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Metal nanoparticles: Phytotoxicity on tomato and effect on symbiosis with the *Fusarium solani* FsK strain

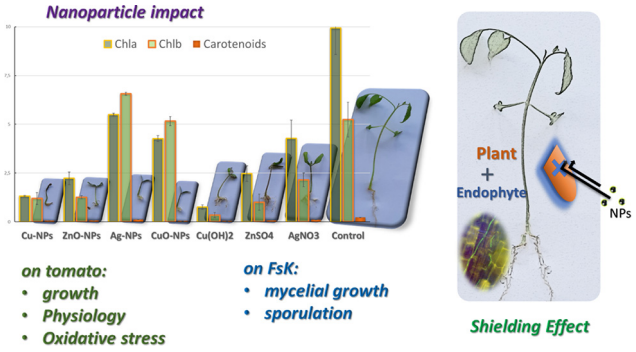
Anastasios A. Malandrakis [a,b,*], Nektarios Kavroulakis [c], Marianna Avramidou [d], Kalliope K. Papadopoulou [d], Georgios Tsaniklidis [c], Constantinos V. Chrysikopoulos [a]

[a] School of Environmental Engineering, Technical University of Crete, 73100 Chania, Greece
[b] Pesticide Science Laboratory, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece
[c] Hellenic Agricultural Organization "ELGO-Dimitra", Institute for Olive Tree, Subtropical Plants and Viticulture, Agrokipio-Souda, 73164 Chania, Greece.
[d] Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis 41500, Larissa, Greece

HIGHLIGHTS

- Nanoparticles (NPs) and counterparts were differentially phytotoxic to tomato plants.
- Oxidative stress levels, growth and photosynthesis were affected by NP treatments.
- NPs and counterparts did not affect FsK colonization of tomato roots.
- A mutual protection from NPs was observed in plants colonized with FsK.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of copper (Cu-NPs, CuO-NPs), silver (Ag-NPs) and zinc oxide (ZnO-NPs) nanoparticles (NPs) on plant growth, physiological properties of tomato plants and their symbiotic relationships with the endophytic *Fusarium solani* FsK strain was investigated. Fungitoxicity tests revealed that the FsK strain was significantly more sensitive to Cu-NPs and ZnO-NPs than CuO-NPs and Ag-NPs both in terms of mycelial growth and spore germination. All NPs were more toxic to FsK compared to their bulk counterparts except for $AgNO_3$, which was 8 to 9-fold more toxic than Ag-NPs. Apart from $AgNO_3$, NPs and bulk counterparts did not affect the number of germinated tomato seeds even in higher concentrations, while root length was significantly reduced in a dose dependent way in most cases. Dry weight of tomato plants was also significantly reduced upon treatment with NPs and counterparts with most pronounced effects in the cases of $AgNO_3$, Cu-NPs, ZnO-NPs, and $ZnSO_4$. Root and shoot length of grown tomato plants was also affected by treatments while differences between NPs and bulk counterparts varied. A marked oxidative stress response was recorded in all cases of NPs/bulk counterparts as indicated by increased MDA and H_2O_2 levels of treated plants. Treated plants had significantly reduced chlorophyl-a and carotenoid levels compared to the untreated control. NPs and counterparts did not affect FsK colonization of roots indicating a possible shielding effect of tomato plants once the endophyte was established inside the roots. Vice versa, a possible alleviation of CuO-NPs, ZnO-NPs, and $ZnSO_4$ toxicity was observed in the presence of FsK inside tomato roots in terms of plant dry weight. The results suggest that phytotoxicity of NPs in tomato treated plants should be considered before application and while both FsK and tomato are sensitive to NPs, their reciprocal benefits may extent to resistance towards these toxic agents.

* Corresponding author at: School of Environmental Engineering, Technical University of Crete, 73100 Chania, Greece.
E-mail address: tasmal@aua.gr (A.A. Malandrakis).

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1. Introduction

Heralded by Richard Feynman's infamous "There's plenty of room in the bottom", nanotechnology initiated a new era in science with numerous applications and virtually infinite possibilities (Feynman, 1960). Research focusing on nanotechnology applications in agriculture is rapidly gaining ground primarily driven by the promising potential of nanoparticles (NPs) for optimized efficacy of inputs and reduction of pesticide/xenobiotic footprint in the environment (Kah et al., 2018; Baker et al., 2017). Controlled release, enhanced bioavailability, target-specific delivery and improved residual action are some of the advantages of nanoparticles used as alternative pesticides or nutrient carriers (Kah et al., 2018; Pandey et al., 2018). Furthermore, their combined use with synthetic pesticides has demonstrated synergism in a number of cases achieving enhanced effectiveness against plant pests with lower doses (Malandrakis et al., 2019, 2020a, 2020b). Under this scope, NPs are proposed as suitable candidates to be used as novel, environmentally compatible pesticide alternatives (Baker et al., 2017; Pandey et al., 2018; Kah et al., 2018; Sun et al., 2018). However, certain environmental concerns such as fate in ecosystems and effects on non-target organisms including humans should be addressed before their wider commercial release (Noori et al., 2020; Baker et al., 2017).

Plants, being an essential part of all ecosystems, are expected to interact directly or indirectly with nanoparticles, that could potentially inflict toxicity, accumulate via uptake or disturb interactions of plants with beneficial/symbiotic organisms (Ma et al., 2010; Courtois et al., 2019; Lewis et al., 2019). Phytotoxicity can result from physical or chemical interaction of NPs with root or other plant tissues via a number of physiological and biochemical mechanisms including membrane interactions, ion release, production of reactive oxygen species (ROS), inactivation of enzymes or DNA disruption (Karami Mehrian et al., 2016; Ma et al., 2010; Noori et al., 2020). Standard indicators of phytotoxicity include seed germination, root elongation, plant biomass, and chlorophyl content (Ma et al., 2010; Larue et al., 2014; Karami Mehrian et al., 2016; Noori et al., 2020; Ristroph et al., 2017). A number of studies evaluating phytotoxicity of metal NPs have been conducted reporting adverse effects on various aspects of plant growth and physiology in numerous plant species (K.E. Li et al., 2015; de la Rosa et al., 2021). Reports are often conflicting although a consensus is obvious: toxicity threshold of NPs towards plants is species dependent and each case should be evaluated separately.

Tomato is a vegetable crop with great popularity and economic importance worldwide with significant nutritional value (Akanbi-Gada et al., 2019; Karami Mehrian et al., 2016). Previous studies evaluating phytotoxicity of tomato caused by silver, zinc oxide, titanium oxide, copper, and ferric NPs are available although the comparative toxicity with their bulk counterparts is limited (Chen et al., 2020; Karami Mehrian et al., 2016; Akanbi-Gada et al., 2019; Noori et al., 2020; Sun et al., 2020). Limited are also the studies about the indirect impact of NPs on the plant performance via their interaction with the rhizospheric/endophytic soil microorganisms. The importance of a potential disruption of plant-beneficial microbe interactions by NPs is evident in cases of N_2 fixing, phosphate solubilizing bacteria, arbuscular mycorrhizae and plant growth promoting microorganisms (Wang et al., 2016). FsK is a non-pathogenic *Fusarium solani* strain which colonizes roots and induces plant response mechanisms against both pathogens and pests, mediated by the ethylene signaling pathway (Kavroulakis et al., 2007; Garantonakis et al., 2018; Kavroulakis et al., 2018). The sensitivity of FsK against a variety of fungicides, commonly used in agricultural practice was tested and the compatibility of this strain in integrated disease management programs was proposed (Malandrakis et al., 2018). A key question besides phytotoxicity risks posed by NPs, is whether they can affect the survival and colonization of FsK and its symbiotic interactions with the tomato plant.

