

Effect of Silver Nanoparticles on Seed Germination of Crop Plants

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Abstract—The use of engineered nanomaterials has increased as a result of their positive impact on many sectors of the economy, including agriculture. Silver nanoparticles (AgNPs) are now used to enhance seed germination, plant growth, and photosynthetic quantum efficiency and as antimicrobial agents to control plant diseases. In this study, we examined the effect of AgNP dosage on the seed germination of three plant species: corn (*Zea mays* L.), watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) and zucchini (*Cucurbita pepo* L.). This experiment was designed to study the effect of AgNPs on germination percentage, germination rate, mean germination time, root length and fresh and dry weight of seedlings for the three species. Seven concentrations (0.05, 0.1, 0.5, 1, 1.5, 2 and 2.5 mg/ml) of AgNPs were examined at the seed germination stage. The three species had different dose responses to AgNPs in terms of germination parameters and the measured growth characteristics. The germination rates of the three plants were enhanced in response to AgNPs. Significant enhancement of the germination percentage values was observed after treatment of the watermelon and zucchini plants with AgNPs in comparison with untreated seeds. AgNPs showed a toxic effect on corn root elongation, whereas watermelon and zucchini seedling growth were positively affected by certain concentrations of AgNPs. This study showed that exposure to AgNPs caused both positive and negative effects on plant growth and germination.

Keywords—*Citrullus lanatus*, *Cucurbita pepo*, seed germination, seedling growth, silver nanoparticles, *Zea mays*.

I. INTRODUCTION

NANOTECHNOLOGY is a branch of science that is related to nanomaterials, which help overcome the limitations of size. The interactions of nanomaterials with plants have not been fully elucidated. There have been different and often conflicting reports on the absorption, translocation, accumulation, biotransformation, and toxicity of nanoparticles in various plant species. The effects of silver nanoparticles (AgNPs) are still under investigation [1], [2]. The impact of AgNPs on higher plants appears to depend on the species and age of the 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 was found to inhibit seed germination in *Hordeum vulgare* and reduced shoot length in flax (*Linum usitatissimum*) and barley (*Hordeum vulgare*) [3].

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A AgNP dosage from 0.2 to 1.6 mg/L was also found to inhibit seed germination, lipase activity, and soluble and reducing sugar content in *Brassica nigra* germinating seeds and seedlings [4]. However, AgNPs were shown to have no significant effects on seed germination, root length, or shoot length of the castor bean plant, *Ricinus communis* L. [5] or *Vicia faba* [6] even at higher concentrations of AgNPs. 100 mg/L AgNPs was found to have no significant effect on seed germination in *Cucumis sativus* or *Lactuca sativa* [7]. Other studies have indicated that AgNPs can promote the growth of *Brassica juncea* [8], *Panicum virgatum*, *Phytolacca Americana* [9], *Phaseolus vulgaris* or *Zea mays* [10]. Seed germination in *Boswellia ovalifoliolata* [11] and *Pennisetum glaucum* [12] have been shown to be positively affected by treatment with AgNPs.

Recent studies have reported that a plant's response to AgNPs, enhancement or inhibition of growth, depends on the AgNP dosage. Exposure to specific concentrations of AgNPs could enhance plant growth compared with non-exposed plants, whereas higher and lower concentrations could affect plant growth negatively [13]-[15]. Of the reported AgNP concentrations used (0, 25, 50, 100, 200 and 400 ppm); a 50-ppm treatment has been determined to be optimal for eliciting a growth response in *Brassica juncea* seedlings. The fresh weight, root and shoot length, and vigour index of seedlings are positively affected at this concentration. This dose was found to induce a 326% increase in root length and a 133% increase in the vigour index of the treated seedlings [8]. Using 10 mg/L of polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) also found to increase root elongation in *Eruca sativa* [16]. Treatment of *Arabidopsis thaliana* plants with 1 or 2.5 mg/L of AgNPs was found to increase seedling biomass, whereas treatment with higher concentrations was found to decrease seedling biomass [13]. Growth inhibition in the aquatic plant *Lemna gibba* has been demonstrated, with a significant AgNP concentration-dependent decrease in frond numbers [17].

Reference [9] examined the responses of eleven wetland plants to AgNPs (20-nm PVP-AgNPs and 6-nm gum arabic coated AgNPs (GA-AgNPs)) using two methods of exposure: direct exposure in simple pure cultures, and soil exposure for seeds planted in homogenized field soils. In the direct exposure experiments, PVP-AgNPs had no effect on germination, whereas 40 mg/L of GA-AgNPs 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, the germination effects were less pronounced. The plant growth response differed between the

taxa, with *Lolium multiflorum* growing more rapidly under GA-AgNP exposures and all other taxa showing significantly reduced growth under GA-AgNP exposure. By contrast, PVP-AgNPs significantly inhibited the growth of only one species. The AgNP toxicity was also found to be concentration dependent.

