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Accumulation and Uptake of Silver Nanoparticles during Seed germinations of selected annual crop plants

Harajyoti Mazumdar*

Department of Biotechnology, Gauhati University, Guwahati, India.

***Corres.author: harajyoti.25@gmail.com**

Abstract: Toxicity of silver nanoparticles in seed germination and seedling growth of plants is a serious problem in environment. Rice (*Oryza sativa*, variety: Jaya), Mustard (*Brassica campestris*, variety: M-27) and Green gram (*Vigna radiata*, variety: K-851) were used as test seeds in this study. Silver nanoparticles were prepared by standard chemical reduction method .The accumulation of these nanoparticles inside seeds of test plants has been reported in this study by AAS (Atomic Absorption Spectrophotometer) analysis. A gradual increase in the accumulation of Ag nanoparticle in seeds was obvious with increasing Ag nanoparticle concentrations. Seeds of *O.sativa* presented relatively low Ag nanoparticle accumulation levels in our study. Seeds of *O.sativa* and *V.radiata* showed an improvement in Ag accumulations at all concentration of Ag nanoparticle solution within three days of treatment. We have observed that the level of Ag nanoparticle found in test seeds increased with increase in concentrations. Uptake of Ag particles was observed more at 1000 µg/mL than 500 µg/mL and 50 µg/mL of Ag nanoparticle solution in all test plants.
Key words: Nanoparticles, Reduction, concentration, Accumulation and AAS (Atomic Absorption Spectrophotometer).

1. Introduction

According to a nanotechnology consumer products inventory, there were about 580 products in the database till October 2007, which was an increase of 175 % from those recorded in March 2006. The maximum category, about 61 % of products, belonged to health and fitness which includes clothes, cosmetics and sunscreens. Comparatively food and beverages products make 11 % of the database, most commonly used nanoparticles being carbon and silver. Short term influence of silica, palladium, gold and copper nanoparticles on a soil microbial community and the germination of lettuce seeds are investigated at different concentrations of nanoparticles. Shoot and root ratio of Lettuce seeds was reported significant reduction compared to control when exposed to nanoparticle solution. No significant effect of nanoparticles on the soil was found on the number of colony forming units, peak areas of methyl ester of fatty acids in the FAME profile or on the total soil community metabolic fingerprint ($P>0.05$)[1]. Effects of Aluminum oxide (nAl_2O_3), silicon dioxide ($nSiO_2$), magnetite (nFe_3O_4), and zinc oxide (nZnO) were also reported on *Arabidopsis thaliana*. Out of the four selected nanoparticles ZnO particle has caused adverse effect on seed germination, root elongation and number of leaves of *Arabidopsis thaliana*[2]. Effect of silver nanoparticles on reduction of biomass and transpiration rate was also reported in *Cucurbita pepo*. The adverse effect on *C. pepo* was more prevalent in nanoparticles than bulk silver

solutions (4.4 to 10 times more)[3]. Uptake of silver nanoparticles was reported in *Brassica juncea* and *Medicago sativa* plants. Depending upon exposure periods and concentration, they showed different uptake of particles inside the cells. *B. juncea* when exposed to 1000 ppm of $AgNO_3$ showed an uptake of 12.4 % wt. of silver after 71 hours. While *M. sativa* showed 13.6 % wt. of silver at 10,000 ppm of $AgNO_3$ solution after 24 hours of exposure[4]. Bioaccumulation of nanoparticles increased with increase in concentrations of growth media and their bioavailability to test plant was calculated by the bioaccumulation factor. Effect of copper nanoparticles on zucchini plants showed inhibition of root length in seedling compare to control[5].

2. Materials and methods

2.1 Synthesis of Ag Nanoparticles:

Silver nanoparticles were synthesized by chemical reduction method with slight modifications. The process involves total conversion of ions into particles in presence of stabilizing agents. Silver nitrate solution was reduced in distilled water by sodium borohydride in presence of Tween-20 (surfactant). The solution get changed to yellow colour indicates formation of nanoparticle after vigorous shaking.

2.2 Seed sterilization and seed germination:

Surface sterilization of seeds was done by immersing them into 10% sodium hypochlorite solutions for ten minutes[6]. After this seeds were rinsed with distilled water for three times. The germination process was done by putting a piece of filter paper (Whatman No.1) in sterilized petriplate after seed soaking and 5 mL of test medium was added into it. Ten sterilized seeds were transferred into each petriplate and they were placed in an incubator at 30ºC[7].

2.3 Treatment of seeds with silver nanoparticle solution:

Different concentrations of silver nanoparticle were prepared. 5 mL of each silver nanoparticle concentration was added to surface sterilized seeds. The seeds were placed over moist filter paper (Whatman No.1) in sterilized petriplate at 30ºC for incubations[7].