Under this light, the scope of this study was to evaluate: (a) the effect of silver, copper, copper oxide, and zinc oxide NPs and their bulk counterparts on growth (seed germination, root elongation and dry weight) and physiology (oxidative stress and photosynthetic pigment content) of tomato plants, (b) their fungitoxic activity against the tomato-endophyte FsK, and (c) the possible impact of NPs/counterparts on root colonization and symbiotic interactions between FsK and tomato plants.

2. Materials and methods

2.1. Nanoparticles, reagents and fungicides

Silver [Ag-NPs] (<100 nm particle size), zinc oxide [ZnO-NPs] (particle size <50 nm), copper [Cu-NPs] (particle size 25 nm), copper oxide [CuO-NPs] (particle size <50 nm) nanoparticles (NPs) as well as zinc sulphate [$ZnSO_4$] and silver nitrate [$AgNO_3$] used in this study were purchased from Sigma-Aldrich, MO, USA. A copper hydroxide containing a commercial fungicide (Copperblau-N 50 WP) used as a bulk counterpart of copper NPs was purchased from NITROFARM (Greece). Stock solutions-suspensions of commercial fungicide, reagents and nanoparticles used in fungitoxicity and phytotoxicity bioassays were prepared using distilled-sterilized water. Appropriate quantities of stock solutions were added aseptically to sterilized growth medium prior to inoculation or seed placement. In order to prevent their aggregation, nanoparticle suspensions were subjected to sonication for 30 min with Transonic 420 (Elma, Germany) before use. Zeta potentials and hydrodynamic diameter measurements for the nanoparticles (see Table S1) were measured with a zetasizer (Nano ZS90, Malvern Instruments, Southborough, MA) in triplicate.

2.2. Fungal isolate and culture conditions

The fungitoxic effect of silver, copper and zinc containing NPs and their bulk/ionic counterparts against the biocontrol agent FsK, a previously characterized, non-pathogenic *F. solani* tomato fungal endophyte, was evaluated (Kavroulakis et al., 2007). The fungal isolate was grown on sterilized potato-dextrose-agar medium (PDA) and maintained in growth chambers in the dark at 25 °C or at 4 °C for long term storage.

2.3. Fungitoxicity tests

2.3.1. Effect of NPs on FsK mycelial growth

The fungitoxic effect of NPs and their bulk/ionic counterparts on the fungal strain FsK was assessed in vitro utilizing poison agar bioassays. The inhibitory effect of antifungal agents was determined by measuring colony radial growth on PDA medium containing appropriate concentrations of NPs or their counterparts. In order to obtain fungitoxicity-curves, concentrations of 0, 5, 10, 50, 100, 250, 500 and 1000 μg mL^{-1} Cu-NPs, CuO-NPs, Ag-NPs, ZnO-NPs, $Cu(OH)_2$, $AgNO_3$, and $ZnSO_4$ were added in sterilized PDA medium, which was poured in Petri dishes and left to solidify. After the growth medium cooled, inoculum consisting of a 5-mm mycelial plug cut from the edge of 5-day old FsK colonies grown on PDA was placed in the center of the plate with the mycelium facing down in direct contact with the medium. Plates were transferred for incubation in growth chambers at 25 °C in the dark for 7 days. Following inoculation and incubation procedures described above, mean colony diameters were then measured and percent inhibition was calculated using the formula: % inhibition = 100 − (mean colony diameter of treated/mean colony diameter of untreated control) × 100. In order to compare sensitivities of FsK to metal NPs and bulk counterparts, EC_{50} values (effective concentration causing 50% inhibition of mycelial growth) were calculated. Three replicate plates were used per concentration while all tests were repeated twice.

2.3.2. Effect of NPs on FsK spore germination

The potential of NPs and their bulk counterparts to inhibit FsK spore germination was assessed in vitro on PDA. Conidial suspensions of the

fungal strain were obtained by inoculating 250 mL glass flasks containing Potato Dextrose Broth (PDB) with four 5-mm mycelial plugs, cut from the edge of rapidly growing colonies. Following incubation for 4 days in the dark at 25 °C in growth chambers under continuous shaking at 200 rpm, conidia were then harvested by filtration using a cheese cloth and the concentration of spores was determined using a haemocytometer. The spore concentration was adjusted to 100 conidia/100 μL by serial dilutions in sterilized-distilled water and 100 conidia were spread on the surface of petri dishes containing PDA amended or not with the appropriate nanoparticle/counterpart concentrations. Concentrations of 0, 1, 5, 10, 25, 50, 100, 250, 500 and 1000 μg/mL of each metal NP or bulk counterpart were used to obtain fungitoxicity-curves. Three replicate dishes of each compound concentration were incubated for 2 days in the dark at 25 °C. The number of forming colonies was counted and the percent inhibition of colony formation was calculated by the formula: % inhibition = 100 − (mean number of colonies of treated/mean number of colonies of untreated control) × 100.

EC_{50} values based on relative percent inhibition were calculated for each compound. The experiment was conducted twice.

2.4. NPs phytotoxicity tests

2.4.1. Germination assays

Tomato seeds (*Solanum lycopersicon*, cv. ACE 55) used in germination assays were surface disinfected in a 2.5% NaOCl water solution, rinsed trice with distilled, sterilized water and then air dried. Germination was assessed in 15-mL Falcon centrifuge tubes filled with 10 mL water agar (WA) medium amended with concentrations of 0, 10, 100 and 1000 μg/mL of copper, silver and zinc oxide NPs as well as their bulk/ionic counterparts $Cu(OH)_2$, $ZnSO_4$ and $AgNO_3$ under aseptic conditions. In each tube, one seed was placed on the surface of the treated or untreated WA using a sterilized forceps and exercising sufficient pressure to ensure contact with the medium. Tubes were covered with aluminum foil and incubated for 6 days in a growth chamber at 25 °C with a 16 h:8 h day:night photoperiod. Twenty tubes per treatment were used and the germination experiment was repeated twice. At the end of the experiment, the number of germinated seeds and root length (length of the longest root) in each treatment was recorded and germination percentage (GP%) was calculated according to the formula:

$$GP\% = \text{mean number of germinated seeds of treatment} / \text{mean number of germinated seeds of the control} \times 100 \quad (1)$$

2.4.2. Impact of NPs on plant growth

The impact of NPs and their bulk/ionic counterparts on tomato plant growth was evaluated in terms of root and shoot length and dry weight of treated tomato seedlings grown on artificial growth medium. Sterilized Hornum-Agar (HA) medium (40 g/L NH_4NO_3, 30 g/L KNO_3, 30 g/L $MgSO_4 \cdot 7H_2O$, 10 g/L $NaH_2PO_4 \cdot H_2O$, 2 g/L Fe-EDTA (9% Fe), 120 mg/L $MnSO_4 \cdot H_2O$, 120 mg/L H_3BO_3, 40 mg/L $CuSO_4 \cdot 5H_2O$, 40 mg/L $ZnSO_4 \cdot 7H_2O$ and 8 mg/L $Na_2MoO_4 \cdot 2H_2O$ and 0.8% agar diluted 1:100 in tap water and pH adjusted to 6.8) was selected to provide essential nutrients for tomato plants and achieve a uniform distribution of NPs during the plants growth. After autoclaving, 500 mL of HA medium amended with appropriate concentrations of metal nanoparticles and reagents was poured in aluminum containers 20 × 15 × 10 cm [length × width × height] and left to solidify. Concentrations of 5, 10, 50, 100, 500 and 1000 μg/mL Cu-NPs, CuO-NPs, Ag-NPs, ZnO-NPs, $Cu(OH)_2$, $AgNO_3$ and $ZnSO_4$ were used in the bioassays. Control treatments consisted of unamended HA medium. In each container, ten 4-day old pre-germinated tomato seeds were equally distributed on the surface of the solidified medium. Containers were covered with transparent lids and incubated in a growth chamber at 25 °C with a 16 h:8 h day:night photoperiod and 70% RH for 2 weeks. Root and shoot length as well as total dry weight were recorded after that period. Two containers per treatment were used and the experiment was repeated twice.

