



ASSESSMENT OF SILVER NANOPARTICLE-INDUCED MORPHOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL ALTERATIONS IN WHEAT ROOTS

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ABSTRACT – Silver nanoparticle (Ag NP) is one of the most widely used nanoparticles in consumer products due to its antimicrobial properties. The wide usage of Ag NPs has led to their increased release into the environment and it may affect plants physiological and biochemical functions. Therefore, this study has been performed to reveal the dose-dependent (0, 0.5, 1, 5, 10 and 20 ppm) effects of 10-nm-sized Ag NPs on wheat (*Triticum aestivum*) roots after 15 d, correlating with the appearance of various biochemical and physiological stress responses. The effects of Ag NPs were examined using different experiment such as root elongation, uptake of Ag NPs, TEM analysis, lignin accumulation, callose deposition, H₂O₂ content, lipid peroxidation, activities of non-enzymatic and enzymatic antioxidants. The result showed that 10 nm-sized Ag NPs caused the inhibition of root elongation over 5 ppm, dose-dependent accumulation of Ag⁺, ultrastructural changes, lignin accumulation, callose deposition, increase of H₂O₂ content and lipid peroxidation and SOD, CAT, GPOX, APOX and GR enzymes activities altered as compared to control. As a result, 0.5 and 1 ppm Ag NPs demonstrated hormetic response, but over 5 ppm reduced plant root growth inducing the plant antioxidant defence systems.

KEYWORDS: SILVER NANOPARTICLES, WHEAT, BIOACCUMULATION, CALLOSE, LIGNIN, ROS, ANTIOXIDANT DEFENSE

INTRODUCTION

Engineered nanoparticles (NPs) are extensively used in various fields such as electronic, communication, medicine, wastewater treatment, cosmetic, agriculture and food packaging technology. According to “Global Nanotechnology Market” report, it has been forecasted that global nanotechnology market will grow around 17.7% between 2016-2022 years and expected that the technological advancements in nanotechnology market will be more common in the fields of health, agriculture, environmental and energy technologies. If the use of NPs increases at the expected pace, the environment will be inevitably affected by NP accumulation (<https://www.researchandmarkets.com/research/4dhfwh/global>).

Among the other NPs, silver NPs (Ag NPs) are one of the most widely preferred one (~24%) in consumer products in

consequence of its unique optical, electrical and thermal properties which incorporated into wide range products such as molecular diagnostic and photonic devices (Cox et al., 2017). Recently Ag NPs are also preferred for its antimicrobial properties which provide protection against bacteria. Global consumption of Ag NPs is estimated to rise rapidly due to their increasing industrial demand (Syafiuddin et al., 2017). The vast use of the Ag NPs as well as other NPs increase risk of creating nano-waste that may cause adverse effects on the living organisms and environment. The toxicity of silver nanoparticles was proven by several researches on animal, bacteria, human, algae and plant (Choi & Hu 2008; Asharani et al., 2008; Miao et al., 2009; van der Zande et al., 2012; Mustafa et al., 2015; Mustafa et al., 2016; Tripathi et al., 2017).

Plants are the prominent component of the ecosystems, and because of being a sessile organism they have an important role to determine the impacts and chain reactions of NPs in the terrestrial environment. The preliminary studies of Ag NPs effect mostly focus to plant growth parameters (El-Temsah & Joner 2012; Yin et al., 2012; Yasur & Rani 2013). In some of these studies interaction of different concentrations of Ag NPs with aqueous suspensions and soil conditions are also compared (El-Temsah & Joner, 2012). Dimkpa et al. (2012) demonstrated the effects of 10 nm Ag NPs (0.5–5 mg kg⁻¹) on seedling growth, accumulation of oxidized glutathione, and induced expression of metallothionein gene in wheat. Karimi & Mohsenzadeh (2017), showed that 20 nm sized Ag NPs affected the physiological parameters in wheat roots and leaves. Recently, Abdelsalam et al. (2018) also verified the genotoxic effects of 25–70 nm sized Ag NPs on wheat roots. It has been known that the reason of the different effects depends on nanoparticles size, shape, surface coating, concentration, exposure times and plant growth medium. Besides, the recent studies revealed that the effects of Ag NPs may vary on different plant species (Tripathi et al., 2017). Hence, the toxicity mechanism of Ag NPs becomes important to evaluate the effect on the environment and to take precautions to prevent possible damage of biological organisms. Although there are relevant case studies concerning similar parameter, detailed and comparative studies are still to be needed.

Therefore, the aim of the present study was to investigate the dose-dependent effects of 10 nm Ag NPs on one of the most important agronomic plants wheat (*Triticum aestivum* L.) roots with uptake of Ag NPs, ultrastructural changes, oxidative stress and alternative stress responses such as lignin accumulation and callose deposition to understand the potential impacts with the goal in mind for environmental monitoring.

MATERIALS AND METHODS

Plant material and Ag NPs treatment

Triticum aestivum (cv Demir 2000) seeds were obtained from The Field Crops Central Research Institute (Ankara-Turkey). The seeds were surface-sterilized and germinated on moistened filter paper in Petri dishes under a controlled growth room conditions (23 ± 2°C temperature, 45–50% relative humidity and a light intensity of 5000 lux day/night: 16/8). The wheat seedlings which reached 0.5–1 cm elongation were used for treatment and transferred into white sand with dimension range of 0.5–1 mm. Before the treatment, sand was washed with distilled water and dried. Prior to cultivating the

seedlings, 70 g sand was added in each vials mixed with 2.5 ml different concentrations of 10 nm sized Ag NPs (0.5, 1, 5, 10, 20 ppm) which were prepared from an aqueous stock solution (0.02 mg/ml) of commercial Ag NPs (Sigma-Aldrich 730785). No sonication needed because of the homogeneous solution. The dose determination was performed according to the former studies and our preliminary tests. The control (distilled water) and Ag NPs treated groups were leaved to growth in controlled growth room for 15 days. All of the seedlings were watered twice a week with 10 ml distilled water. No nutrient solution was added to avoid interaction of NPs with nutrient components. After 15 days, roots length of each plant was measured as we described previously (Yanik & Vardar, 2015). Control and treated roots collected and stored at -80 °C until analysis. Twenty seeds were used for each experimental group and all experiments were conducted in triplicate. Experiments were presented as mean of the replicates ± standard deviations (SD). Results were analyzed by using one-way variance (ANOVA), and significant differences were compared based on the Tukey's HSD post hoc test ($p < 0.05$).