2.4 Atomic Absorptions Spectrophotometer (AAS) analysis:

Atomic absorption – Flame (F-AA) was used to quantify the silver nanoparticles content in seeds at different intervals of nanoparticle treatment. All the experiments were done at Department of Chemical engineering, IIT Guwahati (Varian, Model no-AA240FS). The sample preparation for detections was prepared as followed at Department of Chemical engineering, IIT Guwahati. Analyses were conducted in triplicate. Seeds treated with different concentrations at different time intervals (1, 3, 5 and 7 days) during seed germinations were also analyzed. Treated seeds were first rinsed with tap water followed by distilled water. The samples were sliced into small pieces and stored in refrigerator at 5ºC. Samples of 3.0 g were oven dried at 70ºC for 24 hours and dry weight of sample was calculated. The dried sample was then placed in muffle furnace at 700ºC for 48 hours. The ash was dissolved in 5 mL of 20% HCL and the solutions were transferred to 500 mL beaker. The porcelain dishes were rinsed twice with 1 mL of conc. HCL and three times with 15 mL of 10% HCL. Samples were covered with watch glasses and heated on a hot plate until boiling. 10% HCL was added to avoid sample drying. Samples were then cooled at room temperature. Immediately, 1 mL of HNO_3 and 3 mL of HCL were added. Samples were filtered into 100 mL volumetric flasks after cooling. Final volume will be adjusted with 10% HCL.

3. Results and Discussion

$NaBH_4$ was found to be effective in controlling the uniformity of particle size distribution and for stabilizing nanosized metal particles. Excess of $NaBH_4$ was used as a reducing agent in the preparations of silver nanoparticle in double distilled water with slight modification in the standard method. Thus, the numbers of Ag^+ that would present in the solution in additions to the nanoparticle would be significantly small. $NaBH_4$ (6.6×10^{-3} M) was used as reducing agent for synthesis of silver nanoparticles in LB media[8, 9]. Polyoxyethylene sorbitan monolaurate (ester) is the trade name of Tween-20 and is usually a nonionic surfactant. 0.1 % Tween-20 was

used during preparation to prevent aggregation of particle in double distilled water. Surfactant was used not only to prevent aggregation of particles but it also reduces surface tension and brings about ready wetting of tissues [10]. Nanotoxicity has been the research focus of many publications including several reviews[11-14]. Nanoparticles such as SWCNT and TiO_2, has been widely used as test materials to reveal their toxicity mechanisms. However, available information on this topic was too scarce to reach at any consequences of nanotoxicity and its mechanism.

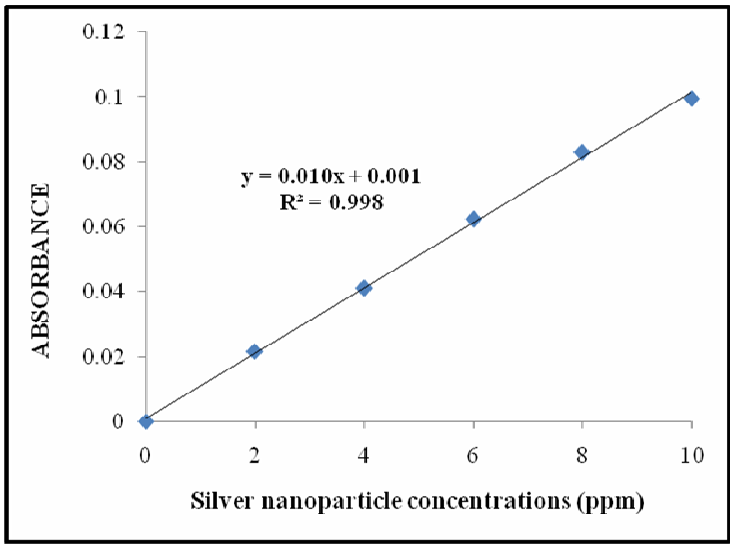


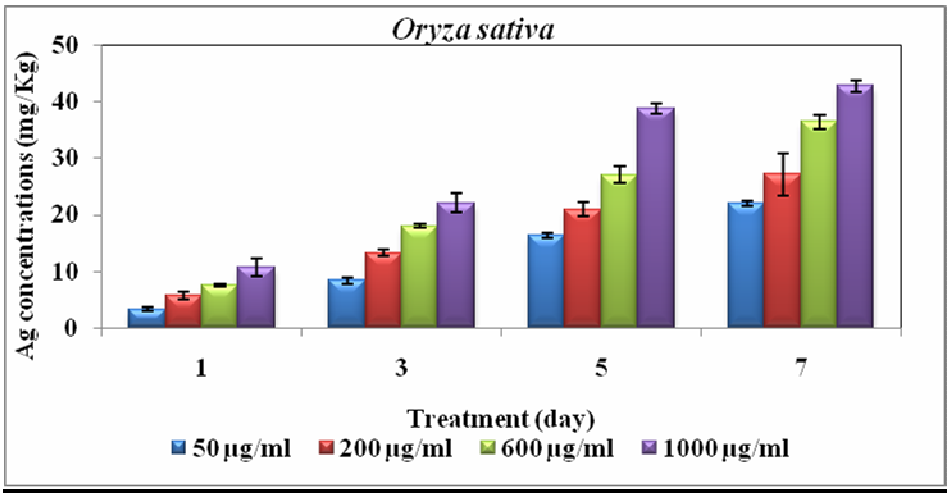
Figure 1: Standard curve of Ag nanoparticle solutions for Atomic Absorption Spectrophotometer analysis.

