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# Effect of Silver Nanoparticles on Seed Germination of Crop Plants

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## ABSTRACT

Engineered nanomaterials have increased for their positive impact in improving many sectors of economy including agriculture. Silver nanoparticles (AgNPs) have been implicated nowadays to enhance seed germination, plant growth, improvement of photosynthetic quantum efficiency and as antimicrobial agents to manage plant diseases. In this study, we examined effect of AgNPs dosage on seed germination of three plant species; corn (Zea mays L.), watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) and zucchini (Cucurbita pepo L.). Therefore, this experiment designed to study the effect of AgNPs on germination percentage, germination rate, mean germination time, root length, fresh and dry weight of seedling for the three spices. Seven concentrations (0.05, 0.1, 0.5, 1, 1.5, 2 and 2.5 mg/L) of AgNPs were examined at seed germination stage. The results showed that the three spices revealed different dosage response to AgNPs on germination percentage and the measured growth characters. Germination rate values were enhanced for the three plants in response to AgNPs. Significant enhancement in germination percentage values for watermelon and zucchini plants were observed by treatment with AgNPs in comparison with nontreated seeds. AgNPs showed toxic effect on corn roots elongation whereas watermelon and zucchini seedling growth were positively affected by certain concentration of AgNPs. This study showed that exposure to AgNPs caused both positive and negative effects on plant growth and germination.

## Keywords

Silver nanoparticles, seed germination, seedling growth, Cucurbita pepo L., Zea mays L., and Citrullus lanatus (Thunb.) Matsum. & Nakai.

## Abbreviations

AgNPs: Silver nanoparticles, PVP-AgNPs: polyvinylpyrrolidine-coated AgNPs, GA-AgNPs: gum arabic coated AgNPs

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## INTRODUCTION

Nanotechnology is a branch of science which is related to nano materials helps in overcoming the limitations of size and can change the outlook of the world regarding science. The interactions of nanomaterials with plants have not been fully elucidated. There are different and often conflicting reports on the absorption, translocation, accumulation, biotransformation, and toxicity of nanoparticles on various plant species. Silver nanoparticles (AgNPs) is one of the nanomaterials that its effects are under investigation [1]–[2]. The impact of AgNPs on higher plants appears to depend on the species and age of plants, the size and concentration of the nanoparticles, the experimental conditions such as temperature, and the duration and method of exposure. For instance, 10 mg/L AgNPs inhibited seed germination in *Hordeum vulgare* and reduced shoot length in *Linum usitatissimum* and *Hordeum vulgare* [3]. AgNPs dosage from 0.2 to 1.6 mg/L also inhibited seed germination, lipase activity, soluble and reducing sugar contents in Brassica nigra germinating seeds and seedlings [4]. However, AgNPs had no significant effects on seed germination, root, and shoot length of castor bean, Ricinus communis L. [5] and Vicia faba [6] even at higher concentrations of AgNPs. 100 mg/L AgNPs had also no significant effect on seed germination in *Cucumis sativus*, and *Lactuca sativa* [7]. Other studies indicate a positive role for AgNPs in the promotion of plant growth in *Brassica juncea* [8], *Panicum virgatum, Phytolacca Americana* [9], *Phaseolus vulgaris and Zea mays* [10]. Seed germination was positively affected by treatment with AgNPs in Boswellia ovalifoliolata plant [11] and Pennisetum glaucum [12].

Recent studies reported that plant response to AgNPs either enhancement or inhibition of growth depends on AgNPs dosage. Of serial of concentrations, exposure to specific concentrations of AgNPs could enhance plant growth compared with nonexposed plants, while higher and lower concentrations effect plant growth negatively [13]-[14]- [15]. Of used AgNPs concentrations (0, 25, 50, 100, 200 and 400 ppm), 50 ppm treatment being optimum for eliciting growth response in *Brassica juncea* seedlings. Fresh weight, root and shoot length, and vigor index of seedlings is positively affected. This dose induced a 326 % increase in root length and 133 % increase in vigor index of the treated seedlings [8]. Using 10 mg/L of polyvinylpyrrolidine-coated AgNPs (PVP-AgNPs) also found to increase root elongation in Eruca sativa [16]. Treatment Arabidopsis thaliana plants with 1 and 2.5 mg/L of AgNPs increased seedling biomass, whereas treatment with higher concentrations decreased seedling biomass [13]. Growth inhibition in the aquatic plant Lemna gibba was demonstrated by a significant decrease of frond numbers dependent on AgNPs concentration [17].