2.4.3. Physiological analysis

Physiological response of tomato seedlings grown on HA medium treated with selected concentrations of each NP or bulk counterpart was analyzed. Tomato plants were grown as described previously in aluminum containers containing HA medium amended with NPs and reagents at concentrations determined by the growth inhibition experiments causing 50% inhibition of growth in terms of dry weight. Specifically, concentrations of 300 μg/mL Cu-NPs, 1000 μg/mL CuO-NPs, 800 μg/mL $Cu(OH)_2$, 1000 μg/mL Ag-NPs, 200 μg/mL $AgNO_3$, 250 μg/mL ZnO-NPs and 300 μg/mL $ZnSO_4$ were used. After 3 weeks incubation in a growth chamber at 25 °C with a 16 h:8 h day:night photoperiod and 70% RH, plant tissues were harvested and analyzed in physiological experiments. Two containers per treatment were used and the experiment was repeated twice.

2.4.3.1. Extraction for lipid peroxidation and H_2O_2 assays. In order to evaluate the oxidative stress response of tomato plants caused by metal NPs and their bulk counterparts, hydrogen peroxide (H_2O_2) levels were determined. Specifically, 150 mg of plant material (FW) was reduced in fine powder with liquid nitrogen and homogenized in 4 mL 0.1% trichloroacetic acid (TCA) at 4 °C by vigorous vortexing. After centrifugation at 6500 rpm for 15 min at 4 °C, the supernatant was used for the determination of both lipid peroxidation levels and H_2O_2 concentration (Sotiras et al., 2019).

2.4.3.2. Hydrogen peroxide assay. Hydrogen peroxide accumulation was measured spectrophotometrically as described by Tsaniklidis et al. (2020) with some modifications. The reaction mixture consisted of 0.25 mL plant extracts, 0.25 mL of 0.1 M potassium-phosphate buffer (pH 7.0), and 0.5 mL of 1 M KI. The reaction color was developed for 45 min in darkness and absorbance was measured at 390 nm. Hydrogen peroxide levels were calculated using a calibration curve prepared with eight known concentrations of H_2O_2. Transformation formula: y = 102.5 + 0.0569x (mmol/g fw).

2.4.3.3. Thiobarbituric acid reactive substances (TBARS)/lipid peroxidation assay. Lipid peroxidation was measured as malondialdehyde (MDA) byproduct content determined by reaction with 0.5% 2-thiobarbituric acid in 20% TCA (*w*/*v*). For each assay, 1 mL of plant extracts, 2 mL of 20% (w/v) TCA and 2 mL of 0,5% (w/v) 2-thiobarbituricacid (TBA) were used. The mixture was heated at 95 °C for 30 min and afterwards was cooled in ice. The concentration of MDA was calculated from the difference of the absorbance at 532 and 600 nm using the Beer–Lamberts equation (extinction coefficient of MDA was 155 $mM^{-1} * cm^{-1}$) (Heath and Packer, 1968).

2.4.3.4. Photosynthetic pigments. Pigment content of treated and control tomato plants was measured in leaves following the method described below. 150 mg of fresh leaf sample was added in an Eppendorf tube containing 1.8 mL of cold acetone (80%) and vortexed vigorously. Tubes were then incubated in the dark for 1 h, while being vortexed every 15-mins. Subsequently, tubes were centrifuged at 6.500 rpm for 5 min at 4 °C. Chlorophyll and carotenoid concentrations were determined spectrophotometrically at absorbance wavelengths of 470, 647, and 663 nm by using the equations described by Lichtenthaler and Buschmann (2001):

$$[\text{Chl a}] = 12.25A_{663} - 2.79A_{647} \quad (2)$$

$$[\text{Chl b}] = 21.5A_{647} - 5.1A_{663} \quad (3)$$

$$[\text{Car}] = \{1000A_{470} - 1.82\,[\text{Chl a}] - 85.02\,[\text{Chl b}]\}/198 \quad (4)$$

2.5. Effect of NPs on the association between tomato plants – FsK

In order to investigate the potential impact of NPs on the beneficial association between the endophytic FsK *F. solani* strain and tomato plants, pot experiments with soil substrate treated with selected concentrations of nano or bulk metals were conducted

2.5.1. Plant material and inoculation

Tomato seedlings (*Solanum lycopersicon*, cv. ACE 55) originated from tomato seeds surface sterilized in 2.5% NaOCl and sown directly into pots. Each pot contained 400 cm^3 of peat amended with 0.8 g/L of a NPK fertilizer (20-20-20). Pots were covered with aluminum foil and transferred in a controlled-environment growth chamber at 20–25 °C with a 16 h photoperiod at 65% RH for a week until the emergence of young tomato seedlings.

FsK conidial suspensions used as inoculum in the *in-planta* experiments were acquired according to the following procedure: A 5-mm mycelial plug cut from the edge of a rapid growing FsK colony, was transferred in PDA containing Petri dishes and incubated for 6 days at 25 °C in the dark for conidiation. Conidia were harvested by scraping the colony, transferring the collected mycelium/conidial mass in distilled-sterilized water and sieving using a cheese cloth in order to remove mycelial fragments. The resulting suspension was then centrifuged at 4000*g* and conidia were re-suspended in an appropriate volume of 0.85% NaCl to achieve the desired inoculum concentration using a haemocytometer. One week after sowing, FsK inoculum was applied in the soil of tomato seedlings as water drench with 10^4 conidia per cm^3 of potting mix.

2.5.2. Application of NPs on FsK inoculated/non-inoculated tomato plants

One week after tomato seedlings were inoculated with FsK, metal NPs and bulk/ionic counterparts were applied as water suspensions (100 mL total water volume per pot) in the soil by drenching in appropriate concentrations. In the control treatment an equal amount of distilled water was used. NP containing suspensions were sonicated for 30 min before drenching to deter particle aggregation. Concentrations of NPs and counterparts applied were selected making sure that they were sublethal to FsK and additionally based on their mean dry weight inhibitory concentration determined in tomato toxicity experiments described above. Specifically, concentrations of 300 μg/mL Cu-NPs, 1000 μg/mL CuO-NPs, 800 μg/mL $Cu(OH)_2$, 250 μg/mL ZnO-NPs, 300 μg/mL $ZnSO_4$, 1000 μg/mL Ag-NPs and 200 μg/mL $AgNO_3$ were used in the experiments. The experiment included two subsets: a set of NPs/bulk counterpart treated tomato plants inoculated with and an identical set not inoculated with FsK. Each treatment consisted of 4 pots containing 4 tomato plants. The whole experiment was repeated twice.

2.5.3. Tomato root tissue harvesting and DNA extraction

The effect of NPs and their counterparts on FsK colonization of tomato root tissues was examined by comparing control and metal-treated tomato plants. Whole roots collected from 16 plants per treatment were washed to remove soil and then dried in sterilized filter paper. Genomic DNA was extracted from root tissue samples using the "NuncleoSpin® Plant II genomic DNA extraction" kit (MACHEREY-NAGEL GmbH & Co. KG, Duren, Germany) according to the manufacturer's protocol.

