Environment and Ecology 37 (3B) : 1107—1113, July—September 2019
Website: environmentandecology.com ISSN 0970-0420

Phytotoxicity and Stimulatory Impact of Silver Nanoparticles on Seedling Growth of Moth Bean

Dhawale V. P., K. C. Mohite, S. D. Kulkarni

Received 17 April 2019; Accepted 20 May 2019; Published on 10 June 2019

Abstract Silver nanoparticles (AgNPs) are one of the most widely used nanoparticles and expected to enter natural ecosystem. We have investigated the effects of AgNPs on plant growth parameters such as % Germination, root length, shoot length, biomass, seedling vigor index (SVI) of an important pulses, moth beans *(Vigna aconitifolia)*. Three concentrations of AgNPs viz. 50, 100, 150 mg/L were used to test the growth parameters. Among the treatments, application of 100 mg/L concentration of AgNPs proved best by giving the highest values for percent seed germination, root length, shoot height and seedling vigor index. A significant negative influence on biomass and root length was observed for all seeds in compared to those of control germination. Application of 150 mg/L AgNPs concentrations decreased mean germination time in comparison to control. Overall, this study has shown that direct exposure of germinating seeds to AgNPs may cause phytotoxicity and underscores the need for eco-responsible disposable of wastes and sludge containing nanoparticles.

Keywords Phytotoxicity, Silver nanoparticles, Moth bean, Seedling growth.

Dhawale V. P*.
Post Graduate, Department of Physics, New Arts Commerce and Science College, Parner, Ahmednagar 414302, India

K. C. Mohite
Post Graduate, Department of Physics, C.T. Bora College, Shirur, Pune 412210, India

S. D. Kulkarni
Post Graduate, Department of Environmental Science, New Arts Commerce and Science College, Ahmednagar 414001, India
e-mail: vijaya.dhawale2009@gmail.com
*Corresponding author

Introduction

Distinct properties of nanomaterials (Ă100 nm) such as large surface area to volume ratio, small size and high reactivity enable them to find applications in various industrial sectors and our daily lives (Handford et al. 2014). There has been rising demand for nanotechnology-based products in recent years. Nanotechnology has many applications in the field of agricultural research, such as in reproductive science and technology disease prevention and various other plant treatments, the transfer of agricultural and food waste to energy and other helpful by-products through enzymatic nanobioprocessing and various other plant treatments using nanocides (Carmen et al. 2003). Silver nanoparticles, is one of the most commonly used in the field of agriculture due to their antimicrobial and safety potential associated with human and environmental use. Silver nanoparticles have been used to create new consumer products, drugs, various food and other medical products.

Efficient seed germination and early seedling establishment are important processes in commercial agriculture. Rapid and uniform seedling emergence leads to successful plant establishment. Some reports have shown that both positive and negative impact on seed germination and seedling growth. Unique properties of nanoparticles can be used to improve seed germination and crop performance. Gubbins et al. (2011) demonstrated that AgNPs could inhibit the growth of *Lemna minor*. Only few studies on vascular plants showed that AgNPs have deleterious effects on plant growth (Stamploulis et al. 2009). Pulate et al. (2011), Patllola et al. (2012) found that nanoparticles have different levels of toxicities which may be size and shape dependent and have ability of penetrate the cell walls. Phytotoxicity studies reported both positive and negative effects of nanoparticles on higher plants as seed germination, cell division, root elongation, growth and metabolic processes (Carlson et al. 2008). Lin and Xing (2007) evaluate phytotoxicity of five types of metallic nanoparticles in six plant species and showed that seed germination was not affected except for the inhibition of nanoscale zincoxide on *Zea mays* and nanoscale zinc on *Lolium multiflorum.* They found that inhibition of root growth varied significantly among nonoparticles and plants and that it was partially correlated to nanoparticle concentrations. Kumar et al. (2011) investigated that AgNPs had cytotoxic and genotoxic impacts on *Allium cepa* meristems.