Reference [9] examined responses of eleven wetland plants to AgNPs–20-nm PVP-AgNPs and 6-nm gum arabic coated AgNPs (GA-AgNPs) by two methods of exposure; direct exposure in simple pure culture and soil exposure for seeds planted in homogenized field soils. In the direct exposure experiments, PVP-AgNP had no effect on germination while 40 mg/L GA-AgNP exposure significantly reduced the germination rate of three species and enhanced the germination rate of one species. The magnitude of inhibition was always greater for GA-AgNPs than for PVP-AgNPs. In the soil exposure experiment, germination effects were less pronounced. The plant growth response differed by taxa with Lolium multiflorum growing more rapidly under GA-AgNP exposures and all other taxa having significantly reduced growth under GA-AgNP exposure while PVP-AgNPs significantly inhibited the growth of only one species. AgNPs toxicity was also concentration dependent.

As AgNPs effect, the enhancement of plant growth with low concentration and the inhibition with higher concentration were seen also with exposure to other nanoparticles. Exposure to low concentration of multi walled carbon nanotubes (MWCNTs) has enhanced growth of maize seedlings compared with higher concentrations [18]. Treatment with 8 g/L of nanosilicon dioxide ($nSiO_2$) was positively affect tomato seed germination [19]. The increase in the anatase nanoparticles (nTiO2) concentration caused to a significant increase in the percentage of germination, germination rate index, radicle and plumule length, fresh weight and vigor index of seedlings in Capsicum annum L., while the best concentration of nTiO2 was 7.5% [20]. nTiO2 promoted photosynthesis and nitrogen metabolism and improved growth of spinach [21].

Here, we have focused on effect of AgNPs on seed germination and plant growth for three crop plants that exposed to different concentrations of AgNPs. Determining dose-response of plant for nanoparticles is expected to improve our ability in using nanoparticles to increase global crop yields and enable plant defenses against pathogens. Moreover, using optimum dose of nanoparticles might minimize the risk of nanoparticles in plant environment.

## MATERIALS AND METHODS

### AgNPs preparation

AgNPs (silver nanopowder, 99.99%, 20 nm) were purchased from U.S. Research Nanomaterials (Houston, TX). Different doses of AgNPs were prepared for the germination experiment. Seed germination of three plants was tested in response to AgNPs by planting seeds in the presence of increasing concentrations of the AgNPs (0.05, 0.1, 0.5, 1, 1.5, 2 and 2.5 mg/L). Control plants were grown in distilled water only.

### Seed Germination Experiment

Seeds were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility [30], then they were soaked in distilled water for two hours, and then soaked in serial of prepared AgNPs concentration suspension for about 2hrs after being rinsed four times with distilled water. One piece of filter paper was put into each 100 mm X 15 mm Petri dish, and 5 ml of a test solution was added. Seeds were transferred onto the filter paper, with 10 seeds per dish and 1 cm or larger distance between each seed [31]. Petri dishes were covered and sealed with tape, incubated room temperature. The germination was halted after 12 days, except zucchini plant, which its germination halted after 16 days. Seed germination rate and mean germination time were calculated, seedling dry and fresh weight, and root length were measured.

## Seed Germination Measurement

The final germination percentage was calculated based on total number of germinated seeds at the end of experiment. The measurements were carried out according to International Rules for Seed Testing [32]. Germination indices were calculated using the following equations [33]-[34]-[35].

Germination percentage (GP %) = (Gf/n) × 100

where, Gf is the total number of germinated seeds at the end of experiment and n is the total number of seed used in the test.

Mean Germination Time (MGT) = Σ NiDi/n

where, Ni is number of germinated seeds till ith day and Di is number of days from start of experiment till ith counting and n is total germinated seeds.

Germination rate (GR) = Σ Ni/ΣTiNi

where, Ni is the number of newly germinated seeds at the time of Ti.

