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RESEARCH PAPER

Physiological Effects of Silver Nanoparticles and Silver Nitrate Toxicity in *Triticum aestivum*

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Abstract Nowadays, nanoparticles (NPs), especially silver nanoparticles (AgNPs), are introduced in a growing number of commercial products and their production is released into the environment and may adversely influence on organisms. Up to now, limited studies are available about toxicity effects of NPs on higher plants. In this work, the effects of AgNPs in comparison with silver nitrate ($AgNO_3$) on some physiological parameters of the wheat (*Triticum aestivum)* were investigated. Silver nanoparticles and $AgNO_3$ at 10 and 100 mg^{-1} L concentrations significantly decreased the fresh and dry weight of roots and shoots. The results showed that AgNPs and $AgNO_3$ decreased plant tissue chlorophyll "*a*" and "*b*," carotenoid and total protein contents of the leaves significantly. Both $AgNO_3$ and AgNPs treatments also increased the amount of proline, lipid peroxidation and catalase activity of wheat seedling tissues. Results of this work revealed that exposure to silver nanoparticles and silver ions might cause negative aspects and toxicity problems in plants.

Keywords Physiological effects · Silver nanoparticles toxicity · Wheat

1 Introduction

Heavy metal toxicity has become a universal threat to all life forms including plants, animals and eventually humans. The unwanted growth of toxic heavy metals, mainly due to different anthropogenic activities, leads to heavy metal pollution that can have disastrous and unexpected effects on ecosystems (Das et al. 1997; Duruibe et al. 2007; Foy et al. 1978; Jarup 2003; Prasad 2004; Roesijadi 1992). Today design, production, optimization, and application of nanoparticles are interesting areas of research. The reason for this interest is the different physical and chemical properties of particles in the nanoscale (Stone et al. 2010) that are increasingly used in diverse fields such as biomedical sciences, medicine, drug delivery, gene therapy, cell targeting, magnetic, optic, mechanic, catalysis and electric devices (Bao et al. 2013; Daniel and Astruc 2004; Luo et al. 2006; Ramalingam et al. 2012; Sharma et al. 2012; Yoo et al. 2011). As for improvement of any kind of new technology, there are concerns about the potentially adverse effects of nanoparticles on human, animal, plant and the general environment. Existing investigations suggest that a complete understanding of the potential for health or environmental risks of nanoparticles does not exist (Morgan 2005). Increasing applications of nanoparticles highlights require elucidating biological effects and nanotoxicity of nanoparticles in organisms (Fabrega et al. 2011; Shaw and Handy 2011; Stampoulis et al. 2009). Indeed, in spite of the considerable number of studies on the toxicity of nanoparticles in animal and bacteria, limited studies are available in higher plants (Ma et al. 2010; Monica and Cremonini 2009). Silver ions are one of the most toxic heavy metals and silver nanoparticles (AgNPs) are one of the most common nanomaterials that have a tendency to be released into the environment. Both silver ions and silver nanoparticles are toxic, but there is some evidence to show that AgNPs toxicity depends on the release of silver ions (Beer et al. 2012; Kittler et al. 2010). Nanomaterials risk assessment of the environment must be estimated based on

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investigations to clarify all related aspects of the concern, but it is difficult for nanomaterials as few studies have been done. Many effects of nanomaterials on ecosystems remain unknown (Dupuy and Mills 2004; Holden et al. 2012). Negative effects of AgNPs the same as Ag ions in plant are significant, such as degradation of the plasma membrane and changes in membrane permeability; failure of the proton motive force and inhibition of the ATP synthesis; inhibition of enzyme activity by binding to –SH groups of amino acids; denature ribosome and inhibiting protein synthesis; creation of reactive oxygen species (ROS) and damaging vital macromolecules (Kaegi et al. 2011; Kumari et al. 2009a; Monica and Cremonini 2009; Nair et al. 2010). Toxicity of silver nanoparticles on plants depends on particle's properties such as size, shape, aggregation state, surface coatings, concentration, exposure time and the types of compounds. In addition, type, age and development stage of plants have significant impacts on plant resistance against toxicity of nanoparticles. To date, there have been only a few reported studies of the impact of AgNPs on plants such as *Phaseolus vulgaris* (Najafi et al. 2014), *Sorghum bicolor* (Lee et al. 2012), *Lemna gibba* (Oukarroum et al. 2013), *Lolium multiflorum* (Yin et al. 2011), *Arabidopsis thaliana* (Geisler-Lee et al. 2012; Kaveh et al. 2013), *Allium cepa* (Kumari et al. 2009b), *Eruca sativa* (Vannini et al. 2013) and *Oryza sativa* (Mazumdar and Ahmed 2011). It has been shown that AgNPs can have positive and negative effects on plant growth. Recently, more and more innovative applications for nanomaterials have been proposed and evaluated. In recent years, silver nanoparticles have become more widely used in various technologies and incorporated into a wide range of consumer products that take advantage of their attractive optical, conductive and antibacterial properties. In the present study, we experienced some physiological parameters of young wheat plants affected by two concentrations of AgNPs and $AgNO_3$.

