ms.

2.8. Scanning Electron Microscopy coupled with an X-Ray energy dispersive micro-analyzer

A SEM analysis was conducted on leaves of treated and control plants to localize the Zn content on the leaf surface. Fully expanded leaves on the second branch from the top of the plant were collected at the end of the experiment and dehydrated using hexamethyldisilazane (HMDS; Sigma-Aldrich Corporation, St. Louis, MO, USA) as a drying agent, which can dry organic materials without the negative effects of surface tension. The leaves were placed in four changes of HMDS during a 48-h period, and then allowed to air dry. After dehydration, a gold coating was applied using a sputter coater (Cressington 108, Cressington Scientific Instruments, Watford, UK) to apply 20 nm of gold on the specimens to eliminate surface charging. Samples were observed under a scanning electron microscope (Tescan Vega 3, Tescan USA Inc., Warrendale, PA, USA). The mapping of the position of elements and the spectrum of elements in the samples was conducted with an Oxford Aztec X-Ray software (EDS Software – AztecEnergy; Oxford Instruments plc., Abingdon, Oxfordshire, UK). All microscopic investigations were performed at the Microscopy and Imaging Center, Texas A&M University, College Station, TX, USA.

2.9. Statistical analysis

Data was subjected to the analysis of variance (ANOVA) by completely randomized design. One-way ANOVA was performed and means separation between treatments were obtained using Tukey's test. Data was analyzed using the Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA).

3. Results

3.1. Plant biomass

ZnO NPs positively affected the fresh weight (FW) of roots and leaves (Fig. 2 A and C), increasing the FW by 37% (root) and 95% (leaves) when compared to control. No significant effects of ZnO NPs were reported on the stem FW (Fig. 2 B). Conversely, $ZnSO_4$ negatively affected the FW. A decrease in root (15%), stem (26%) and leaves (8%)

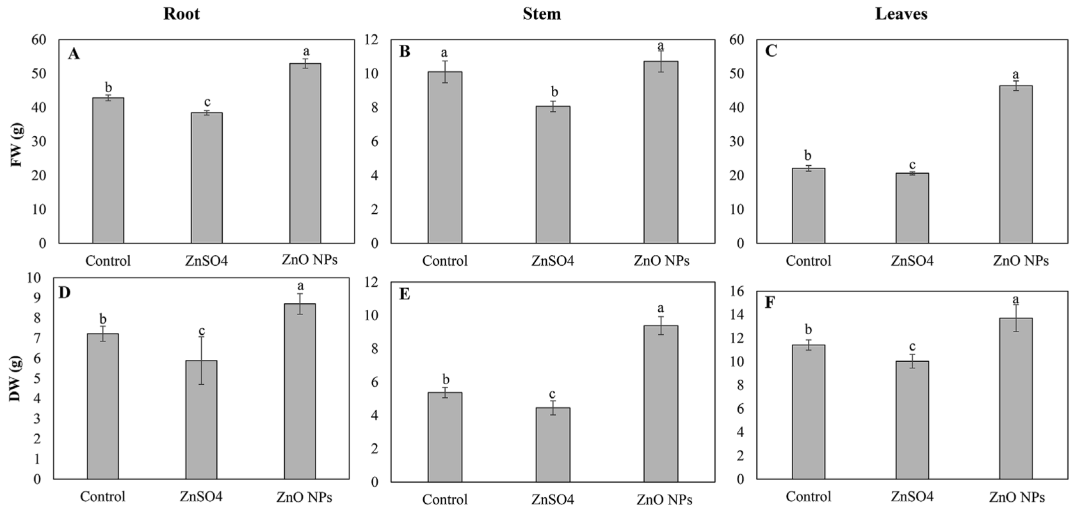

Fig. 2. Fresh and dry weight of root, stem and leaves of *Coffea arabica* L. foliar fertilized with ZnSO4 and ZnO NPs. Means labeled by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n = 6). Two-way ANOVA results are reported in tables. (A): Root FW, (B): Stem FW, (C): Leaves FW, (D): Root DW, (E): Stem DW, (F): Leaves DW.

biomass was observed for all treated plants (Fig. 2 A, B and C).

A similar pattern was found for DW. ZnO NPs lead to an increase of the DW of roots (28%), stem (85%) and leaves (20%), when compared to the controls. However, $ZnSO_4$ treated plant showed a decrease in roots (19%), stem (16%) and leaves (10%) DW (Fig. 1 C, D and E).

