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**ORIGINAL ARTICLE**

# Physiological Analysis of Silver Nanoparticles and $AgNO_3$ Effect to *Brassica napus* L.

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**KEYWORDS**

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Seedling Biomass;
Seedling length

**ABSTRACT:** In this study, the effects of different concentrations of bulk and nano-sized Ag on seed germination and seedling growth of canola were investigated in a randomized completely design with four replications. The experimental treatments included four concentrations of bulk $AgNO_3$ (10, 100, 500 and 1000 ppm), four concentrations of nanosized Ag (10, 100, 500 and 1000 ppm), and the control without Ag. Results indicated that among the canola germination indices only mean germination time and germination index were not affected by treatments. The TTC tests showed all root tips were colored red. It is concluded that bulk $AgNO_3$ treatments inhibited germination indices of canola more than nano sized Ag**.**

## INTRODUCTION

The huge proceed on nanoparticle investigation field has been made important effects on many of environmental aspects. Nano-sized Ag is one of the most notable particles in nanobiotechnology. Nano-sized Ag particles can be changed to the Ag that is a heavy metal, in environment. Besides, in the case of high concentrations of silver in soil, this metal will be collected in various organs of the plants. However, by changing the morphology of the plant the production will be harmful for human consumptions. Many scientists have considered nanotechnology as the next logical roadmap in all sciences [1]. Nanoparticles are accepted as compound with diameter less than 100 nm [2]. Particles in such diameter (<100 nm) fall in the transitional zone between individual atoms or molecules. In this condition the physical and chemical characteristics of the material (e.g., conductivity, reactivity, and optical sensitivity) [3], can be modified by the corresponding bulk material. Nanometer-sized particles have also shown special toxicity and they are usually more toxic than the bulk material of larger size [4]. Therefore, such materials generate adverse biological effects in living cells.

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However, applications of nanomaterials might be limited in terms of concerns about toxicity. Nano-sized silver is one of the most extensive nanomaterials, and, they are identified in more than 250 materials as of May 2012 [5]. These nanoparticles inclined to be released into the environment through materials use and distribution [6]. Fabrics including nano-sized Ag washed with distilled water can flow freely Ag particles to wastewater [7]. Everyone may drain about mg of silver into wastewater per day [8]. AgNPs have risk potential for aquatic systems for example, toxic effects of AgNPs on development of Japanese medaka (*Oryza latipes*) [9] and *Daphnia magna* [10] have been reported. The ions of Ag are one of the most poisonous heavy metals [11], and its toxicity depends on released silver ions [11]. However, the role of AgNP toxicity is more than toxic ions released into the environment [9, 5].

The impact of AgNPs on plants has recently aroused a great deal of interest. Treatments of 500 and 100 $mgL^{-1}$ Ag nanoparticles decreased biomass and transpiration rates of *Cucurbita pepo* 57 and 41%, respectively, in comparison with the control and bulk Ag treatments [12]. Kumari et al. [13] examined effects of Ag nanoparticles on the onion root cells reported some cell division abnormalities. Toxic effects of nano-sized Ag on *Lolium multiflorum* have been reported by Yin et al. [14]. They showed that Ag nanoparticles coated with gum Arabic could be more toxic than $AgNO_3$. Nano-sized Ag could decrease the growth and development of *Lemna minor* [15, 16]. Other experiments are reported in Table 1. These findings suggest that plants, as an major element of the ecosystem should be considered when determining the general poisonous effects of nano-sized particles in the environment. Even though the poisonousness of nano-sized Ag to plants has been studied, the functional mechanism of these nanoparticles has not been evaluated.

In the present study, canola (*Brassica napus* L.) has been chosen as an oil seed plant to investigate how plants respond to AgNP at the germination and seedling growth stages, and determining difference in the effect of AgNPs and $AgNO_3$.