2.5.4. Quantification of FsK colonization using qPCR

The effect of NPs on the symbiotic relationships of FsK with tomato plants was evaluated by quantifying the presence (colonization) of FsK inside treated and non-treated tomato roots using Real Time qPCR. *F. solani* ITS region-specific primers FFsITS (5′-TGGTCATTTAGAG GAAGTAA-3′) and RFsITS (5′-GGTATGTTCACAGGGTTGATG-3′), were used for the Real Time PCR assay. Copy numbers of the ITS gene in total DNA samples extracted from root tissues of FsK-inoculated plants were evaluated using an external standard curve as previously described (Garantonakis et al., 2018). Data were means of two technical replicates for each of three biological replicates. Values were normalized to ng of total DNA isolated.

3. Statistical analysis

The NPs and counterparts EC_{50} values for the FsK strain were calculated by regressing the relative inhibition of mycelial growth against the Log_{10} compound concentrations. The same analysis was conducted for the determination of the IC_{50} values of metal compounds in the tomato plant toxicity experiments. Statistical differences between treatments in fungitoxicity and phytotoxicity experiments were evaluated by analysis of variance and the resulting means were separated according to Tukey's HSD test ($\alpha = 0.05$). Correlations of growth and physiological parameters of NPs/counterpart treated tomato plants were evaluated using Pearson correlation coefficients. The SPSS v20 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

4. Results

4.1. Fungitoxic activity of NPs in vitro

The fungitoxic activity of metal nanoparticles in comparison with their bulk/ionic counterparts in terms of mycelial growth and spore germination inhibition of the FsK *F. solani* strain was evaluated in vitro. A dose-dependent response of FsK to metallic compounds tested was observed both in radial growth and spore germination. The respective EC_{50} values for each treatment are shown in Fig. 1. Among NPs tested, Cu-NPs

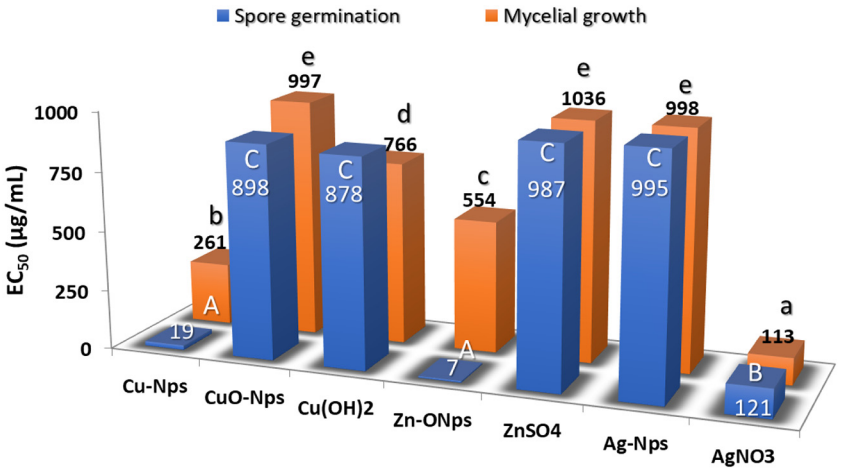

Fig. 1. Mean sensitivity of FsK to NPs and their respective bulk/ionic counterparts in terms of mycelial growth and spore germination. Between treatments, bars marked by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

Table 1
Effect of NPs and their respective bulk/ionic counterparts on the germination of tomato seeds.

Treatment	Germinated seeds (%) (mean[a] ± SD[b])			Root length (%) (mean ± SD)		
	10[c]	100	1000	10	100	1000
Ag-NPs	100.00 a	114.29 ± 10.40 b	100.00 b	48.55 ± 2.65 a	53.95 ± 4.50 bc	38.21 ± 4.54 b
$AgNO_3$	85.71 ± 0.42 a	71.43 ± 3.17 a	57.15 ± 4.69 a	65.64 ± 4.37 b	65.64 ± 2.46 c	13.49 ± 1.67 a
Cu-NPs	97.55 ± 1.05 a	100.00 b	80.75 ± 12.35 b	62.76 ± 2.05 b	21.80 ± 3.11 ab	15.27 ± 4.55 a
CuO-NPs	100.00 a	110.25 ± 11.5 b	85.71 ± 1.12 b	86.72 ± 3.69 bc	40.00 ± 5.14 b	35.63 ± 2.80 b
$Cu(OH)_2$	100.00 a	100.00 b	86.52 ± 5.23 b	75.43 ± 6.16 b	12.72 ± 2.61 a	14.53 ± 5.55 a
ZnO-NPs	71.43 ± 2.14 a	100.00 b	100.00 b	84.10 ± 3.45 bc	67.01 ± 2.28 c	14.29 ± 2.49 a
$ZnSO_4$	100.00 a	100.00 b	100.00 b	96.14 ± 3.17 c	76.97 ± 1.15 d	10.65 ± 2.00 a

[a] Expressed as percent of the control treatment after 14 days incubation at 22 °C ($n = 3$).
[b] Standard deviation of the means ($n = 10$).
[c] Numbers indicate concentration of NPs/bulk counterparts in μg/mL of active ingredient.

and ZnO-NPs were the most toxic against FsK both in mycelial growth and spore germination inhibition experiments. EC_{50} values calculated for Cu-NPs and ZnO-NPs were 260.8 and 554.4 μg/mL in mycelial growth and 18.6, 6.9 μg/mL in the spore germination tests respectively (see Fig. 1). FsK was significantly less sensitive to CuO-NPs and Ag-NPs with EC_{50} values close to 1000 μg/mL both in mycelial growth and spore germination assays. ZnO-NPs and Cu-NPs were 2 to 4 times more toxic to FsK compared to their counterparts $ZnSO_4$ and $Cu(OH)_2$ respectively in terms of mycelial growth while differences were dramatically more profound in the case of spore germination (141 and 46 times more toxic respectively-see Fig. 1). On the contrary, CuO-NPs were less or equally toxic with $Cu(OH)_2$ while $AgNO_3$ was approximately 8 times more toxic than AgNPs in all bioassays (see Fig. 1).

4.2. Phytotoxicity tests

4.2.1. Impact of NPs on tomato seed germination

The impact of metal NPs and their bulk counterparts on germination and root length of tomato seed was evaluated in vitro. Percent germination, root length and percent germination index rates of tomato seed treated with 10, 100 and 1000 μg/mL of metallic compounds compared to the untreated control seeds are presented in Table 1. Addition of NPs or their bulk/ionic counterparts did not affect the number of germinated seeds compared to the control even in the highest concentrations (1000 μg/mL). The only exception was observed in the case of $AgNO_3$ which exhibited a dose dependent decrease in the seed germination rate with a maximum inhibition of 43% at the highest concentration. In contrast, a significant, dose dependent impact on root elongation of germinating seeds was observed in all treatments (see Table 1). Addition of NPs or their bulk counterparts negatively affected root elongation of tomato seeds even at the lowest (10 μg/mL) concentration. Inhibition of root length at the 1000 μg/mL concentration ranged between 60 and 90% compared to the control treatment (see Table 1). The less toxic compounds in terms of root elongation were Ag-NPs and CuO-NPs with an inhibition rate of approximately 60–65% while the remaining NPs and counterparts exhibited inhibition rates close to 85–90% at the highest tested concentration.

