Foliar Application of Low-concentration CuO, SiO_2, and ZnO Nanoparticles Fails to Enhance Thermotolerance in Tomato Seedlings

Chungkeun Lee[1] and Daniel I. Leskovar[1]

KEYWORDS. abiotic stress, antioxidant enzyme, chlorophyll fluorescence, heat stress, growth chamber, reactive oxygen species

ABSTRACT. Nanoparticles have been shown to enhance salinity and drought stress tolerance in various crops, but their potential to enhance thermotolerance remains less studied. This study evaluated the effects of foliar applications of CuO, SiO_2, and ZnO nanoparticles on the thermotolerance of tomato (*Solanum lycopersicum*) seedlings. 'Celebrity' tomato seedlings were treated weekly with 10 ppm CuO, 50 ppm SiO_2, or 50 ppm ZnO nanoparticles for 4 weeks and subjected to either optimal (26/19 °C, day/night) or heat stress (38/30 °C) conditions in growth chambers. Under heat stress, all nanoparticle treatments improved chlorophyll fluorescence (Fv/Fm), and SiO_2 nanoparticles reduced reactive oxygen species (ROS) accumulation by 9.6%. However, nanoparticle treatments did not enhance shoot and root biomass, leaf pigment and proline content, and enzymatic and nonenzymatic antioxidant capacity. Additionally, CuO and SiO_2 nanoparticles reduced leaf transpiration rates by 27.7% and 30.5%, respectively, potentially limiting evaporative cooling under heat stress. In conclusion, although nanoparticles influenced specific physiological responses, they did not improve the overall thermotolerance in tomato seedlings.

Nanoparticles have been commonly applied in agriculture due to their unique properties compared with bulk materials (>100 nm), owing to their small size (1 to 100 nm). Numerous studies have demonstrated the beneficial effects of nanoparticle application in enhancing plant abiotic stress tolerance, whereas contrasting results have also been reported for their toxicity with reduced plant growth and crop productivity, particularly at high concentrations (Ma et al. 2010). The effects of nanoparticles can vary depending on plant species, specific abiotic stress conditions, and the characteristics of the nanoparticles used. Copper oxide (CuO), silicon dioxide (SiO_2), and zinc oxide (ZnO) nanoparticles have been widely studied for their benefits in mitigating drought and salinity stress in various crops, demonstrating increased plant biomass, gas exchange rate, and antioxidant capacity (El-Saadony et al. 2022). However, research on their thermotolerant effects is limited, particularly in tomatoes. Tomato plants have an optimal growth temperature range of 20 to 30 °C (Camejo et al. 2005) and experience heat stress above this range, presenting challenges for growers in hot climate regions. This study aimed to evaluate the potential of CuO, SiO_2, and ZnO nanoparticles to confer thermotolerance in tomato seedlings through improved physiological responses. We hypothesized that foliar application of nanoparticles enhances plant biomass by improving photosynthetic efficiency and antioxidant capacity of tomato seedlings under heat stress.

Received for publication 12 Feb 2025. Accepted for publication 7 Apr 2025.

Published online 17 Jul 2025.

[1]Texas A&M AgriLife Research and Extension Center, Texas A&M University, Uvalde, TX 78801, USA

D.I.L. is the corresponding author. E-mail: d-leskovar@tamu.edu.

https://doi.org/10.21273/HORTTECH05629-25

Materials and methods

'Celebrity' tomato (*Solanum lycopersicum* L.; Clifton Seed Company, Faison, NC, USA) seedlings were grown in 200-cell trays (TR200A model, Speedling, Ruskin, FL, USA) filled with a growing medium (LM-CB, Lambert Peat Moss Inc., Riviére-Ouelle, QC, Canada) in a greenhouse with supplemental fluorescent lighting (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density). The seedlings were fertigated once a week with 50 mg $N \cdot L^{-1}$ using Peters Professional water-soluble fertilizer (20N–8.7P–16.6K; ICL Specialty Fertilizers, Tel Aviv, Israel) and transplanted into square plastic pots (810 cm^3; BWI Companies, Inc., Nash, TX, USA) containing 100 g of LM-CB growing media 7 weeks after sowing.

Thereafter, the seedlings were placed in growth chambers (GEN1000, Conviron Ltd., Winnipeg, MB, Canada) set at 26/19 °C (16/8 h, day/night) for optimal and 38/30 °C for heat stress temperatures. Relative humidity was maintained at 60% and light intensity at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Plants were fertigated with 100 mg $N \cdot L^{-1}$ of 20N–8.7P–16.6K fertilizer for the first 2 weeks, and 200 mg $N \cdot L^{-1}$ for the final 2 weeks.

