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Toxicity Evaluation of Nano Silver on Faba Bean Germination and Seedling Development

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The target of this research is to study the toxic effect of silver nanoparticles (AgNPs) on seed germination and seedling growth of faba bean (*Vicia faba* L.). Seeds of *V. faba* were soaked in 50, 100, 200 and 400 mg/L of chemically synthesized AgNPs for 24 h. The shoot length was significantly reduced upon exposure to 200 mg/L of AgNPs compared to the control. There was no significant difference in root elongation, although the number of lateral roots decreased significantly at 200 and 400 mg/L compared to the control. Seedling vigor index decreased only at 200 mg/L. Conclusively, AgNPs were toxic to seedling development of *V. faba* at high concentrations. This study also showed that the number of lateral roots is a useful index to evaluate the effect of nanomaterials on early seedling growth.

Key words: broad bean, nano silver, plant growth, lateral root number, vigor index

INTRODUCTION

Food production is suppressed by biotic and abiotic stresses. Although transgenetically or chemically synthesized nutrition offer solutions, they incur disadvantages affecting plant growth through direct or indirect mechanisms. Currently, an ever–expanding scope of uses includes nanomaterials, particularly agricultural nano–regulators, nano–pesticides and nano–fertilizers. Also, nanomaterials have been utilized industrially for a wide assortment of material coatings which incorporates different areas, for example hardware vitality, contact activity and meds. Silver nanomaterials have a significant influence in business utilization in the field of pharmaceutical and other clinical sciences (Samuel and Guggenbichler, 2004; Nowack and Bucheli, 2007; Vigneshwaran *et al*., 2007; Benn and Westerhoff, 2008; Abdelghany *et al*., 2018). Since the applications of the nanomaterials competed with other common materials in the field of agriculture in the last decade, they gained a lot of interest and many research activities reported their effects, or probably their side effects, on plants as well as their obscure mechanism of interaction (Stampoulis *et al*., 2009; Thabet *et al*., 2019; Galal *et al*., 2020; Zhao *et al*., 2020).

Literature available shared information about both positive and negative impacts of nanomaterials on plant germination and growth (Roohizadeh *et al*., 2015; Abdel Latef, *et al*., 2018; Thabet *et al*., 2019). Among these, silver nanoparticles (AgNPs) found to defend plants against pathogenic and insecticidal invaders (Mishra *et al*., 2014; Cromwell *et al*., 2014; Zhao *et al*., 2020) and were reported as a toxic material not only on animal and human cells (Wijnhoven *et al*., 2009; Quadros and Marr, 2010) but also on plant cells (Yin *et al*., 2012; Prakash *et al*., 2015; Galal and Thabet, 2018; Pastelín–Solano *et al*., 2019). AgNPs also affect plant growth such as *Cucurbita pepo* (Stampoulis *et al*., 2009), *Lemna minor* (Gubbins *et al*., 2011), *Oryza sativa* (Nair and Chung, 2014), *Triticum aestivum* (Vanninia *et al*., 2014) and *Vicia faba* (Galal and Thabet, 2018). Essentially, less data is accessible on the potential components of the poisonous quality of AgNPs from *in vivo* investigations and researchers had some apprehensions concerning genotoxic effect and oxidative stress related to AgNPs on plant cells (Galal and Thabet 2018; Zhang *et al*., 2018). About 800 tons of AgNPs are used globally (Geisler–Lee *et al*., 2014). As the AgNPs applications will keep on developing, there is still a lot that should be comprehended in terms of their aggregation in the environment and their latent capacity for long–term consequences for people and other organisms (Abdelghany *et al*., 2018). Therefore, this up–to–date research tries to cover this demand for inquiry to determine the toxic effect of AgNPs on germination and growth of faba bean *V. faba*, as an example of one of the most consumable legumes for humans and animals. Here, *V. faba*, as a rich source of protein and carbohydrate and as a plant model to study toxicity, was chosen to evaluate the effect of AgNPs on its germination and growth.