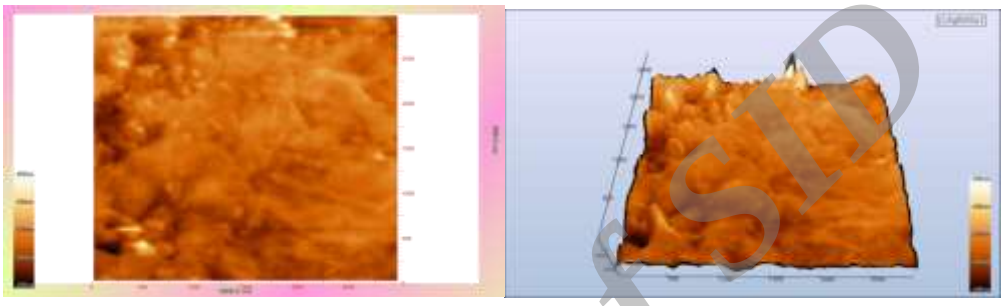
**Table 1**. The effects of nano silver on plants growth

| Plant | Effects on growth | Reference |
|---|---|---|
| **Cucumis sativus and Triticum aestivum** | Ag NPs and Ag+were toxic to the two plants. | Cui et al. , 2014 |
| **Ricinus communis** | Silver nanoparticles had no significant effects on seedling growth, while the silver in bulk form inhibited the seed germination. | Yasur & Rani , 2013 |
| **Sprodela polyrhiza** | AgNPs and AgNO3 significantly decreased plant tissue nitrate–nitrogen content and Chlorophyll | Jiang, 2012 |
| **Arabidopsis thaliana.** | Immersion in AgNP suspension inhibited seedling root elongation | Lee , 2013 |
| **Eleven Wetland Plants** | $AgNO_3$ did not decrease the growth of tested plants while Ag nanoparticles significantly suppressed the growth of one plant | Yin , 2012 |

## MATERIALS AND METHODS

### *Description of Materials*

Canola seeds were taken from the Pakan Bazr Company, Isfahan Province, Iran. Nano-sized Ag powder was
provided by Nutrient Company The diameter and topography of Ag nanoparticles (Figure 1 and 2) were measured by scanning tunneling microscope (STM, Nama-SS-6 model) and atomic force microscope(AFM) in the Central Laboratory of Ferdowsi University of Mashhad, Iran. X-ray diffraction (XRD) pattern of Ag nanoparticles was shown in Figure 3. XRD measurement showed that the used Ag nanoparticles were made by Ag and trace of Cu. Bulk $AgNO_3$ was supplied by Merk Company.