3.2. Photosynthetic parameters

Net photosynthesis rate (Fig. 3A) did not vary over time for ZnO NPs treated plants. However, an increase of 55% was measured at D40 when compared to control. Other minor changes were found during the experiment, especially in the initial stages. As for the stomatal conductance (Fig. 3B), a decrease in g_s by about 30% was noticed at D20 for $ZnSO_4$ treated plants. Conversely, an increase by more than 55% was recorded for both control and ZnO NPs-treated plants at D30, when compared with the $ZnSO_4$ treated plants. Finally, an increase of more than 90% was observed for the ZnO NPs-treated plants at D40, when compared to controls. Differences in g_s between the Zn treatments were significant at D20, D30 and D40 (Fig. 3B). Overall, no significant differences were detected between different treatments for F_m/Fv. (Fig. 3C). No significant differences in SPAD readings were detected among the treatments (Fig. 3D).

3.3. Zinc assimilation

Zn content in leaves increased in both treatments (Fig. 4A). Noticeably, ZnO NPs treated leaves contained a higher Zn content (1267.1 ± 367.2 mg/kg DW) when compared to $ZnSO_4$ treated plants (344.1 ± 106.2 mg/kg DW), while control plants had only a small amount of Zn in their leaves (53.6 ± 18.9 mg/kg DW). No significant differences were found in the concentration of Zn in stems and roots (Fig. 4 B and C). The Zn content in the soil used for the experiment was 17.8 ± 3.2 mg/kg soil dry weight.

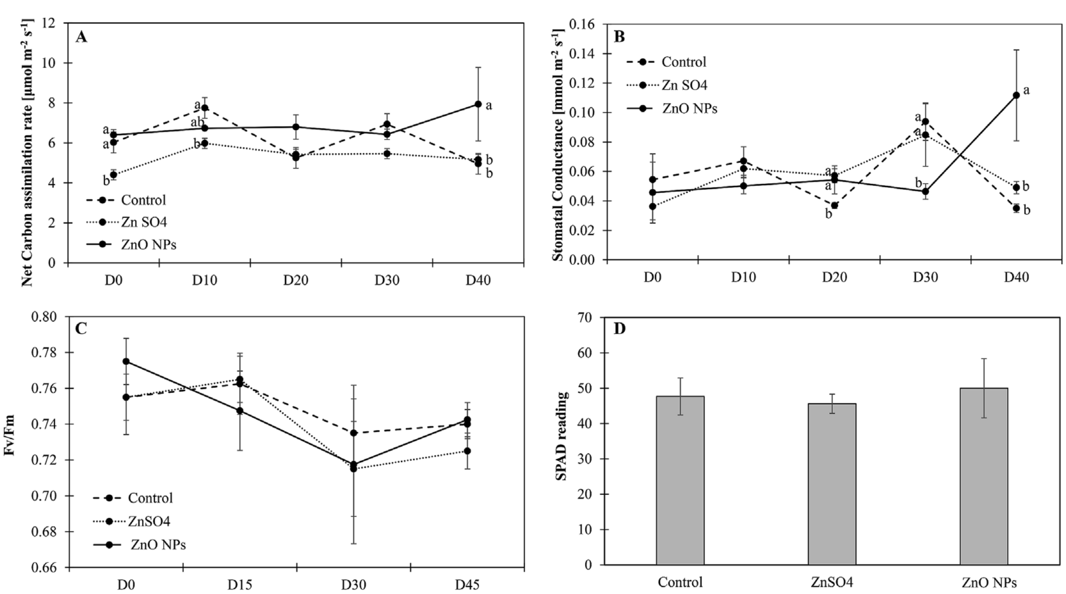

Fig. 3. Net Carbon assimilation rate (A), Stomatal conductance (B), Fv/Fm ratio (C) measured at different days after sowing (*e.g.,* D12 means twelve days after sowing). SPAD Reading (D). All the measurements are referred to *Coffea arabica* L. plants foliar fertilized with ZnSO4 and ZnO NPs. Means followed by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n = 6).