A completely randomized design with five plant replicates per treatment was used. Treatments included foliar sprays of nanoparticle water dispersions: 10 ppm CuO (25 to 55 nm), 50 ppm SiO_2 (30 nm) and 50 ppm ZnO (30 to 40 nm), all purchased from US Research Nanomaterials, Inc. (Houston, TX, USA). Distilled water was used as a control, and 0.05% Tween 20 was added as a surfactant to all treatments. Nanoparticle treatments were applied weekly, beginning 3 d after transplanting (DAT), with heat stress imposed at 4 DAT.

Plant growth and physiological measurements were taken at 30 DAT. Shoot fresh weight (FW) and root dry weight (DW) were recorded and leaf transpiration rate was measured using an LI-600 porometer (LI-COR Biosciences, Lincoln, NE, USA). Leaf chlorophyll and carotenoid contents were estimated following the spectrophotometric method of Lichtenthaler and Buschmann (2001). Chlorophyll fluorescence (Fv/Fm), proline and reactive oxygen species (ROS) concentration, nonenzymatic antioxidant capacity (FRAP) and antioxidant enzyme activities were measured as described in Lee et al. (2023), using the second fully expanded leaf.

All experimental data were subjected to a two-way analysis of variance (ANOVA) to examine the effects of temperature, nanoparticles and their interactions using R software (version 4.2.0). Differences between treatment means were determined using Duncan's multiple range test at a significant level of P = 0.05 with the "agricolae"

Table 1. **Plant growth, physiology, and antioxidant capacity of tomato seedlings treated with foliar application of CuO, SiO_2, and ZnO nanoparticles under heat stress.**

Temp	Trt	Shoot FW (g)	Root DW (g)	Chl ($mg \cdot g^{-1}$ FW)	Car ($mg \cdot g^{-1}$ FW)	Proline ($\mu mol \cdot g^{-1}$ FW)	FRAP (mmol $Fe^{2+} \cdot g^{-1}$ FW)	SOD ($U \cdot mg^{-1}$ protein)	CAT ($U \cdot mg^{-1}$ protein)
Optimal	Control	21.7 a[i]	0.272 a	3.14 b	1.25 bc	1.63 b	0.386 b	14.76 ab	12.76 ab
	CuO	21.7 a	0.230 abc	3.14 b	1.19 c	1.45 b	0.366 b	12.57 ab	11.78 ab
	SiO_2	22.3 a	0.218 bcd	3.32 b	1.34 bc	1.81 b	0.385 b	15.61 a	14.17 a
	ZnO	23.1 a	0.244 ab	3.52 b	1.40 b	1.42 b	0.370 b	11.78 ab	10.18 ab
Heat	Control	12.1 b	0.178 d	6.38 a	2.13 a	7.99 a	0.628 a	11.13 b	10.56 ab
	CuO	11.5 b	0.170 d	5.62 a	1.99 a	7.55 a	0.546 a	14.10 ab	10.77 ab
	SiO_2	12.4 b	0.186 cd	6.22 a	2.06 a	7.77 a	0.623 a	11.05 b	9.04 b
	ZnO	12.4 b	0.178 d	6.06 a	2.05 a	8.33 a	0.593 a	11.76 ab	10.79 ab

Temp = temperature; Trt = treatment; FW = fresh weight; DW = dry weight; Chl = total chlorophyll; Car= carotenoid; FRAP = ferric reducing antioxidant power; SOD = superoxide dismutase; CAT = catalase.

[i] Means denoted by different letters indicate significant differences between treatments within a column at $P \leq 0.05$.

package. The assumptions of normality and homogeneity of variances were checked using the Shapiro–Wilk test and Levene test (from the "car" package), respectively. The effect size of treatment differences were calculated using Hedge's g with the "effsize" package.

Results and discussion

The foliar application of CuO, SiO_2, and ZnO nanoparticles had minimal effects on the growth and physiology of tomato seedlings. There were no significant differences between the nanoparticle treatment groups and control plants in shoot FW, leaf pigment and proline content, nonenzymatic antioxidant capacity (FRAP), and superoxide dismutase (SOD) and catalase (CAT) activities under either optimal temperature or heat stress conditions (Table 1). Root DW in SiO_2 nanoparticle-treated seedlings was lower than that of the control at optimal temperature, whereas no significant differences were observed under heat stress. Although many studies have reported enhanced thermotolerance with nanoparticle applications under heat stress, including increased plant biomass, leaf pigment content and antioxidant enzyme activities in tomato, wheat, and sorghum (Azmat et al. 2022; Djanaguiraman et al. 2018; Haghighi et al. 2014), such beneficial effects were not observed in this study, suggesting that low concentrations of CuO, SiO_2, and ZnO nanoparticles may not be effective as foliar treatments for improving plant growth or antioxidant capacity in tomato seedlings.