MATERIALS AND METHODS

The present study was conducted at the Laboratory of Insect Natural Enemies, Faculty of Agriculture and at the Ultramicroscopy Research Center, Kyushu University, Fukuoka, Japan.

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Seed materials

Seeds of faba bean (*V. faba* L.) were obtained from Canada (Kokusaipet food, Kobe, Japan).

Characterization of AgNPs

AgNPs used in this study were chemically synthesized by the electrical explosion method using the inorganic metal salt of $AgNO_3$ (US Research Nanomaterials, Inc., Houston, USA). The shape and size of the nanoparticles were characterized using the transmission electron microscope (TEM) micrographs (Helmy and Mekawey, 2014). Further characterizations were done to authorize the elemental identity of nanomaterials by elemental analysis using the energy dispersive X–ray (EDX) as well as electron diffraction (ED).

TEM analysis

Samples for TEM were prepared by placing a drop of well dispersed AgNPs solution onto conventional carbon coated copper TEM grids (150 μm meshes, Plano GmbH, Germany), allowing the drop to dry overnight in a desiccator before imaging. The TEM images of the samples were obtained using an accelerating voltage of 200 kV, using TEM (PHILIPS TECNAI–G2 20, Japan). Three images with multiple AgNPs were taken to have a clear representation of its morphology and particle size.

EDX spectra analysis

For TEM–EDX spectra analysis, a sample of AgNPs solution was investigated using TEM (Tecnai–G2 20, FEI, Japan). The EDX spectra were used to examine the elemental chemical composition of the nanoparticles sample solution. Surface binding elements of the sample nanoparticles were analyzed with X–ray photoelectron spectroscopy. The nanoparticles excited by an electron beam showed the peak values percentage of Ag elements in comparison to other surface binding elements.

ED analysis

ED coupled with the conventional TEM (JEM–2100HCKM, JEOL, Tokyo, Japan) was used to characterize the diffraction pattern of tested nano metals. Samples for electron diffraction were prepared by placing a drop of well dispersed nanoparticles solution separately onto conventional carbon coated copper TEM grids (150 μm meshes), allowing the drop to dry overnight in a desiccator before imaging. Images of the ED analyses of the samples were obtained using an accelerating voltage of 200 kV.

Application of AgNPs

Randomly selected seeds of *V. faba* with good appearance were sterilized for 3 min in sodium hypochlorite solution (2.5%), presoaked in distilled water for 3 h. Then, groups of 60 seeds were immersed for 24 h in 50, 100, 200 and 400 mg/L of AgNPs in addition to distilled water as the control experiment (0 mg/L). Finally, 10 seeds replicated six times for each treatment in randomized complete design were investigated for germination and seedling growth. Seeds were washed by distilled water before investigation to remove the residual amounts of treatments.

Seed germination

Ten seeds per replication were allowed to germinate in a Petri dish (six dishes/treatment) lined with cotton moistened with distilled water at 25 ± 1°C. Seeds were observed every 24 h (seed considered germinated when the radicle was at least 3 mm in length). Three germination indices were derived according to Ranal and Santana (2006) and Ranal *et al*. (2009) as follows:

Final germination percentage (G%);

$$G\% = \frac{\sum n_i}{N},$$

where n_i is the number of germinated seeds on day i and N is the total number of seeds in each experimental unit.

Mean germination time (MGT);

$$MGT = \frac{\sum n_i t_i}{\sum n_i},$$

where t_i is the time in days from seeding to germination on day i.

Coefficient of germination velocity (CGV);

$$CGV = \frac{\sum n_i}{\sum n_i t_i} \times 100$$

Seedling growth

After five days seeds from each Petri dish were moved to pots filled by peat moss and left to grow in a growth room (25°C and 16 h light). After two weeks shoot and root lengths were measured by a ruler and lateral root number were counted as the mean of five seedlings per replication. The seedling vigor index (SVI) was calculated according to Dahindwal *et al*. (1991) as follows:

$$SVI = G\ \% \times \text{mean seedling length},$$

where the seedling length is the sum of root and shoot lengths.