Vigna aconitifolia, commonly known as moth beans is a popular Indian crop cultivated as a forage and cover crop. It is also grown in Pakistan, United States, Thailand, Australia and other parts of Asia. Moth beans are an excellent source of proteins and other essential minerals and vitamins. These are considered to be effective in the prevention of constipation heart diseases, high blood pressure, high cholesterol, osteoporosis and low energy.

Rapid and uniform seedling emergence leads to successful plant establishment, as a deep root system is formed before the upper layers of the soil dry out, harden or reach supra-optimal temperatures (Chen and Arora 2013). The unique properties of nanoparticles can be used to improve seed germination and crop performance. This use of the positive impacts of nanoparticles may be useful approach to decrease use of chemical agents in agriculture that would help to lower environmental pollution. Poor seed germination is a common occurrence in pulses and there are no studies on the effects of nanoparticles on *Vigna aconitifolia.* This study was therefore carried out to investigated possible phytotoxicity and /or beneficial stimulatory effects of AgNPs concentrations on moth bean seed and seedling growth.

Materials and Methods

Nanoparticle preparation

The sol-gel method was used for preparation of silver nanoparticles. silver nitrate ($AgNO_3$, 99.9%) and sodium oleate ($C_{18}H_{33}NaO_2$, 99%) were purchased from Molychem and Modern industries respectively. It was used eithout further purification. IM silver nitrate ($AgNO_3$) and 1M sodium oleate ($C_{18}H_{33}NaO_2$) solution in deionized water were prepared. Sodium oleate was added in silver nitrate solution and stirred at 20^0C on a magnetic stirrer for 2 h. After that filtered by Whatmann filter paper, dried mixture and heated in furnace at 300^0C for 4 h (Kim et al. 2004).

Preparation of nanoparticles suspension

Nanoparticles stock suspensions (1000 mg/L) was prepared by pre-weighed AgNPs in deionized water (DI-water) and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min. For further stabilization of nanoparticle suspension, 10% (v/v) polyethlene glycol (PEG-400) a dispersant was added (Zhang et al. 2007). The suspensions were sonicated again for at least 1 min before use.

Seeds germination and exposure

Seeds of moth bean *(Vigna aconitifolia)* were purchased from the local market. The seeds were stored in dark under room temperature. All the seeds were checked for their viability by suspending them in deionized water. The seeds which settle to the bottom were selected for further study. Seeds were immerzed in 10% sodium hypochloride for 10 minutes to ensure surface sterility (USEPA 1996). After rinsing three times with DI-water, seeds were soaked in deionized water (control) and different concentrations of AgNPs suspension solution. To investigate the promotory and inhibitory effects of nanomaterials on moth bean, three concentrations (50, 100, 150 mg/L) were prepared. Seeds were soaked in each suspensions (50, 100, 150 mg/L) for 2 h. One piece of a moisture germination paper was into each 100 mm × 15 mm petri dish. In each petri dish 5 ml of test medium of AgNPs (50, 100 and 150 mg/L) were added. Seeds were then transferred onto the moisture germination paper, with 10 seeds per dish and 1 cm of larger distance between each seed (Yang and Watts 2005). Petri dishes were covered and sealed with tape and allowed to germinate. All concentrations of AgNPs and the control were tested at the same time to ensure uniform conditions of light and temperature across all tests. Seeds were considered germinated when the radical showed at least 2 mm in length (ISTA 2009). The dry weight of seedling was recorded and expressed in gram after oven drying at 70^0C for 72 h.

Germination percentage was calculated when no further germination took place. Mean germination time was calculated based on Mathews and Khajeh-Hosseini (2007) (Eq. (1).

$$\text{MGT} = \frac{\sum FX}{\sum F} \quad (1)$$

Where F is the number of seeds newly germinated at the time of X and X is the number of days from sowing. After germination, the length of roots and shoots for all treatments and related controls (untreated) were measured.