The only significant benefits observed from nanoparticle application in this study were on leaf chlorophyll fluorescence (Fv/Fm) and ROS concentration. Under heat stress, Fv/Fm

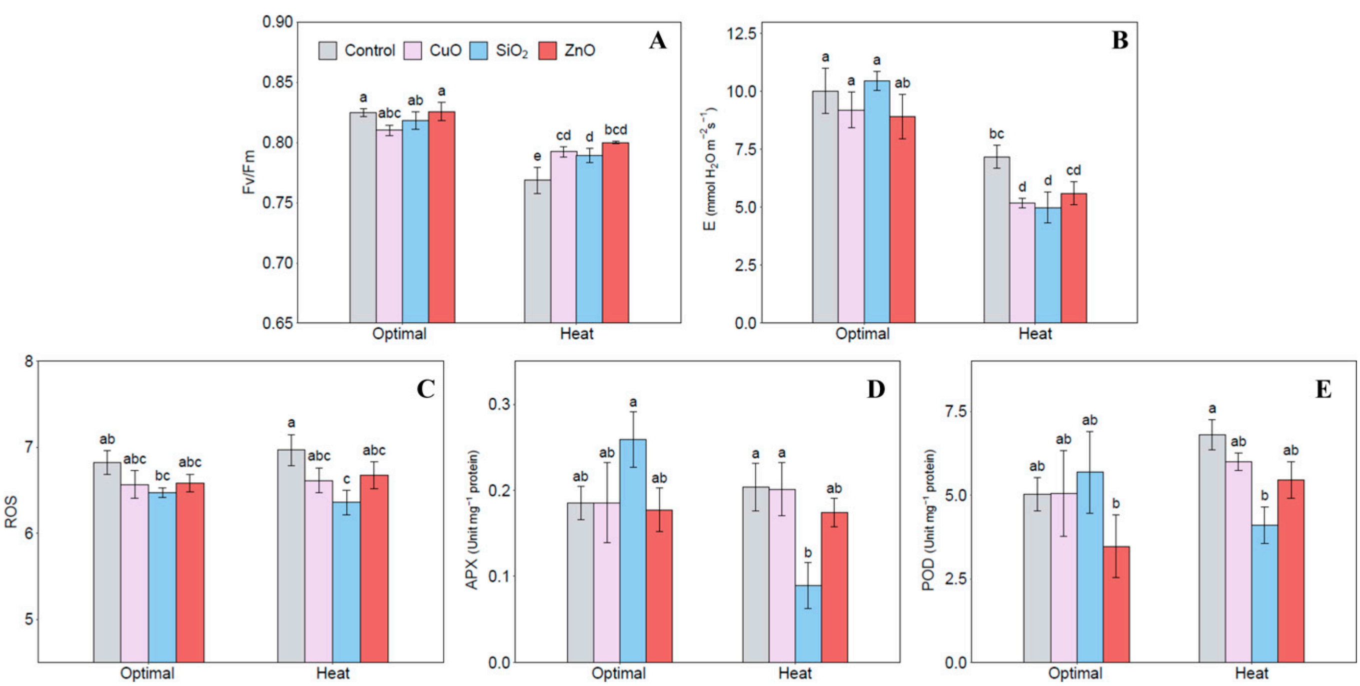

Fig. 1. **(A) Fv/Fm, (B) transpiration rate, (C) ROS content, (D) APX, and (E) POD activities in tomato leaves treated with foliar application of CuO, SiO_2, and ZnO nanoparticles under heat stress. Different letters indicate significant differences between treatments at $P \leq 0.05$. Error bars indicate the standard error of the mean. Fv/Fm = chlorophyll fluorescence; E = transpiration rate; ROS = reactive oxygen species; APX = ascorbate peroxidase; POD = guaiacol peroxidase.**