Ag-3D Ag- 2D

**Figure 1**. Image of nanosized Ag by STM

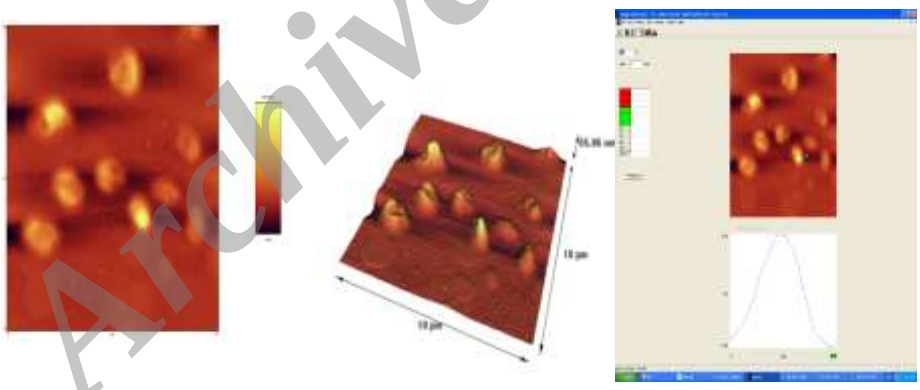


**Figure 2**. Image of nanosized Ag by AFM

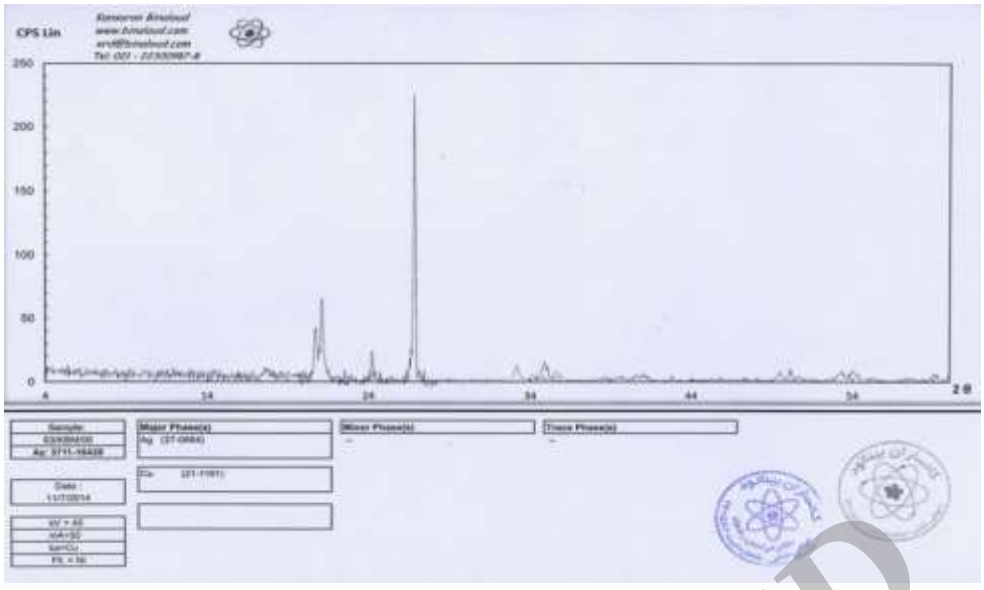



**Figure 3.** XRD analysis of nanoAg particles

***Experimental Design and Data Observation***

In order to study the effect of different concentrations of bulk and nanosized Ag on seed germination of canola, a randomized completely design with four replications was carried out. Four concentrations of bulk Ag (10, 100, 500, 1000 ppm) and four concentrations of nanosized Ag (10, 100, 500, 1000 ppm) used as treatments and distilled water as control. The experiment was conducted in germinator with temperature of 25±1°C and 30% humidity.

One hundred seeds of similar size were randomly selected and placed on wet paper in Petri dishes, and then 5 ml of each treatment was added to each Petri dish and distilled water was added to Petri dishes as control. All concentrations of Ag and the control were run at the same time and therefore under like conditions. Germinated seeds were counted every day for one week. Seeds were considered as germinated when the radicle length was at least 1 mm, the seeds considered germinated. In the present research, some germination factors were measured such as Germination Rate (GR), Germination percentage (GP, %), Relative germination percentage (RGP), Mean germination time (MGT),

Germination index (GI) and Weighted germination index (WGI). These parameters were also calculated from the formulas proposed by [17-19].

$$GP = 100 \times GN / SN \quad (1)$$

GN is the total number of germinated seed; SN is the total number of seeds tested

$$RGP = GP \text{ treatment} / GP \text{ control} \times 100. \quad (2)$$

$$GI = (\sum \langle\!\langle (N-i) \rangle\!\rangle \times Gi) \times 100 / (N \times GN) \quad (3)$$

i is the number of days since the day of sowing and Gi is the number of seeds germinated on day I.

$$N' \times WGI = [N \times n1 + (N-1) \times n2 + (N-2) \times n3 + \ldots] / (N) \quad (4)$$

n1, n2, …, n60 are the number of seeds that germinated on first, second…. ;N is total days of experiment;N´ is the total number of seeds placed in incubation

$$GR = 100 \times \sum_n^i Si/Gi \quad (5)$$

i is the number of days since the day of sowing and Gi is the number of seeds germinated on day I.

Vigor index = germination% × seedling length (root + shoot) (6)

After one week, plumule and radical length of seedlings were measured using a ruler. For dry biomass to be weighed the 7-day seedlings were first weighed; then, they dried in oven at 70 °C for 72 h and were weighed after that.

***TTC assay for root cells***

Triphenyl tetrazolium chloride, TTC, or simply tetrazolium chloride (with the formula 2, 3, 5-triphenyl-2H-tetrazolium chloride) is a redox indicator commonly used in biochemical experiments especially indicate cellular respiration. TTC is used to differentiate between metabolically active and inactive tissues.

Five mL of 0.5% solution of TTC was added to test tubes containing root tips in 35 °C, and kept in the dark for 5 h. Then TTC solution After 5 h in the dark, the TTC solution was separated with a syringe and root tips were washed with distilled water. The root tips with red color were considered to be living and others were dead [20].

## STATISTICAL ANALYSIS

To detect the significance of differences of variables statistical analysis was performed employing one way ANOVA test using SPSS software (Chicago, IL, USA).

