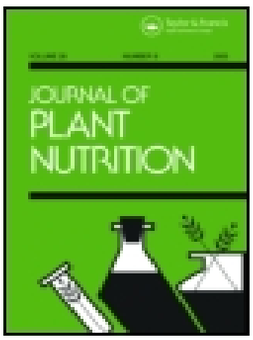

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# Physiological and molecular responses of black cumin (*Nigella sativa* L.) seedlings to silver nanoparticles

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**ABSTRACT**

Due to their anti-bacterial and anti-fungal properties, silver (Ag) nanoparticles (AgNPs) are increasingly being used in consumer products. In this study, the effects of applying different concentrations of AgNPs (0, 2.5, 5, 10, 20, 40 and 80 mg $L^{-1}$) on the growth, leaf concentrations of Ag, micro and macro-elements, leaf concentrations of malondialdehyde (MDA) and $H_2O_2$, total antioxidant activity and activity of antioxidant enzymes in leaf extracts and leaf expression of phenylalanine ammonia-lyase (*PAL*) and chalcone synthase (*GSH*) genes were investigated in black cumin (*Nigella sativa*) seedlings. Results showed that AgNPs treatments led to increases in Ag concentrations in leaves and roots, with the accumulation in roots being slightly higher than leaves. AgNPs treatments at concentrations over 20 mg $L^{-1}$ reduced growth, biomass production and accumulation of macro- (P, Mg and Ca) and micro-elements (Zn, Cu, Mn and B) and increased total antioxidant activity and MDA and $H_2O_2$ contents. Application of AgNPs also increased the activity of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) in leaf extracts when compared to the controls. The activity and expression of PAL and CHS enzymes also increased significantly with AgNPs treatment. Results indicate that the oxidative stress induced by AgNPs in black cumin plants is counteracted by increases in the activity of antioxidant systems and the expression and activity of PAL and CHS enzymes.

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**KEYWORDS**
Antioxidant enzyme activity; black cumin; chalcone synthase; phenylalanine ammonium-lyase; silver nanoparticles

## Introduction

Silver (Ag) nanoparticles (AgNPs) are used in different fields because of their antibacterial and antifungal activities, and the application of AgNPs with controlled structure is increasing rapidly. Other industrial applications of AgNPs include cosmetic formulations, pharmaceutic, water filtration, catalytic systems and biomedical sciences (Albrecht, Evans, and Raston 2006). Since nanoparticles can cause high toxicity, a proper knowledge of the properties of nanoparticles, including their uptake, accumulation, interactions and impact on bio-systems, is urgently needed (Navarro et al. 2008). When AgNPs enter water and soil environments, Ag well as other accompanying metals can cause toxicity in many organisms, including plants (Nowack 2009). Therefore, the phytotoxicity of AgNPs is one of the major concerns for the use of this type of nanoparticle in agriculture. Some reports of beneficial effects of AgNPs have been published. For instance, foliar application of AgNPs improve growth and yield of cucumber (Shams, Ranjbar, and Amiri 2013),

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and showed that the use of AgNPs increased the growth of *Brassica juncea* (Sharma et al. 2012). However, other studies can have as a negative effect the growth of wheat (Vannini et al. 2014) and rice (Nair and Chung 2014). In tomato plants, increases in the activity of superoxide dismutase (SOD) enzyme and decreased contents of photosynthetic pigments have been reported with AgNPs treatment (Song et al. 2013).

Black cumin (*Nigella sativa* L.) is a widely used medicinal plant that belongs to the Ranunculaceae family. It is used as an aromatic and flavoring plant, as well as for its medicinal properties (Cheikh-Rouhou et al. 2007). The pharmacological functions of black cumin are due to the presence of secondary metabolites, including seed fixed volatile oils (stearic, palmitic, oleic and linoleic acids), as well as phenolic compounds in roots and shoots (Matthaus and Ozcan 2011). Black cumin is widely used to remedy illnesses such as tumors, diabetes, irritation, asthma, high blood pressure, digestive disturbances and women disorders (Ramadan 2007).

The aim of the present study was to assess the effects of adding different concentrations of AgNPs on the growth, biomass, macro and micro-element concentrations in black cumin seedlings. Oxidative stress was also studied by measuring the leaf concentrations of malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) as well as the total antioxidant activity and the activities of antioxidant enzymes SOD, ascorbate peroxidase (APX) peroxidase (POX) and catalase (CAT) in leaf extract. Finally, the effects of AgNPs on the activity and expression level of chalcone synthase (*CHS*) and phenylalanine ammonia-lyase (*PAL*) genes were also assessed.

## Materials and methods

### AgNPs suspensions and characterization

Silver nanoparticles (AgNPs), obtained from US Research Nanomaterials, Inc. were suspended in double distilled water using an ultrasonic device (250 model, He-Yu Technology Co., Taiwan) at 40 kHz and 100 W for 30 min. Purity and specific surface area (SSA) were 99% and 18–22 $m^2\ g^{-1}$, respectively. The average size of AgNPs was estimated to be in the range 5–30 nm using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Figure 1).