2 Materials and Methods

2.1 Plant Materials and Growth Conditions

Seeds of wheat (*Triticum aestivum* L. var. Chamran) were obtained from Zarghan Agricultural Research Center, Iran. These seeds were kept in a cool and dark place in the laboratory. Seeds were surface-sterilized by soaking in 5% (w/v) sodium hypochlorite (NaOCl) for 10 min. They were washed three times with distilled water and air-dried on filter papers. Seeds were allowed to germinate in the dark at 25 °C on wet papers in the plate. Five-day-old seedlings (20) were transferred into small plastic containers filled with perlite and Hoagland nutrient solution (pH 6.2). Wheat seedlings were grown in the growth chamber set at 16 h/8 h light–dark periods. Three replicates were used for each treatment.

2.2 Silver Nitrate and Silver Nanoparticle Treatments

Silver nanoparticles with average sizes of 20 nm and 99.99% purity were purchased from US Research Nanomaterials, Inc. [USA] (Fig. 1). Using Hoagland nutrient solution as a solvent, two concentrations (10 and 100 mg^{-1} L) of AgNPs and $AgNO_3$ were prepared. The dissolved particles were dispersed by a high-power probe-type Sonicator (Misonix, QSonica LLC, Newton, USA) for 30 min. Hoagland nutrient solution was used as a control. Wheat seedlings (21-day old) were collected one week after the beginning of treatments, washed with double-distilled water and used for analyses. Parameters analyzed were roots and shoots, fresh and dry weight, chlorophyll and carotenoid pigments, catalase activity, lipid peroxidation, proline and leaves total protein contents.

2.3 Seedlings Fresh and Dry Weight

After washing with distilled water, wheat seedlings were blotted dry on tissue papers, and after taking their fresh weights, they were dried at 70 °C for 48 h for dry weight analysis.

2.4 Photosynthetic Pigment Measurement

The contents of photosynthetic pigments were determined according to Wellburn and Lichtenthaler (1984). Fresh leaf tissue (200 mg) was weighted and powdered using liquid nitrogen. After adding 80% acetone, the volume was brought to 25 mL. The resulting solution was centrifuged at 4800 rpm for 20 min. The supernatant was used for

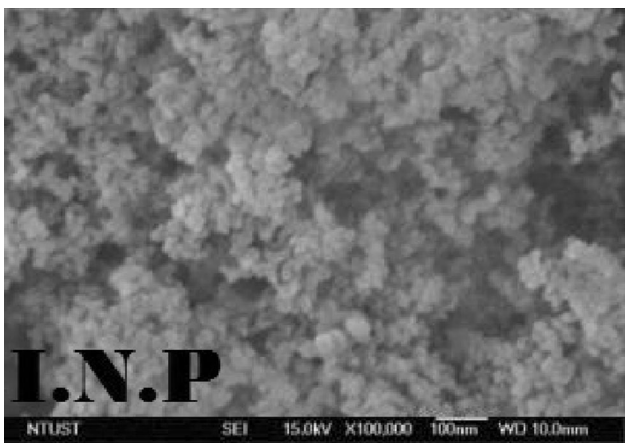

Fig. 1 Scanning electron microscopy (SEM) image of silver nanoparticles (average size was 20 nm)

measuring the chlorophyll *a*, *b* and carotenoid. The absorbance of the clear supernatant was read at 645 nm (chlorophyll *b*), 663 nm (chlorophyll *a*), and 470 nm (carotenoid).

2.5 Protein Determination

Soluble protein was quantified according to Bradford (1976). Samples were homogenized in 0.1 M Na-phosphate buffer (pH 7; 1:5 w/v). After adding the reagent, absorbance was recorded at 595 nm and the concentration was calculated using a calibration curve made with bovine serum albumin. Protein concentrations were determined after realizing a standard curve.

2.6 Proline Determination

Free proline contents were measured by the method of Bates et al. (1973). Fresh leaf tissue (100 mg) was homogenized in 3% (w/v) sulphosalicylic acid, and proline was estimated by ninhydrin reagent (0.125 g of ninhydrin in 2 mL orthophosphoric acid 6 M, and 3 mL of acetic acid). The earned chromophore was extracted from liquid phase with toluene, and remarking organic layer was read at 520 nm. Proline concentrations were determined after realizing a standard curve.