For the germination rate and root and shoot growth investigation, seeds were allowed to germinate for 10 days. Seed germination and percent (FGP) of each treatment was calculated. Seedling root length and shoot length was measured. A seed was considered to have germinated when radical emerged from the seed coat according to the following equation (Ellis and Robert 1981, Ruan et al. 2002).

$$\text{FGP} = \frac{\text{No. of germinated seeds after 4 days}}{\text{Total number of germinated seeds}} \times 100$$

Germination Index (GI)

Germination index was calculated according to the following equation

$$\text{GI} = \frac{\text{Germination percentage of each treatment}}{\text{Germination percentage in the control}} \times 100$$

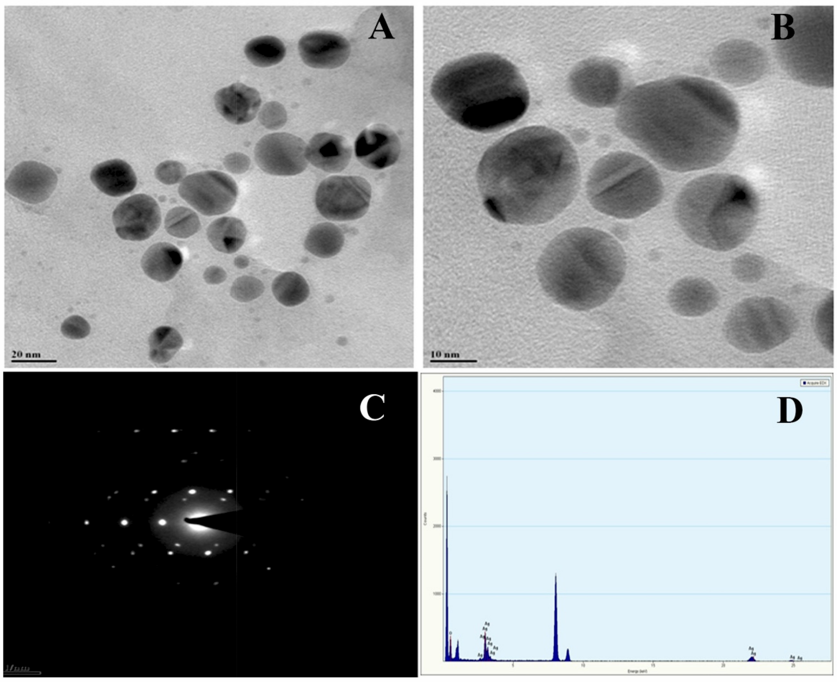

Fig. 1. Transmission electron micrographs (TEM) (A, B), selected are electron diffraction pattern (C) and EDAX of Ag nanoparticles (D).

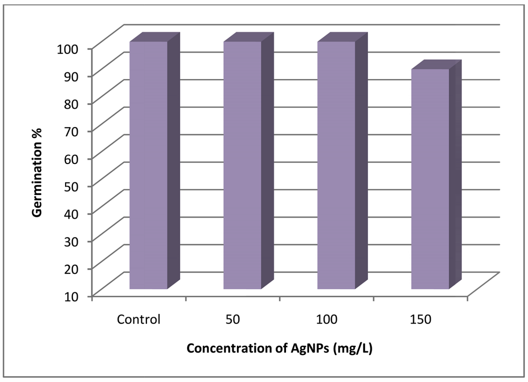

Fig. 2. Effect of different concentrations of AgNPs on germination percent of *Vigna aconitifolia*.