### Plant materials, growth conditions and experimental design

The seeds of black cumin (*Nigella sativa* L.) were surface-sterilized and sown in pots (10 cm diameters and 15 cm length, 5 seeds per pot) filled with autoclaved river sand. The pots kept in a greenhouse, with a 14/10 h light/dark cycle and at 25/18 °C. After germination, only one seedling per pot was kept. The treatments with AgNPs started 15 days after germination by irrigating every other day with 150 mL of distilled water containing 0, 2.5, 5, 10, 20, 40 or 80 mg $L^{-1}$ of AgNPs.

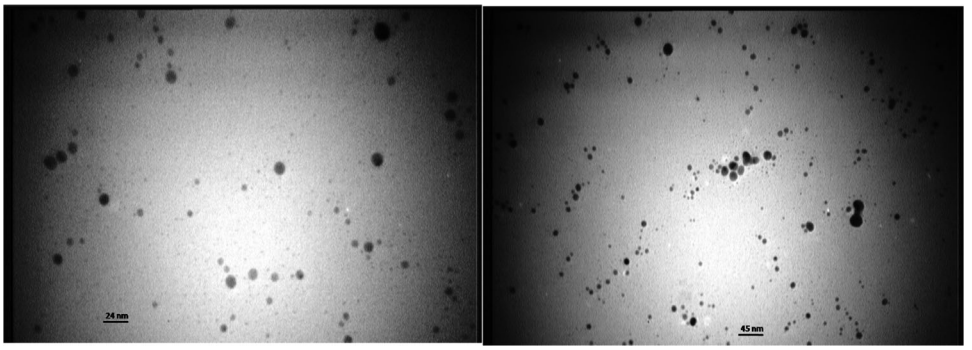


**Figure 1.** Images of the transmission electron microscope (TEM).

A half strength Hoagland solution (150 mL per pot) was used to irrigate all pots once a week. After 4 weeks, plants were harvested and stored in a freezer (−80 °C) before measuring biochemical and molecular traits.

### Elemental analysis

For elemental analysis, dried leaf or root tissue (0.1 g dry weight -DW-) was sampled at the end of the experiment and digested in a $HNO_3$:$H_2O_2$ solution (1:4 ratio, v:v). Digests were placed in polypropylene centrifuge tubes and the volume made up to 15 mL with water. Tissue concentrations of Ag, micro and macro-elements were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; Optima 4300 DV, Perkin Elmer, Waltham, MA, USA).

### Estimation of malondialdehyde (MDA), $H_2O_2$ and total antioxidant activity

At the end of the experiment, the leaf content of MDA was determined using the thiobarbituric acid (TBA) method and an extinction coefficient of 155 $mM^{-1}cm^{-1}$ (Heath and Packer 1968). Leaf $H_2O_2$ contents were measured as in Velikova, Yordanov, and Edreva (2000). After homogenizing fresh leaves with trichloroacetic acid 1% (w/v) and centrifuging at 12000 g (10 min), 0.5 mL of supernatant was supplemented with 1 mL 1 M KI and 0.5 mL 10 mM K phosphate buffer (pH 7.0) and the absorbance was recorded at 320 nm with a spectrophotometer (Carry 300, Varian, California, USA). Total antioxidant activity was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to Brand-Williams, Cuvelier, and Berset (1995).

### Estimation of antioxidant enzymes

To extract soluble proteins, fresh leaf material (0.5 g) was sampled at the end of the experiment and homogenized in 2 mL of K phosphate buffer pH 7.8 containing 0.5% polyethylene glycol 4000, 0.1 mM EDTA (ethylenediaminetetraacetic acid), 1% PVP (polyvinylpyrrolidone) and 2 mM dithiothreitol. Total protein was measured according to Bradford (1976). To measure SOD activity, the absorbance of a reaction mixture containing 100 µL of extract, 100 µL of methionine (13 mM), 100 µL of riboflavin (13 µM), 2.3 mL of 25 mM sodium phosphate buffer (pH 6.8), 100 µL of 63 µM nitroblue tetrazolium was read at 560 (Giannopolitis and Ries 1977). The activity of CAT was determined from the decrease in absorbance of $H_2O_2$ at 240 nm according to Bailly et al. (1996). To determine POD activity, the absorbance of a reaction mixture containing 100 µL of enzyme extract, 500 µL of 20 mM guaiacol, 1.4 mL of K phosphate buffer (50 mM, pH 5.0), 500 µL of 40 mM $H_2O_2$ was recorded. The activity of POD enzyme was calculated by determining the absorbance increase at 240 nm for 2 min (Chance and Maehly 1955). The activity of APX enzyme was determined by measuring the decrease in absorbance at 290 nm during time according to Nakano and Asada (1981).