2.7 Catalase Determination

Catalase (CAT) activity was determined by decomposition of H_2O_2 and was measured by assessing the decrease in absorbance at 240 nm (Aebi 1984). The reaction mixture contained 200 mM KPO_4 buffer (pH 7.0), 30 mM H_2O_2 and enzyme extract. Catalase activity was calculated through Aebi formula, and H_2O_2 decomposed g^{-1} FW min^{-1} was defined as a unit of CAT.

2.8 Lipid Peroxidation

The lipid peroxidation was measured in the leaf tissues by estimating the malondialdehyde (MDA) as an indicator of lipid peroxidation. Malondialdehyde was assayed by thiobarbituric acid reactive substances contents (Heath and Packer 1968).

2.9 Statistical Analysis

The experimental designs were randomized complete block, and each value reported is the average of three repeats. The raw data were imported into Microsoft Excel 2007 program for calculations and graphic representation. SPSS (version 16.0) software was used for analysis of variance. Quantitative changes in parameters were analyzed through analysis of variance (one-way ANOVA), with Duncan's multiple range tests at $p \leq 0.05$ to find out significant differences among treatments. All results are presented as the mean ± standard deviation (SD).

3 Results and Discussion

3.1 Effects of AgNPs and $AgNO_3$ on Plant Growth

After 1 week of silver ions and silver nanoparticles exposure, the fresh and dry weights of root and shoot of *T. aestivum* L. were measured (Tables 1, 2 or Figs. 2, 3). Data represent the mean ± standard deviation (SD). Data with different letters are significantly different.

A clear and significant growth inhibition was observed in wheat plants exposed to $AgNO_3$ and AgNPs treatments. Results showed that shoot fresh weight, root fresh weight and shoot + root fresh weight in all treatments led to a significant decrease according to the increase in the concentration of $AgNO_3$ and AgNPs compared with the control, with the exception of treated plants at 10 mg^{-1} L $AgNO_3$ concentration. Shoot + root fresh weight of $AgNO_3$- and AgNPs-treated plants decreased about 52 and 51%, at 10 mg^{-1} L concentration and 68 and 66% at 100 mg^{-1} L concentration compared with the control, respectively. The maximum reduction in the root fresh weight was observed at 100 mg^{-1} L $AgNO_3$. Shoot + root dry weight of $AgNO_3$- and AgNPs-treated plants was decreased about 57 and 46%, at 10 mg^{-1} L concentration and 69 and 58% at 100 mg^{-1} L concentration compared to the control, respectively. Maximum reduction in root dry weight was observed at 100 mg^{-1} L concentration of both $AgNO_3$ and AgNPs, and its value was not significantly different.

As the results showed, AgNPs and $AgNO_3$ reduced the fresh and dry weights (biomass) of wheat approximately in all concentrations (Tables 1, 2 or Figs. 3, 4). Our results supported the outcomes found in the study of the effects of heavy metals and nanoparticles on other plant (Fritioff et al. 2005; Glick 2003; Jiang et al. 2012; Oukarroum et al. 2012). However, for fresh and dry weight, the values of $AgNO_3$ were lower than AgNPs; therefore, growth inhibition of $AgNO_3$ was significantly stronger than AgNPs. $AgNO_3$ and AgNPs possibly by the decrease in leaf chlorophyll and photosynthesis led to a significant decrease in dry and fresh weights of wheat root and shoot.

3.2 Effects of AgNPs and $AgNO_3$ on Contents of Photosynthetic Pigments

Contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoid) in wheat are presented in Table 3 and Figs. 5 and 6. A significant decrease in chlorophyll *a*, *b* and carotenoid contents in plants (1-week

Table 1 Contents shoot fresh weight, root fresh weight, shoot + root fresh weight (mg) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

Concentration (mg L^{-1})	Shoot fresh weight	Root fresh weight	Shoot + root fresh weight
Control	0.435 ± 0.021a	0.109 ± 0.005a	0.5457 ± 0.015a
$AgNO_3$ 10	0.183 ± 0.011b	0.081 ± 0.003b	0.264967 ± 0.014b
$AgNO_3$ 100	0.120 ± 0.006c	0.058 ± 0.003c	0.179607 ± 0.002d
AgNPs 10	0.182 ± 0.005b	0.089 ± 0.004a	0.27209 ± 0.005b
AgNPs 100	0.120 ± 0.005c	0.069 ± 0.004b	0.1901 ± 0.001c

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$)

Table 2 Contents of shoot dry weight, root dry weight and shoot + root dry weight (mg) *in T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for period of 7 days

