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Influence of silver nanoparticles on seedlings of *Vigna radiata* (L.) R.Wilczek

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ABSTRACT

Nanoparticles(NPs), especially the engineered ones have many applications in the fields of pharmaceuticals, consumer products, cosmetics, transportation, energy and agriculture, thus, have an impact on many sectors of economy. In spite of this positive influence of the nanotechnology, the effect of release of nanoparticles into the environment is not clearly understood, especially, the potential health and environmental risks associated with them. Also, there are few studies available on the general consequences of the interactions of nanoparticles with higher plants. The present investigation was carried out to determine the effect of silver nanoparticles on different parameters such as seed germination and seedling biology of *Vigna radiata* (mung bean), belonging to Fabaceae, an economically important leguminous plant. Seeds of *V. radiata* were cultured under *in vitro* conditions on MS nutrient medium (Murashige and Skoog's medium, strength 1/20) fortified with filter sterilized silver nanoparticles at concentrations of 10 ppm (parts per million), 20 ppm, 50 ppm and 100 ppm. MS medium without nanoparticles served as the control. Addition of silver nanoparticles at a concentration of 10 ppm enhanced percent seed germination in comparison to the basal medium. Also, the shoots and roots of the seedlings raised attained maximum average length on this level of silver nanoparticles tried as compared to the control. The response obtained on other concentrations tried was less. These preliminary observations suggest that nanoparticles at a low level may promote seedling growth in the selected plants. If such positive observations are made with other plants as well, the results can be used for increasing the crop productivity.

Key words: *in vitro*, Murashige and Skoog's medium, silver nanoparticles, *Vigna radiate*

INTRODUCTION

Nanobiotechnology is a new emerging field of research which correlates the effect of nanotechnology on biological systems. It is a known fact that nanotechnology has a significant effect on agriculture and main areas of the food industry. We know that nanoparticles have an impact on many sectors of economy and trade, including consumer products, loom, pharmaceutics, cosmetics, transportation, energy and agriculture etc. These particles are measured in the scale of nanometer size which is one-billionth of a meter.

These small sized nanoparticles can modify the physiochemical properties of the materials, which can lead to adverse biological effect on living cells. According to a projection, nanoparticle industry will reach $1 trillion in 2015 from earlier $4 billion in 2005(1). They have been used in so many systems and in such large quantities that it is imperative that they are released into the ecosystem. There is a large difference between our understanding of nanotechnology and its impact on environment. There is very little data and research regarding the effects of engineered nanoparticles on terrestrial crops especially agricultural crops.

There are so many ways of exposure to human beings like the use of nanoparticles in medicine to pesticides. Due to all these reasons interest in environmental impact of nanoparticles has rapidly increased over the past few years. Nanoparticles as carriers of insecticides/pesticides/manures and/or fertilizers has resulted in improved plant protection but has led to their indiscriminate use and entry into the ecosystem. Also, another mode of entry is the synthesis and reckless disposal of nanoparticles in the laboratories. There are needs to have proper checks and regulations for determining the safety limits of nanoparticles.

There have been very few studies on effect of nanoparticles on plants and there are no clear cut guidelines for determining the toxicity of nanoparticles on plant systems (2). Out of the very few studies that are there, one study found that the mixture of nanoscale SiO_2 (silicon dioxide) and TiO_2 (titanium dioxide) hasten germination and growth in soya bean (3). Nanoscale TiO_2 is also known to promote photosynthesis, and growth of spinach according to one report (4,5). It has been observed in some experiments that aluminum (Al) particles did not inhibit the growth of *Phaseolus vulgaris* and *Lolium perenne*(6). Phytotoxicities of NPs on cabbage and carrot have been reported (7). Also, at concentrations greater than 2000 ppm, NPs were toxic to radish, rape, and rye grass (8). *Cucurbita maxima* growing in an aqueous medium containing magnetic nanoparticles could absorb, move and accumulate in the plant tissues (9) but on the other hand *Phaseolus limensis* was not able to absorb NPs. This highlights a very important point that different plants have different response to the same nanoparticles. Going by the same trend, aqueous TiO_2 (size 25 nanometers and 100 nanometers) in willow cuttings did not show any harmful effects (10). Application of slow/controlled release fertilizer coated by nanomaterials improved grain yield (11). There was an inconsequential increase in protein content and a decrease in soluble sugar content in wheat compared to NPK (Nitrogen, Phosphorous, Potassium) chemical fertilizer. This is a step in the direction of finding new methods to increase the productivity of crops. There are reports of developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana* (12) and phytotoxicity of ZnO(Zinc oxide) nanoparticles to rye grass(13) .