Determination of Ag⁺ ion uptake and microscopic observations

Bioaccumulation of Ag⁺ ions in the roots was measured according to our previous study (Yanik & Vardar, 2015). The sample roots were dried at 80 °C, grinded with a microhammer cutter and filtered. 0.3 g sample were mixed with 8 ml 65% HNO₃ in Teflon vessels and mineralized in a microwave for 5 min at 145 °C, 5 min at 165 °C, and 20 min at 175 °C, respectively. After filtering with whatman filters the volume were completed to 50 ml and measured by ICP-OES (PerkinElmer-Optima 7000 DV).

For characterization of Ag NPs, wheat roots were fixed in 6% glutaraldehyde in 0.1 M phosphate-saline buffer (pH 7.8) for 16 h. The dehydrated samples were embedded in Epoxy resin using propylene oxide. Ultrathin sections (~70 nm) were contrasted with uranyl acetate and lead citrate, and examined with a JEOL JEM 1011 transmission electron microscope (TEM) confirming NP size, morphology and uptake.

Determination of lignin and callose deposition

To determine the lignin and callose accumulation, fresh roots were cut and rinsed in distilled water. For lignin staining, samples were incubated in acid phloroglucinol solution for 5 min. The roots were analysed with stereomicroscope (Leica EZ4). For callose staining, roots were stained with aniline blue for an hour in the dark and rinsed in 0.1 M K₃PO₄ (pH 8). The roots were monitored at 455 nm wavelength with KAMERAM software, assisted by a KAMERAM fluorescent camera and an Olympus BX-51 fluorescence microscope.

Determination of total proline content

Total proline content was determined by the method of Bates (1973). 50 mg roots were homogenized in sulfosalicylic acid (3% w/v) and centrifuged at 5,000 g for 10 min. The supernatant (2 ml) was mixed with equal quantities of glacial acetic acid-ninhydrin reagent, and boiled for 1 h at 100°C. The solution was mixed with 5 ml toluene on ice. The absorbance was measured at 520 nm.

Determination of hydrogen peroxide content and lipid peroxidation

Hydrogen peroxide (H₂O₂) contents were determined according to Junglee et al., (2014). Roots (300 mg) were homogenized with 2 ml extraction buffer containing 0.5 ml 0.1% (w/v) TCA, 1 ml KI (1 M) and 0.5 ml potassium phosphate buffer (10 mM) in the dark and centrifuged at 12,000 g for 15 min at +4°C. The supernatants were kept for 20 min in dark. A positive control was prepared with H₂O instead of KI for tissue coloration background. The absorbance of the supernatants was measured at 390 nm.

Lipid peroxidation was determined by the thiobarbituric acid (TBA) assay which measures the amount of malondialdehyde (MDA) according to procedure of Cakmak & Horst (1991). The roots were homogenized with 2 ml 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 g for 20 min at room temperature. 0.5 ml supernatant was mixed with 2 ml 0.6% (w/v) TBA in 20% (w/v) TCA. The mixture was heated at 95 °C for 30 min and transferred to ice. The samples were centrifuged at 12,000 g for 10 min. The absorbance of the supernatants was measured at 532 and 600 nm.

Determination of antioxidant activity

Control and Ag NPs exposed roots were extracted according to Lee & Lee (2000) to evaluate antioxidant enzyme activity. 0.5 g roots were homogenized with extraction buffer including 0.1 mM EDTA, 1% Polyvinylpyrrolidone (PVP) (w/v), 0.5% Triton X-100 (v/v), 100 mM PBS (pH 7.8) at +4 °C. The homogenates were centrifuged at 18,000 g for 20 min at +4 °C. The supernatants were used for measurement of guaiacol peroxidase (GPOX), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) activity. For ascorbate peroxidase (APOX) activity, 0.5 g roots were extracted with homogenization buffer containing 0.1 mM EDTA, 2 mM ascorbate, 2% polyvinylpyrrolidone (PVPP), 50 mM PBS (pH 7) at +4 °C and centrifuged at 14,000 rpm for 30 min at +4 °C (Nakano and Asada, 1981). The supernatant was used for APOX activity. GPOX activity was measured using the method of Birecka et al., (1973). The reaction mixture

containing 1.5 ml substrate buffer (0.1 M PBS pH 5.8, 5 mM H₂O₂, 15 mM guaiacol) and 10 µl enzyme extract were measured immediately for 2 min at 470 nm.

CAT activity was determined according to Cho et al., (2000). 37.5 µl enzyme extract was added to assay buffer (200 mM phosphate buffer pH 7.0, 72 mM H₂O₂). Absorbance was measured immediately for 2 min at 240 nm. SOD activity was evaluated by the method of Cakmak and Marschner (1992). Supernatant (2 µL) and 2 ml reaction mixture (0.1 M phosphate buffer pH 7, 2 M Na₂CO₃, 0.5 M EDTA, 300 mM L-methionine, 7.5 mM NBT, 0.2 mM riboflavin) was kept under white fluorescent lamps (15 W) for 10 min in test tubes. The absorbance was measured at 560 nm. GR activity was assayed according to method of Foyer & Halliwell (1976) by following the decrease in absorbance due to NADPH oxidation at 340 nm. The reaction mixture consisted of 25 mM PBS (pH 7.8), 1.2 mM NADPH, 5 mM glutathione (oxidized form, GSSG) with supernatant (100 µL) in a 1 ml volume. The absorbance was measured immediately for 3 min at 340 nm. APOX activity was measured based on the method of Nakano & Asada (1981). The reaction mixture consisted of 50 mM PBS (pH 7.0), 5 mM ascorbate, 1 mM EDTA, 1 mM H₂O₂ and supernatant (100 µl) in a 1 ml volume. The absorbance was measured immediately for 2 min at 290 nm.