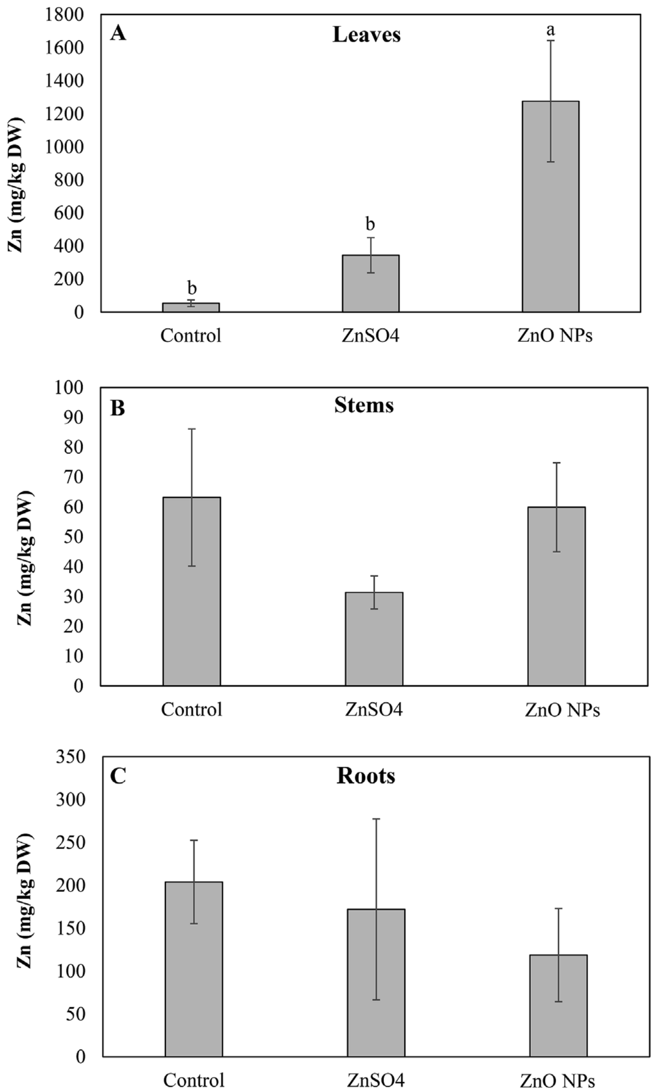

Fig. 4. Zinc content in leaves (A) stem (B) and roots (C) of *Coffea arabica* L. plants foliar fertilized with ZnSO4 and ZnO NPs. Means followed by different letters are significantly different by Tukey's post-hoc test ($p < 0.05$). Error bars represent the standard deviation (n = 6).

3.4. Scanning Electron Microscopy (SEM) and X-Ray energy dispersive analyses

Plant treated with $ZnSO_4$ had more Zn on their surface (Fig. 5D) when compared to ZnO NPs treated plants (Fig. 5E). The amount of Zn detected on the surface of control plants was less than the amount detected on $ZnSO_4$ plants (Fig. 5B). The Zn maps were taken at the exact same position as showed in the SEM photographs (Fig. 5A, C and E).

4. Discussion

Among a wide range of possible applications of nanotechnology in agriculture, development of novel nano-agrochemicals is one of the most explored areas (Subramanian et al., 2015). While some concerns have been expressed regarding the potential risks of new products (Nhan et al., 2015), many foresee a great potential of nano-fertilizers to support the necessary increase in global food production in a sustainable way (Kah et al., 2018). Emphasis has been towards the improvement of application methods of micro-nutrients and nano-fertilizers and integrating them into crop systems.

Due to the significant role of Zn in coffee composition and quality, this investigation aimed to explore the use of a new and efficient delivery method of Zn by studying the physiological impact of nano-fertilizer on coffee in comparison to traditional fertilizer application methods.