The enhancement of plant growth at a low concentration of nanoparticles and inhibition at a higher concentration has also been observed with exposure to other nanoparticle types. Exposure to low concentrations of multi-walled carbon nanotubes (MWCNTs) has been shown to enhance the growth of maize seedlings, in contrast to exposure to higher concentrations [18]. Treatment with 8 g/L of nanosilicon dioxide (nSiO₂) was found to positively affect tomato seed germination [19]. An increase in the anatase nanoparticle (nTiO₂) concentration was found to cause a significant increase in the percentage of germination, the germination rate index, the radicle and plumule length, the fresh weight and the vigour index of *Capsicum annum* L. seedlings, while the optimal concentration of nTiO₂ was 7.5% [20]. nTiO₂ also promoted photosynthesis, nitrogen metabolism and growth of spinach [21].

Here, we focused on the effect of AgNPs on the seed germination and plant growth of three crop plants that were exposed to various concentrations of AgNPs. Determining the dose response of plants to nanoparticles is expected to improve our ability to use nanoparticles to increase global crop yields and to boost plant defences against pathogens. Moreover, using the optimum dose of nanoparticles might minimize the risk of the presence of nanoparticles in the plant environment.

II. RESULTS

The increasing use of nanoparticles in daily products is of great concern, particularly when the positive and negative impacts of nanoparticles on the environment are not known. Hence, in this study, we investigated the impact of AgNP application on the seed germination and seedling growth of three crop plants: corn, watermelon and zucchini. The germination rate of corn seeds significantly increased after treatment with higher AgNP concentrations, whereas no significant effect was found on the corn germination percentage or mean germination time. The highest germination rate for corn seeds, which was 6.5 seeds/ day, was observed after exposure to 1.5 mg/ml of AgNPs. By contrast, watermelon and zucchini plants exposed to AgNPs showed significantly enhanced germination percentages and germination rates compared with untreated seeds. The highest germination percentage (73.33%) and highest germination rate (1.59 seeds/ day) for watermelon were recorded at 2 mg/ml AgNPs. For zucchini plants, the highest germination percentages (86.67% and 90%) and highest germination rates (1.68 and 1.66 seeds/ day) were recorded at 0.5 and 2.5 mg/ml of AgNPs, respectively. Among the three plant species, a significant change in mean germination time was observed only for zucchini seeds, the germination time of which increased significantly at 1.5 and 2.5 mg/ml of AgNPs, translating to later germination than untreated plants.

TABLE I

INFLUENCE OF AGNPs CONCENTRATIONS ON GERMINATION PERCENTAGES, GERMINATION RATES AND MEAN GERMINATION TIMES FOR CORN, WATERMELON AND ZUCCHINI PLANTS

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

* Representing significant effects at 0.05% probability level.

M = Mean, SD =Standard Deviations, GP =Germination Percentage, GR = Germination Rate and MGT = Mean Germination Time, mg = milligram, ml = milliliter, cm = centimeter, % = percentage.

TABLE II
INFLUENCE OF AGNPs CONCENTRATIONS ON ROOT LENGTHS, SEEDLING FRESH AND DRY WEIGHTS OF CORN, WATERMELON AND ZUCCHINI PLANTS

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

* Representing significant effects at 0.05% probability level.

M = Mean, SD = Standard Deviations, RL = Root Length, FW =Seedling Fresh Weight, DW = Seedling Dry Weight, mg = milligram, ml = milliliter, cm = centimeter.

AgNPs had a toxic effect on corn seedling as shown in the root length values. Significant decreases in the root length were observed at all AgNP concentrations, especially at 1.5 and 2 mg/ml, which led to lengths of 7.30 and 7.58 cm, respectively. The seedling fresh weights of corn treated with certain AgNP concentrations were significantly higher than those of untreated plants, as shown for the 2 mg/ml AgNP treatment (154 mg), whereas no significant effect on seedling dry weight was observed. The root lengths of watermelon and zucchini plants were positively affected by AgNPs. For watermelon, a significant change in the root length values was found at higher AgNPs concentrations, whereas large root length values were observed at low AgNP concentrations for zucchini. The largest mean root length for watermelon (11.4 cm) was observed at 2 mg/ml of AgNPs; and for zucchini (14.48 cm), at 0.5 mg/ml of AgNPs. The seedling fresh weight for watermelon increased at higher concentrations of AgNPs, whereas the dry weight decreased significantly at certain concentrations of AgNPs. The highest value for watermelon seedling fresh weight (373.5 mg) was observed at 2 mg/ml of AgNPs. The fresh and dry weight values for zucchini increased significantly at certain concentrations of AgNPs. The highest seedling fresh weight (1088.89 mg) was recorded after exposure to 0.1 mg/ml of AgNPs, whereas the highest seedling dry weight (124.36 mg) was observed for seedlings treated with 0.05 mg/ml of AgNPs.