Concentration (mg L^{-1})	Shoot dry weight	Root dry weight	Shoot + root dry weight
Control	0.053 ± 0.002a	0.011 ± 0.0003a	0.064 ± 0.002a
$AgNO_3$ 10	0.020 ± 0.0007b	0.008 ± 0.0002b	0.028 ± 0.0009b
$AgNO_3$ 100	0.012 ± 0.0003c	0.007 ± 0.0002c	0.020 ± 0.0003d
AgNPs 10	0.026 ± 0.001b	0.009 ± 0.0002a	0.035 ± 0.0008b
AgNPs 100	0.020 ± 0.0007c	0.006 ± 0.0002b	0.027 ± 0.0005c

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$)

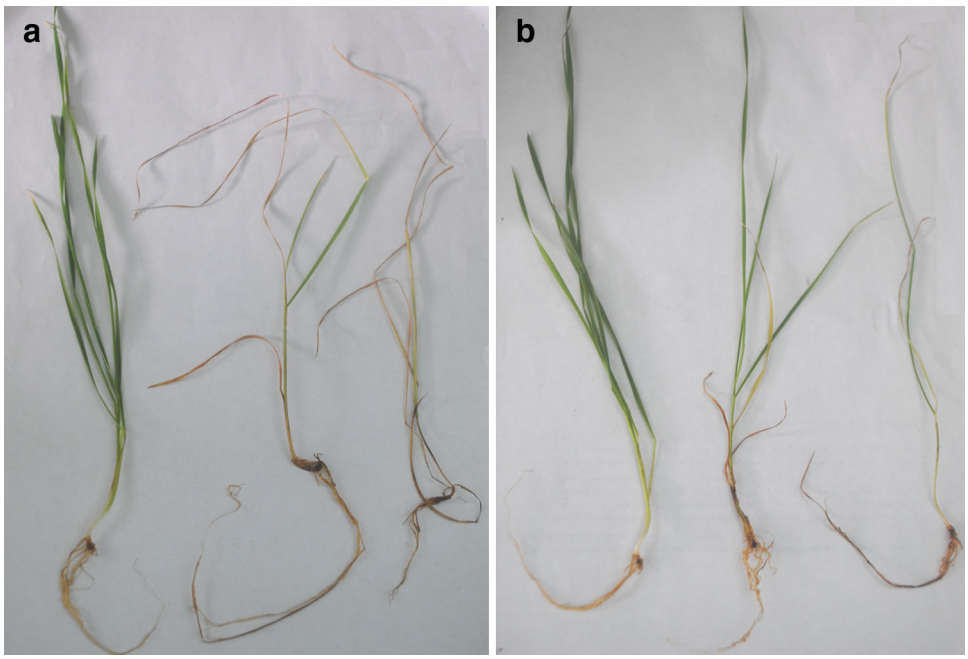

Fig. 2 Color changes in leaves (chlorosis) of wheat exposed to **a** $AgNO_3$ at 0, 10, and 100 mg^{-1} L concentrations and **b** AgNPs at 0, 10, and 100 mg^{-1} L concentrations (*left* to *right*, respectively)

exposed to silver ions and silver nanoparticles treatments) compared with the control was observed. Wheat leaves showed signs of chlorosis after exposure to treatments according to the increase in silver ions and silver nanoparticles concentration, while the control plant appeared to be healthy and green (Fig. 2). The total chlorophyll contents of $AgNO_3$- and AgNPs-treated plants decreased around 48 and 31%, at 10 mg^{-1} concentration and 77 and 64% at 100 mg^{-1} concentration compared with the control, respectively. The same result was observed in the contents of carotenoids. The carotenoid contents of $AgNO_3$- and AgNPs-treated plants decreased approximately 67 and 50%, at 10 mg^{-1} concentration and 79 and 68% at 100 mg^{-1} concentration compared with the control, respectively. The decrease in the chlorophyll and carotenoid in $AgNO_3$ treatments was significantly more than that of the AgNPs at the same concentration.

As shown in Table 3 and Figs. 5 and 6, photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoid) decrease significantly in wheat on exposure to $AgNO_3$ and

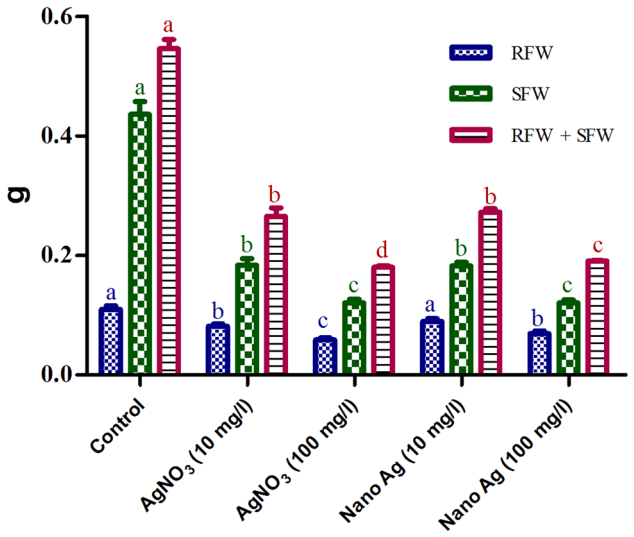

Fig. 3 Contents shoot fresh weight, root fresh weight, shoot + root fresh weight (mg) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for period of 7 days (g: gram)

