Comparative study on the effects of silver nanoparticles and silver ions on the disturbance of antioxidant system in roots of tobacco plants

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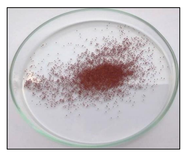
INTRODUCTION

Antimicrobial properties of silver and enhanced reactivity when applied in the form of nanoparticles (AgNPs) led to increased utilization of AgNPs in consumer products. Their release into environment directly affects biological systems and indirectly human health, as AgNPs can be transported through plants into the food chain[1,2]. AgNPs can impose detrimental effects on plants, mainly through excess generation of reactive oxygen species (ROS), leading to induction of oxidative stress[3]. In this work, *in vitro* grown tobacco (*Nicotiana tabacum*, L.) plants were exposed to AgNPs stabilized with cetyltrimethylammonium bromide (CTAB) or polyvinylpyrrolidone (PVP) coating and to ionic silver ($AgNO_3$), applied in the same concentrations (25, 50 and 100 µM). The aim was to compare AgNP and $AgNO_3$ impact by investigating ROS generation and subsequently changes in the antioxidant system in roots of tobacco plants in correlation to Ag accumulation upon exposure to ionic- and nano-silver.

MATERIALS AND METHODS

TREATMENTS:

TOBACCO SEEDS



Cultivation on solid Murashige Skoog (MS)[4] medium **60 DAYS**

ADULT PLANTS

$AgNO_3$ treatment	AgNPs treatment	Control (MS ½)

25, 50, or 100 µM

7 DAYS

ANALYSES:

1. Concentration of accumulated Ag measured by ICP MS (ELAN DRC-e)

2. ROS content measured spectrophotometrically from root extracts

3. Soluble proteins extraction → **EXTRACTED PROTEINS**

- Spectrophotometric enzyme assays
- Native polyacrylamide gel electrophoresis followed by treatment with specific substrates
- Immunoblotting assay (after only 100 µM treatment)

RESULTS

Table 1. Concentration of Ag measured in roots of adult tobacco plants. The results represent the mean value of 6 replicates ± standard error. Values marked with different letters represent signiicant difference ($p \leq 0.05$) according to Duncan test. Value < 0.0001 µg g^{-1} represents instrument quantification bound.

Treatment	ADULT PLANTS
	ROOT
	Ag concentration (µg g^{-1} fresh weight)
Control	< 0.0001[a]
25 µM AgNPs 50 µM AgNPs 100µM AgNPs	1247.4 ± 122.0[b] 1395.2 ± 351.5[b] 1742.2 ± 192.8[bc]
25 µM $AgNO_3$ 50 µM $AgNO_3$ 100µM $AgNO_3$	1121.5 ± 136.2[b] 1450.9 ± 436.2[b] 1747.4 ± 150.0[bc]

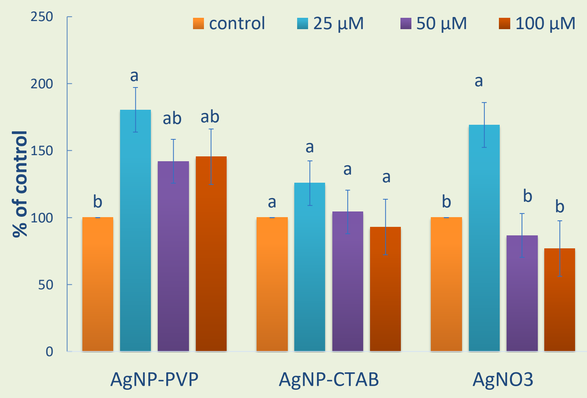


Figure 1. Levels of **ROS** in tobacco roots and leaves after exposure to 25, 50 and 100 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

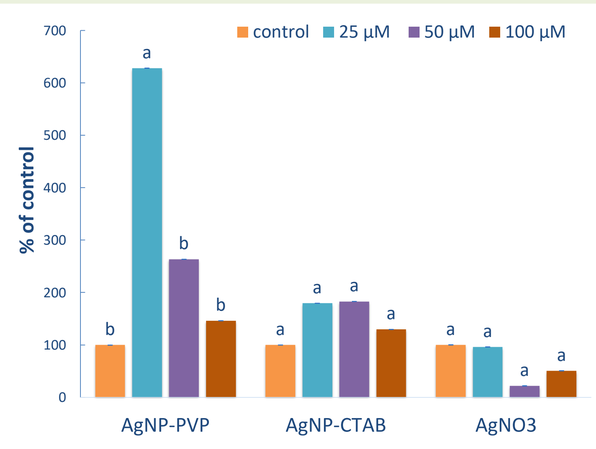


Fig. 2. Activity of **APX** in tobacco roots after exposure to 25, 50 and 100 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