Statistical analyses

The effect of concentration and replicate on G%, SVI and CGV was tested with general linear models, followed by Dunnett tests for post hoc comparisons with the control. The effect of concentration and replicate on root and shoot lengths was tested with generalized linear models with normal distributions and log–link functions, followed by post hoc tests with Bonferroni correction of P value (P = 0.0125). The effect of concentration and replicate on lateral root number was tested with a generalized linear model with a Poisson distribution and log–

link function. The effect of concentration and replicate on MGT was tested with a parametric survival analysis with a Weibull distribution. We used JMP13.2.1 software.

RESULTS

Characterization of AgNPs

AgNPs were recognized to have a spherical shape with a size of 19.8 ± 5.0 nm (mean ± SD, n = 16) in diameter (Fig. 1). Selected area electron diffraction spots that corresponded to the [from inside to outside of the central ring] planes of the face–centered cubic structure of elemental AgNPs were clearly seen (Fig. 2). The ED pattern showed intense peaks in the whole spectrum of AgNPs. Additionally, EDX analysis shows the peak in ED region confirming the presence of elemental silver (Ag) (Fig. 3). The presence of strong signals consistent with elemental silver were observed, along with weak signals from copper (Cu) atoms (Fig. 3).

Regarding the selected area electron diffraction showing the characteristic crystal planes of elemental silver ions produced by AgNPs, the four intense peaks observed in the spectrum of silver ions produced by AgNPs agree with the Bragg reflection (the angles for coherent and incoherent scattering from a crystal lattice) of silver nanocrystals reported in literature (Lu *et al*., 2003). This further confirms that AgNPs were formed in the colloidal solution by a direct chemical method in the form of nanocrystals, also stated by Shaligram *et al*. (2009) who confirmed that the diffraction pattern indicated the crystalline structure of tested metal nanoparticles. Thus, the ED spectrum analysis confirmed the presence of AgNPs in a crystalline form. The Cu signals observed accompanying the strong Ag signals were likely caused by the X–ray emission from the copper substrate used in the EDX analysis (Mohammed *et al*., 2009; Li *et al*., 1999).

Effect on germination.

Table 1 illustrates that AgNPs could not affect germination process *in vitro* as there were no significant differences among tested concentrations (50, 100, 200 and 400 mg/L) on G% (F_4 = 1.24, P = 0.325), CGV (F_4 =

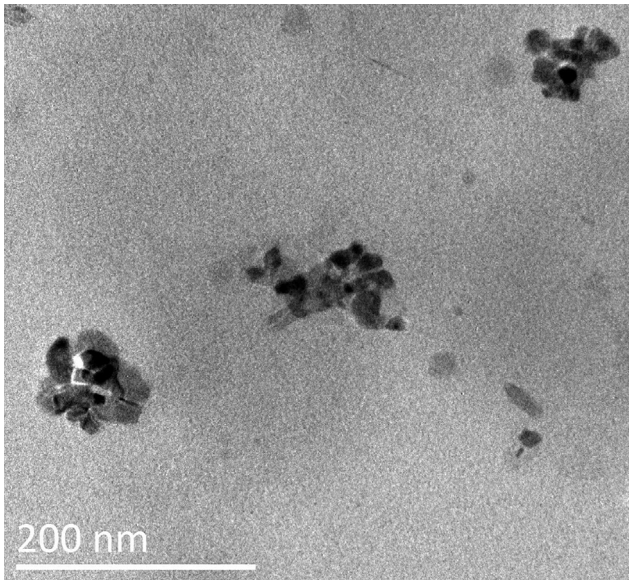

Fig. 1. TEM micrograph of chemically synthesized silver nanoparticles (AgNPs) (scale bar: 200 nm) showed the spherical shape with a mean diameter of 19.8 nm.