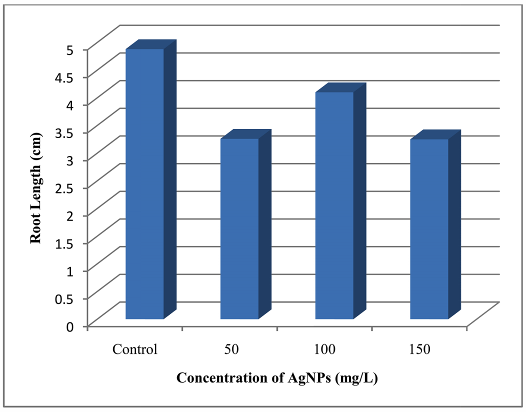

Fig. 3. Effect of different concentrations of AgNPs on root length of *Vigna aconitifolia.*

Seedling Vigor Index (SVI)

It was calculated according to the following formula

$$SVI = \frac{\text{Average shoot length}}{\text{Average root length}} \times \text{Germination percentage}$$

Results and Discussion

The transmission electron microscopy (TEN) image Figure 1 (A, B), selected area electron diffraction (SAED) pattern of AgNPs and EDAX are shown in Figure 1 (C, D). The TEM micrographs indicated that the AgNPs shows spherical morphology and their average particle size were found to be 17.3 nm by ImgeJ software EDAX spectrum shows the chemical composition of AgNPs. Result indicates the presence of Ag as the main element. This analysis confirms that AgNPs are effectively composed of Ag with no contamination. The SAED pattern of AgNPs shows that the rings are composed of dots suggesting the crystalline nature of these particles.

The effect of AgNPs on germination of moth beans at 50,100 and 150 mg/L concentration was examined. The obtained data clearly revealed slightly negative effects on seed germination percent as compared to the control (untreated) (Fig. 2). These results indicating that AgNPs had insignificant toxicological effect on seed germination. This may be due to the selective permeability of seed coats that does not allow nanoparticle material to pass through it (Lin and Xing 2007, Wierzbicka and Obidzinska 1998). These findings agree with recent reports stating that seed germination for different species was not affected by nAl_2O_3 (Yang and Watts 2005, Lee et al. 2010). Burklew et al. (2012) have been stated that the seed coat of tobacco seeds were most likely permeable to the Al_2O_3 nanoparticles, therefore the germination rate was not affected.

Regarding root growth exposure to AgNPs has a marked reduction in root length where the highest average growth was 4.1 cm after treatment with 100 mg/L concentration as compared to the control (4.88 cm). However minimum root length was observed in 3.25 cm in 150 mg/L AgNPs concentration (Fig. 3). These findings are consistent with other studies which reported that exposure of some seeds to several nanomaterials significantly reduced root growth (Yin et al. 2012). Dietz and Herth (2011) stated that nanoparticles may have to penetrate cell walls of plasma membranes of epidermal layers in root to intervascular tissues explaining why to root exposure was strong. Seed coats, which can have selective permeability play a very important role in protecting the embryo from harmful external factors. The process of seed germination and a root growth is rapid and widely used acute phytotoxicity test owing to sensitivety. simplicity, low cost and suitability for unstable chemicals. Pollutants as nanoparticles could penetrate root system causing obviously growth inhibition, may not affect seed germination if they cannot

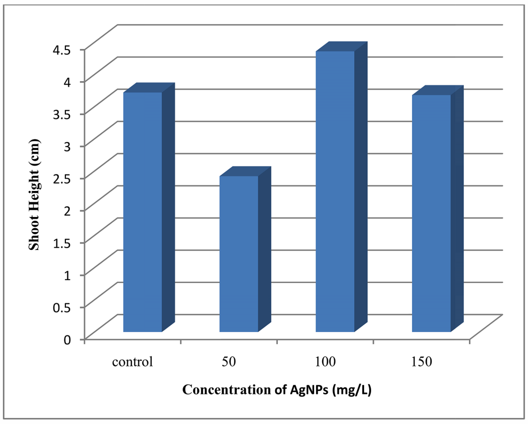

Fig. 4. Effect of different concentrations of AgNPs on shoot height of *Vigna aconitifolia.*