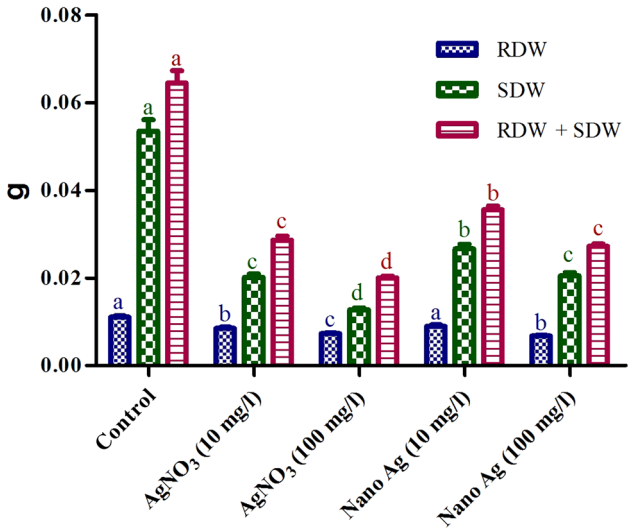

Fig. 4 Contents shoot dry weight, root dry weight, shoot + root dry weight (mg) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days (g: gram)

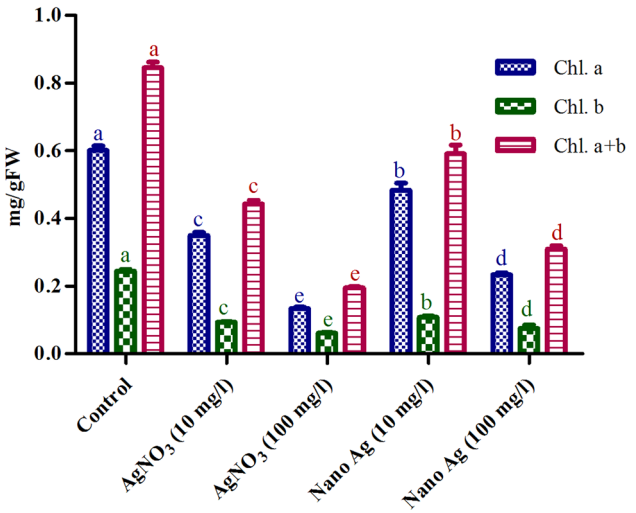

Fig. 5 Contents of chlorophyll *a*, *b*, and chlorophyll *a* + *b* (mg g^{-1} FW) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

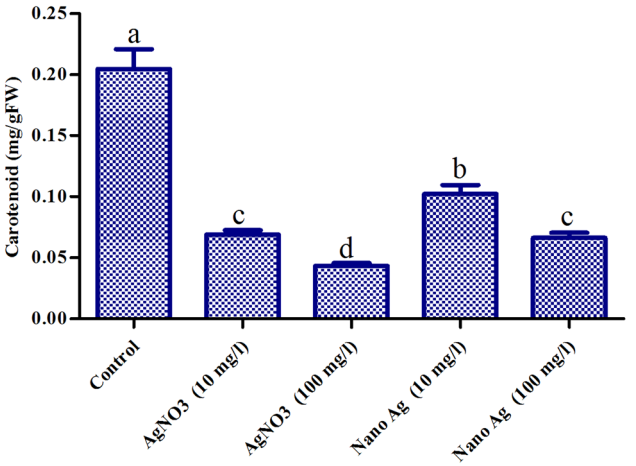

Fig. 6 Contents of carotenoids (mg g^{-1} FW) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

Table 3 Contents of chlorophyll *a*, *b*, chlorophyll *a* + *b* and carotenoids (mg g^{-1} FW) in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

Concentration (mg L^{-1})	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	Carotenoids
Control	0.601 ± 0.013a	0.244 ± 0.005a	0.845 ± 0.016a	0.204 ± 0.016a
$AgNO_3$ 10	0.349 ± 0.009c	0.093 ± 0.001c	0.443 ± 0.010c	0.068 ± 0.003c
$AgNO_3$ 100	0.133 ± 0.003e	0.061 ± 0.002e	0.195 ± 0.003e	0.043 ± 0.002d
AgNPs 10	0.483 ± 0.021b	0.107 ± 0.004b	0.591 ± 0.025b	0.102 ± 0.007b
AgNPs 100	0.234 ± 0.005d	0.075 ± 0.009d	0.309 ± 0.010d	0.066 ± 0.004c