The present study shows effect of silver nanoparticles on *Vigna radiata* seedlings under *in vitro* conditions. The data will help in understanding the toxic limit of silver nanoparticles used in industry for commercial purposes which are intentionally or unintentionally released in ecosystem.

The mung bean (Figure IA and B) commonly known as green gram is an economically important leguminous plant belonging to the family Fabaceae used for production of bean sprouts (14). This plant can be grown successfully in extreme environmental conditions of high temperatures, low rain fall, and poor soils with few economic inputs. Many of these species produce multiple edible products, and these products provide subsistence farmers with a food supply throughout the growing season as well as dry seeds that are easy to store and transport. For example, tender shoot tips and leaves of cowpeas can be consumed as soon as the plants reach the seeding stage and immature pods and seeds can be consumed during the fruiting stage. Harvested dry seed of all of the Vigna crops can be consumed directly, and seeds of several of the crops are commonly used to make flour or produce sprouts. Plant residues can be used as fodder for farm animals. Vigna food products exhibit many excellent nutritional attributes and these products provide a needed complement in diets comprised mainly of roots, tubers, or cereals (15).

Figure I: *Vigna radiata* : (A) Seeds. (B) A flowering and fruiting plant.

METHODOLOGY

Preparation of silver (Ag) nanoparticles

Physical and chemical both methods can be used to prepare Ag nanoparticles. We have preferred to use chemical reduction. There are many reductants that can be used like borohydride, citrate, ascorbate and elemental hydrogen. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. The reduction of various complexes with Ag^+ions leads to the formation of silver atoms (Ag^0), which is followed by agglomeration into oligomeric clusters which lead to the formation of colloidal Ag particles. According to studies, use of a strong reductant such as borohydride, resulted in small particles that were somewhat monodispersed, but the generation of larger particles was difficult to control. Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but the size distribution was far from narrow. It is important to use protective agents such as Polyvinylpyrrolidone(PVP) to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation. The most common strategy is to protect the nanoparticles with protective agents that can be adsorbed or bind onto the nanoparticle surface, avoiding their agglomeration. 100 ml of standard solution of silver nitrate (10mg in 100ml water) was prepared. Then 50 ml solution of sodium citrate (0.5g in 50ml distilled water) was prepared. Silver nitrate solution was poured in the beaker and the beaker was put on magnetic stirrer for half an hour. Slowly, 4ml of sodium citrate was added to 100ml of silver nitrate solution, using pipette. The colour change was observed after half an hour. After the colour changes to grey, the stirring was stopped. Tween-20 was added as a stabilizer of nanoparticles.

Preparation of MS medium (Murashige and Skoog's medium)
Murashige and Skoog's semi-solid (MS) medium at a strength of 1/20 was prepared(16). Sucrose at 3% level was added as carbon source to the nutrient medium. For solidification, 0.8% agar was added. The pH of the medium was adjusted to 5.8.

Incorporation of silver NPs (Nanoparticles) into the nutrient medium
Nanoparticles are thermolabile, so they cannot be autoclaved. Therefore, silver NPs were sterilized by passing the solution, through sterilized Millipore filtration system having a pore size of 0.3 µm attached with clinical syringe. Then equal amount of the medium containing NPs was dispensed into culture tubes. These steps were carried out in a Laminar Air Flow

Chamber. The different concentrations of silver NPs tried were 10 ppm, 20 ppm, 50 ppm and 100 ppm. MS medium without NPs was considered as the control.

Sterilization of seeds

The seeds (Figure IA) of *V. radiata* were procured from a local nursery(17). They were first washed with teepol for ten minutes. Then, they were surface sterilized with 0.5% mercuric chloride for eight minutes and finally washed with sterilized double distilled water 2-3 times in a Laminar Air Flow Chamber.

Culture conditions

The cultures of *V. radiata* were maintained at a temperature of 26±2^{0}C and illumination of 80 µ mol sec^{-1} m^{-2} of 8 hours light and 16 hours dark in the culture room.

In vitro studies

For raising the cultures, two seeds per culture tube were inoculated. The various parameters studied were percentage of seed germination, day of emergence of leaves, number of leaves, leaf characteristics, shoot length, day of appearance of the radical, root length and number of root laterals. The observations were taken at an interval of two days. The final observation was taken after ten days of inoculation.

RESULTS

On comparing the observations of different media, it was noted that highest percentage of seed germination was on 10ppm silver NPs containing medium as compared to basal medium as well as other levels of NPs tried. The average length of root and number of root laterals was also maximum in this medium (Figure II). This concentration of silver NPs also promoted shoot growth as maximum average shoot length was obtained with shoots showing green and well-spread leaves (Figure III). On other media, the shoots and roots were comparatively smaller in length. Average root length was more on basal medium as compared to 20 ppm, 50 ppm and 100 ppm, but less than 10 ppm concentration of silver NPs fortified MS medium (Figure IV A). The *in vitro* developed plants of *V. radiata* were transferred to pots containing vermiculite where they survived well (Figure IV B).