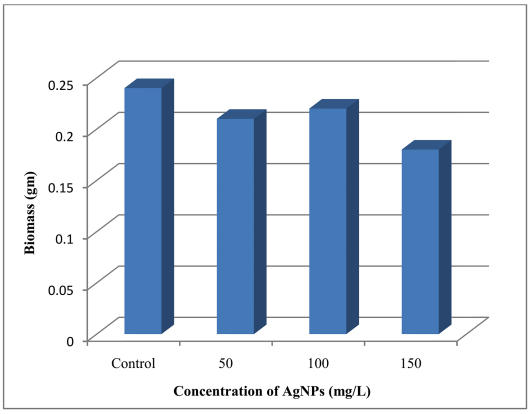

Fig. 6. Effect of different concentrations of AgNPs on biomass of *Vigna aconitifolia.*

pass through seed coats. This may explain that seed germination in this study was not greatly affected by nanoparticles.

Seeds with high vigor is important for crop production because it cannot significantly enhance seeding establishment but also improve the capability to compete against weeds at seedling stage. Huang et al. (2004) stated that seedling vigor is the ability of seed to rapidly from water or soil mainly reference to seed germination rate and early seedling growth. A significant reduction on SVI of various nanoparticle concentration was observed as compared to control

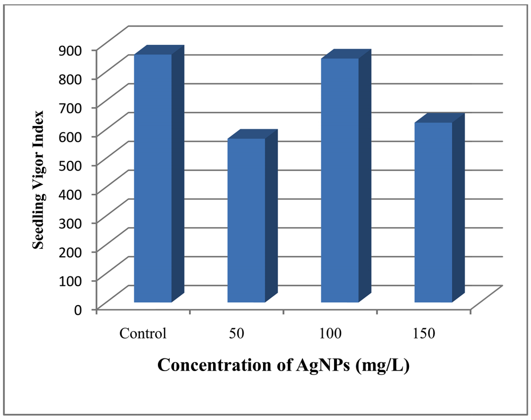

Fig. 5. Effect of different concentrations of AgNPs on seedling vigor index of *Vigna aconitifolia*.

(Fig. 5). Highest seedling vigor index was produced in 100 mg/L AgNPs concentration which was 486 followed by 623.7 and 568 in 150 mg/L and 50 mg/L AgNPs concentrations respectively.

Shoot, root and biomass was significantly affected by AgNPs concentrations. Application of AgNPs in 50 mg/L concentration greatly decreased shoot height (2.42 cm) compared to control seeds (3.72 cm). The highest shoot length (4.36 cm) was observed in 100 mg/L concentration showing significant positive effect (Fig. 4). The greatest biomass was found in 100 mg/L AgNPs concentration (0.22 g). Application of 150 mg/L AgNPs concentration greatly decreased biomass (0.18 g) compared to the control seeds (0.24 g), but at the concentrations of 100 mg/L (0.22 g) did not demonstrate a marked reduction in biomass (Fig. 6). It is probable that increasing the concentration of AgNPs induced aggregation of particles and resulted in clogging of pores that interrupted water uptake by seeds. In addition, application of 100 mg/L concentration of AgNPs increased moth beans seeding root length, shoot height and biomass. However, a significant decrease was observed at 150 mg/L concentration of AgNPs. It seems that AgNPs could stimulate process of seed germination just like water and oxygen uptake results in, improved seed germination percentage but in later growth stages seedling might respond as different. Lin and Xing (2007) confirmed the phytotoxicity of nano-Al and Al_2O_3 significantly affected root elongation of rye

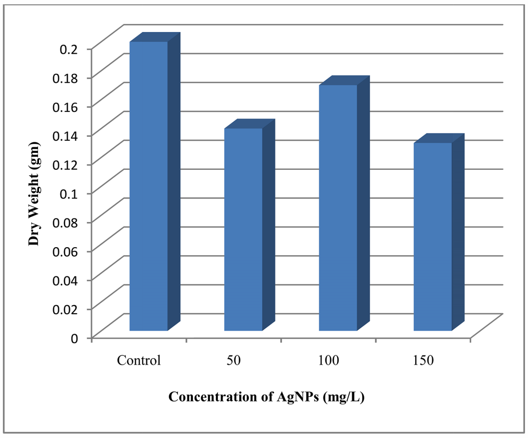

Fig. 7. Effect of different concentrations of AgNPs on dry weight of *Vigna aconitifolia.*

grass and corn respectively whereas nano-Al facilitated root growth in radish and rape. Barnea et al. (2009) stated that although root length and weight are not standardized in toxicity tests, they may be helpful to compare the toxicity effects after seed exposure to nanoparticles since low values can be related to non-acute toxicological effects.