values were significantly higher in all nanoparticle-treated groups compared with the control (Hedge's g: CuO = 1.45, SiO_2 = 1.13, ZnO = 2.14), with a significant decrease in the control under heat stress compared with optimal conditions (P = 0.024 for temperature × nanoparticle interaction), whereas ROS concentration was significantly lower in SiO_2 nanoparticle-treated seedlings than in the control (Hedge's g: 1.75) (Fig. 1A and C). These results indicate that CuO, SiO_2, and ZnO nanoparticles may help protect photosystem II (PSII) from heat stress damage, with SiO_2 nanoparticle specifically showing potential in promoting ROS scavenging. Similar results have been reported that Si (47 ppm) and ZnO (60 and 90 ppm) nanoparticles improved Fv/Fm in wheat and alfalfa seedings, respectively, under heat stress (Kareem et al. 2022; Younis et al. 2020). Also, SiO_2 nanoparticles (50 ppm) reduced hydrogen peroxide concentration in potato and pea plants under drought stress (Al-Selwey et al. 2023; Sutulienė et al. 2021) and in rice plants under salinity stress (Abdel-Haliem et al. 2017). On the basis of our findings, it is speculated that the decreased ascorbate peroxidase (APX) and guaiacol peroxidase (POD) activities in SiO_2 nanoparticle-treated seedlings under heat stress (Hedge's g: APX = 2.54, POD = 2.39) (Fig. 1D and E) could be attributed to reduced ROS levels, which inactivated the upregulation of these enzyme activities. However, this study could not identify the specific antioxidants responsible for ROS scavenging, indicating the need for further research to clarify these mechanisms.

In contrast to the beneficial effects, nanoparticle treatments adversely affected the leaf transpiration rate under heat stress; seedlings treated with CuO and SiO_2 nanoparticles had significantly lower transpiration rates than the control (Hedge's g: CuO = 2.15, SiO_2 = 1.77) (Fig. 1B). Under heat stress, transpiration contributes to leaf temperature regulation via evaporative cooling (Sharma et al. 2015), suggesting that decreased transpiration in nanoparticle-treated seedlings may have intensified heat stress. Because stomata are primary pathways for foliar nanoparticle uptake (Sidhu et al. 2024), repeated nanoparticle applications may have physically blocked stomatal pores (Hong et al. 2021), leading to reduced transpiration rates. Alternatively, nanoparticles may have induced stomatal closure by regulating related genes and metabolites under heat stress (Guo et al. 2024).

Overall, the heat stress responses observed in tomato seedlings treated with CuO, SiO_2, and ZnO nanoparticles were inconclusive, making it difficult to determine whether the nanoparticles mitigated or exacerbated heat stress. The positive and negative effects of nanoparticle applications observed in Fig. 1 also appeared insufficient to have a significant impact on plant growth (Table 1). However, the effects of nanoparticles can vary depending on factors such as plant species, developmental stages, nanoparticle characteristics (e.g., ion type, size, synthesis method), and application methods. Also, the relatively low nanoparticle concentrations and the limited number of plant replicates in this study could have restricted a thorough understanding of their effects. Therefore, further studies are needed to identify the specific conditions under which nanoparticles enhance plant thermotolerance, as well as to evaluate a wider range of nanoparticle concentrations and plant developmental stages.

Conclusion

Tomato plants are highly susceptible to heat stress, yet the potential of nanoparticles for enhancing their thermotolerance remains unclear. This study demonstrated that foliar application of low concentrations of CuO, SiO_2, and ZnO nanoparticles failed to enhance overall thermotolerance in tomato seedlings, as evidenced by similar levels of plant biomass, leaf pigment, proline, and antioxidant capacity, along with reduced leaf transpiration compared with the control. Although all nanoparticle treatments improved chlorophyll fluorescence (Fv/Fm) and SiO_2 nanoparticles reduced ROS accumulation, these physiological benefits were insufficient to mitigate heat stress damage. To assess fully the effect of nanoparticles on thermotolerance, additional studies are required, particularly by selecting different application methods, higher nanoparticle concentrations, and their long-term effects during the reproductive stage.

References cited

Abdel-Haliem ME, Hegazy HS, Hassan NS, Naguib DM. 2017. Effect of silica ions and nano silica on rice plants under salinity stress. Ecol Eng. 99:282–289. https://doi.org/10.1016/j.ecoleng.2016.11.060.

Al-Selwey WA, Alsadon AA, Alenazi MM, Tarroum M, Ibrahim AA, Ahmad A, Osman M, Seleiman MF. 2023. Morphological and biochemical response of potatoes to exogenous application of ZnO and SiO_2 nanoparticles in a water deficit environment. Horticulturae. 9(8):883. https://doi.org/10.3390/horticulturae9080883.