## RESULTS

Once the canola seeds were plated, it took approximately three to five days for them to germinate. After one week, the germination percentage of the canola seeds were calculated for each treatment for the control group the germination percentage was 96.6%, therefore almost all of the canola seeds germinated. The seeds of treatments of 500 and 1000 ppm bulk $AgNO_3$ did not germinated. The minimum germination percentage (40%) was found 100 ppm concentration bulk Ag (Table 2). The highest germination rate (25.1%) was shown in the control treatment that was not different with other treatments significantly.

Control treatment had minimum mean germination time (3.68 day), and treatment of 100 ppm Ag bulk had the maximum value (4.36 day). Then treatment of 100 ppm concentration Ag bulk increased this factor by 15% in compared to the control. Although there was some variation between the mean germination times of canola seeds under treatments of nano and bulk Ag were different and this difference was statistically significant between control and 1000 ppm nano-Ag with 100 bulks AgNO3. In the media containing 10 nano and bulk Ag, The relative germination percentage of treatments 10 nano and bulk Ag (96.6 and 80 respectively) were statistically significant and higher than others (Table 2). Treatments of 100 and 500 ppm nano-Ag showed maximum and minimum value of germination index (44.38 and 42.38 respectively) all treatments of nano and bulk Ag had no significantly difference with the control (Table 2). Different concentrations of nano-Ag did not significantly affect the weighted germination index of canola seeds was not significantly affected by nano and bulk Ag, but treatments of 10 and 100 ppm of bulk Ag decreased significantly this factor in compared to the control.

The effect of nano-Ag treatments on plumule length was not significant, but bulk $AgNO_3$ treatments had a significant effect on this character. Radicle length at all of treatments of nano and bulk Ag was lower than control. The lowest radicle length was achieved at 100 ppm bulk Ag (Table 3). All studied treatments had no significant effect on seedling fresh biomass except of 500 ppm bulk Ag and 100 ppm nano treatment. The minimum seedling fresh weight was found in 100 ppm bulk Ag. Experimental treatments no affected seedling dry biomass significantly except of 500 ppm bulk Ag and 100 ppm nano treatment. The minimum seedling dry biomass (0.011 g) was found in 100 ppm concentration bulk Ag, and the highest was shown in 10 ppm $AgNO_3$ treatment (0.0239 g). Therefore, 100 ppm concentration bulk Ag treatment reduced seedling dry biomass by 45% in comparison to untreated control, (Table 2). Vigor index was affected significantly by bulk and nanosized Ag concentrations (Table 3). The lowest vigor index was shown in 100 ppm $AgNO_3$ that reduced vigor index by 33% in comparison with the control (Table 3). The TTC assay revealed that different concentrations of bulk and

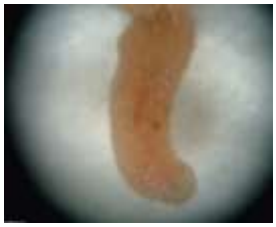
nano Ag had no effect on root tip cells. After 24 hours of treatments, all root tips were colored red (Figure 4).