### Estimation of phenylalanine ammonium-lyase (PAL) and chalcone synthase (CHS) activity

Fresh leaf material (0.5 g) was sampled at the end of experiment and homogenized with 2 mL of 150 mM Tris-HCl buffer (pH 8.5) containing 15 mM $\beta$-mercaptoethanol. After centrifugation at 12000 g for 20 min, the supernatant was used for the determination of PAL activity according to Ochoa-Alejo and Gómez-Peralta (1993), measuring the cinnamic acid formation. After incubation of the reaction solution (100 µL of enzyme extract, 800 µL of L-phenylalanine, 1100 µL of buffer and 2 mL of extraction buffer) at 37 °C for 1 hour, 6 M HCl was added, the products were

Table 1. Sequences of primers used in the qPCR reactions.

| Gene name | Accession No. | 5′-primer-3′ | Tm |
|---|---|---|---|
| CHS | nm-121396.4 | F: CGCATCACCAACAGTGAACAC | 63.40 |
| | | R: TCCTCCGTCAGATGCATGTG | 63.89 |
| PAL | AY07936.1 | F: GCAGTGCTACCGAAAGAAGTG | 64.07 |
| | | R: CGACCTACATTCCTTGATCCTG | 63.61 |
| $\beta$-actin | NM_001338359.1 | F: CTTGCACCAAGCAGCATGAA | 60.89 |
| | | R: CCGATCCAGACACTGTACTTCCTT | 64.21 |

extracted with ethyl acetate, followed by evaporation to eliminate the extracting solvent. After dissolving the solid residue in 0.05 M NaOH, the absorbance of cinnamic acid was read at 290 nm.

To measure the CHS activity, fresh leaves (0.4 g) were sampled at the end of experiment and homogenized in 2 mL of 1 mM 2-mercaptoethanol dissolved in 0.1 M borate buffer pH 8.8. After adding 0.1 g Dowex $1 \times 4$ resins and letting rest for 10 min, the solution was centrifuged at 12000 g for 15 min. Then, 0.2 g resin was added to the supernatant in a new tube and letting rest for 10 min. The supernatant was collected after centrifugation at 12000 g for 15 min and mixed with 10 mM K cyanide in 1.9 mM Tris-HCI buffer pH 7.8. After adding chalcone dissolved in 10 $\mu$L ethylene glycol monomethyl to the enzyme extract, the reaction solution was incubated at 30 °C for 1 min and the absorbance was read at 370 nm using a spectrophotometer.

### Gene expression analysis

In order to examine the expression of *PAL* and *GHS* genes, plant material sampled at the end of the experiment. Frozen leaf tissue (0.1 g) was used to extract RNA using the TRIzol protocol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Two µg of total RNA after DNase*I* treatment, were used for first-strand cDNA synthesis using 10 mM dNTPs, oligo (dT) primers and reverse transcriptase according to manufacturer's instructions (Thermo Scientific, Germany). The qPCR (Quantitative real-time PCR) was carried out with a thermal cycler (C1000$^{TM}$, BioRad, Hercules, CA, USA), using 2 µL of cDNA template, 5 µL Maxima SYBR Green/ROX qPCR Master Mix (2X, Thermo Scientific, Waltham, MA, USA), 0.3 µM primers and nuclease-free water in total volume of 10 µL. The PCR program was 3 min at 95 °C, 15 s at 95 °C for denaturation, 45 s at 60 °C for annealing, 25 s at 72 °C for extension (40 cycles). The $\beta$-actin gene was used as a housekeeping gene for normalization and the $2^{-\Delta\Delta ct}$ method was used for data analysis (Livak and Schmittgen 2001). The experiment included three independent biological replicates with three technical replications for each biological replication. Primer3 online and OLIGO5 analyzer software packages were used to design and check primers, respectively (Table 1).

### Statistical analysis

All experiments were repeated three times and each mean was calculated from three independent biological replicates. Data were analyzed using SAS 9.1.3 software (SAS Institute, Inc., SAS Campus Drive, Cary, NC, USA) and means were compared using with a least significant difference (LSD) test at the 5% level.