Azmat A, Tanveer Y, Yasmin H, Hassan MN, Shahzad A, Reddy M, Ahmad A. 2022. Coactive role of zinc oxide nanoparticles and plant growth promoting rhizobacteria for mitigation of synchronized effects of heat and drought stress in wheat plants. Chemosphere. 297:133982. https://doi.org/10.1016/j.chemosphere.2022.133982.

Camejo D, Rodriguez P, Morales MA, Dell'Amico JM, Torrecillas A, Alarcon JJ. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J Plant Physiol. 162(3):281–289. https://doi.org/10.1016/j.jplph.2004.07.014.

Djanaguiraman M, Belliraj N, Bossmann SH, Prasad PV. 2018. High-temperature stress alleviation by selenium nanoparticle treatment in grain sorghum. ACS Omega. 3(3):2479–2491. https://doi.org/10.1021/acsomega.7b01934.

El-Saadony MT, Saad AM, Soliman SM, Salem HM, Desoky E-SM, Babalghith AO, El-Tahan AM, Ibrahim OM, Ebrahim AAM, Abd El-Mageed TA, Elrys AS, Elbadawi AA, El-Tarabily KA, AbuQamar SF. 2022. Role of nanoparticles in enhancing crop tolerance to abiotic stress: A comprehensive review. Front Plant Sci. 13:946717. https://doi.org/10.3389/fpls.2022.946717.

Guo S, Hu X, Yu F, Mu L. 2024. Heat Waves Coupled with Nanoparticles Induce Yield and Nutritional Losses in Rice by Regulating Stomatal Closure. ACS Nano. 18(22):14276–14289. https://doi.org/10.1021/acsnano.3c13165.

Haghighi M, Abolghasemi R, da Silva JAT. 2014. Low and high temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment. Sci Hortic. 178:231–240. https://doi.org/10.1016/j.scienta.2014.09.006.

Hong J, Wang C, Wagner DC, Gardea-Torresdey JL, He F, Rico CM. 2021. Foliar application of nanoparticles: Mechanisms of absorption, transfer, and multiple impacts. Environ Sci: Nano. 8(5):1196–1210. https://doi.org/10.1039/D0EN01129K.

Kareem HA, Hassan MU, Zain M, Irshad A, Shakoor N, Saleem S, Niu J, Skalicky M, Chen Z, Guo Z, Wang Q. 2022. Nanosized zinc oxide (n-ZnO) particles pretreatment to alfalfa seedlings alleviate heat-induced morpho-physiological and ultrastructural damages. Environ Pollut. 303:119069. https://doi.org/10.1016/j.envpol.2022.119069.

Lee C, Harvey JT, Nagila A, Qin K, Leskovar DI. 2023. Thermotolerance of tomato plants grafted onto wild relative rootstocks. Front Plant Sci. 14:1252456. https://doi.org/10.3389/fpls.2023.1252456.

Lichtenthaler HK, Buschmann C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current protocols in food analytical chemistry. 1(1):F4.3.1–F4.3.8. https://doi.org/10.1002/0471142913.faf0403s01.

Ma X, Geisler-Lee J, Deng Y, Kolmakov A. 2010. Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. Sci Total Environ. 408(16):3053–3061. https://doi.org/10.1016/j.scitotenv.2010.03.031.

Sharma DK, Andersen SB, Ottosen CO, Rosenqvist E. 2015. Wheat cultivars selected for high Fv/Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. Physiol Plant. 153(2):284–298. https://doi.org/10.1111/ppl.12245.

Sidhu AK, Sharma M, Bhickchand Agrawal S, Pradip Bhavsar P, Samota MK. 2024. Nanomaterial strategies for enhancing plant resilience in the face of temperature stress. CABI Agric Biosci. 5:60. https://doi.org/10.1186/s43170-024-00255-w.

Sutulienė R, Ragelienė L, Samuolienė G, Brazaitytė A, Urbutis M, Miliauskienė J. 2021. The response of antioxidant system of drought-stressed green pea (*Pisum sativum* L.) affected by watering and foliar spray with silica nanoparticles. Horticulturae. 8(1):35. https://doi.org/10.3390/horticulturae8010035.

Younis A, Khattab H, Emam M. 2020. Impacts of silicon and silicon nanoparticles on leaf ultrastructure and TaPIP1 and TaNIP2 gene expressions in heat stressed wheat seedlings. Biol Plant. 64:343–352. https://doi.org/10.32615/bp.2020.030.