Fig. 2. Selected area electron diffraction showing the characteristic crystal planes of elemental silver ions produced by silver nanoparticles (AgNPs).

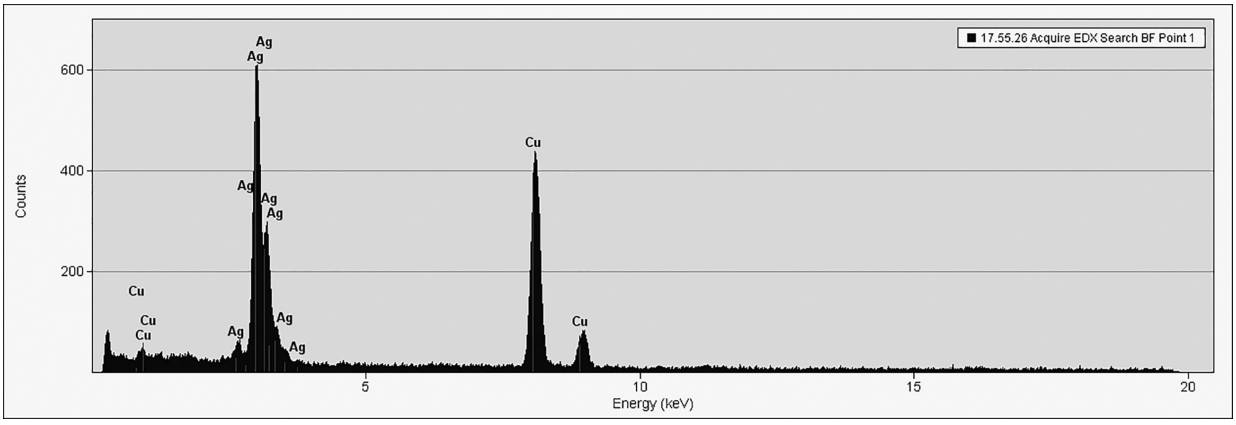

Fig. 3. Energy–dispersive X–ray spectrum (EDX) of elemental silver ions produced by direct chemical reduction method of silver nanoparticles (AgNPs).

Table 1. Germination percentage, coefficient of germination velocity (CGV), and mean germination time (MGT) (mean ± SE) of *Vicia faba* treated with Ag nanoparticles (AgNPs) of different concentrations

AgNPs concentration (mg/L)	G (%)	CGV	MGT (days)
0	95.00 ± 3.42	61.48 ± 6.94	1.72 ± 0.18
50	95.00 ± 2.24	64.58 ± 5.45	1.60 ± 0.12
100	93.33 ± 2.11	61.70 ± 4.65	1.68 ± 0.15
200	88.33 ± 3.42	59.42 ± 5.45	1.74 ± 0.15
400	96.50 ± 2.23	73.19 ± 7.97	1.45 ± 0.16

0.73, $P = 0.585$) and MGT ($\chi^2_4 = 3.77$, $P = 0.439$) compared to control (0 mg/L). Variable effects of replication were observed for G% ($F_5 = 0.40$, $P = 0.845$), CGV ($F_5 = 0.65$, $P = 0.662$) and MGT ($\chi^2_5 = 12.23$, $P = 0.032$).

Effect on seedling growth

Root length was not different among AgNPs concentrations ($\chi^2_4 = 7.53$, $P = 0.11$) (Fig. 4a), although it was significantly different among replications ($\chi^2_5 = 24.50$, $P = 0.0002$). In contrast, shoot length was significantly different among AgNPs concentrations ($\chi^2_4 = 12.2$, $P = 0.0157$) (Fig. 4b) and replications ($\chi^2_5 = 17.21$, $P = 0.0041$) and only 200 mg/L shortened shoot length significantly (7.94 cm in the control, to 4.07 cm). Also, the number of lateral roots differed significantly among concentrations ($\chi^2_4 = 20.92$, $P = 0.0003$) and replications ($\chi^2_5 = 19.21$, $P = 0.0018$) to reach the lowest number (8.08 lateral roots, compared to 15.26 lateral roots in the control) at 200 mg/L while 400 mg/L decreased it to 9.40 lateral roots (Fig. 4). These results reflected on SVI to reach a lower value (8.99 compared to 17.89 in the control) only at 200 mg/L (Dunnett test $P = 0.027$) (Fig. 5).