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$)

Table 4 Contents of proline, lipid peroxidation, catalase and total protein in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

Concentration (mg L^{-1})	Proline	Lipid peroxidation	Catalase	Total protein
Control	25.943 ± 0.591d	31.206 ± 1.359c	0.010 ± 0.001d	5.407 ± 0.703a
$AgNO_3$ 10	32.663 ± 1.242b	42.693 ± 1.074b	0.016 ± 0.001b	3.924 ± 0.534b
$AgNO_3$ 100	36.263 ± 1.343a	58.476 ± 1.860a	0.024 ± 0.002a	2.779 ± 0.534c
AgNPs 10	28.266 ± 1.414c	35.213 ± 2.004c	0.013 ± 0.0006c	4.711 ± 0.699ab
AgNPs 100	34.456 ± 1.272ab	43.493 ± 2.017b	0.018 ± 0.001b	3.776 ± 0.562bc

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$)

AgNPs. In addition, declines in leaf total chlorophyll content lead to chlorosis. Chlorosis is the most common sign of toxicity of heavy metals (Pandey and Sharma 2002). Also, the declines in total chlorophyll and carotenoid contents can be regarded as general responses related to metal toxicity (Chandra et al. 2009; MacFarlane and Burchett 2001; Radic et al. 2010; Ralph and Burchett 1998). It seems that the decrease in chlorophyll contents in tissues of metal treated plants is dependent on several factors such as disturbance in the synthesis of pigments (Shweta and Agrawal 2006), pigments degradation (Prasad et al. 2001; Somashekaraiah et al. 1992), direct inhibition of enzymatic steps coupled with chlorophyll biosynthesis, protein composition of photosynthetic membranes (Mysliwa-Kurdziel et al. 2004; Prasad and Strzałka 1999) and avoiding the arrangement of photoactive protochlorophyll reductase enzyme complex and aminolevulinic acid (ALA) synthesis (Oncel et al. 2000; Stobart et al. 1985). These results supported the findings of other researchers (Lagriffoul et al. 1998; Oukarroum et al. 2012; Ralph and Burchett 1998; Saison et al. 2010; Wei et al. 2010).

3.3 Effects of AgNPs and $AgNO_3$ on Contents of Proline

Proline contents of treated and untreated wheat are shown in Table 4 and Fig. 7. According to the increase in $AgNO_3$ and AgNPs concentration, the proline contents of leaf increased significantly compared to the control. The maximum increase in proline contents was observed at 100 mg^{-1} L in both $AgNO_3$ and AgNPs. Proline contents of $AgNO_3$- and AgNPs-treated plants increased approximately 25 and 8%, at 10 mg^{-1} L concentration and 39 and 32% at 100 mg^{-1} L concentration compared with the control, respectively.

Results indicated that the accumulation of proline in leaves of wheat increases significantly with rising $AgNO_3$ and AgNPs concentration. Proline as an amino acid is an important osmolyte and accumulates in a broad range of organisms ranging from bacteria to higher plants on exposure to abiotic stress throughout the adaptation to a diversity of types of environmental stress, such as drought, cold, salinity, high temperature, nutrient lack and exposure

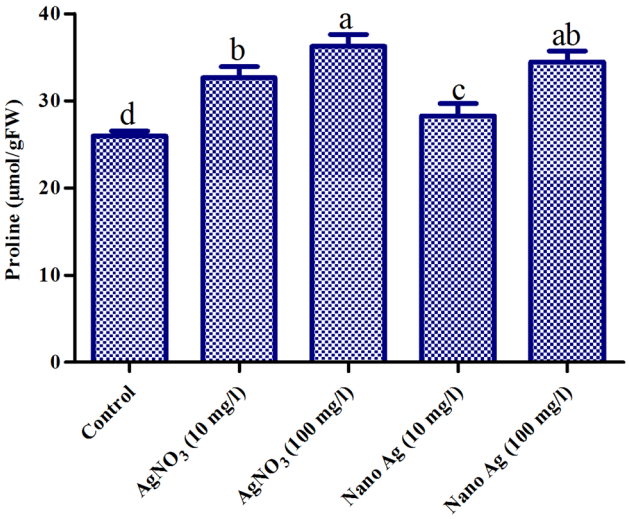

Fig. 7 Contents of proline in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

to heavy metals (Ashraf and Foolad 2007b). Proline in various ways, including acting as a metal chelator (Sharma and Dubey 2005), detoxification of reactive oxygen species (ROS) such as hydroxyl radical and singlet oxygen (Szabados and Savouré 2010) and osmoprotectant (Ashraf and Foolad 2007a; Tamayo and Bonjoch 2001), protection for the enzymes against denaturation and stabilization of protein synthesis (Sanchez-Partida et al. 1992; Shah and Dubey 1997) alleviates heavy metal toxicity.