RESULTS

Ag NPs uptake and characterization

Control and Ag NPs treated roots were analysed with ICP-OES for determining possible uptake of Ag released from Ag NPs. Ag content in plant roots were quantified as 1.37 µg/g in 0.5 ppm, 3.05 µg/g in 1 ppm, 14.88 µg/g in 5 ppm, 39.43 µg/g in 10 ppm and 62.99 µg/g in 20ppm. According to ICP-OES results, uptake of Ag showed a significant elevation with increasing concentration of Ag NPs. In consideration of these results, visualization of Ag NP accumulation inside the cells was analysed by TEM (Figure 1). Ag NPs exposure resulted in bioaccumulation in wheat roots confirmed by both of ICP-OES and TEM. According to TEM results cellular deformations were also evident principally at 10 and 20 ppm (Figure 2).

Effects of Ag NPs on root growth

To evaluate the effects of Ag NPs root growth of wheat seedlings were measured. Our results revealed that Ag NPs inhibited the root growth (Figure 3). Although root length was slightly increased by 9% in 0.5 ppm Ag NPs exposure,

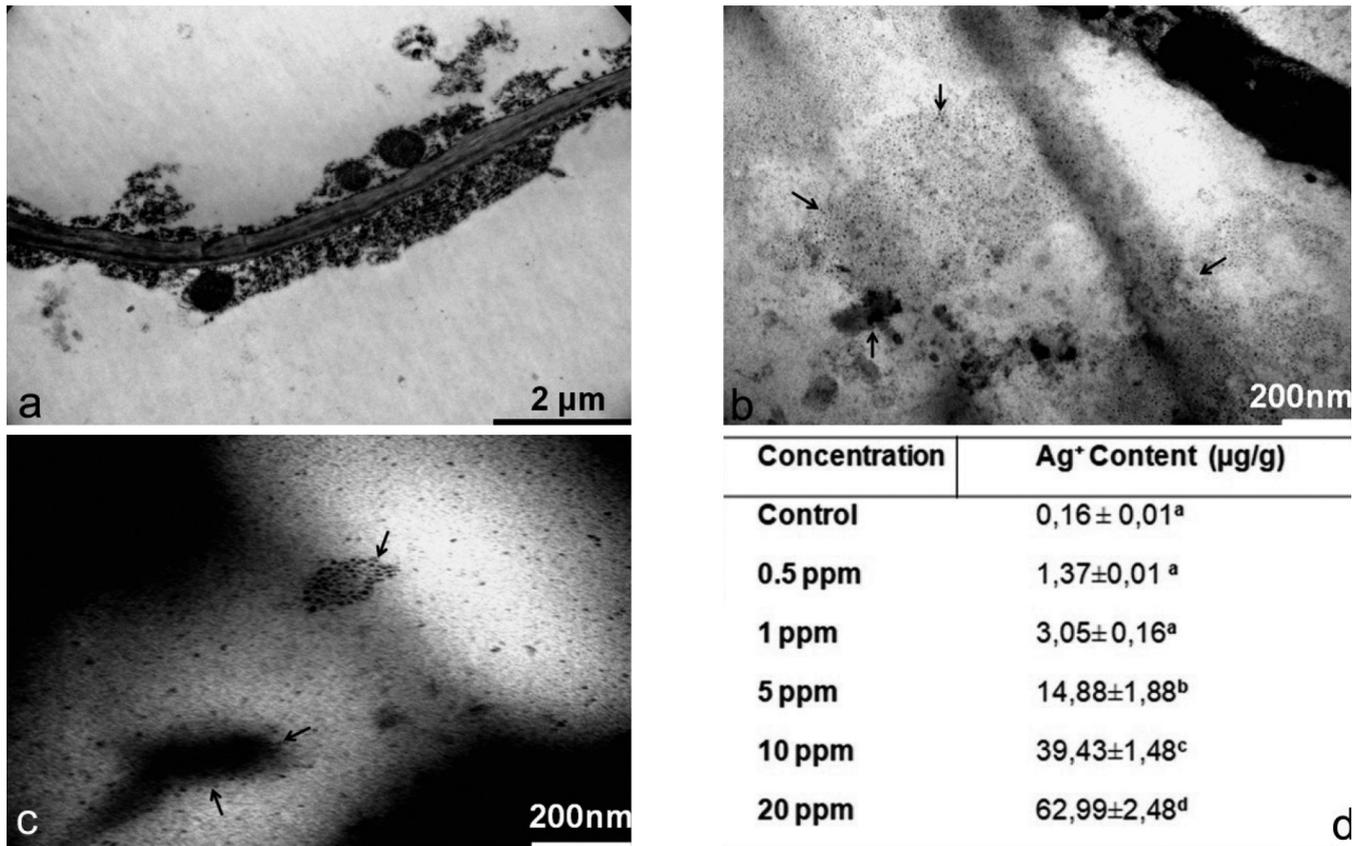


Figure 1. Electron micrograph of control (a), 10 ppm (b), 20 ppm (c) (arrows—NPs accumulation), Ag content (μg/g) in roots of wheat plants after treatment with Ag NP (d). All data are significantly different from the control at $P < 0.05$ level. Values represent means ± SD.

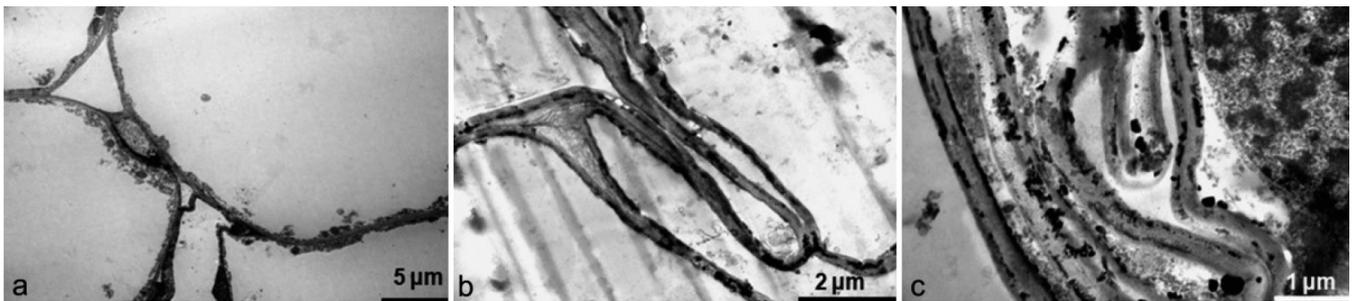


Figure 2. Ultrastructure of wheat root cells. Control (a), 10 ppm (b), 20 ppm (c).

there was no significant difference in 1 ppm in contrast to control. However, root length was reduced by 8%, 25% and 39% in 5, 10 and 20 ppm Ag NPs, respectively. These results showed that dose-dependent inhibition of root elongation occurred at 5 ppm and higher doses in wheat.