The result shows the accumulated weight of Ag particle per dry weight of plant (mg/Kg) as functions of both Ag concentrations and exposure time. **Fig 1** reveals that there exist a correlation between exposure time, Ag concentrations and Ag content in consistent forms. It was observed that *O. sativa* seeds uptake more than two times of Ag particle within three days at 50 µg/mL, 200 µg/mL and 600 µg/mL of Ag nanoparticle solution. After 3rd day, the Ag content of *O.sativa* seeds was increased to 8.33±0.55 mg/Kg, 13.3±0.62 mg/Kg, 18.03±0.32 mg/Kg and 22.06±1.65 mg/Kg at 50 µg/mL, 200 µg/mL, 600 µg/mL and 1000 µg/mL of Ag nanoparticle concentration respectively. The Ag content on *O.sativa* seeds was found to be even more after 5th day due to continuous uptake of Ag particles from Ag nanoparticle solution. *O.sativa* seeds showed an increase in Ag uptake from 3.30±0.34 mg/Kg to 22.0±0.50 mg/Kg when exposed to 50 µg/mL Ag nanoparticle solution within 7 days of treatment. More than five times silver uptake was reported in *O. sativa* seeds at 200 µg/mL and at 600 µg/mL of Ag nanoparticle concentration within 7 days of treatment. However within 7 days, *O. sativa* seeds showed four times more uptake of Ag content when exposed to 1000 µg/mL of Ag nanoparticle solution.

V.radiata seeds, after 1st day of treatment reported an Ag uptake of 4.50±0.36 mg/Kg, 7.03±0.28 mg/Kg, 9.60±0.20 mg/Kg, 13.06±0.23 mg/Kg and 15.90±0.72 mg/Kg Ag particles at 50 µg/mL, 400 µg/mL, 800 µg/mL, 1200 µg/mL and 1600 µg/mL of Ag nanoparticle concentrations respectively. *V.radiata* seeds reported four times more accumulation of Ag particle at 50µg/mL and 400 µg/mL of Ag nanoparticle concentration within 5 days of treatment. It was also observed that *V.radiata* seeds uptake more than three times within 5 days at 800 µg/mL, 1200 µg/mL and 1600 µg/mL concentration of Ag nanoparticle concentration. More than six times increase in Ag uptake by *V.radiata* seed was reported within 7 days, when exposed to 50 µg/mL concentration. Ag uptake was increased to 35.63±0.75 mg/Kg and 44.56±1.48 mg/Kg at 400 µg/mL and 800 µg/mL Ag concentration respectively after 7th day of treatment. However 1200 µg/mL and 1600 µg/mL Ag nanoparticle concentration exposed to *V.radiata* seeds showed more than four times Ag uptakes within 7 days of treatment.

. Exposure of *B.campestris* seeds to 50 µg/mL of Ag concentration reported an increase in Ag uptake from 3.46±0.35 mg/Kg to 13.96±0.86 mg/Kg within three days of treatment. After 3rd day, Ag uptake by

B.campestris seeds was increased to 17.5±0.36 mg/Kg, 21.43±0.61 mg/Kg and 24.86±2.08 mg/Kg when exposed to 400 µg/mL, 800 µg/mL and 1200 µg/mL of Ag nanoparticle solution respectively. *B.campestris* seeds reported seven times increase in particle uptake at 50 µg/mL within 7 days. Exposure of *B.campestris* seeds to 400 µg/mL and 800 µg/mL of Ag nanoparticle solution resulted an uptake of 34.3±0.50 mg/Kg and 41.60±0.36 mg/Kg respectively, after 7 days of treatment. Moreover 1200 µg/mL of Ag nanoparticle showed an increase in Ag uptakes by more than three times within 7 days of exposure period. The highest Ag concentration found in *B.campestris* seeds was 15.90±0.72 mg/Kg of Ag particle at 1600 µg/mL of Ag nanoparticle solution after 1st day of exposure. The Ag content in seeds of *B.campestris* increased in plants exposed to 1600 µg/mL of Ag nanoparticle solution when compared to the other Ag nanoparticle concentrations (50 µg/mL-1200 µg/mL) after 3rd day of exposure. It was also reported that higher the Ag metal concentration (10000 ppm) in growth media and exposure period, higher will be the accumulation of Ag metal in *B. juncea* and *M. sativa* (Harris and Bali, 2007). In our study, *V.radiata* seeds reported four times more accumulation of Ag particle at 50 µg/mL and 400 µg/mL Ag nanopartide concentrations within 5 days of treatment compare to *B.campestris* and *O.sativa* seeds. A slight increase of Ag particle was observed in seeds of *B.campestris* exposed to 50 µg/mL Ag nanoparticle solutions when compared to *O.sativa* and *V.radiata* after 5th day of treatment. The increase in Ag nanoparticle concentration did not affect the accumulation level of Ag particles in seeds of test plants. The content of Ag particle in seeds was nearly four times higher in *B.campestris* (47.16±1.36 mg/Kg) and *V.radiata* (57.60±1.86 mg/Kg) at the end of the experiment.