**Table 2.** Effect of different concentrations of bulk and nanosized Ag on seed germination of Canola

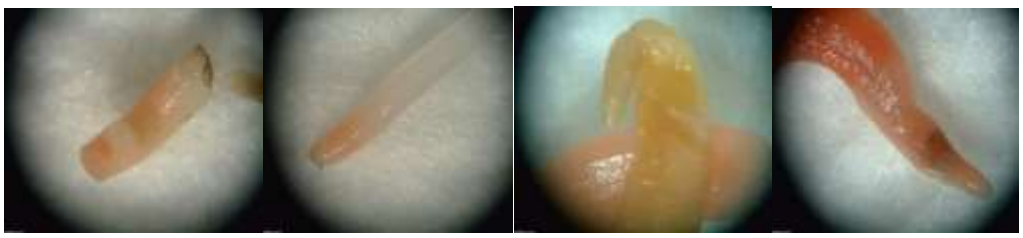
| Concentration(ppm) | Germination (%) | RGP | Germination Rate (%Day$^{-1}$) | MGT(Day) | GI | WGI |
|---|---|---|---|---|---|---|
| **Bulk Ag** | | | | | | |
| **10** | 80$^{bc}$ | 80$^{b}$ | 23.517$^{a}$ | 4.11667$^{ab}$ | 44.0133$^{a}$ | 0.546$^{bc}$ |
| **100** | 40$^{d}$ | 33.3333$^{b}$ | 19.11$^{b}$ | 4.365$^{a}$ | 44.035$^{a}$ | 0.5341$^{c}$ |
| **500** | 0 | 0 | 0 | 0 | 0 | 0 |
| **1000** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Nano Ag** | | | | | | |
| **10** | 93.33$^{ab}$ | 96.6667$^{a}$ | 24.261$^{a}$ | 4.02667$^{ab}$ | 42.4367$^{a}$ | 0.56933$^{ab}$ |
| **100** | 80$^{bc}$ | 73.3333$^{b}$ | 23.914$^{a}$ | 4.05333$^{ab}$ | 44.38$^{a}$ | 0.56163$^{ab}$ |
| **500** | 73.33$^{c}$ | 73.3333$^{b}$ | 23.984$^{a}$ | 4.07667$^{ab}$ | 42.38$^{a}$ | 0.5584$^{abc}$ |
| **1000** | 83.33$^{abc}$ | 79$^{b}$ | 22.662$^{a}$ | 3.709$^{b}$ | 42.8533$^{a}$ | 0.55823$^{abc}$ |
| **Control** | 96.66$^{a}$ | ---- | 25.111$^{a}$ | 3.68233$^{b}$ | 42.85$^{a}$ | 0.574$^{a}$ |

**Table 3.** Effect of bulk and nanosized Ag concentrations on seedling growth of canola

| Concentration(ppm) | VI | Radicle length (cm) | Plumule length(cm) | Seedling Fresh weight(g) | Seedling dry weight(g) |
|---|---|---|---|---|---|
| **Bulk Ag** | | | | | |
| **10** | 8.16$^{c}$ | 3.9$^{bc}$ | 6.3$^{a}$ | 0.7$^{a}$ | 0.023967$^{a}$ |
| **100** | 3.9033$^{e}$ | 0.2667$^{e}$ | 6.3$^{a}$ | 0.31$^{d}$ | 0.0118$^{d}$ |
| **500** | 0 | 0 | 0 | 0 | 0 |
| **1000** | 0 | 0 | 0 | 0 | 0 |
| **Nano Ag** | | | | | |
| **10** | 6.5567$^{d}$ | 2.6$^{b}$ | 4.166$^{c}$ | 0.5833$^{bc}$ | 0.0198$^{b}$ |
| **100** | 9.9764$^{b}$ | 4.466$^{b}$ | 6.2$^{ab}$ | 0.5667$^{bc}$ | 0.0195$^{b}$ |
| **500** | 6.8167$^{d}$ | 4.166$^{b}$ | 4.1$^{c}$ | 0.5$^{c}$ | 0.016467$^{c}$ |
| **1000** | 6.3167$^{d}$ | 3.266$^{cd}$ | 4.3$^{c}$ | 0.5567$^{bc}$ | 0.020067$^{b}$ |
| **Control** | 12.1333$^{a}$ | 7.466$^{a}$ | 5.1$^{bc}$ | 0.6333$^{ab}$ | 0.020433$^{b}$ |

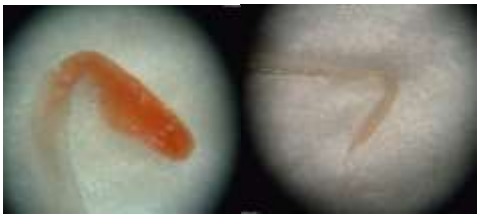


Control



1000n 500n 100n 10n

**Figure 4.** TTC assay for different concentrations of bulk and nano-sized Ag



100 10

**Figure 4.**Continued.

## DISCUSSION

Nanobiotechnology is a new field, with its major focus on human and animal studies. Nevertheless, not many studies have been carried out to determine the poisonous of nanoparticles to organisms that living or growing on the land, especially plants. The mechanisms of inhibitory of nanoparticles to the growth of or poisonous to plants, is greatly not identified and few data on the potential absorption of these materials by plants and their future fate is accessible. Our research showed that seed germination rate of canola did not affect by silver nanoparticles however, bulk Ag had decreased the germination rate. The results of other researches done