After 45 days of treatment, our data showed that ZnO NPs positively affected plant biomass, confirming a major effect on the overall fresh and dry weight. While experiments with ZnO NPs have been conducted on other species and the overall positive interactions have been previously described (Davarpanah et al., 2016; El-Kereti et al., 2013; Panwar, 2012; Tarafdar et al., 2014), the data presented here are the first obtained on coffee. Conversely, traditional treatments with $ZnSO_4$ seemed to hinder the overall plant biomass, as is confirmed when comparing FW and DW data with the control. As a corroboration of these findings, similar results were found in *Cicer arietinum* L. seedlings grown in ZnO NPs and $ZnSO_4$. Previous research (Pavani et al., 2014) showed an increase in FW and DW of seedlings grown under ZnO NPs, whereas seedlings grown in $ZnSO_4$ showed a slower growth. Although Zn is an essential element, it can reduce plant health and performance at phytotoxic concentrations. Symptoms of Zn toxicity can be seen as reduced growth and plant biomass, inhibition of cell elongation and division, wilting (Rout et al., 2009), curling and rolling of young leaves, chlorotic and necrotic leaf tips (Nagajyoti et al., 2010) and root growth inhibition (Sharma et al., 1999). The plants used in this short-term study were not Zn-deficient, which could explain why a significant increase in leaf Zn content was found at the end of the experiment. The different physiological impact of ZnO NPs and $ZnSO_4$ may be attributed to the slow release of Zn^{2+} from ZnO NPs. While ZnO NPs are known for their higher dissolution, previous study suggested that the dissolution of ZnO NPs in water is relatively slow and only about 2% of Zn was dissolved from ZnO NPs in 24 h (Reed et al., 2012). Because the ZnO NPs solution was made fresh in each application, the dissolution was not expected to be high. However, after the NPs were attached to coffee leaf surfaces, Zn ion might be continuously released, providing a long-term source of Zn. Following a previously established method (Wang et al., 2018), the dissolution of ZnO NPs in DI water reached about 30% after five days of mixing.

To gain insight into the effects of $ZnSO_4$ and ZnO NPs on plant physiology, a physiological screening of the plants was conducted during the experiment. The results showed that some aspects of the photosynthetic machinery were improved when coffee plants were exposed to ZnO NPs for more than 30 days. Particularly, positive interactions were found between ZnO NPs and net carbon assimilation rate and stomatal conductance, confirming a role of ZnO NPs in metabolic adjustments. Zn is a cofactor of carbonic anhydrase that increases the content of CO_2 in the chloroplast, and thus also increases the carboxylation capability of the Rubisco enzyme (Salama et al., 2006). Zn can affect the absorption of different macro and micronutrients (Li et al., 2007; Peralta-Videa et al., 2014). In acidic soils, Zn usually causes severe Fe-deficiency chlorosis in dicots. Crops such as lettuce, mustard, and beet are highly susceptible to excessive soil Zn (Chaney and Robson, 1993).

No changes in the F_v/F_m ratio were reported. This ratio reflects the light reemission by chlorophyll molecules when light returns from excited state to ground state and is used as an indicator of the photosynthetic energy conversion in higher plants (Maxwell and Johnson, 2000). The non-significant changes suggested that neither fertilizer formulations changed the plant light energy use efficiency of the photosystem II. Correspondingly, no changes were observed in chlorophyll contents (SPAD readings). A similar study conducted on tomato (*Solanum lycopersicum* L.) presented analogous results, where chlorophylls were largely unaffected by foliar application of ZnO NPs (Raliya et al., 2015).

Leaf uptake and translocation of Zn were also studied. Usually, ZnO NPs enter the leaf system through stomata, cuticle penetration, hydathodes and wounds (Singh et al., 2018). Particularly, most of the Zn