4.2.2. Impact of NPs on tomato plant growth

The effect of metal nanoparticles tested as well as their bulk/ionic counterparts on root and shoot length of tomato plants grown in HA medium is shown in Figs. 2 and 3. Among NPs tested, Cu-NPs were the most toxic followed by CuO-NPs, ZnO-NPs and Ag-NPs in terms of relative root length, with IC_{50} values of 17.88, 52.93, 197.72, and 693.20, respectively (see Fig. 2). In the case of shoot length, IC_{50} values of Cu-NPs, CuO-NPs, ZnO-NPs and Ag-NPs were 41.85, 75.69, 594.21 and 84.44 respectively. Comparison of the impact of NPs with their respective bulk/ionic counterparts on root and shoot development is shown in Fig. 3. In all cases, statistically significant differences were found between metal nanoparticles and their counterparts as indicated by their respective IC_{50} values. Cu-NPs had a significantly higher adverse effect on root and shoot development compared to $Cu(OH)_2$ while in the ZnO-NPs and $ZnSO_4$ case, zinc nanoparticles were more toxic to root than shoot development where the reverse relationship was found (see Fig. 3). CuO-NPs and Ag-NPs were less toxic than their counterparts both in terms of root and shoot development.

Percent mean dry weight of tomato plants treated with 100, 500 and 1000 μg/mL of NPs and bulk/ionic counterparts is shown in Table 2. Reduction of tomato plant dry weight caused by all treatments was dose-dependent in most cases. At the highest concentration, $AgNO_3$ had the most toxic effect causing a 89% reduction in dry weight compared with the control treatment, followed by Cu-NPs, ZnO-NPs and $ZnSO_4$. Ag-NPs, CuO-NPs and $Cu(OH)_2$ which exhibited the less toxic effect on tomato plants in terms of dry weight (see Table 2).

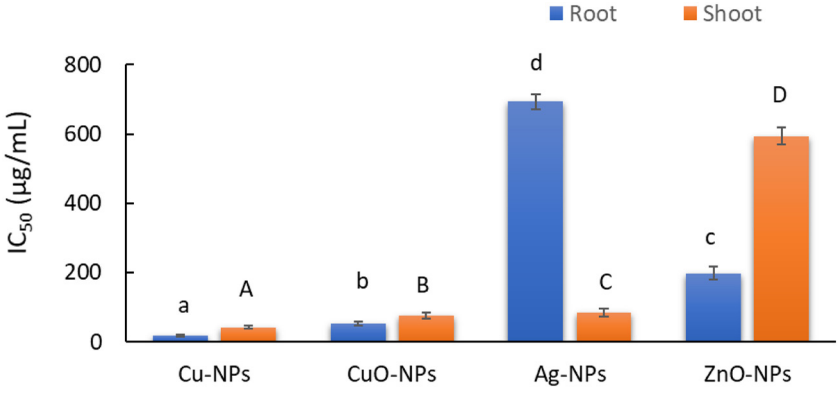

Fig. 2. Comparison of mean toxicity of copper, silver and zinc containing nanoparticles on root and shoot length in tomato plants. Bars marked by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

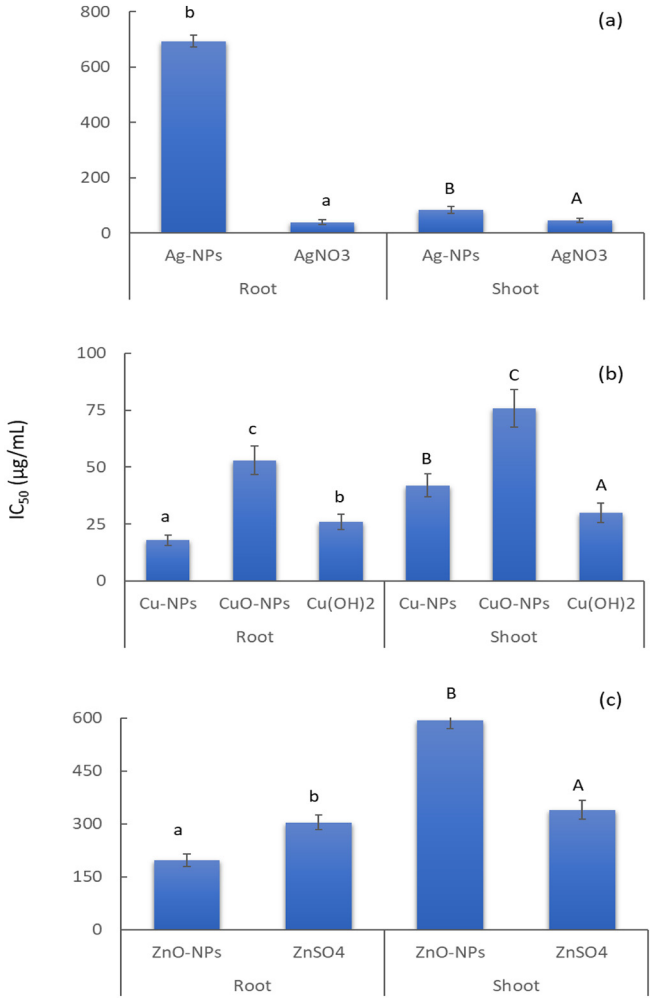

Fig. 3. Effect of (a) Ag-NPs, (b) Cu-NPs and CuO-NPs, and (c) ZnO-NPs compared to their bulk/ionic counterparts $AgNO_3$, $Cu(OH)_2$ and $ZnSO_4$ respectively in terms of IC_{50} (concentration that causes 50% inhibition in length of root/shoot compared to the untreated control). Bars marked by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

4.2.3. Effect of NPs on physiological parameters

Effect of NPs and non-nanoparticle counterparts on lipid peroxidation, an indicator of plant membrane integrity, measured as MDA byproduct was evaluated in comparison with non-treated tomato plants. All treatments except for CuO-NPs and $ZnSO_4$ exhibited higher MDA levels than the control treatment (see Fig. 4a). The highest MDA value was recorded in the case of Cu-NPs (1.39 μmol/g FW) which did not differ statistically from the respective value (1.17 μmol/g FW) of $Cu(OH)_2$. This was also the case for Ag-NPs and $AgNO_3$ (0.94 and 1.07 μmol/g FW), while ZnO-NPs exhibited significantly higher (1.01 μmol/g FW) MDA levels than $ZnSO_4$ (0.87 μmol/g FW) (see Fig. 4a).

Hydrogen peroxide accumulation as a response to oxidative stress potentially caused by NPs and counterpart treatments was determined in tomato plant tissues. Both NPs and counterparts exhibited higher levels of H_2O_2 accumulation than the untreated control (see Fig. 4b). Among NPs, higher levels of H_2O_2 (0.52 μmol/g FW) were recorded in Cu-NPs-treated tomato plants followed by ZnO-NPs (0.44 μmol/g FW), Ag-NPs (0.35 μmol/g FW) and CuO-NPs (0. 17 μmol/g FW) respectively. Cu-NPs and ZnO-NPs treatments resulted in a statistically higher H_2O_2 response than their counterparts $Cu(OH)_2$ and $ZnSO_4$. No statistical difference was found in hydrogen peroxide levels between silver NPs and $AgNO_3$. In contrast, $Cu(OH)_2$ treatment resulted in a higher H_2O_2 response than CuO-NPs (see Fig. 4b).

Table 2
Percent mean dry weight of tomato plants treated with NPs and their respective bulk/ionic counterparts.