= (a/1)+(b-a/2)+(c-b/3)+.....+(n-n-1/N)

## Statistical analysis

Means and standard deviations were derived from measurements on three replicates for each treatment and the related controls. The data obtained from the various treatments were statistically analysed using the t-test at a significance level of 0.5.

# RESULTS

Increasing use of nanoparticles in daily products is of great concern today, especially when their positive and negative impact on environment is not known. Hence, in current research, we have studied the impact of AgNPs application on seed germination and seedling growth of three crop plants; corn, watermelon and zucchini. A significant increasing shown in germination rate for corn seeds by treatment with higher AgNPs concentrations, whereas no significant effect on corn germination percentage and mean germination time (Table 1).

**Table 1. Influence of AgNPs concentrations on germination percentage, germination rate and mean germination time for corn, watermelon and zucchini plants**

| AgNPs concentrations (mg/L) | | | Control | 0.05 | 0.1 | 0.5 | 1 | 1.5 | 2 | 2.5 |
|---|---|---|---|---|---|---|---|---|---|---|
| Corn | GP% | M | 96.67 | 96.67 | 100.00 | 86.67 | 96.67 | 100.00 | 100.00 | 100.00 |
| | | SD | 2.77 | 1.77 | 0.00 | 0.77 | 1.77 | 0.00 | 0.00 | 0.00 |
| | GR (seed/day) | M | 4.81 | 6.40 | 5.39 | 4.97 | 6.11 | 6.50* | 6.25* | 6.25 |
| | | SD | 0.59 | 0.12 | 0.37 | 1.31 | 1.17 | 1.04 | 0.52 | 1.10 |
| | MGT (day) | M | 2.20 | 2.03 | 2.87 | 2.03 | 2.67 | 1.97 | 2.87 | 2.07 |
| | | SD | 0.93 | 0.29 | 0.54 | 0.46 | 1.26 | 1.16 | 1.34 | 1.20 |
| Watermelon | GP% | M | 40.00 | 56.67* | 60.00* | 60.00* | 63.33* | 63.33* | 73.33* | 60.00 |
| | | SD | 1.00 | 3.15 | 3.00 | 1.32 | 1.28 | 2.77 | 5.28 | 2.00 |
| | GR (seed/day) | M | 0.85 | 1.33 | 1.25 | 1.50* | 1.51* | 1.49* | 1.59* | 1.34 |
| | | SD | 0.06 | 0.10 | 0.02 | 0.06 | 0.06 | 0.20 | 0.15 | 0.10 |
| | MGT (day) | M | 2.80 | 3.20 | 3.67 | 2.83 | 3.33 | 2.83 | 2.87 | 3.20 |
| | | SD | 1.09 | 1.04 | 0.42 | 1.31 | 1.45 | 0.10 | 1.03 | 0.23 |
| Zucchini | GP% | M | 56.67 | 66.67 | 73.33 | 86.67* | 73.33* | 83.33* | 66.67* | 90.00* |
| | | SD | 3.12 | 1.28 | 2.82 | 1.28 | 1.55 | 2.82 | 0.77 | 8.00 |
| | GR (seed/day) | M | 0.99 | 1.54* | 1.36* | 1.68* | 1.58* | 1.59* | 1.16 | 1.66* |
| | | SD | 0.06 | 0.03 | 0.05 | 0.02 | 0.01 | 0.04 | 0.02 | 0.02 |
| | MGT (day) | M | 4.03 | 5.03 | 4.43 | 6.03 | 3.57 | 6.23* | 4.90 | 6.87* |
| | | SD | 0.70 | 1.24 | 2.21 | 1.35 | 1.56 | 1.17 | 2.68 | 1.97 |

* Representing significant effects at 0.05% probability level. M (Mean), SD (standard deviations), GP (germination percent), GR (Germination rate) and MGT (mean germination time).