Effects of Ag NPs on lignin and callose deposition

The lignin formation was investigated using phloroglucinol reaction characterized by red coloration in Ag NPs exposed roots. Lignin accumulation was observed only at 10 and 20

ppm. Intensive lignin accumulation determined especially in root cap and maturation zone of roots (Figure 4). Besides morphological observations demonstrated that elongation and maturation zones of roots were swollen at 10 and 20 ppm. The callose deposition was visualized with aniline blue fluorescence staining procedure. If compared to control, yellowish-green fluorescence of callose deposition was visible in all Ag NPs treated samples. Besides the density of the callose deposition increased depending on the Ag NPs concentrations. Callose deposition was monitored evidently in the all parts of the root at 10 and 20 ppm (Figure 5).

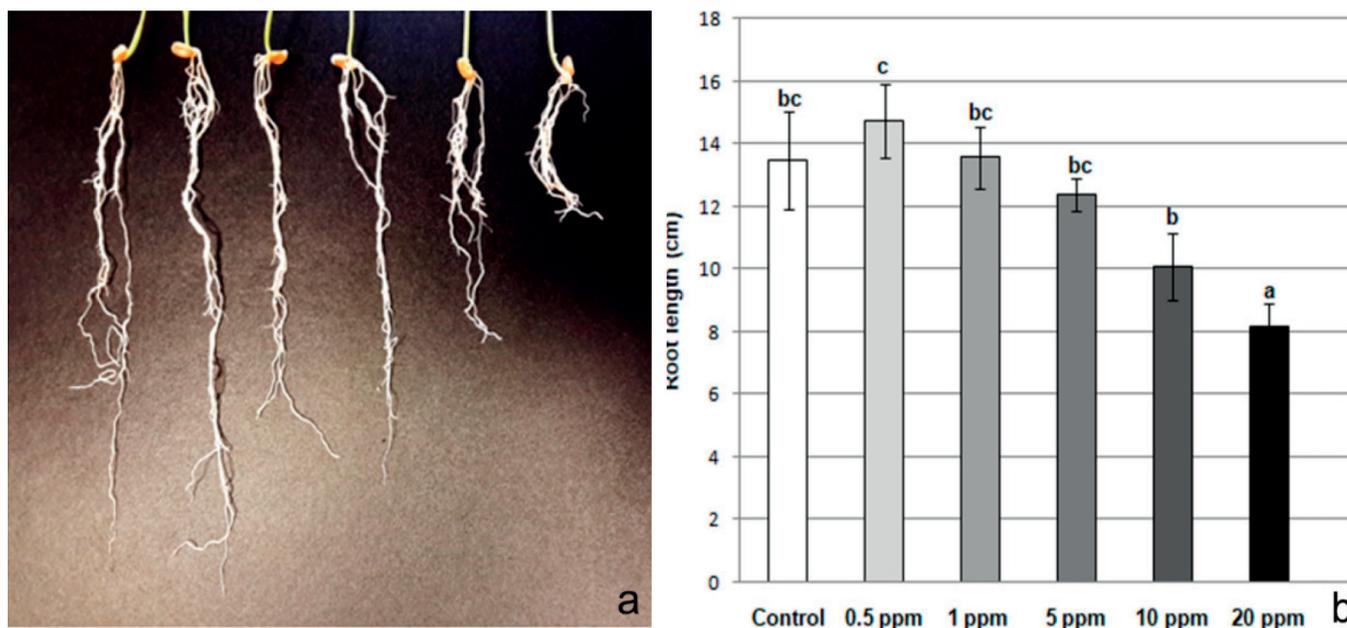


Figure 3. Control and Ag NPs (0.5, 1, 5, 10 and 20 ppm) treated relative root lengths in wheat. Morphological view of wheat roots (a, left to right; control, 0.5, 1, 5, 10 and 20 ppm), Root lengths of wheat (b, left to right; control, 0.5, 1, 5, 10 and 20 ppm). All data are significantly different from the control at $P < 0.05$ level. Values represent means \pm SD.

Effect of Ag NPs on H_2O_2 content and lipid peroxidation

It has been known that H_2O_2 generation is one of the signals of oxidative stress. According to our results the Ag NPs treatments significantly raised H_2O_2 content by 37.87% at 0.5 ppm, 42.14% at 1 ppm, 85.21% at 5 ppm, 85.56% at 10 ppm and 91.45% at 20 ppm if compared to control (Figure 6). Lipid peroxidation is a resultant of ROS accumulation and oxidative damage under stress conditions. It can be determined by measuring MDA level in control and Ag NPs treated roots. Although slight increase in MDA content was observed in 0.5 and 1 ppm, it increased by 14.37% in 5 ppm, 25.62% in 10 ppm and 36.87% in 20 ppm as compared to control (Figure 6).

Effect of Ag NPs on antioxidant activity

In the present study, SOD, CAT, GPOX, APOX, and GR activities were measured to evaluate the impact of the Ag NPs on the antioxidant defence potential related to oxidative stress. According to our studies SOD activity slightly increased only at 5 ppm by 7%. However, it was reduced depending on dose in higher concentrations. The maximum decrease was observed at 20 ppm by 68% (Figure 7).