Treatment	% Dry weight (mean ± SD[a])		
	100[b]	500	1000
Cu-NPs	97.25 ± 3.24 c[c]	45.12 ± 4.39 ab	27.84 ± 7.70 b
CuO-NPs	105.13 ± 0.85 c	76.31 ± 7.02 c	52.55 ± 4.25 c
$Cu(OH)_2$	96.19 ± 6.11 c	85.74 ± 3.90 c	45.07 ± 3.89 c
ZnO-NPs	85.20 ± 5.25 b	55.81 ± 2.88 b	29.69 ± 5.10 b
$ZnSO_4$	78.52 ± 3.88 ab	45.90 ± 9.16 ab	24.83 ± 3.55 b
Ag-NPs	95.70 ± 9.32 c	75.55 ± 4.19 c	55.23 ± 6.30 c
$AgNO_3$	65.79 ± 5.00 a	35.97 ± 5.05 a	11.00 ± 2.12 a

[a] Standard deviation of the means ($n = 4$).
[b] Numbers indicate concentration of NPs/bulk counterparts in μg/mL of active ingredient.
[c] Within columns, means followed by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

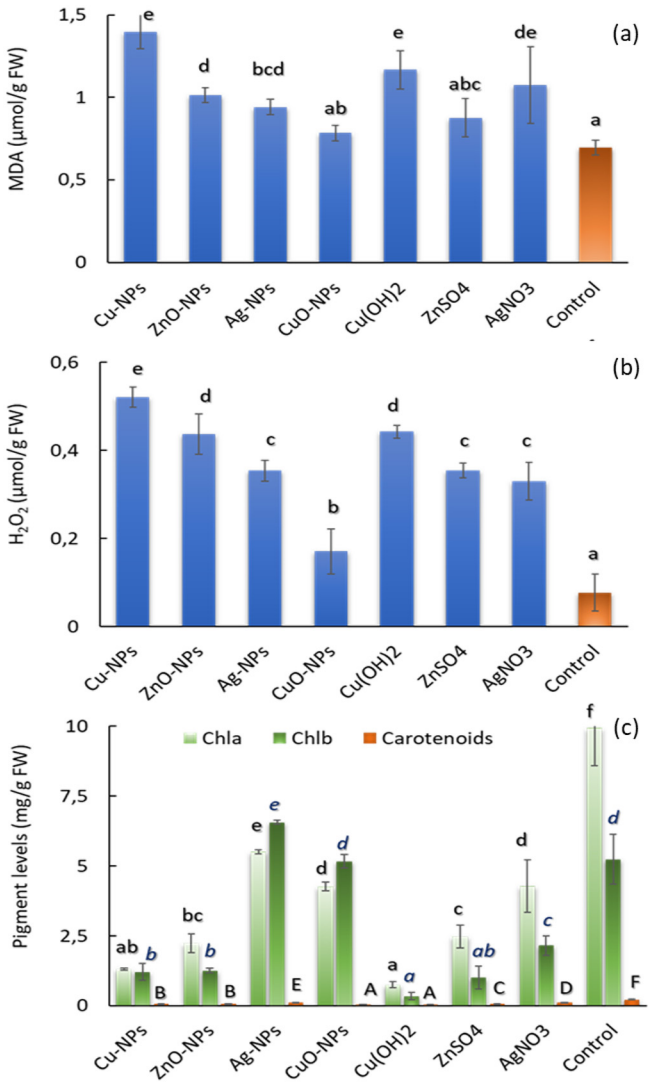

Fig. 4. Effect of metal NPs and their bulk/ionic counterparts on (a) MDA, (b) H_2O_2 and (c) photosynthetic pigments levels of treated tomato plants. Bars marked by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$). Between treatments in the pigment chart (c), capital letters indicate differences in carotenoid levels, small black letters in Chlorophyll-a (Chla) levels and blue italic letters in Chlorophyll-b (Chlb) levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

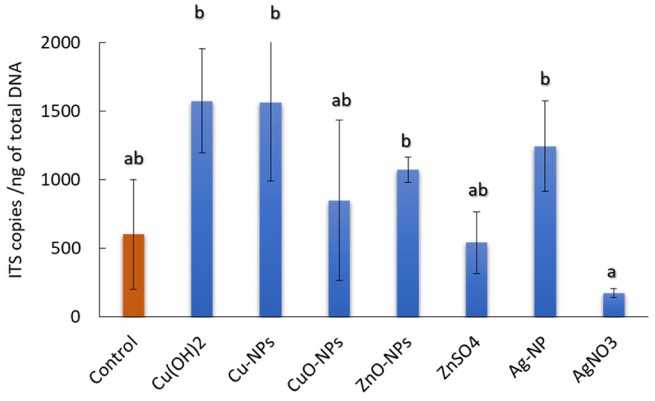

Fig. 5. Effect of nanoparticles application on fungal (FsK) colonization within root tissues. Quantification of fungal colonization within root tissues by qPCR using primers specific for ITS gene (primary axis). Bars marked by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

The photosynthetic response potential of tomato plants to NPs and counterpart treatments was evaluated by measuring Chl-a, Chl-b, and carotenoid concentrations. Chl-a content was significantly reduced compared to control in all treatments indicating that both nano and bulk metals exerted a high stress potential that could negatively affect growth and development of tomato plants (see Fig. 4c). Nano-size did not seem to affect Chl-a content in the case of copper and zinc containing treatments while a significant decrease on Chl-a content was observed in $Cu(OH)_2$ and $AgNO_3$ compared to CuO-NPs and Ag-NPs treatments respectively. A similar significant ($P < 0.01$) decrease in Chl-b levels was recorded in the case of Cu-NPs (1.2 mg/g FW), ZnO-NPs (1.25 mg/g FW), $Cu(OH)_2$ (0.34 mg/g FW), $ZnSO_4$ (1.00 mg/g FW) and $AgNO_3$ (2.15 mg/g FW) treatments compared to the control (5.24 mg/g FW). In contrast treatment of tomato plants with CuO-NPs and Ag-NPs (5.16 and 6.56 mg/g FW respectively) resulted in no significant decrease in Chl-b content compared to the control. All treatments had an adverse effect on the carotenoid content of tomato plants. Plants exhibited a 50 ($AgNO_3$) to 83% (CuO-NPs) reduction in the carotenoid levels when exposed to metal NPs and their bulk/ionic counterparts compared to the untreated control (see Fig. 4c).

4.3. Effect of NPs on the symbiotic relationship between tomato plants – FsK

The ability of endophytes to colonize host plants is essential for the establishment of a successful symbiotic relationship. Sub-lethal doses of NPs and their bulk/ionic counterparts applied as root drenches were used to evaluate potential adverse effects of treatments on tomato root colonization of FsK. Quantitative real time PCR was used for the quantification of root colonization and the results were expressed in terms of copy numbers per ng of FsK DNA. Although considerable variability was observed between treatments, no statistically significant difference was found between the tomato roots treated with NPs/counterparts and the untreated control (see Fig. 5). No differences in colonization of FsK were found between NPs and their bulk/ionic counterparts with the exception of $AgNO_3$, which resulted in significantly less FsK DNA levels present in tomato roots than Ag-NPs (see Fig. 5). This probably indicates a shielding effect exerted by tomato root tissue on the FsK strain once the endophyte is established inside them. The lack of systemic action of metallic compounds could be the reason for the absence of significant differences in tomato root colonization compared with the untreated control.