In addition, proline supports mitochondrial oxidative phosphorylation for protecting natural generation of ATP (Ashraf and Foolad 2007b; Siripornadulsil et al. 2002) and acts as an inhibitor of lipid peroxidation (Hara et al. 2003; Mehta and Gaur 1999). Our results were also similar to results obtained by other investigators (Jiang et al. 2012; John et al. 2009; Kastori et al. 1992; Mehta and Gaur 1999).

3.4 Effects of AgNPs and $AgNO_3$ on Lipid Peroxidation

The effect of special treatment of silver ions and silver nanoparticles with respect to the amount of lipid peroxidation was significant (Table 4; Fig. 8). The level of MDA

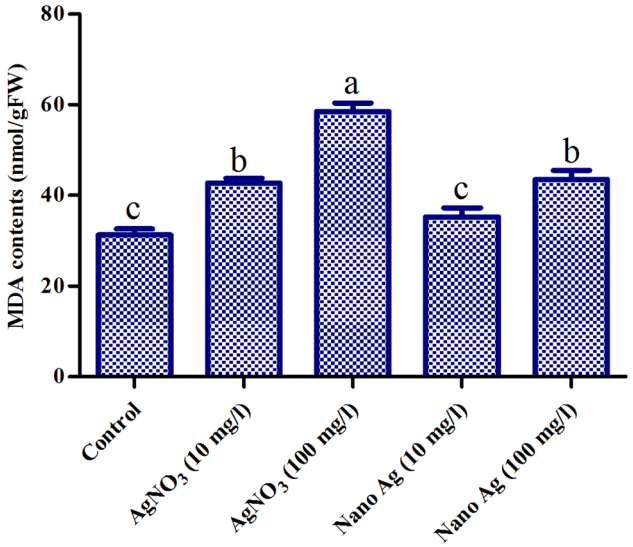

Fig. 8 Contents of lipid peroxidation in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

formation indicates the level of free radical production and lipid peroxidation (Dexter et al. 1989; Mak and Weglicki 1988). The smallest amount of lipid peroxidation was realized on control, most of which was at concentration 100 mg^{-1} L of $AgNO_3$. The increase in the lipid peroxidation in $AgNO_3$ treatments was significantly more than that of the AgNPs of the same concentration. The difference between lipid peroxidation of concentration 10 and 100 mg^{-1} L of silver ions and 100 mg^{-1} L of silver nanoparticles was significant compared with the control. Results showed no significant effect on lipid peroxidation of low concentration (10 mg^{-1} L) of AgNPs. The amount of lipid peroxidation of $AgNO_3$ and AgNPs treated plants increased approximately 36 and 12%, at 10 mg^{-1} L concentration and 87 and 39% at 100 mg^{-1} L concentration compared with the control, respectively.

Malondialdehyde (MDA) contents, a product of lipid peroxidation, are considered as an indicator of oxidative damage and peroxidation of membrane lipids in plants (Nacif de Abreu and Mazzafera 2005; Xu et al. 2006). The cell membrane is usually the main site of the attack by any heavy metal in a plant cell. In our experiments, significant increases in MDA concentration with increasing the $AgNO_3$ and AgNPs concentration were observed that indicate a negative effect of heavy metals on membrane integrity and permeability. Produced free radicals as a result of $AgNO_3$ and AgNPs can attack the unsaturated fatty acid side chains of membrane lipids and cause the formation of lipid hydroperoxides (Halliwell and Chirico 1993). This result was similar to the consequences obtained by other investigators (Gallego et al. 1996; Ghosh et al. 2010; Panda et al. 2003; Sayes et al. 2005; Zhang et al. 2007).

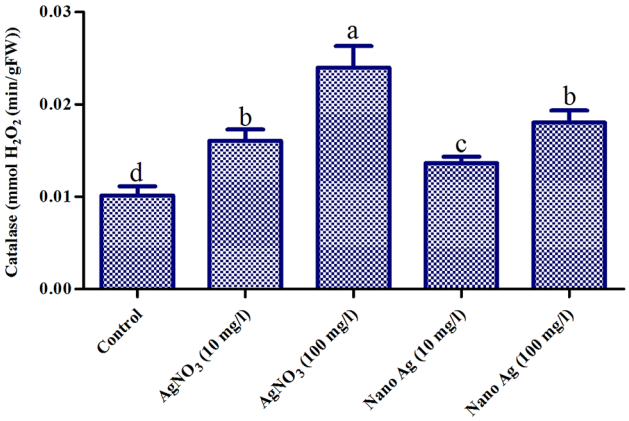

Fig. 9 Contents of catalase activity in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