## Results and discussion

### Silver uptake and morphological features

The leaf and root Ag concentrations showed progressive increases with increasing AgNPs doses. The highest Ag concentrations in the roots and leaves (180–220 µg $g^{-1}$) were observed at 80 mg

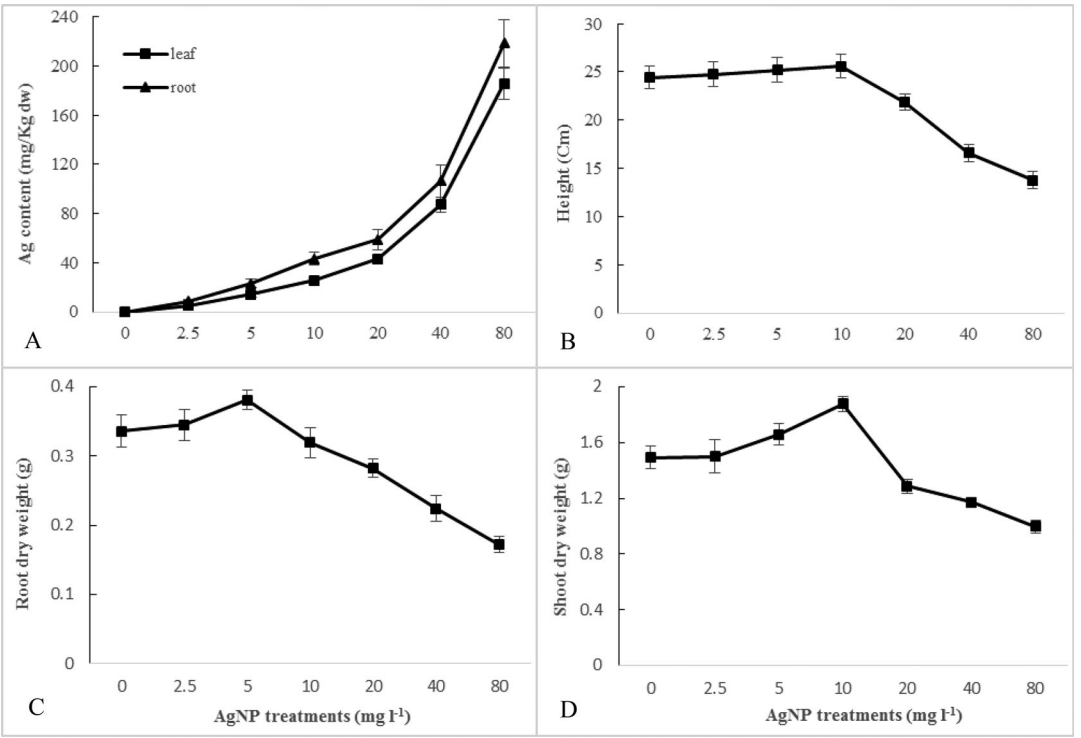


**Figure 2.** Leaf and root Ag concentration (A; in mg/Kg DW), plant height (B; in cm), root dry weight (C; in g) and shoot dry weight (D; in g) in *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

$L^{-1}$ AgNPs (Figure 2A). Plant height was not affected at AgNPs concentrations in the range 2.5 to 10 mg $L^{-1}$, but was reduced by 11, 32 and 44% with the 20, 40 and 80 mg $L^{-1}$ AgNPs treatments when compared to the controls (Figure 2B). In the case of the root DW, it was not affected at AgNPs concentrations at 2.5 mg $L^{-1}$, increased significantly at 5 mg $L^{-1}$ and decreased with 20, 40 and 80 mg $L^{-1}$. The highest reduction, 49%, was observed with 80 mg $L^{-1}$ (Figure 2C). Shoot DW was not affected at 0.5 mg $L^{-1}$ AgNPs, increased by 11 and 26% at 5 and 10 mg $L^{-1}$ AgNPs, respectively and decreased by 14, 22 and 33%, at 20, 40 and 80 mg $L^{-1}$ AgNPs, respectively when compared to the controls (Figure 2D).

Nanoparticles, such as AgNPs, are widely used in agricultural systems due to their physicochemical properties, including the ability to promote electron exchange, high catalytic capabilities and high surface area to volume ratio. The results showed that application of AgNPs at high concentrations (20, 40 and 80 mg $L^{-1}$ AgNPs) had an inhibitory effect on the growth and biomass of black cumin plants. These results are in line with those obtained by Geisler-Lee et al. (2012) and Thuesombat et al. (2014) in *Arabidopsis thaliana* and *Oryza sativa*, respectively. Hsiao et al. (2015) showed that AgNPs can release silver ions in the cell, and Ag can inhibit the perception of ethylene (Beyer 1976). Silver ions decrease plant growth by reducing the uptake of macro and microelements on *Raphanus sativus* (Zuverza-Mena et al. 2016). Increasing the doses of AgNPs enhanced the absorption and accumulation of Ag ions in the roots and leaves of *Portulaca oleracea* (Zare et al. 2020), *Raphanus sativus* (Zuverza-Mena et al. 2016) and *Allium cepa* (Cvjetko et al. 2017). Therefore, increasing the accumulation of Ag as a heavy metal has a negative effect on the vital parts of the plant cell, including the photosynthetic apparatus (Rastogi et al. 2019), thus reducing the growth and yield of the plant.