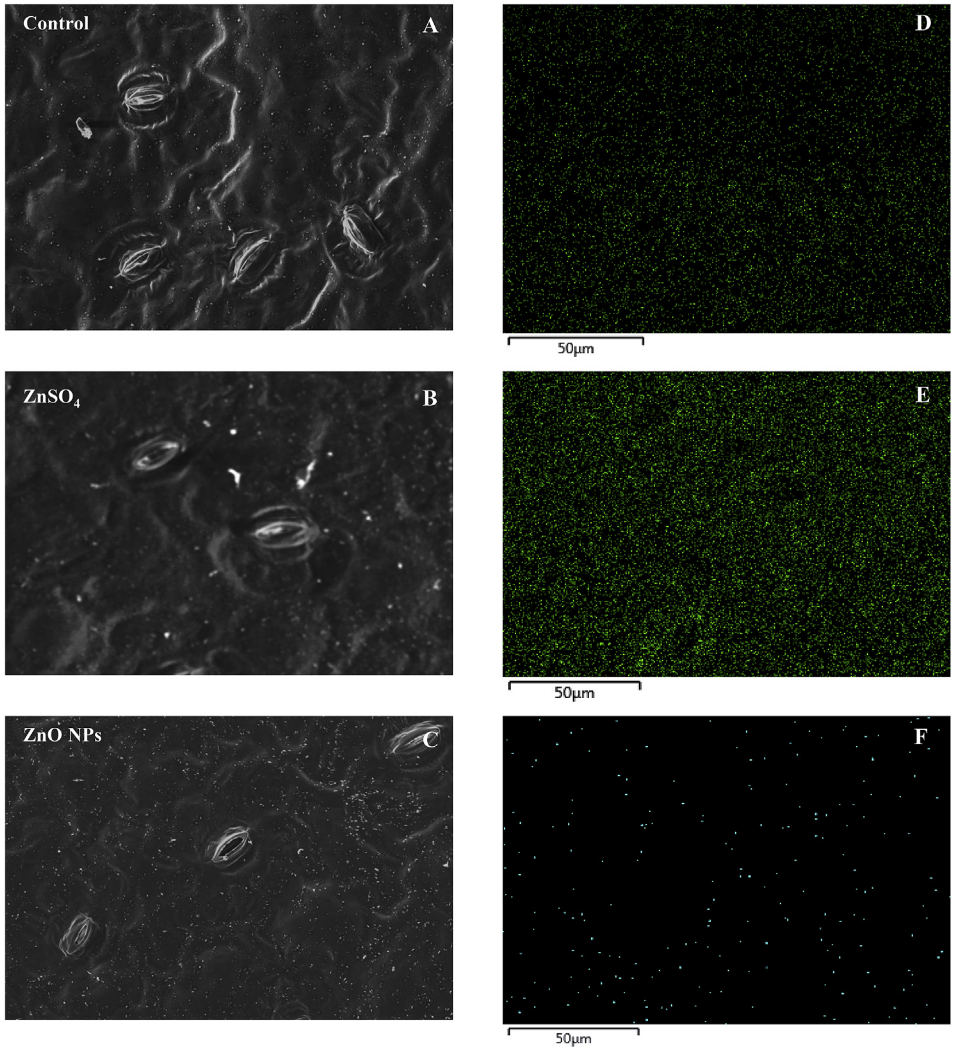

Fig. 5. Scanning Electron Microscopy (SEM) images of leaf surface of *Coffea arabica* L. plants non-fertilized (control, A) and foliar fertilized with ZnSO4 (B) and ZnO NPs (C). X-Ray microanalysis Zn maps of control (D), ZnSO4 (E) and ZnO NPs (F) leaves. Zn maps were taken on the exact same position as the SEM images.

forms are absorbed through the cuticle and/or the stomates. This can be seen in the results, where ZnO NPs led to a conspicuous increase of Zn in leaf while $ZnSO_4$ did not show any significant accumulation when compared to the control. Interestingly, no Zn translocation to the stem was reported. This may be because Zn, together with Fe or Ca, is one of the elemental nutrients that occur as positively charged cations. The apoplast is dominated by a negative charge, which is caused by free carboxyl groups of galacturonic acids (galacturonic acids are part of the mid-lamellae pectins and primary cell walls) which, in turn, causes the binding and subsequent accumulation of cations in the apoplast, and their translocation into other organs of the plant difficult (Sattelmacher, 2001; Dapkekar et al., 2018; Li et al., 2018; Sturikova et al., 2018).

To conclude, ZnO NPs positively influenced coffee growth and physiology and demonstrated more favorable effects than conventional Zn salts, mostly due to their increased ability to penetrate the leaf. Moreover, despite the high leaf Zn level measured in the treated plants at the end of the experiment, no toxicity effects were observed. Nanoparticle fertilizer represents a novel and efficient method of nutrient delivery to improve plant performance, which is of great importance for achieving more sustainable crop systems around the globe. With these results, there exists an opportunity for ZnO NPs to have a significant impact on coffee fruit set and quality. However, before recommending integrating ZnO NPs application in coffee systems, it may be important to study the impact ZnO NPs have on other nutrients essential to plant health and to the overall rhizosphere ecology.

Contributions

Lorenzo Rossi, Leonardo Lombardini and Xingmao Ma were responsible for design of the experiment and preparation of the manuscript. Lauren Fedenia and Hamidreza Sharifan were responsible for planning, conducting of the experiment, analysis of data and preparation of the manuscript.

Acknowledgment

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