Catalase catalyses the breakdown of H_2O_2 to H_2O and O_2 . In the present study, correlated with H_2O_2 increase, CAT activity was significantly decreased than that of control (Fig 7). However, the activity of GPOX which is one of the H_2O_2 -

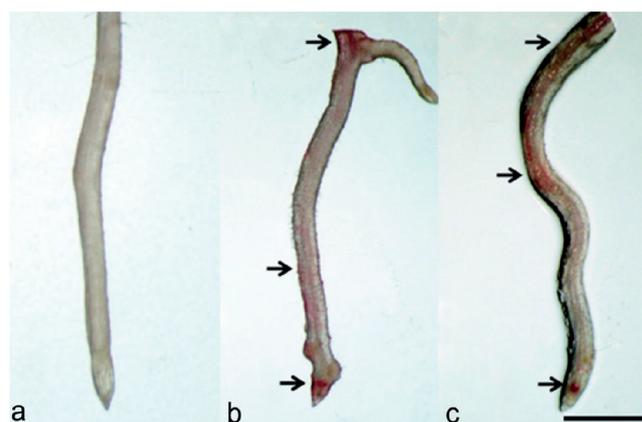


Figure 4. Lignin accumulation of control and Ag NPs treated wheat roots. Control (a), 10 ppm (b), 20 ppm (c) (Arrows show lignin depositions). The bar (50 μ m) in (e) also applies to (a-c).

detoxifying enzymes presented fluctuations. Although GPOX increased in 0.5 and 20 ppm by 42% and 7%, it decreased in 1, 5 and 10 ppm by 8%, 1% and 26%, respectively (Figure 7). APOX is also one of the H_2O_2 detoxifying enzymes. The APOX activity increased in 0.5, 1 and 5 ppm by 17%, 8% and 12%, however no significant change was observed at higher concentrations as compared to the control (Figure 7). One of the plant antioxidant activities GR activity showed no remarkable difference at 0.5 ppm. However, GR activity was decreased in 1, 5, 10 and 20 ppm by 37%, 49%, 49% and 52% (Figure 7). One of the non-enzymatic antioxidant

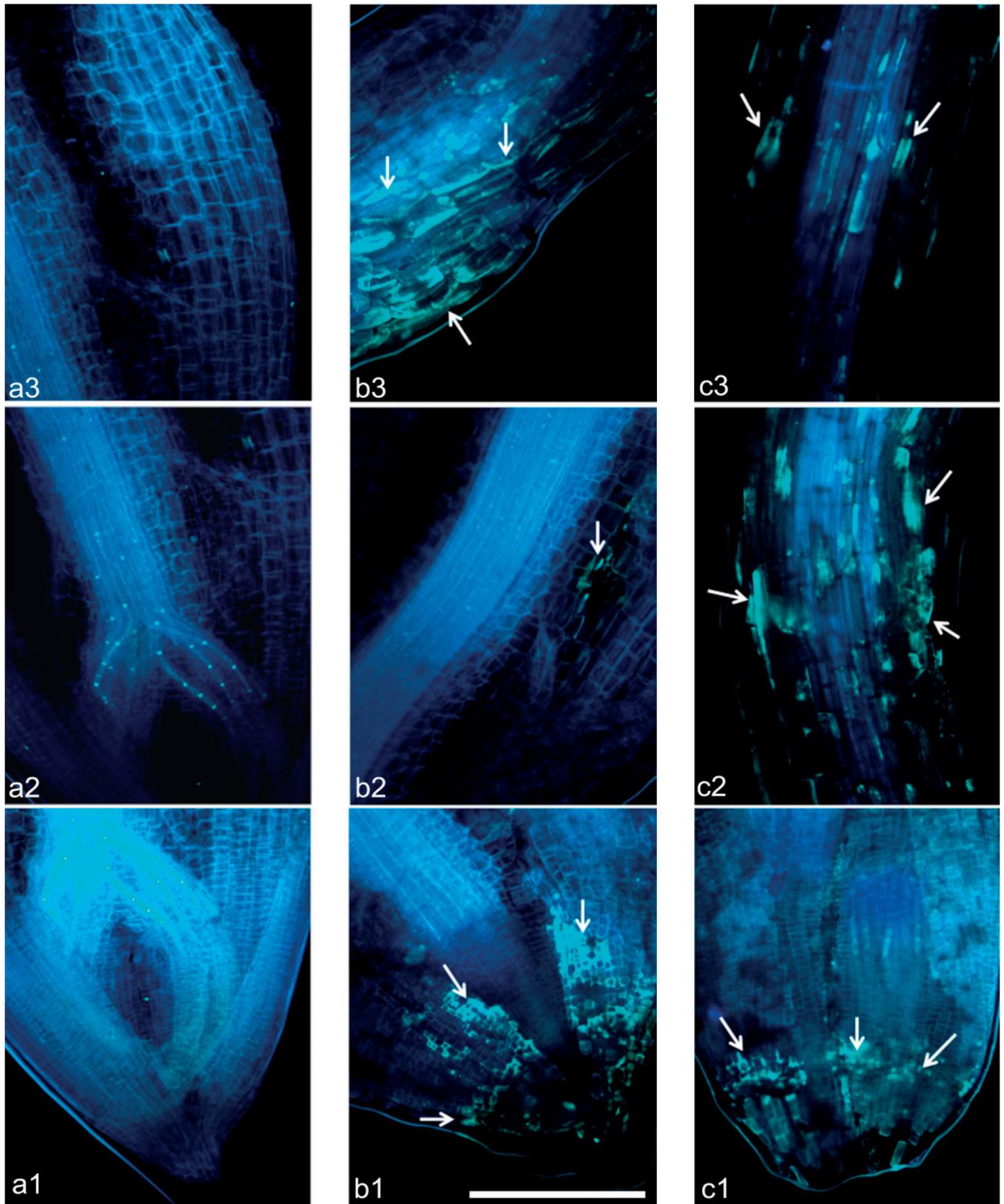


Figure 5. Callose deposition of control and Ag NPs. Control (a), 10 ppm (b), 20 ppm (c). Root cap (1), elongation zone (2), maturation zone (3). (Arrows show callose deposition). The bar (50 μ m) in (b) also applies to (a–c)