The highest germination rate value for corn seeds, which was 6.50 seeds/day, observed with exposure to 1.5 mg/L of AgNPs. However, watermelon and zucchini plants with exposure to AgNPs revealed great response to AgNPs treatments compared with nontreated seeds, shown in significant enhancement in germination percentage and germination rate values. The highest germination percentage value (73.33%) and highest germination rate value (1.59 seeds/day) for watermelon recorded with 2 mg/L AgNPs. For zucchini plant, the highest germination percentage values (86.67and 90%) and highest germination rate value (1.68 and 1.66 seeds/day) recorded with 0.5 and 2.5 mg/L of AgNPs, respectively.

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Among the three plants, the significant response in mean germination time observed only for zucchini seeds, which increased significantly with 1.5 and 2.5 mg/L of AgNPs, and that means later germination in comparison to nontreated plants.

AgNPs had toxic effect on corn seedling shown in root length values. Significant inhibition in root length was observed with all AgNPs concentration, especially with 1.5 and 2 mg/L, which was 7.30 and 7.58 cm, respectively (Table 2). Seedling fresh weight values for corn plants treated with certain AgNPs concentration were significantly higher than nontreated plants as shown with 2 mg/L AgNPs treatment (154 mg), while no significant effect on seedling dry weight with AgNPs treatment. Root length of watermelon and zucchini plants was positively affected by AgNPs. The significant response in root length values shown with higher AgNPs concentration for watermelon, whereas high root length values for zucchini observed with low AgNPs concentrations. The highest root length mean value in watermelon (11.4 cm) observed with 2 mg/L of AgNPs, and for zucchini the highest root length (14.48 cm) observed with 0.5 mg/L of AgNPs. Seedling fresh weight for watermelon increased with higher concentrations of AgNPs, while dry weight decreased significantly with certain concentrations of AgNPs. The highest value for watermelon seedling fresh weight (373.5 mg) observed at 2 mg/L of AgNPs. Zucchini fresh and dry weight values increased significantly by certain concentration of AgNPs. The maximum value for seedling fresh weight (1,088.89 mg) recorded with exposure to 0.1 mg/L of AgNPs, whereas the maximum value for seedling dry weight (124.36 mg) observed for seedling treated with 0.05 mg/L of AgNPs.

**Table 2: Influence of AgNPs concentrations on root length, seedling fresh and dry weight of corn, watermelon and zucchini plants**

| AgNPs concentrations (mg/L) | | | Control | 0.05 | 0.1 | 0.5 | 1 | 1.5 | 2 | 2.5 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Corn** | RL (cm) | M | 11.50 | 9.52* | 7.67* | 8.25* | 8.18* | 7.30* | 7.58* | 8.96* |
| | | SD | 1.67 | 2.65 | 1.09 | 1.81 | 2.28 | 1.63 | 2.79 | 1.87 |
| | FW (mg) | M | 114.44 | 113.37 | 94.67 | 118.26* | 122.67* | 143.75* | 154.00* | 124.00 |
| | | SD | 15.11 | 21.12 | 9.27 | 6.86 | 14.63 | 3.79 | 9.35 | 8.72 |
| | DW (mg) | M | 14.44 | 13.44 | 12.33 | 15.51 | 17.93 | 12.33 | 13.33 | 14.33 |
| | | SD | 1.39 | 0.51 | 1.03 | 2.27 | 2.00 | 2.52 | 2.08 | 0.58 |
| **Watermelon** | RL (cm) | M | 4.37 | 4.04 | 2.64 | 4.88 | 9.62* | 6.34* | 11.41* | 8.46* |
| | | SD | 1.91 | 2.16 | 1.87 | 1.06 | 2.41 | 1.42 | 2.62 | 1.28 |
| | FW (mg) | M | 298.61 | 225.00 | 263.14 | 305.56 | 343.44 | 337.14* | 373.50* | 285.58 |
| | | SD | 14.98 | 11.06 | 5.38 | 6.71 | 10.95 | 1.50 | 10.98 | 5.54 |
| | DW (mg) | M | 32.94 | 21.19 | 24.00* | 29.71 | 29.58 | 29.21* | 31.53* | 27.25* |
| | | SD | 0.76 | 1.69 | 1.29 | 0.01 | 1.63 | 2.71 | 1.75 | 1.23 |
| **Zucchini** | RL (cm) | M | 6.90 | 8.79* | 13.28* | 14.49* | 9.88 | 12.94 | 8.94 | 8.79 |
| | | SD | 1.59 | 1.35 | 2.53 | 1.44 | 2.07 | 1.19 | 3.97 | 2.79 |
| | FW (mg) | M | 556.11 | 818.17 | 1088.89* | 824.42* | 855.14* | 974.33* | 467.78 | 712.39 |
| | | SD | 33.32 | 44.82 | 55.61 | 18.29 | 37.02 | 10.36 | 47.76 | 55.71 |
| | DW (mg) | M | 67.04 | 124.36* | 118.02* | 108.69* | 95.28 | 101.11 | 121.90* | 116.33 |
| | | SD | 6.44 | 6.40 | 20.59 | 4.84 | 9.37 | 18.36 | 15.93 | 12.66 |