III. DISCUSSION

Germination is important for determining the density of the final plant if planted seeds germinate completely and vigorously [22]. AgNPs have been used in agriculture to improve crop yield. This study showed that three crop species had different responses to AgNPs in terms of germination

percentage and the measured growth characteristics. Our results indicated that exposure to AgNPs had significant effects on the seed germination and seedling growth of corn, watermelon and zucchini plants. The optimum dose of AgNPs for watermelon plants, as determined from our results, is 2 mg/ml, which is the dose that led to the highest germination percentage and germination rate. Exposure to 0.5 or 2.5 mg/ml of AgNPs appeared to be enough to enhance zucchini seed germination. The highest germination percentage and germination rate were observed at the aforementioned concentrations; however, compared with untreated plants, the mean germination time increased at all AgNP concentrations, which represents later germination. The same results were obtained by [11] who found that AgNPs significantly increased the germination percentage of seeds of *Boswellia ovalifoliolata* but increased the germination time. In contrast to our observations, seed germination in *Vicia faba* [6] and *Arabidopsis thaliana* [14] were not found to be affected by exposure to AgNPs.

The best effect of exposure to AgNPs was observed for corn germination: all germination parameters were enhanced at higher AgNP dosages. It is probable that nanoparticles penetrate the seed coat and exert a beneficial effect on the process of seed germination. Based on studies of nanoparticles' effects on seed germination mechanisms, it is possible that nanoparticles increase water absorption by seeds [23], increase nitrate reductase enzyme levels, increase the seeds abilities to absorb and utilize water and fertilizer, promote seed antioxidant systems [24], reduce antioxidant stress by reducing H_2O_2 , superoxide radicals, and malonyldialdehyde content, and increase the activities of some enzymes (such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase) [25]. These changes improve seed germination in some plant species.

The effect of AgNPs on the seedling growth of the three species studied appeared not to be related to its effect on germination parameters. AgNPs had a toxic effect on the root length of corn seedlings, whereas the root lengths of watermelon and zucchini plants were positively affected by AgNPs. Shorter corn root lengths were also observed by [10]. He demonstrated that the mean root lengths of corn and common bean plants decreased with exposure to of AgNPs at concentrations higher than 60 ppm, which is equal to 0.06 mg/ml, and increased at AgNP concentrations lower than this concentration (which are lower than the concentrations applied in our experiment). Similarly to our watermelon and zucchini plants, the root lengths of *Vicia faba* [6] and *Eruca sativa* [16] plants were increased in response to AgNP treatment. The effect of AgNPs on corn plants in this study has also been observed on *Pennisetum glaucum* plants, in which seed germination was promoted in response to AgNPs while seedling root elongation was inhibited [12].

The seedling fresh weight increased with AgNP treatments for the three species tested in this study. The seedling dry weight for zucchini increased at certain concentrations of AgNPs, whereas the seedling dry weight for watermelon decreased significantly. Increased seedling growth was observed for zucchini plants after treatment with AgNPs. This result disagrees with the results of a study by [26]) that found that the biomass of zucchini plants was reduced after exposure to AgNP concentrations from 1 to 1000 mg/L. Increases in the seedling fresh and dry weight in response to AgNPs were also shown by [10], [27]. By contrast, AgNPs were not found to have significant effects on the seedling growth of *Ricinus communis* L [5]. AgNPs were also found to inhibit the growth of *Lemna minor* [28] and rice (*Oryza sativa* L.) [29].

IV. MATERIALS AND METHODS

A. AgNPs Preparation

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

B. Seed Germination Experiment

Seeds were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility [30], They were soaked in distilled water for two hours, rinsed four times with distilled water; and then soaked in a series of prepared AgNP suspensions for approximately 2 hours. One piece of filter paper was placed into a 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 farther separating each seed [31]. The Petri dishes were covered and sealed with tape and incubated at room temperature. The germination was halted after 12 days, except for zucchini

plants, for which germination was halted after 16 days. The seed germination rate and mean germination time were calculated, and the seedling dry and fresh weight and root length were measured.

C. Seed Germination Measurement

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

$$\text{Germination Percentage (GP \%)} = (\text{Gf}/n) \times 100 \quad (1)$$

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.

$$\text{Mean Germination Time (MGT)} = \sum \text{NiDi}/n \quad (2)$$

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

$$\text{Germination Rate (GR)} = \sum \text{Ni}/\sum \text{TiNi} \quad (3)$$

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

$$\text{GR} = (a/1) + (b-a/2) + (c-b/3) + \dots + (n-n/1/N) \quad (4)$$

D. 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.

V. CONCLUSION

Exposure to nanomaterials can encourage earlier plant germination and improve plant production. Our results indicated that exposure to AgNPs had significant effects on the seed germination and seedling growth of corn, watermelon and zucchini plants. The germination rate of the three species was enhanced in comparison with untreated plants at all tested concentrations of AgNPs. The germination percentage improved for watermelon and zucchini at all AgNP concentrations, despite an increase in mean germination time for zucchini plants. AgNPs had a toxic effect on corn seedlings, as shown by the root length values, whereas the fresh weight increased at higher concentrations of AgNPs. The seedling growth of watermelon plants improved with AgNP exposure, whereas the dry weight decreased at all concentrations. Zucchini plants showed the greatest enhancement in seedling growth at low concentrations of AgNPs, as shown on all growth parameters. The outcomes of this study are useful for determining the biocompatibility of AgNPs and for identifying potential agricultural applications for nanoparticles in crop improvement and food production.

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