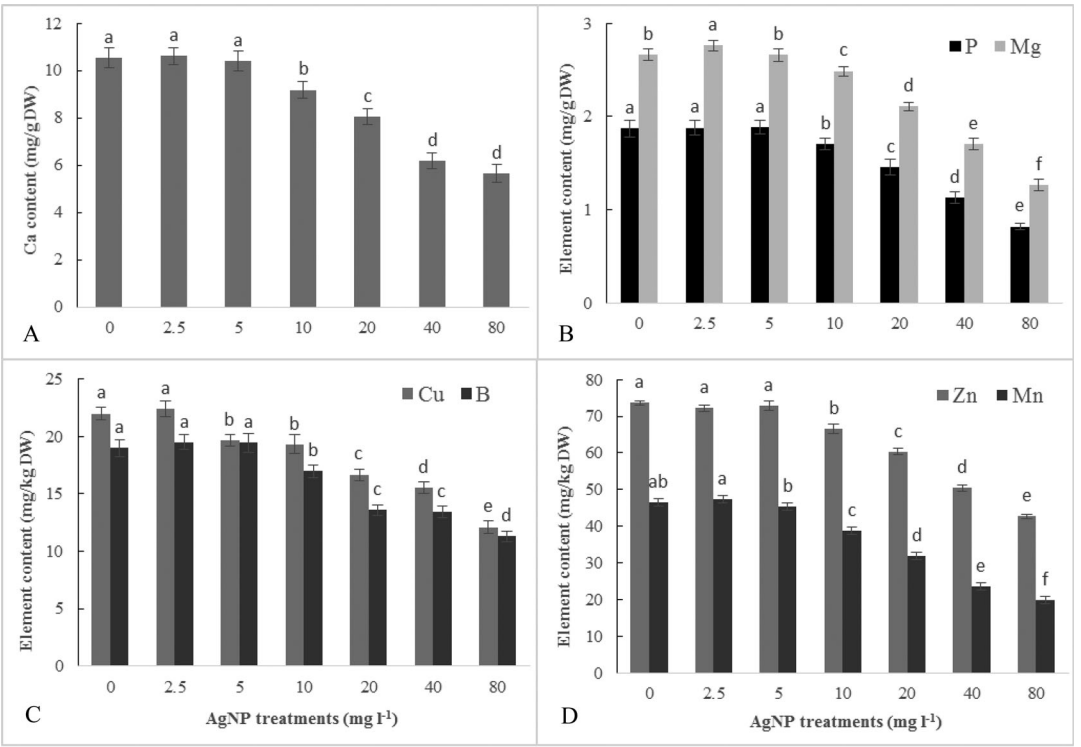


**Figure 3.** Leaf concentrations (in µg $g^{-1}$) of Ca (A), P and Mg (B), Cu and B (C) and Zn and Mn (D) in *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

### *Leaf macro- and micro-nutrient concentrations*

Treatment with AgNPs reduced the leaf concentrations of P, Ca and Mg. Increasing the concentration of AgNPs reduced the leaf concentration of both P and Ca in leaves, and the highest reduction was observed with the highest AgNPs concentration. The concentrations of P and Ca reduced by 56 and 46%, respectively at 80 mg $L^{-1}$ AgNPs when compared to the controls (Figure 3A, B). The leaf concentration of Mg significantly increased under 2.5 mg $L^{-1}$ AgNPs compared to the controls but decreased at higher levels (10, 20, 40 and 80 mg $L^{-1}$). The highest decline detected at 80 mg $L^{-1}$ AgNPs by 52.5% (Figure 3B). The results of micronutrient concentrations showed that application of AgNPs treatments decreased the concentrations of Zn, Cu, B and Mn elements in the leaves compared to controls plants, and the lowest concentrations of Zn, Cu, Mn and B was observed at 80 mg $L^{-1}$ of AgNPs (Figure 3C, 3D). The effects of nanomaterials on the uptake of nutrient elements in plants were shown in various studies. Trujillo-Reyes et al. (2014) showed that Cu/CuO and Fe/$Fe_3O_4$ nanoparticles changed the leaf concentration of Mn and Zn in lettuce plants. In another study, Servin et al. (2013) also showed that n$TiO_2$ improved the uptake of K, Mg and Ca in cucumber plants. Hong et al. (2015) examined the effects of CuNPs on macronutrients uptake in lettuce and alfalfa plants, and showed that CuNPs changed the concentration of some elements such as Cu, Mg and K. Our results showed that AgNPs, especially at high concentrations, reduced the leaf concentration of macro-elements (P, Ca and Mg) and microelements (Mn, Zn, Cu and B) in black cumin plants. These results are in line with the findings of Zuverza-Mena et al. (2016) in *Raphanus sativus*. The AgNPs-induced decreases in elemental concentrations can be due to the inhibition of ionic channels and thereby reducing their absorption by increasing the concentration of Ag. In addition, it has been shown that Ag ion