Significant reduction in dry weight was observed as compared to control, the results are graphically illustrated in Fig. 7. In general, lower mean germination time shows the carlier germination. In the present study moth bean seeds were exposed to 50, 100 and 150 mg/L AgNPs concentrations. The results revealed that seeds in 50 and 100 mg/L AgNPs concentrations obtained the lowest mean germination

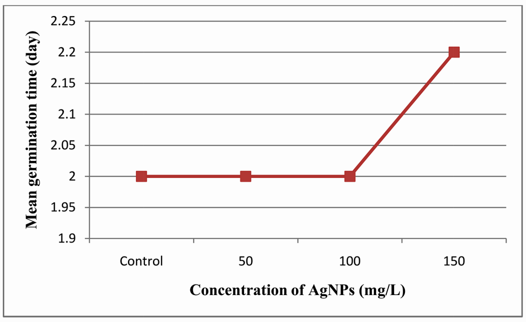

Fig. 8. Effect of different concentrations of AgNPs on mean germination time of *Vigna aconitifolia* seed.

time (2 d) same as that of control. However higher AgNPs concentration (150 mg/L) did not improve mean germination time (Fig. 8). Zheng et al. (2005) stated that the considerable effect of nanosized TiO_2 in spinach germination in tests was probably because of small particle size, which allowed nanoparticles to penetrate the seed during the treatment period, exerting its enhancing function during growth. Feizi et al. (2013) observed that the mean germination time in 40 ppm concentration of nanosized TiO_2 treatment reduced by 31.8% and of bulk TiO_2 treatment (40 ppm concentration) reduced by 21% in comparison with the control.

Conclusion

Seed germination and seedling growth is a rapid and widely used phytotoxicity test owing to sensitivity, simplicity, low cost and suitability for unstable chemicals. Seed coat play an important role in protecting the embryo from harmful external factors. Pollutants as nanomaterials may not affect seed germination but could penetrate root system causing obviously root growth inhibition. Our findings indicate that germination percentage was not adversely affected after all treatments whereas root growth, shoot height, biomass and seedling vigor index have negative impact after treatment with the various concentrations of AgNPs as compared to the control. It was observed that low and higher AgNPs concentrations have more adverse effects as compared to intermediate concentration on seedling growth (100 mg/L).

Overall, the study demonstrates the adverse effects of AgNPs on seedling growth, which underscores the necessicity of taking remedial measures in the disposal of wastes and sludge containing the nanoparticles and calls for further research for assessing the potential impacts of manufactured nanoparticles on agriculture and environmental systems.

Acknowledgement

Smt Dhawale V.P. is thankful to University Grants Commission (WRO), Pune, India for providing the Teacher Fellowship under faculty improvement program.

References

Barnea R, Casals E, Colón J, Font X, Sánchez A, Puntes V (2009) Evaluation of the ecotoxicity of model nanoparticles. Chemosphere 75 : 850—857.

Burklew CE, Ashlock J, Winfrey WB, Zhang B (2012) Effects of aluminum oxide nanoparticles on the growth, development, and microRNA expression of tobacco *(Nicotiana tabacum)*. PLoS ONE 7 (5) : e34783.

Carlson C, Hussain SM, Schrand AM, Brayditch-Stolle LK, Hess KL, Jones R, Schlager JJ (2008) Unique cellular interaction of silver nanoparticles : Size dependent generation of reactive oxygen species. J Physical Chem 112 (43) : 13608—13619.