DISCUSSION

The studied concentrations (50, 100, 200 and 400 mg/L) of AgNPs (19.8 nm) showed no impact on *V. faba* seed germination in terms of G%, CGV and MGT. Similarly, different particle sizes (20–80 nm) and concentrations up to 534.72 mg/L of AgNPs did not affect germination of *Arabidopsis thaliana* in hydroponic conditions (Geisler–Lee *et al.*, 2013). These results agree with AgNPs effect at 1, 10 and 40 mg/L (20 nm) on 11 wetland plant species (Yin *et al.*, 2012) and on *Cucurbita pepo* at higher concentrations (1000 mg/L) and with a larger particle size (100 nm) (Stampoulis *et al.*, 2009). In contrast, AgNPs (25 nm) increased faba bean G% at 50 mg/L possibly owing to the reduction of toxic Ag ion by sodium citrate (Galal and Thabet, 2018). In terms of seedling growth in this study, tested AgNPs limited shoot development at 200 mg/L and the number of lateral roots at 200 and 400 mg/L. This contrasts to lower concentrations of AgNPs (25, 50 and 75 mg/L) that had no effect on the shoot length but increased root length (Galal and Thabet, 2018). In contrast, 50 mg/L of AgNPs (20 nm) shortened *Vigna radiata* root and shoot lengths (Prakash *et al.*, 2015). Different results at same concentration may be due to manufacturing technique (biologically or chemically synthesized), application method, the growth medium, and/or probably genetic differences between plants according to species or variety used (Zhao *et al.*, 2020). This is the first record for the effect of nanoparticles on the number of lateral roots. The number of lateral roots can be a more sensitive index of early seedling growth than root length. The limited shoot growth reflecting on lowering of SVI at 200 mg/L may be due to the small size of AgNPs as plants uptake AgNPs more easily than bulk silver, inducing higher impact on cells with accumulated smaller particles (Geisler–Lee *et al.*, 2013; Ivask *et al.*, 2014). Also, possible diffusion of Ag ions from the particle surface may also add to the AgNPs toxicity (Geisler–Lee *et al.*,