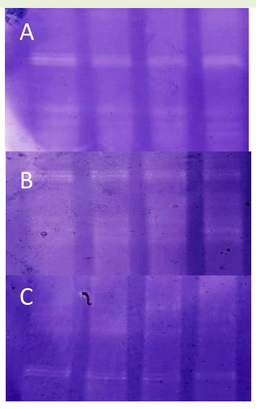


Fig. 3**. APX activity and isoforms** in tobacco roots after conducted native PAGE; 1- control; 2 - 25 µM, 3 - 50 µM , 4- 100 µM AgNPs: **A**- AgNP-PVP, **B**- AgNP-CTAB, **C**- $AgNO_3$

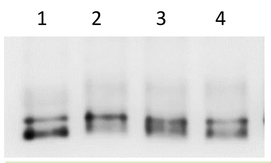


Fig. 4. Immunoblotting assay with **anti-APX** antibody after treatment with 100 µM AgNPs or $AgNO_3$. 1- control, 2- AgNP-PVP, 3- AgNP-CTAB, 4- $AgNO_3$

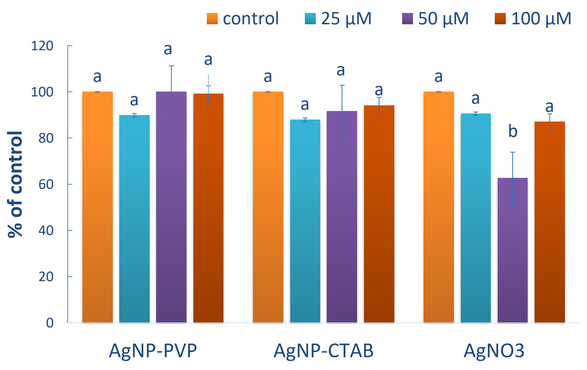


Fig. 5. Activity of **PPX** in tobacco roots after exposure to 25, 50 and 100 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

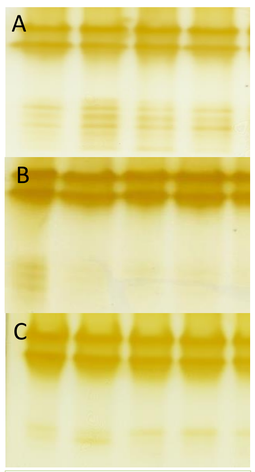


Fig. 6**. PPX activity and isoforms** in tobacco roots after conducted native PAGE; 1- control; 2 - 25 µM, 3 - 50 µM , 4- 100 µM AgNPs: **A**- AgNP-PVP, **B**- AgNP-CTAB, **C**- $AgNO_3$

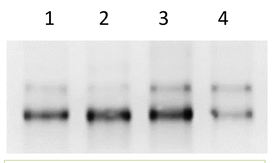


Fig. 7. Immunoblotting assay with **anti-HRP** antibody after treatment with 100 µM AgNPs or $AgNO_3$. 1- control, 2- AgNP-PVP, 3- AgNP-CTAB, 4- $AgNO_3$

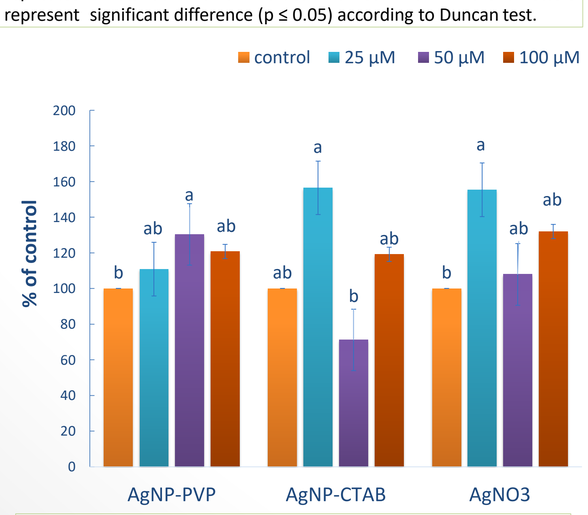


Fig.8. Activity of **SOD** in tobacco roots after exposure to 25, 50 and 100 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