with nano-sized and bulk Ag were disagreeing. They showed that nano-sized Ag did not affect the seed germination and seedling growth but $AgNO_3$ decreased the plant growth. Stampoulis et al. reported that different types of nano and bulk compounds such as carbon nanotubes, copper, zinc, silver and silisium with 1000 ppm concentration did not affect seed germination of zucchini [12]. Musante and White examined the effect of nano-sized Ag on seed germination traits on *Cucurbita pepo* and revealed that nano Ag inhibited biomass (66%) and transpiration (84%). Also they measured

concentration of Ag ions in suspension and showed that Ag ions in nano-sized Ag suspension was higher than the bulk Ag [21]. The results of this research showed that concentration of 500 ppm nano-sized Ag were significantly inhibited seed germination of *C. pepo*. Interestingly, in the present research, canola seeds was not affected by high concentrations of nano Ag (1000 ppm), whereas Mazumdar and Ahmed [22] used 1,000 µg $L^{-1}$ for rice seed germination and showed inhibitory effects. Perhaps, thicker seed coat of canola seed is responsible for this phenomena which decrease the uptake of the nanoparticles into the seed.

Although many studies indicated Ag nanoparticles had an effect on other plant species such as ryegrass, barley and squash, tomato, cucumber and maize [12, 21, 23], in this research no toxic effect was observed on canola seed germination, oppositely, bulk Ag treatments inhibited the seed germination at these concentrations. Overall, the toxicity or stimulatory effct of nanoparticles is dependent on plant species, nanoparticle size and used concentrations[ 12, 14 and 24], similarly, the effects of Ag nanoparticle in this research could be species-specific.

There was an uptake of Cu content in mung bean and wheat seedlings uptake Cu [25] but, in canola seeds, Ag nanoparticles had no major effect on seed germination characteristics. Lin and Xing [26] examined the effects of multi-walled carbon nanotube, aluminum alumina, zinc, and zinc oxide on seed germination and root growth of radish, rape ryegrass, lettuce, corn, and cucumber and showed that except nano-Zn on rye grass and nano-ZnOon corn, at 2,000 mg $L^{-1}$, seed germination of other plants did not affected.

Barrena et al. showed that nanoparticles of gold, silver and iron had low toxicity on lettuce and cucumber, and the presence of stabilizers was due to this effect [27]. In seed germination tests on cucumber seeds using Ag-solvent (only NaBH4), AgNPs showed reduced germination index and root growth at

100 μg $mL^{-1}$ whereas similar concentrations of AgNPs showed no significant differences in lettuce seeds. Lower toxic effects of AgNP compared with NP-free solvent solutions at same concentrations were attributed to the property of adsorption of solvent molecules at NPs surface, henceforth, decreasing the effective concentrations of $NaBH_4$ [27]. The very small size of NPs is believed to cause higher toxicity in plants. El-Temsah and Joner studied the effect of different sizes of Ag nano-sized on flax, barley, and rye grass growth and showed that silver nanoparticles with 2 nm diameter had more poisnous effect than nanoparticles with 5 and 20 nm diameters [28]. Studies by Yin et al. [14] demonstrated that exposure to GA-AgNPs or $AgNO_3$ had significantly affected seed germination rates for multiple plant species, while exposure to PVP–AgNPs had no measurable effects on germination of 11 wetland plants except for one plant. Several other studies too reported the toxic effects of silver ions and silver nanoparticles, and different reasons were given.

While examining the acute toxic effects of AgNPs in different organisms, it has been suggested that the toxic effects of silver nanoparticles are firstly due to the presence of nanoparticles and secondly by the release of $Ag^+$ ions from nanoparticles and the free radicals generated during the AgNP suspension [29]. The toxicity in daphnids depends on the surface coatings which influence the dissolution of AgNP into soluble Ag [30].

## CONCLUSIONS

Silver nanoparticles had no impact on canola seed germination indices when the seeds treated with silver nanoparticles, while the bulk form of silver($AgNO_3$) had retarded the seedling growth. The toxicity of $AgNO_3$ might be due to ionic form silver that had affected the canola seed and roots are main sites for the absorption of the NPs, hence, the first site to be affected was root growth when compared with other plant organs.

From the above results, we can conclude that AgNP have lesser toxicity in comparison to other particles in canola seeds and may be used for agriculture with tested profile at permissible levels. In view of the present findings it is suggested that variations in seed germination in its form of occurrence, i.e., whether in nano-form or bulk form can serve as useful biomarkers in ecotoxicological tests with silver nanoparticles.

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