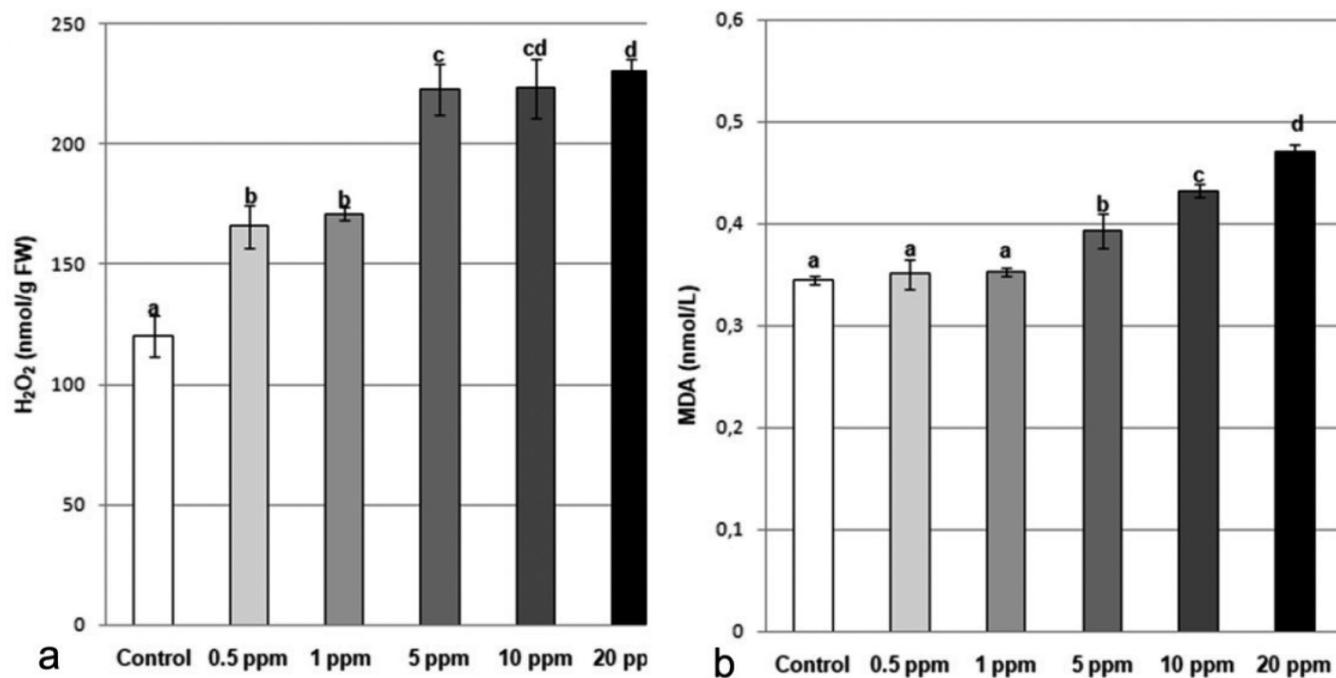


Figure 6. H₂O₂ content (a) and Lipid peroxidation (b) of control and Ag NPs treated wheat roots. The data with different letter are significantly different according to Tukey's post-hoc HSD test for independent samples at $P < 0.05$. The bars on columns represent means \pm SD

proline was evaluated after Ag NPs treatment. Proline content was increased in 0.5, 1, 5 ppm and 10 ppm by 17%, 37%, 40% and 14%, however it was decreased in 20 ppm by 19% in comparison to control (Figure 7).

DISCUSSION

The wide range usage of Ag NPs may induce an increased disposal and accumulation in the environment leading to adverse effects (Anjum et al., 2013; Gupta et al., 2018). Thus the long term effects of Ag NPs should be clarified concerning aquatic, terrestrial, and atmospheric ecosystems (Tripathi et al., 2017). Anjum et al. (2013) suggested that soil accumulates more Ag NPs released from waste-water management systems, biosolid application, accidental release and Ag NPs based fertilizers. At this stage plants are the first affected organisms from accumulated NPs in soil. In the present study uptake of Ag NPs was evidenced by ICP-OES and TEM. ICP-OES results showed that Ag which possibly released from Ag NPs accumulated in plant root tissues depending on the concentration. Besides, TEM micrographs confirmed the accumulation of NPs in the form of black dots in the cytoplasm. Dimkpa et al. (2013) reported that Ag NPs accumulated in roots and transported to the shoots in wheat. Nair & Chung (2014c) have conducted a

comparative study on roots of *Arabidopsis thaliana* by the method of ICP-MS showing the accumulation of Ag⁺ after Ag NPs and AgNO₃ exposure. These results reveal that, Ag NPs treatment results in Ag⁺ accumulation in root and shoots of different species of plants indicating the importance of assessing the cumulative effects of Ag NPs.

The growth parameters are essential to understand the impacts of NPs on plants. According to our morphological parameters Ag NPs exposure reduced root length in 5 ppm and higher doses of Ag NPs as compared to control. The results concerning root growth inhibition in 5, 10 and 20 ppm support the previous studies performed by different concentrations, sizes and surface coatings of Ag NPs in *Lolium multiflorum*, *Vigna radiata* and *T. aestivum* (Dimkpa et al., 2013; Nair & Chung, 2015; Zuverza-Mena et al., 2016). However, phytostimulatory effect in 0.5 and 1 ppm suggest hormetic response (Gupta et al., 2018; Kozumbo & Calabrese, 2019).

Lignin as a biopolymer compound is a component of plant cell wall. It has important roles such as increasing cell wall rigidity, transportation of minerals through the xylem, barrier against to pest and pathogens and response to various environmental stresses (Liu et al., 2018). Therefore, lignin accumulation attracts attention to reveal its role on plant defence metabolism. There are some reports concerning dose dependent lignin accumulation after CuO, CeO₂ and Al₂O₃ NPs exposure (Rico et al., 2013; Nair & Chung 2014b; Yanik & Vardar, 2015). In this study, lignin accumulation