DISCUSSION

Of the different levels of silver nanoparticles added to MS medium, 10 ppm concentration proved to be the best as optimum response in terms of percent seed germination and overall seedling growth was obtained at this level. Similar results were reported on common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) wherein small levels of silver nanoparticles stimulated the growth of the plantlets, while the higher concentrations had an inhibitory effect(18). The variation in the response was attributed to the accumulation and uptake of silver NPs by the roots. Also, the accumulation and uptake of NPs was dependent on the exposure concentration.

A remarkable increase of chlorophyll content was reported in mungbean(19). In another study, a positive effect of silver NPs on seedling growth of *V. radiata* was observed and the enhanced uptake of water and nutrients by the treated seeds was suggested as the influencing factor(20).

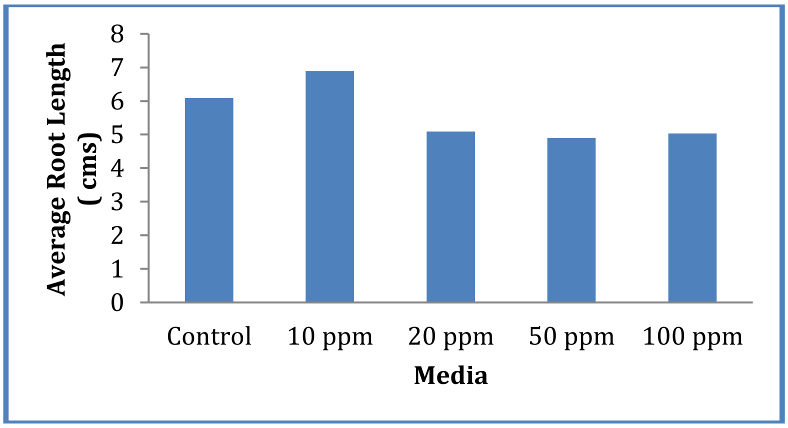

Figure II: Effect of silver NPs on average root length of *Vigna radiata* seedlings.

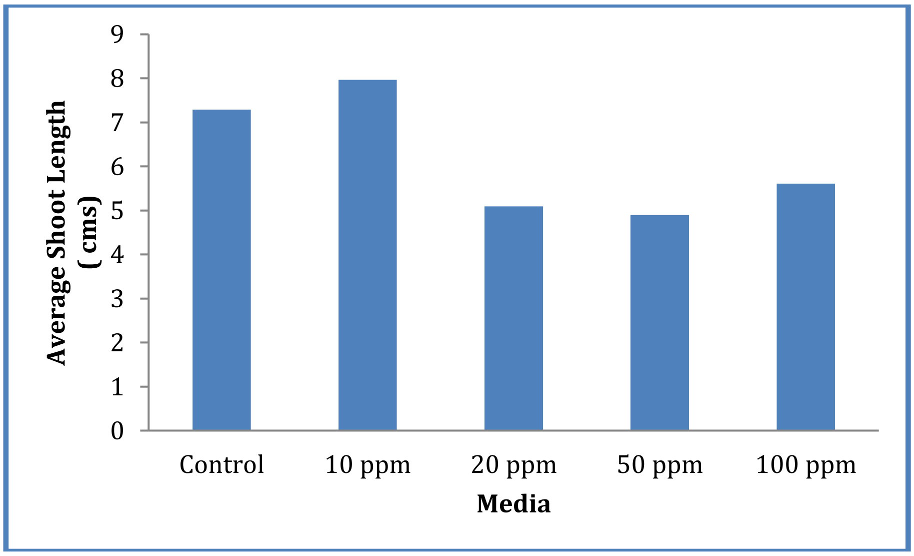

Figure III: Effect of silver NPs on average shoot length of *Vigna radiata* seedlings.

Figure IV *Vigna radiata*: (A) Plants raised on different concentrations of silver NPs supplemented to MS medium after 10 days of culture. (B) *In vitro* raised plant transferred to vermiculite.

CONCLUSIONS

The present investigation demonstrated the effect of silver nanoparticles on mung bean. Supplementation of MS medium with silver nanoparticles affects the growth at different concentrations. An enhanced per cent seed germination with increased root and shoot length over control was obtained at low level of NPs tried whereas higher concentrations either inhibited the growth or the response was comparatively less.

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