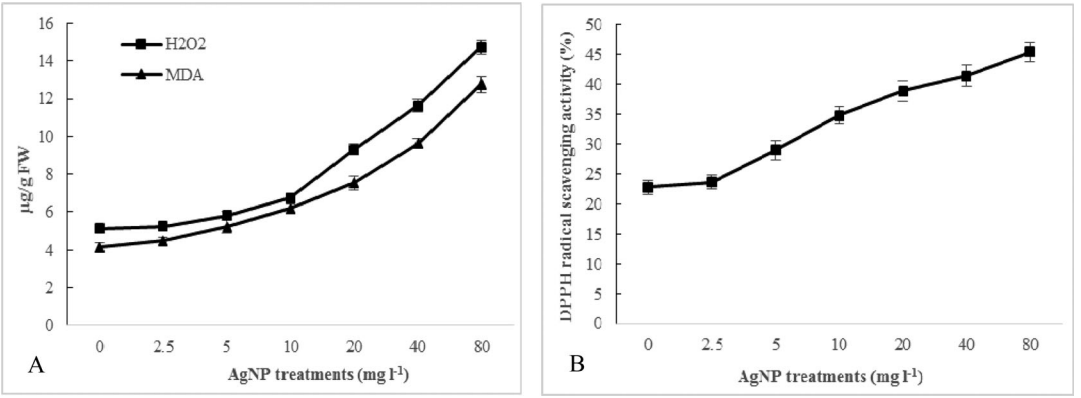


**Figure 4.** Leaf concentrations of $H_2O_2$ and MDA (A, in µg $g^{-1}$ FW), and DPPH radical scavenging activity (B, in %) in *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

damages cell membranes and disrupts cell ion homeostasis in cells (Magesky and Pelletier 2015), therefore affecting negatively nutrients uptake. Since the mechanisms of the effect of the AgNPs on the accumulation of nutrient elements are unclear, further research is needed to understand the role of AgNPs in nutrient uptake.

### *The contents of hydrogen peroxide ($H_2O_2$), lipid peroxidation, total antioxidant and activity of antioxidant enzymes*

A progressive increase was observed in $H_2O_2$ contents with increasing AgNPs concentration, and the highest increase was recorded by 187% at 80 mg $L^{-1}$ AgNPs when compared the controls (Figure 4A). The leaf MDA content of black cumin plants at 5 mg $L^{-1}$ of AgNPs began to rise and reached maximum $H_2O_2$ contents at 80 ppm AgNPs (Figure 4A). With the increasing of AgNPs concentration, total antioxidant enhanced as shown in Figure 4B. Total antioxidant increased by 27, 53, 71, 82 and 99% at 5, 10, 20, 40 and 80 mg $L^{-1}$ AgNPs, respectively compared to the controls (Figure 4B).

Heavy metals, such as Ag, generate a variety of free radicals that cause oxidative stress in plants. Therefore, the heavy metals-induced oxidative stress damages the various parts of the cells, especially cell membranes, and causes the oxidation of membrane lipids (Zhang et al. 2005). The results showed that the increase in the AgNPs concentration enhanced contents of $H_2O_2$, MDA and DPPH scavenging activity compared to control treatment, indicating the toxicity of AgNPs on the growth of black cumin plants. Karami-Mehrian, Heidari, and Rahmani (2015) and Nair and Chung (2014) obtained similar results from the toxicity of AgNPs in tomato and rice seedlings, respectively.

An increasing trend observed in SOD activity with increasing AgNPs concentration, and the highest SOD activity obtained at 80 mg $L^{-1}$ AgNPs when compared to the controls (Figure 5A). Application of AgNPs significantly increased the activity of APX enzyme at all AgNPs levels, except at 2.5 mg $L^{-1}$ AgNPs, compared to non-AgNPs treatments. However, the highest activity of APX enzyme recorded by 193% at 80 mg $L^{-1}$ AgNPs compared to the controls (Figure 5B). The activity of CAT enzyme in the leaves of black cumin plants at 5 mg $L^{-1}$ AgNPs began to rise and reached maximum CAT activity at 40 mg $L^{-1}$ AgNPs, then, decreased at 80 mg $L^{-1}$ AgNPs (Figure 5C). An increasing trend observed in the activity of POD enzyme with increasing AgNPs concentration, and the highest increase recorded at 80 mg $L^{-1}$ AgNPs when compared to the controls (Figure 5D). The SOD enzyme reduced the production of radical hydroxyl by scavenging the anion superoxide, which, as a highly reactive compound, damages important macromolecules