In an attempt to investigate the potential of FsK to alleviate phytotoxicity caused by NPs and their bulk/ionic counterparts, growth parameters such as fresh, dry weight and shoot length of treated tomato plants were measured in the presence or absence of FsK. In non-inoculated tomato plants, no significant differences were found between treatments with xenobiotics and the control in terms of fresh weight and shoot length (see Table 3). On the contrary, the dry weight was significantly affected by treatment of tomato plants with NPs and counterparts in the absence of FsK. Specifically, CuO-NPs, ZnO-NPs, $ZnSO_4$ and $AgNO_3$ significantly reduced the dry weight of tomato plants compared with the untreated control (see Table 3). When plants were inoculated with FsK, significant differences in fresh weight and shoot length were only recorded between NPs/counterpart treatments and the control only in the case of $AgNO_3$, which exhibited a dramatic reduction (43 and 57% respectively). In contrast, FsK-inoculated plants did not suffer any significant dry weight loss compared with the control in all NPs/counterparts cases except for $AgNO_3$. The later exhibited a significant decrease (41%) in dry weight compared with the control (see Table 3).

5. Discussion

In this study, the potential of copper, silver and zinc oxide NPs to cause phytotoxicity or interfere with the association between tomato plants and their endophytic partner *Fusarium solani* FsK strain was investigated via plant growth, physiological analysis and colonization bioassays.

Tomato seeds sown on growth medium containing all tested NPs germinated successfully and in rates similar or even higher than the control treatment even at the highest 1000 μg/mL concentration. This was also the case for the NPs bulk/ionic counterparts except for $AgNO_3$, which inhibited seed germination in a dose-dependent manner. In contrast, tomato-seedling root length was significantly reduced in a dose dependent way in most cases of NPs and bulk counterparts. Ag-NPs and CuO-NPs had the less negative effects on root elongation and were significantly less toxic than their counterparts $AgNO_3$ and Cu $(OH)_2$ respectively. The remaining NPs were equally toxic with their bulk counterparts in terms of root elongation. Khaldari et al. (2021)

Table 3
Effect of metal NPs and counterparts on fresh, dry weight and shoot length of tomato seedlings in the presence or absence of the endophytic FsK *F. solani* strain.

Treatment	Fresh weight[a] (mean ± SD[c])		Dry weight[a] (mean ± SD)		Shoot length[b] (mean ± SD)	
	−FSK	+FSK	−FSK	+FSK	−FSK	+FSK
Control	16.81 ± 3.24 a[d]	16.18 ± 2.01 b	1.46 ± 0.42 d	1.20 ± 0.21 bc	54.5 ± 10.62 a	61.25 ± 8.06 b
Cu-NPs	17.10 ± 2.53 a	16.29 ± 2.43 b	1.19 ± 0.27 bcd	0.90 ± 0.25 abc	62.38 ± 11.28 a	65.75 ± 12.05 b
CuO-NPs	14.13 ± 3.05 a	14.05 ± 2.33 b	0.65 ± 0.32 ab	0.75 ± 0.19 ab	65.5 ± 2.65 a	63.00 ± 9.56 b
$Cu(OH)_2$	16.98 ± 0.92 a	16.33 ± 1.47 b	1.27 ± 0.13 cd	0.93 ± 0.03 abc	59.25 ± 6.9 a	65.00 ± 5.10 b
ZnO-NPs	15.39 ± 5.85 a	15.34 ± 3.4 b	0.77 ± 0.32 abc	1.09 ± 0.29 bc	61.63 ± 17.01 a	58.13 ± 8.08 b
$ZnSO_4$	17.55 ± 3.31 a	15.40 ± 1.02 b	0.81 ± 0.03 abc	1.17 ± 0.13 bc	70.25 ± 7.63 a	57.75 ± 6.5 b
Ag-NPs	14.10 ± 1.47 a	14.83 ± 1.62 b	0.95 ± 0.12 abcd	1.32 ± 0.3 c	55.00 ± 9.28 a	50.50 ± 1.22 ab
$AgNO_3$	10.45 ± 2.73 a	7.18 ± 3.52 a	0.49 ± 0.18 a	0.5 ± 0.34 a	53.13 ± 3.22 a	35.50 ± 11.82 a

[a] Mean fresh and dry weight measured in grams.
[b] Shoot length measured in mm.
[c] Standard deviation of the means (n = 3).
[d] Within columns, means followed by the same letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$).

observed negative effects of green synthesized CuO-NPs on tomato root elongation even at the lowest concentration, while seed germination was only affected at the highest over a number of concentrations ranging from 4 to 4000 μg/mL. Chen et al. (2020) reported an increase on seed germination of tomato grown in iron, zinc and copper NPs-amended Murashige–Skoog (MS) nutrient medium compared with the control, although in lower concentrations. Similar reports on the effect of Ag-NPs on tomato plants indicate that silver NPs may not be a limiting factor for seed germination in concentrations up to 5000 μg/mL but significantly reduce root elongation in doses as low as 25 μg/mL (Cox et al., 2016; Song et al., 2013; Karami Mehrian et al., 2016).

Growth experiments of tomato plants grown on HA medium amended with metal NPs and their counterparts revealed a significant toxic effect of treatments on biomass, root and shoot length. Specifically, dry weight of plants was significantly reduced upon treatment with NPs and counterparts in concentrations over 500 μg/mL with most pronounced effects in the case of $AgNO_3$, Cu-NPs, ZnO-NPs and $ZnSO_4$. Cu-NPs resulted in a stronger reduction in tomato dry weight than $Cu(OH)_2$ while $AgNO_3$ were 5 times more toxic than Ag-NPs in terms of dry weight. Root and shoot length of grown tomato plants was also affected by treatments with NPs and their bulk counterparts. Cu-NPs incorporation to the growth medium resulted the highest root and shoot length inhibition among NPs tested, followed by CuO-NPs and ZnO-NPs. Shoot length was less negatively affected by NPs than their respective bulk counterparts in all cases. This was also the case for CuO-NPs and Ag-NPs as far as root length is concerned. On the contrary, Cu-NPs and ZnO-NPs resulted a more pronounced reduction in root length than $Cu(OH)_2$ and $AgNO_3$ respectively.

Tomato plant biomass significantly decreased (59–78%) upon exposure to CuO-NPs while NPs treatment also significantly reduced root (68–75%) and shoot (42–47%) length as compared to untreated controls (Pagano et al., 2016). Phytotoxicity of silver NPs in tomato plants was demonstrated by reduced wet weight, root length, lower chlorophyll contents, higher superoxide dismutase activity and less fruit productivity (Song et al., 2013; Noori et al., 2020). A significant reduction in root and shoot length of *S. lycopersicon* was also observed upon treatment with ZnO-NPs as well as its bulk counterpart form (Ahmed et al., 2019). In the present study, NP treatments had a more pronounced toxic effect in root rather than in shoot length as indicated by the respective IC_{50} values. The only exception was in the case of Ag-NPs where relative shoot length inhibition was greater than the one observed in roots. A probable higher translocation factor of Ag-NPs towards the shoot could be responsible for this enhanced toxicity. This could be attributed to the PVP coating of Ag-NPs which results in a negative charge of this NP in contrast to Cu-NPs and ZnO-NPs which were positively charged. Koelmel et al. (2013) has reported a surface charge-dependent bioaccumulation of Au-NPs in various rice organs with negatively charged NPs being more toxic and mostly accumulating in the above ground rice organs. Additionally, Noori et al. (2020) have reported a significantly higher translocation factor (TF) of AgNP-exposed tomato plants compared to $AgNO_3$ which results in the release of positively charged Ag^+ ions. On the contrary, positively charged ZnO-NPs were found to attach to negatively charged soybean and tomato roots resulting in a low translocation factor (Zn shoot to root concentration ratio) (Ristroph et al., 2017; Akanbi-Gada et al., 2019).