3.5 Effects of AgNPs and $AgNO_3$ on Catalase Activity

A significant increase in catalase activity was observed in response to the increase of $AgNO_3$ and AgNPs concentrations (Table 4; Fig. 9). The highest value of catalase activity was recorded at 100 mg^{-1} L $AgNO_3$. Catalase activity contents of $AgNO_3$- and AgNPs-treated plants increased approximately 60 and 30%, at 10 mg^{-1} L concentration and 240 and 80% at 100 mg^{-1} L concentration compared with the control, respectively. The increase in the amount of catalase activity in treatments was significantly more than (twofold at low concentration and threefold at high concentration) that of the AgNPs at the same concentration.

The result showed that the catalase activity of leaves significantly increased in response to the increase of $AgNO_3$ and AgNPs concentrations. Both AgNPs and $AgNO_3$ induce antioxidant enzyme, but this induction in $AgNO_3$ treatments was significantly more than the AgNPs at the same concentration. The activities of antioxidant enzyme have generally increased during abiotic stress, such as chilling, drought, high temperature, salt and heavy metal stress (Baker and Orlandi 1995; Mittler 2002), and correlated with enhanced cellular protection of reactive oxygen species. Catalase is an important antioxidant which protects plants by suppressing oxidative injury and assists as a reactive species scavenger. These consequences were similar to the results obtained by other researchers (Du et al. 2011; Gallego et al. 1996; Krishnaraj et al. 2012; Zhang et al. 2007).

3.6 Effects of AgNPs and $AgNO_3$ on Contents of Total Protein

According to increasing concentrations of $AgNO_3$ and AgNPs, total protein content was decreased. Protein

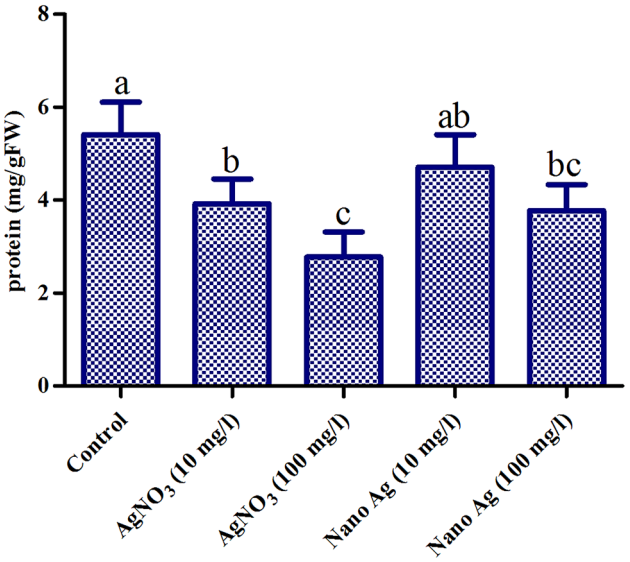

Fig. 10 Contents of protein in *T. aestivum* plants subjected to $AgNO_3$ and AgNPs stress for a period of 7 days

contents of $AgNO_3$- and AgNPs-treated plants decreased by 28 and 23%, at 10 mg^{-1} L concentration and by 49 and 31% at 100 mg^{-1} L concentration compared with the control, respectively. Amount of total protein in response to the AgNPs at 10 mg^{-1} L concentration showed no significant change as compared to control. The decrease in the total protein by $AgNO_3$ was more than by AgNPs at the same concentration (Table 4; Fig. 10).

Under heavy metals and nanoparticles, oxidative stresses lead to generation of reactive oxygen species and degeneration of protein (Choi and Hu 2008; Rana 2008; Wan et al. 2012; Xia et al. 2008). The results showed that in most cases, $AgNO_3$ had more negative effects than AgNPs. Although both dissolved silver and AgNPs can provoke the production of reactive oxygen species, AgNPs may have direct toxic effects (Yin et al. 2011). While toxicity of AgNPs to plants is obvious, their negative effects and mechanisms on higher plants have not been completely characterized (Jiang et al. 2012).

4 Conclusion

Our study focused on the potential effect of $AgNO_3$ and silver nanoparticles on wheat. Overall, findings of this work revealed that exposure to silver nanoparticles and silver ions might cause negative aspects and toxicity problems in plants. Although silver ions and nanoparticles have many positive aspects in life, overuse and lack of knowledge about the environmental impacts can cause damage in the environment, especially to human, animals and plant health. Therefore, to better understand the toxicity effects of Ag ions and AgNPs further experiments should be performed and, at the same time, essential precautions whether in the production or consumption of these materials must be taken.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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