Carmen IU, Chithra P, Huang Q, Takhistov P, Liu S, Kokini JL (2003) Nanotechnology : A new frontier in food science. Food Technol 57 : 24—29.

Chen K, Arora R (2013) Priming memory invokes seed stress-tolerance. Environm Experim Bot 94 (1) : 33—45. DOI : org/10. 1016/j.envexpbot.2012.03.005

Dietz KJ, Herth S (2011) Plant nanotoxicology. Trends in Pl Sci 16 : 582—589. doi: 10.1371/journal.pone.0047674

Ellis RH, Robert EH (1981) The quantification of aging and survival of orthodox seeds. Seed Sci Technol 9 : 373—409.

Feizi H, Kamali M, Jafari L, Moghaddam R (2013) Phytotoxicity and stimulatory impacts of nanosized and bulik titanium dioxide on fennel (*Foeniculum vulgare* Mill).Chemosphere 91 : 506—511.

Gubbins EJ, Batty LC, Lead JR (2011) Phytotoxicity of silver nanoparticles to *Lemna minor* L. Environm Poll 159 : 1551—1559.

Handford CE, Dean M, Henchion M, Spence M, Elliott CT, Campbell K (2014) Implications of nanotechnology for the agric-food industry : Opportunities, benefits and risks. Trends food Sci Technol 40 (2) ; 226—241.

Huang Z, Yu T, Su L, Yu SB, Zhang ZH, Zhu YG (2004) Identification of chromosome regions associated with seedling vigor in rice. Yi Chuan Xue-Bao 31 : 596—603.

ISTA (2009) ISTA rules. International Seed Testing Association. Zurich, Switzerland.

Kumar N, Shah V, Walker VK (2011) Perturbation of an arctic soil microbial community by metal nanoparticles. J Hazard Mater 190 : 816—822.

Lee CW, Mahendra YS, Zodrow YK, Li YD, Tsai YY-C, Braam J, Alvarez PJJ (2010) Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis Thaliana. Environ Toxicol Chem 29 : 669—675.

Lin D, Xing B (2007) Phytotoxicity of nanoparticles : Inhibition of seed germination and root growth. Environ Poll 150 : 243—250.

Matthews S, Khajeh-Hosseini M (2007) Length of the lag period of germination and metabolic repair explain vigor differences in seed lots of maize (*Zea mays*). Seed Sci Technol 35 : 200—212.

Patllola AK, Berry A, May L, Tehounwou PB (2012) Genotoxicity of silver nanoparticles in *Vicia faba* : A pilot study on the environmental monitoring of nanoparticles. Int J Environ Res Public Hlth 9 (5) : 1649—1662.

Pulate PV, Ghurde MU, Deshmukh VR (2011) Cytological effects of the biological and chemical silver-nanoparticles in *Allium cepa* L. Int J Innovat Bio-Sci 1 : 32—35.

Ruan S, Xue Q, Tylkowska K (2002) The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soil. Seed Sci Technol 30 : 61—67.

Stamploulis D, Sinha SK, White JC (2009) Assay-dependent phytotoxicity of nanoparticles to plants. Environ Sci and Tech 43 : 9473—9479.

US Environmental Protection Agency (USEPA), Ecological effects test guidelines : Seed germination/root elongation toxicity test. OPPTS 850. 4200. EPA 712-c-96- 154. Washington, DC. Salama, 1996.

Wierzbi.ka M, Obidzinska J (1998) The effect of lead on seed inhibition and germination in different plant species. Pl Sci 137 : 155—171.

Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol Letters 158 : 122—132.

Yin L, Benjamin PC, Bonnie MM, Justin PW, Emily SB (2012) Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. PLoS ONE 7 : 47674.

Zhang LL, Jiang YH, Ding YL, Povey M, York D (2007) Investigation in to the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanoparticle Res 9 : 479—489.

Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biol Trace Element Res 105 : 83—91.