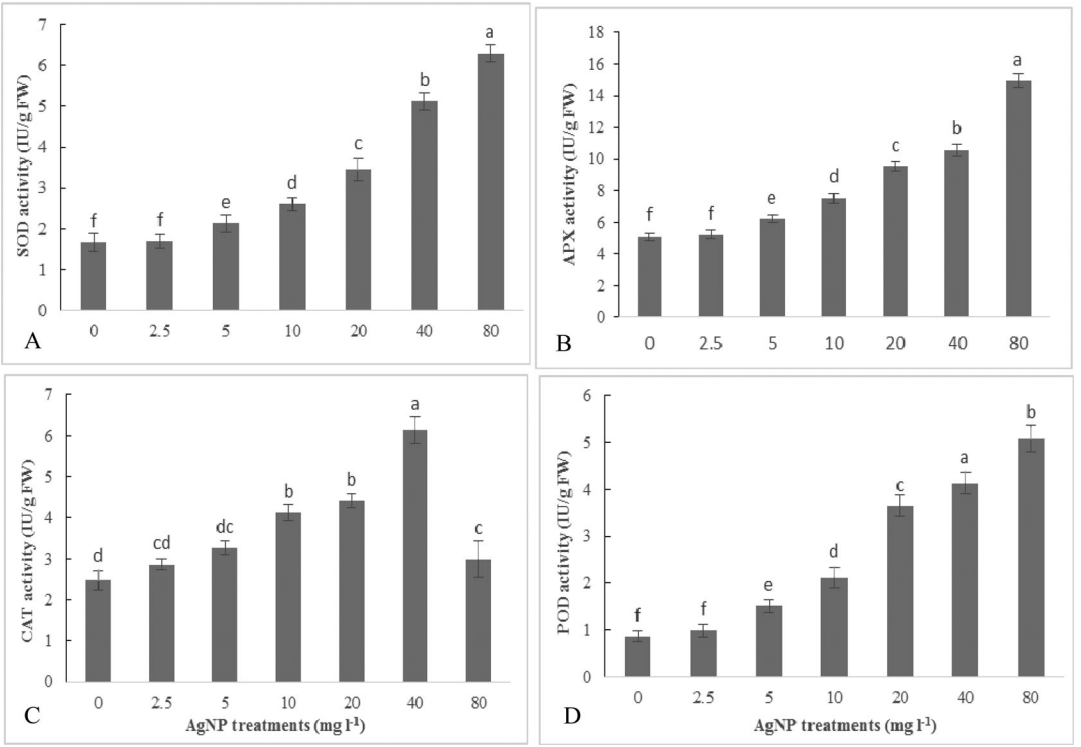


**Figure 5.** Activities (in IU $g^{-1}$ FW) of the enzymes SOD (A), APX (B), CAT (C) and POS (D) in leaf extracts of *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

such as DNA, membranes and proteins (Zare et al. 2020). Sharma et al. (2012) and Karami-Mehrian, Heidari, and Rahmani (2015) showed that the application of AgNPs induced oxidative stress and increased the activity of antioxidant enzymes such as SOD, APX, CAT and POD in mustard and tomato plants, respectively. Increasing the activity of antioxidant enzymes under heavy metal stress has also been shown in several studies (Li et al. 2006; Zhang et al. 2005). The POD enzyme plays an important role in reducing the lipid oxidation and thus retaining the membrane stability under stressful conditions. Increasing the activity of CAT, APX and POX enzymes can play an important role in reducing the negative effects of oxidative stress induced by AgNPs treatments. These results corroborate with the findings of Ghorbanpour and Hatami (2015) and Karami-Mehrian, Heidari, and Rahmani (2015), who indicated that application of AgNPs treatments induced the activity of CAT and POD enzymes in geranium and tomato plants, respectively. Reducing the activity of the CAT enzyme at high concentrations of AgNPs (80 mg $L^{-1}$) may be due to the inactivation of the CAT by the nonspecific degradation of the enzyme proteins or the production of excess free radicals (Filek et al. 2008). The decrease of CAT activity at 80 mg $L^{-1}$ AgNPs indicates that POD, APX and SOD enzymes are more effective than CAT enzyme in diminishing oxidative stress induced by AgNPs in black cumin plants.

## *Activity and expression of CHS and PAL enzymes*

The activity of CHS enzyme in black cumin plants at 10 mg $L^{-1}$ of AgNPs started to rise and reached maximum activity at 40 mg $L^{-1}$ AgNPs, then, decreased by 4% at 80 mg $L^{-1}$ AgNPs when compared to 40 mg $L^{-1}$ AgNPs (Figure 6A). Application of AgNPs significantly increased the activity of PAL enzyme at all AgNPs levels, except at 2.5 mg $L^{-1}$ AgNPs, compared to non-