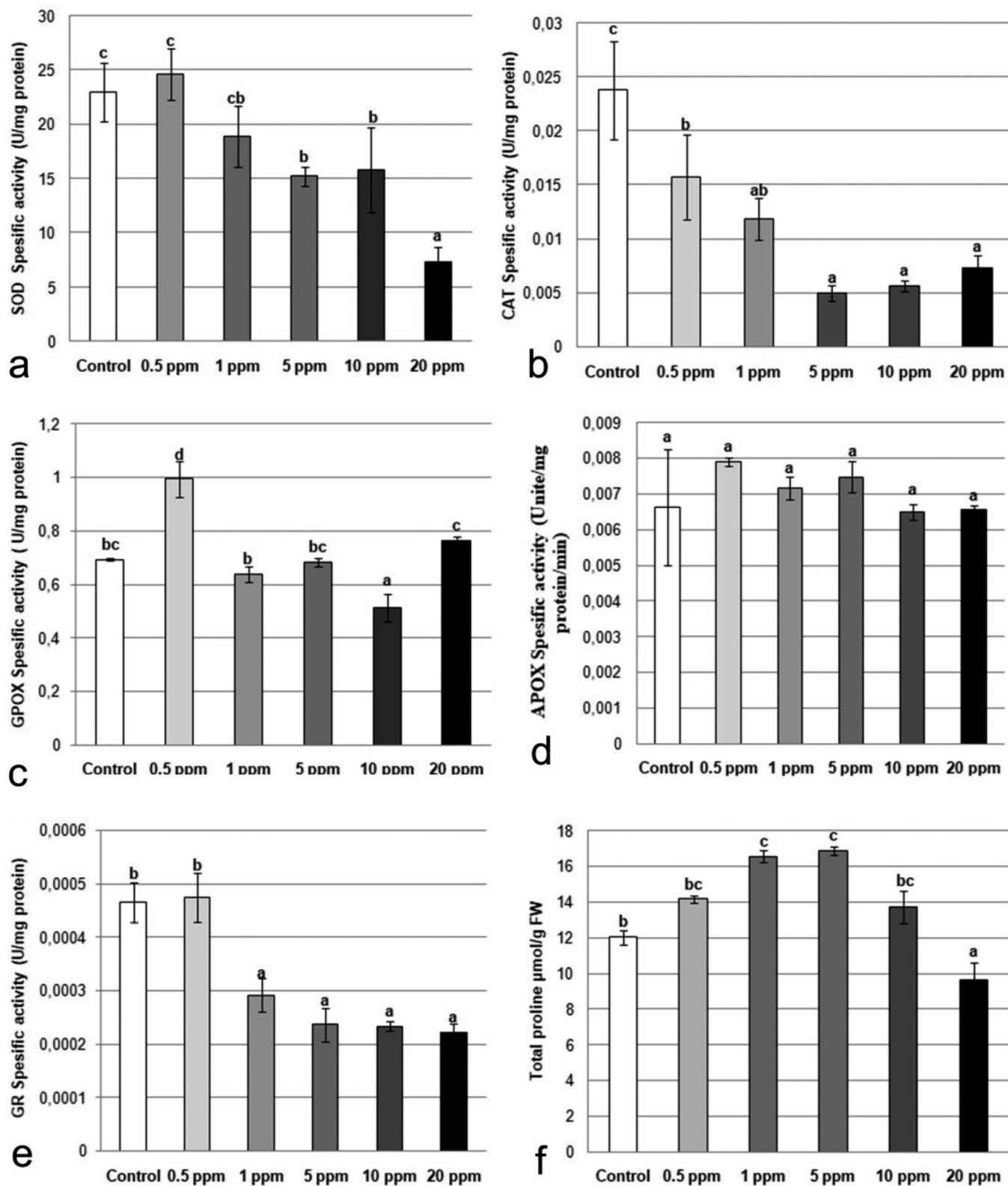


Figure 7. SOD (a), CAT (b), GPOX (c), APOX (d), GR (e) and total proline content (f) of control and Ag NPs treated roots. The data with different letter are significantly different according to Tukey's post-hoc HSD test for independent samples at $P < 0.05$. The bars on columns represent means \pm SD

was clearly observed in higher concentrations and intensive accumulation was seen in root cap and maturation zone of wheat. The present investigation is the first study which reveals the lignin accumulation after Ag NPs exposure.

Callose is a β -1,3-linked polymer that contains some β -1,6-branches and synthesized in the course of plant development and various abiotic-biotic stresses (Ünal et al., 2013; Yanik & Vardar, 2015). In order to better understand the mechanisms between NP effect and callose formation, we investigated callose formation in wheat roots exposed to Ag NPs. According to our results, Ag NP as a stress factor induced callose formation dose dependently. According to previous studies, there are only a few research available reporting callose deposition after NP treatment (Yanik & Vardar, 2015; Nair & Chung, 2017).

The H_2O_2 is generated during oxidative stress and leads to a chain reaction termed lipid peroxidation (Siddique et al., 2012). It is a very stable ROS formation and can cross the cellular membranes accelerating oxidative stress. It also acts as signaling molecules triggering plant tolerance against environmental stresses at low concentrations. On the other hand, at higher concentrations H_2O_2 can inactivate enzymes by oxidizing their thiol groups leading to programmed cell death (Sharma et al., 2012). Based on our results, we observed high accumulation of H_2O_2 inducing oxidative stress. According to Nair & Chung (2014a, 2015) different concentrations of Ag NPs caused H_2O_2 production in *Oryza sativa* and *V. radiata*. Similarly, it has been also reported that Ag NPs trigger ROS accumulation altering cellular functions unfavorably (Tripathi et al., 2017; Cvjetko et al., 2017).

Lipid peroxidation is a resultant of ROS accumulation and oxidative damage under stress conditions (Sharma et al., 2012). Although we observed slight change in 0.5 and 1 ppm Ag NPs treatment, the higher concentrations induced increase in MDA content which is the final product of lipid peroxidation. MDA increase were also reported by several researchers after different dose of Ag NPs exposure (Nair & Chung, 2014a; 2015; Cvjetko et al., 2017; Fayez et al., 2017; Tripathi et al., 2017). However, Gupta et al. (2018) reported MDA reduction after different doses of biogenic Ag NPs (10, 20 and 40 ppm) treatment in *O. sativa*. The researchers correlate their result to growth stimulation.