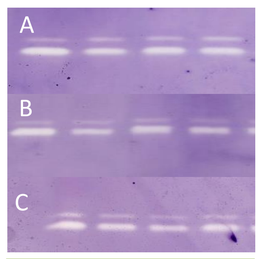


Fig. 9**. SOD activity and isoforms** in tobacco roots after conducted native PAGE; 1- control; 2 - 25 µM, 3 - 50 µM , 4- 100 µM AgNPs: **A**- AgNP-PVP, **B**- AgNP-CTAB, **C**- $AgNO_3$

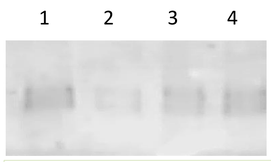


Fig. 10. Immunoblotting assay with **anti-SOD** antibody after treatment with 100 µM AgNPs or $AgNO_3$. 1- control, 2- AgNP-PVP, 3- AgNP-CTAB, 4- $AgNO_3$

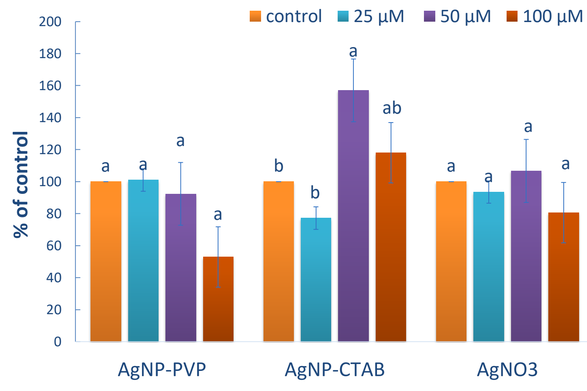


Fig. 11. Activity of **CAT** in tobacco roots after exposure to 25, 50 and 100 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

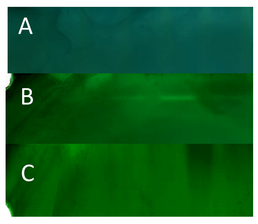


Fig. 12**. CAT activity and isoforms** in tobacco roots after conducted native PAGE; 1- control; 2 - 25 µM, 3 - 50 µM , 4- 100 µM AgNPs: **A**- AgNP-PVP, **B**- AgNP-CTAB, **C**- $AgNO_3$

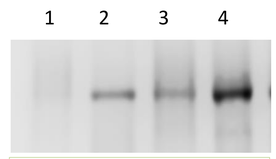


Fig. 13. Immunoblotting assay with **anti-CAT** antibody after treatment with 100 µM AgNPs or $AgNO_3$. 1- control, 2- AgNP-PVP, 3- AgNP-CTAB, 4- $AgNO_3$

CONCLUSIONS

- After both types of treatments similar accumulation of Ag was obtained
- Increase in ROS level was observed in roots only after AgNP-PVP and 25 µM $AgNO_3$ treatment
- Significant changes in SOD activities were observed after all treatments, while CAT activity exhibited significant increase in activity after exposure to AgNP-CTAB
- Changes in PPX and APX isoform patterns were observed upon different treatments, suggesting that the form of applied silver affects the activity of different isoenzymes.
- The results of Immunoblotting assay showed higher abundance of HRP after AgNPs treatment, while all treatments caused increase in expression of CAT
- Obtained results suggest that all Ag treatments induced disturbance in plant`s antioxidant system, which manifested in increased acitivity and expression of antioxidant enzymes, while observed changes were mostly dependant on the form of applied Ag and possibly on the intrinsic properties of AgNP coatings

REFERENCES: 1. Tolaymat, T.M. et. al. *Sci. Total Environ.* **2010**, *408*, 999–1006, doi:10.1016/j.scitotenv.2009.11.003.
2. Rico, C.M. et.al. *J. Agric. Food Chem.* **2011**, *59*, 3485–3498, doi:10.1021/jf104517j.
3. Tkalec,M. et.al. *Compr. Anal. Chem.* **2019**, *84,* 145-198, doi: 10.1016/bs.coac.2019.04.010
4. Murashige, T., Skoog, F. *Physiol. Plant.* **1962**, *15*, 474-497