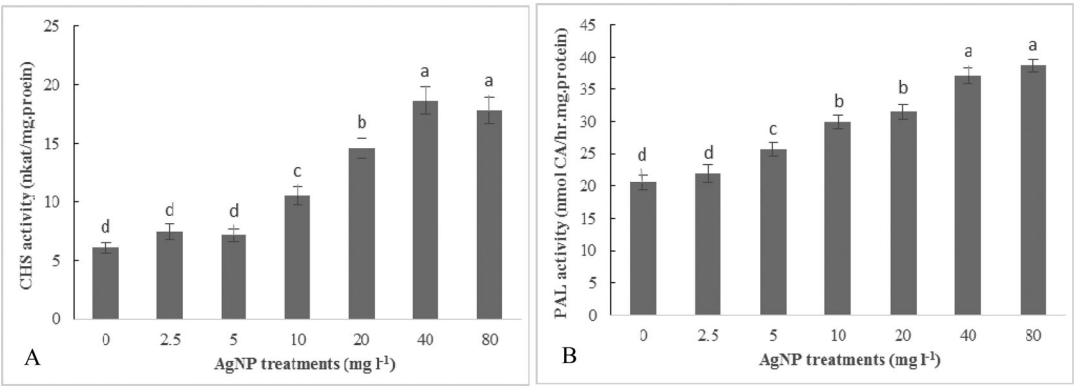


**Figure 6.** Activities of the enzymes CHS (A; in nkat/mg Pr) and PAL (B; in nmol CA/hr mg Pr) in leaf extracts of *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

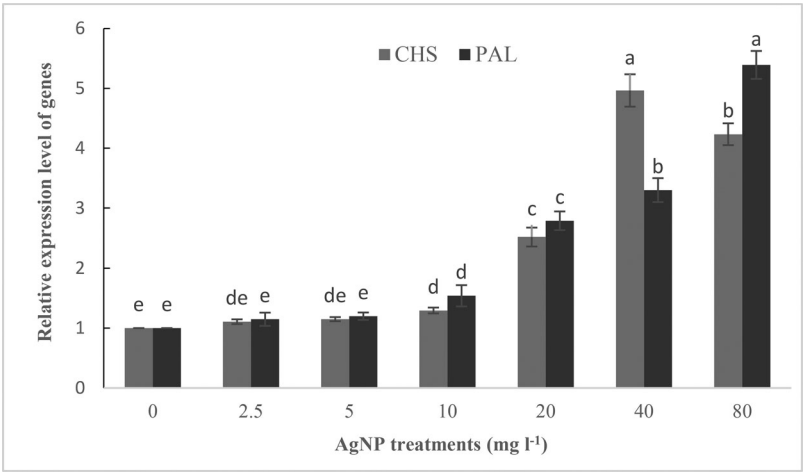


**Figure 7.** Relative expression of the *CHS* and *PAL* genes in leaves of *Nigella sativa* L. plants grown for 4 weeks with different concentrations of AgNPs (in mg $L^{-1}$). Treatments (150 mL/pot) were applied every two days. Values (means ± SD, n = 3) followed by the same letter are not significantly different ($P < 0.05$; LSD test).

AgNPs treatments. However, the highest activity of PAL enzyme recorded by 89% at 80 mg $L^{-1}$ AgNPs when compared to the controls (Figure 6B). As the concentration of AgNPs increased, expression of both *CHS* and *PAL* genes significantly increased in comparison to control treatments. The highest expression of *CHS* and *PAL* genes observed at 40 and 80 mg $L^{-1}$ AgNPs by 4- and 4.4-fold, respectively, more than control treatments (Figure 7). The enzymes PAL and CHS play key role in the production of phenylpropanoid compounds and in the plant responses to biotic and abiotic stresses. The results showed that with increasing AgNPs concentration, the activity and expression level of both PAL and CHS enzymes increased and, these results indicated the role of these enzymes in response to oxidative stress induced by AgNPs. Increases in the activity of PAL and CHS has been reported under various biotic and abiotic stresses, including heavy metal, bacterial or fungal infection, chilling and salinity (Macdonald and D'cunha 2007; Gholizadeh and Baghban-Kohnehrouz 2010; Kosyk et al. 2017; Dao, Linthorst, and Verpoorte 2011). In this study, the effects of different concentrations of AgNPs on the activity and expression level of PAL and CHS enzymes in the black cumin plants were also found. Increasing the expression of *PAL* and *CHS* genes can be in response to cellular damage caused by higher

concentrations of AgNPs. It can be suggested that increasing activity and transcription of *PAL* and *CHS* enzymes in black cumin plants can be related to the role of these enzymes in the plant responses to AgNPs toxicity.

## Conclusion

The results of our study demonstrated that the application of AgNPs had significantly phytotoxic effects in *Nigella sativa*. Treatments with an AgNPs concentration higher than 20 mg $L^{-1}$ led to reduced growth and biomass and induced oxidative stress, as judged by the $H_2O_2$ and MDA leaf concentratins. Increasing ROS by application of AgNPs as well as increased activity of enzymes involved in plant defense system and the up-regulation of *PAL* and *CHS* genes illustrated excess oxidative stress as well as the activation of plants defense mechanisms to decrease the destructive effects of oxidative stress under AgNPs treatments.

## Conflict of interest

Authors declare no conflict of interest.

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