Plants metabolism produces enzymatic and non-enzymatic antioxidants to mitigate the damaging effects of ROS in the course of plant development and under various stress conditions. ROS affects many cellular functions and cause damage to nucleic acids, oxidation of proteins and peroxidation of lipids. The equilibrium between ROS production and detoxification determines the role of ROS if it is a stress factor or a signal factor. Stress-induced ROS accumulation is detoxified by enzymatic antioxidants such as SOD, CAT, GR, APOX and GPOX, and non-enzymatic

antioxidants such as ascorbate (ASH), glutathione (GSH), phenoloids, carotenoids and proline (Gill & Tuteja, 2010). Proline is an amino acid acting as a non-enzymatic antioxidant mitigating the adverse effects of ROS and its accumulation has an importance during adaptive (or hormetic) response such as scavenging ROS, metal chelators acting as a signal molecule in plant defence mechanism (Mattson, 2007; Rana et al., 2017). In this study, we observed an increase in total proline content at 0.5, 1, 5 ppm. Although it was higher from control, proline content started to decrease in 10 and 20 ppm. Similarly, Nair & Chung (2014) showed proline accumulation in roots of *O. sativa* upon exposure to 0.2, 0.5 and 1 ppm Ag NPs. The researchers observed an increase in *V. radiata* exposure to 5, 10, 20, and 50 mg/L of Ag NPs. Girilal et al. (2018) had a comparative study to assess the effects of biologically and chemically synthesized Ag NPs on *Solanum lycopersicum*. They found higher proline accumulation in both chemically and biologically synthesized of Ag NPs. As reported by Chen & Dickman (2005), proline is potential inhibitor of PCD. Therefore, the reduction of proline accumulation may be related to inhibition of plant defence systems to activate PCD. According to our observation, 10 and 20 ppm showed higher proline biosynthesis possibly leading to PCD.

The SOD catalyzes the dismutation of $O_2^{\cdot-}$ to O_2 and H_2O_2 and plays a central role in defense against oxidative stress. According to our result, The SOD activity was decreased dose dependently except in 0.5 ppm. The decreased SOD may be as a result of overproduction of ROS after Ag NPs treatment. Supporting our results Cvjetko et al. (2017) reported that SOD activity was decreased in the higher concentrations (100 and 500 μ M) of exposed Ag NPs in tobacco roots. However, increased SOD activities and upregulation of SOD genes were also reported at different plants species (Yasur & Rani, 2013; Nair & Chung, 2015; Homae & Ehsanpour, 2016; Tripathi et al., 2017). In the conflicting responses in different studies may be related to the exposure time, plant species, concentration or size of the Ag NPs.

CAT is a scavenger enzyme that catalyzes the dismutation of two molecules of H_2O_2 into water and oxygen molecule. In the present study, correlated with H_2O_2 increase, CAT activity was significantly decreased than that of control. Previous studies based on NPs toxicity exhibited both increase and reduce of CAT activity. Although enhanced CAT activity were more common after NPs application (Morales et al., 2013; Nair & Chung, 2014a), depletion of CAT has been also reported (Lopez-Moreno et al., 2016). Nair & Chung (2015) observed that mRNA expression of CAT increased at 10 and 20 ppm Ag NPs, but CAT mRNA level decreased at 50ppm Ag NPs on *O. sativa* roots. Cvjetko et al., (2017) indicated a decline of CAT activity at 500 μ M Ag NPs treatment with respect to 25, 50, 75 and 100 mM in *Nicotiana tabacum*. It is therefore possible that

different type, size, shape, dose and exposure time of NPs may have effect on antioxidant activity as in CAT activity (Sharma et al., 2012). GPOX reduces H_2O_2 to H_2O with aromatic electron donors such as guaiacol and pyrogallol (Sharma et al., 2012). In our observations, GPOX activity showed some fluctuations based on the concentration. The highest reduction was observed at 10 ppm but GPOX activity increased in 0.5 ppm and 20 ppm. Similarly, Fayez et al., (2017) reported that GPOX activity was increased at low concentrations of Ag NPs, but it was approximately equal to control in higher concentrations. Moreover, Yasur & Rani (2013) showed increased GPOX activity on *Ricinus communis*. However, Gui et al., (2015) reported that long-term (30 d) exposure to CeO_2 NPs inhibited activities of SOD and GPOX in lettuce roots.

APOX and GR are the major enzymes of ascorbate-glutathione cycle to recycle ascorbic acid (AsA) and reduced GSH regeneration which also detoxifies H_2O_2 . The APOX and GR also play an essential role in the control of intracellular ROS levels (Sharma et al., 2012). In our results, APOX activity increased up to 5 ppm, and decreased in the higher concentration with respect to control. Besides, whereas GR activity showed no remarkable difference at 0.5 ppm, but at higher concentrations it was decreased dose dependently. Nair & Chung (2014a, 2015), reported that exposure to Ag NPs, cause no significant difference in mRNA expression of APOX genes in roots. Besides, Ag NPs treatment reduced APOX activities in leaves of *S.tuberosum* and roots of *N. tabacum* (Homae & Ehsanpour 2016; Cvjetko et al., 2017). Tripathi et al. (2017) reported that APOX activity increased *Pisum sativum* roots under treatments of Ag NPs. GR is an enzyme of ASH-GSH cycle and plays an essential role in the defence system against ROS by sustaining the reduced status of GSH. The presented study designed to improve our knowledge on clarifying the possible toxicity mechanism of the 10-nm-sized Ag NPs. Our results suggested that exposure over 5 ppm Ag NPs has resulted in adverse effects on morphological, physiological and biochemical characteristics in wheat roots. It should be also considered that lower concentrations of Ag NPs (0.5 and 1 ppm) have stimulatory effects referring adaptive (or hormetic) response. Based on results while 0.5 and 1 ppm Ag NPs constitute a moderate stress in wheat roots, 5 ppm and higher concentrations generate extreme stress. It has been thought that the excess uptake and accumulation of the Ag NPs by plants as a result of several types of industrial applications, may also affect animal and human health. There are many gaps in our knowledge concerning the biological effects of Ag NPs as well as the other NPs and the plants responses to NPs should be under consideration, for protection of biological organisms and environment. The presented results may help to understand the potential impacts of Ag NPs and may assist in terms of use and safe disposal of Ag NPs as the reduction of its detrimental effects in both environmental and agricultural systems.

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