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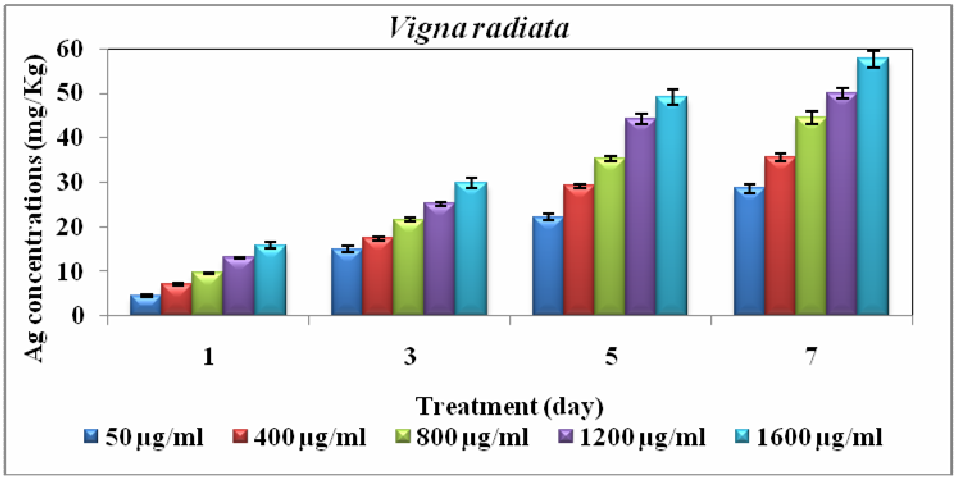

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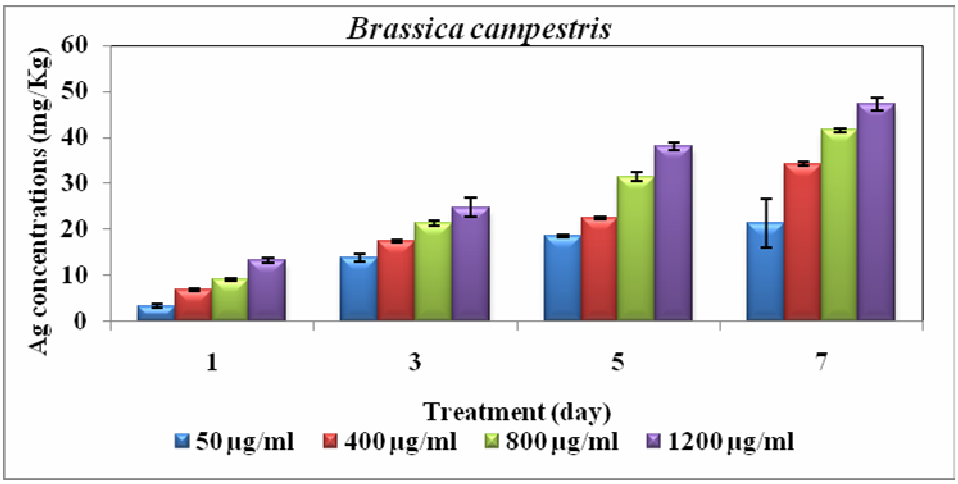

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Figure 2: Ag uptake during germination of treated seeds of (A) *O.sativa*, (B) *V.radiata* and (C) *B.campestris* from different concentration solution after different period of treatments.

O.sativa seeds showed an increase in Ag particle uptake by four-fold when exposed to 1000 µg/mL of Ag nanoparticle solution within 7 days of treatment. *P. radiatus* and *T. aestivum* reported an increase in metal uptake when exposed to 1000 mg/L of growth media (Lee *et al.*, 2008). Reports regarding the accumulation of nanoparticle on seed are very limited, so this kind of study should be quite useful in predicting the uptake of nanoparticle during seed germination.

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