* Representing significant effects at 0.05% probability level. M (Mean), SD (standard deviations), RL (root length) FW (seedling fresh weight) and DW (seedling dry weight).

## DISCUSSION

Germination is important for determining the final plant density if planted seeds germinate completely and vigorously [22]. AgNPs have been implicated nowadays in agriculture to improve crops. The study showed that the three spices revealed different dosage response to AgNPs on germination percentage and the measured growth characters. Our result indicated that exposure to AgNPs had significant effects on seed germination and seedling growth of corn, watermelon and zucchini plants. The best dose of AgNPs for watermelon plant detected by our result is 2 mg/L, which enhanced germination percentage and germination rate for highest values. Exposure to 0.5 and 2.5 mg/L of AgNPs appeared to be proper to enhance zucchini seed germination. Highest germination percentage and germination rate observed with these concentrations but mean germination time increased with all AgNPs concentration compared to nontreated plants, which represents later germination. Same results seen by [11] that AgNPs significantly increased germination value of seeds of Boswellia ovailifoliolata plant, but increased germination time. In contrast to our observations, seed germination was not affected by exposure to AgNPs in Vicia faba [6] and Arabidopsis thaliana [14].

The best effect of exposure to AgNPs shown with corn germination, which revealed great enhancement for all germination parameters with higher AgNPs dosage. It is most probable that nanoparticles could penetrate into the seed coat and exert a helpful effect on the process of seed germination. Based on studies on nanoparticles effect on seed germination mechanism it could state nanoparticles might helped the water absorption by the seeds [23], increase nitrate reductase enzyme, increase seed abilities of absorbing and utilizing water and fertilizer, promote seed antioxidant system [24], reduced anti oxidant stress by reducing $H_2O_2$, superoxide radicals, and malonyldialdehyde content, and increasing some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities [25], result in improve seed germination in some plant species.

The effect of AgNPs on seedling growth for the three plants appeared to be not related to its effect on germination parameters. AgNPs had toxic effect on seedling root length of corn whereas root length of watermelon and zucchini plants were positively affected by AgNPs. Inhibition in corn root length values are confirmed by results obtained from [10] study. It demonstrated that corn and common bean root length mean values decreased with exposure to concentration higher than 60 ppm of AgNPs, which equal to 0.06 mg/L, and increased with AgNPs concentrations less than this concentration, which less than the concentrations applied in our experiment. As shown in watermelon and zucchini plants, root length values enhanced in response to AgNPs treatment for Vicia faba [6] and Eruca sativa [16]. Same effect of AgNPs on corn plant in this study was observed on Pennisetum glaucum plant, which its seed germination promoted in response to AgNPs while seedling root elongation was inhibited [12].

Seedling fresh weight increased with AgNPs treatments for the three plant. Seedling dry weight for zucchini increased with certain concentrations of AgNPs, while dry weight for watermelon decreased significantly. Zucchini plant revealed the great enhancement in seedling growth with treatment by AgNPs. This result disagree with [26] study, which found that the biomass of zucchini plant reduced with exposure to AgNPs concentrations from 1 to 1000 mg/L. Improvement in seedling fresh and dry weight in response to AgNPs confirmed by [10]-[27]. Contrastingly, AgNPs had no significant effects on seedling growth of Ricinus communis L. [5]. AgNPs also inhibited seedling growth of Lemna minor [28] and rice (Oryza sativa L.) [29].

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