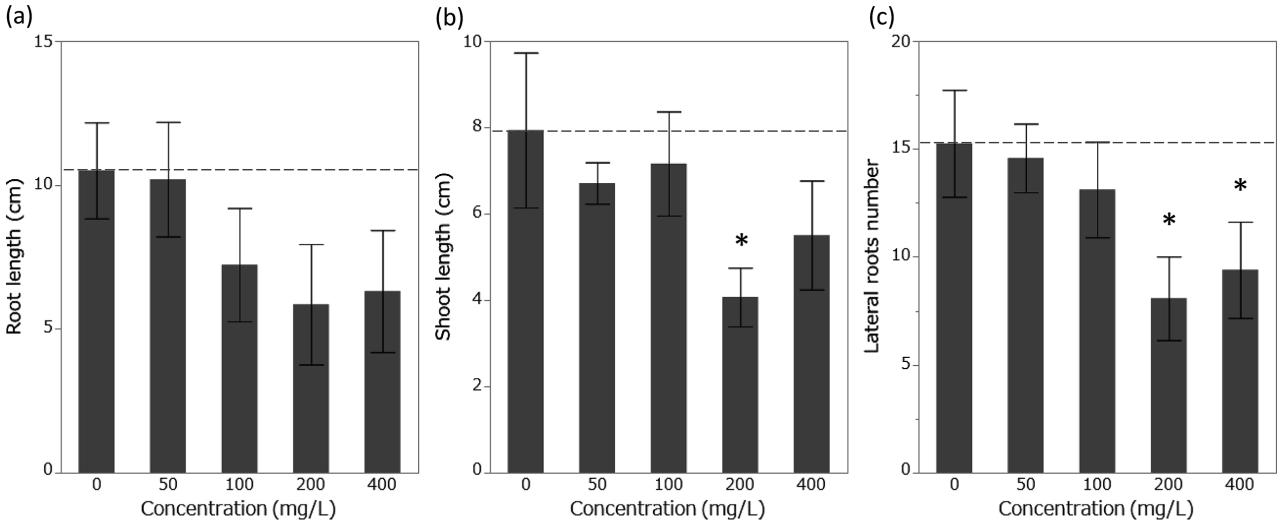

Fig. 4. Root length (cm), shoot length (cm) and lateral roots number (mean ± SE) of *Vicia faba* treated with silver nanoparticles (AgNPs) of different concentrations. An asterisk above a bar indicates a significant difference ($P < 0.01$) from the control (0 mg/L).

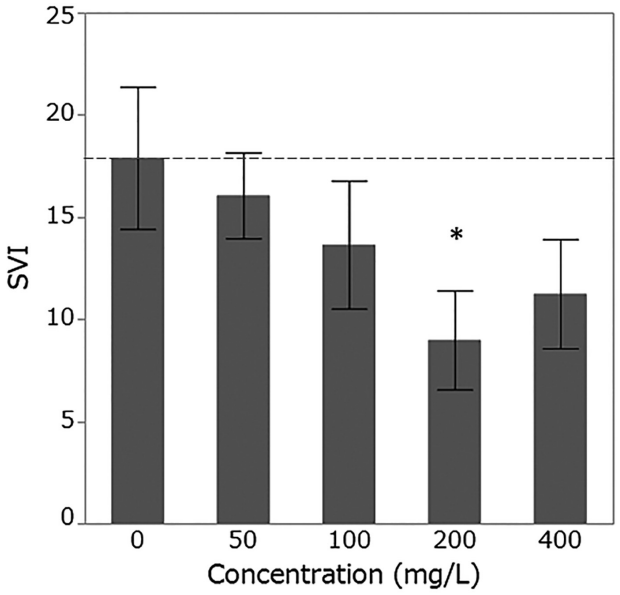

Fig. 5. Seedling vigor index (SVI) (mean ± SE) of *Vicia faba* treated with silver nanoparticles (AgNPs) of different concentrations. An asterisk above a bar indicates a significant difference ($P < 0.05$) from the control (0 mg/L).

2013). At a higher concentration (400 mg/L), it is likely that AgNPs form colloids, which makes the uptake by plants more difficult than at lower concentrations. Direct inhibition of photosynthetic activity of *V. faba* by AgNPs may explain the limited seedling growth (Queiroz *et al*., 2016). Indirect reduction of nutrients by decreasing symbiotic rhizobium in the root may also have affected the seedling development in *V. faba* (Abd–Alla *et al*., 2016).

CONCLUSION

We demonstrated that chemically synthesized AgNPs had a negative effect on seedling growth in faba bean *V. faba* at 200 mg/L and a higher concentration. This study also showed that the number of lateral roots is a useful index to evaluate nanomaterial effects on early seedling growth.

AUTHOR CONTRIBUTIONS

O. A. Galal and M. F. M. El–Samahy conceived the original idea. O. A. Galal designed the experiment and supervised the work. A. F. Thabet did the experiments, E. A. Helmy did the characterization of the material, A. F. Thabet and M. Tuda contributed to the data analysis, preparation of the figures and tables, and the interpretation of the results. A. F. Thabet, M. Tuda and E. A. Helmy wrote the manuscript and conducted the literature search. M. Tuda and O. A. Galal did critical revision of the manuscript. All authors approved the final version.

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