A number of physiological and biochemical mechanisms including membrane interactions, ion release, production of reactive oxygen species (ROS), inactivation of enzymes, disruption of the photosynthetic mechanism or damage to DNA are considered responsible for NPs phytotoxicity (Karami Mehrian et al., 2016; Ma et al., 2010; Noori et al., 2020). In an attempt to investigate the potential involvement of NPs and their bulk counterparts on lipid peroxidation and oxidative stress response of tomato, experiments determining MDA and hydrogen peroxide (H_2O_2) levels of treated and untreated *S. lycopersicon* plants were conducted. Overall, treatment of tomato plants with NPs/bulk counterparts resulted in a marked oxidative stress response both in terms of MDA and H_2O_2 levels which were significantly elevated in treated plants compared to the untreated control. The greatest oxidative response was observed in the case of Cu-NPs (2–8 fold increase in MDA and H_2O_2 levels compared to the untreated control respectively) followed by ZnO-NPs and Ag-NPs. This had an expected impact to the corresponding fresh weight (FW) of tomato plants as revealed by the negative correlation observed between H_2O_2 levels and FW. Comparison between NPs and their bulk/ionic counterparts showed different patterns of oxidative response. Oxidative stress caused by Cu-NPs and ZnO-NPs was greater than the one imposed by their counterparts $Cu(OH)_2$ and $ZnSO_4$ possibly indicating a higher toxicity of those metals related to nano properties. In contrast, treatment with Ag-NPs and CuO-NPs resulted in a lesser or equal oxidative response than that of their counterparts $AgNO_3$ and $Cu(OH)_2$ indicating that the ionic form of those metals are more reactive than the nano form. Similar studies on the oxidative response of Ag-NPs on tomato plants have reported a significant increase of MDA and H_2O_2 levels compared with untreated plants, which were significantly lower than that of plants treated with $AgNO_3$ (Noori et al., 2020; Jiravova et al., 2016). High concentrations of CuO-NPs (>0.5 μM) have been reported to trigger oxidative bursts leading to elevated H_2O_2 levels, challenging oxidative plant-defense mechanisms and resulting in the disruption of membrane integrity/phytotoxicity in barley and rice plants (Shaw et al., 2014). A similar elevation of oxidative stress in terms of H_2O_2 production as response to ZnO-NPs treatment has been reported in tomato plants (Akanbi-Gada et al., 2019). Oxidative response to NPs and counterparts treatments in the present study were correlated with chlorophyl-a and carotenoid levels which were significantly reduced compared to the untreated control. Reduction of photosynthetic pigments was more pronounced in cases with high H_2O_2 and MDA confirming the suggestion that plant biomass and chlorophyll levels are more sensitive indicators of phytotoxicity than seed germination or root elongation (Ma et al., 2010). Photosynthetic pigment level alterations have been associated with metal nanoparticle treatments in tomato plants in various studies, including treatments with copper, zinc and zinc oxide NPs (Noori et al., 2020; Akanbi-Gada et al., 2019; Lopez-Lima et al., 2021).

A number of studies have investigated the potential of metal NPs to suppress both plant pathogens and beneficial/symbiotic microorganisms (S. Li et al., 2015; Oktarina and Singleton, 2020; Malandrakis et al., 2019, 2020a, 2020b, 2021). The importance of the potential toxic action of NPs towards beneficial soil microorganisms including bacteria and fungi derives from the direct implication of these organisms to soil and plant health and productivity, and the agroecosystem/environmental sustainability (Dimkpa, 2014; Ameen et al., 2021). A better understanding of such ramifications requires studies involving combined plant-microbe interactions under the nanoparticle's influence (Ameen et al., 2021). FsK is a root colonizing *Fusarium solani* strain proven to induce tomato plant resistance mechanisms against pathogens and abiotic stress (Kavroulakis et al., 2007; Garantonakis et al., 2018; Kavroulakis et al., 2018). A key question besides tomato phytotoxicity and FsK fungitoxicity risks posed by NPs, is whether they can affect the survival and colonization of FsK and its symbiotic interactions with the tomato plant. In vitro fungitoxicity tests revealed that FsK was significantly more sensitive to Cu-NPs and ZnO-NPs than CuO-NPs and Ag-NPs both in terms of mycelial growth and spore germination. All NPs were more toxic to FsK compared to their bulk counterparts except for $AgNO_3$ which was 8 to 9-fold more toxic than Ag-NPs. A similar fungitoxic profile was reported in another *F. solani* strain exposed in vitro to the above metal NPs and bulk counterparts (Malandrakis et al., 2019).

When applied in sublethal doses in a soil-based tomato-endophyte system, NPs and bulk counterparts did not exhibit any significant effect on FsK colonization of tomato roots. This could be due to the fact that the endophyte was already established inside tomato roots which acted as a barrier, before treatment with metal NPs/counterparts. On the other hand, tomato plants colonized by the FsK strain did not suffer

any adverse effects in terms of dry mass from CuO-NPs, ZnO-NPs and $ZnSO_4$ treatments compared with the untreated control. In the absence of FsK, the above treatments of tomato plants resulted in a significant decrease in dry mass compared with the untreated control. This indicates a potential plant-endophyte mutual protection against NPs and heavy metals with obvious implications on plant health and production. A number of studies have reported toxicity of NPs on beneficial soil fungi including biocontrol agents and other beneficial symbiotes such as arbuscular mycorrhizal fungi (AMF) and their impact on colonization and plant growth. Oktarina and Singleton (2020) have reported a significant fungitoxic effect of Ag-NPs in terms of colony diameter and spore production in the beneficial soil fungus *Trichoderma harzianum* in concentrations of 200, 600 and 1000 μg/mL in growth media. A similar study reported a significant reduction in maize growth and colonization by the (AMF) fungus *Funneliformis mosseae* in the presence of 500 mg/kg of ZnO-NPs and $ZnSO_4$ (S. Li et al., 2015). On the other hand, AM fungi have demonstrated an alleviation potential against the negative effects of Ag-NPs and ZnO-NPs exposure in maize (*Zea mays* L.) and tomato plants (Cao et al., 2020; Noori et al., 2017; Wang et al., 2016).

6. Conclusion

Metal nanoparticles significantly negatively affected growth and physiology of tomato plants as indicated by dry mass, oxidative stress responses and photosynthetic pigment levels. Differences in plant and FsK responses between nano and bulk/ionic counterparts were observed indicating potential differences in the mode of toxic action of the two categories. NPs and counterparts applied in sublethal concentrations did not affect FsK colonization of tomato roots, while a possible alleviation of metal toxicity was observed in the presence of FsK in the case of CuO-NPs, ZnO-NPs0 and $ZnSO_4$. These results suggest that phytotoxicity of NPs in tomato treated plants should be considered before application and while both FsK and tomato are sensitive to NPs and counterparts, their symbiotic benefits can extent to mutual resistance towards these toxic agents.

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CRediT authorship contribution statement

Anastasios A. Malandrakis: Conceptualization, Investigation, Methodology, Writing
Nektarios Kavroulakis: Investigation, Visualization, Reviewing
Georgios Tsaniklidis: Investigation, Reviewing
Marianna Avramidou: Investigation, Reviewing
Kalliope K. Papadopoulou: Investigation, Reviewing
Constantinos V. Chrysikopoulos: Supervision, Visualization, Reviewing and Editing

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: *Fusarium solani* FsK is patented (20070100563/1006119, issued by the Industrial Property Organization to NK and KKP).

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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