



## Original article

Preparation, characterization of silver phyto nanoparticles and their impact on growth potential of *Lupinus termis* L. seedlingsAsma A. Al-Huqail<sup>a</sup>, Maysa M Hatata<sup>b,d</sup>, Arwa A. AL-Huqail<sup>c</sup>, Mohamed M. Ibrahim<sup>d,e,\*</sup><sup>a</sup> King Saud University, Science College, Botany and Microbiology Department, 11451-P.O. Box 2455, Riyadh, Saudi Arabia<sup>b</sup> Taibah University, Faculty of Science, Biology Department, Al Madinah Al Munawara, Saudi Arabia<sup>c</sup> Princess Nourah University, College of Science, Department of Biology, Riyadh, Saudi Arabia<sup>d</sup> Alexandria University, Faculty of Science, Botany and Microbiology Department, 21511 Alexandria, Egypt<sup>e</sup> Stockton University, Natural Sciences and Mathematics School, Galloway, NJ 08205, USA

## ARTICLE INFO

## Article history:

Received 20 May 2017

Revised 21 August 2017

Accepted 22 August 2017

Available online 1 September 2017

## Keywords:

Silver nanoparticles

XRD

*Lupinus termis*

Growth potential

## ABSTRACT

The current study reports rapid and easy method for synthesis of eco-friendly silver nanoparticles (AgNPs) using *Coriandrum sativum* leaves extract as a reducing and covering agent. The bio-reductive synthesis of AgNPs was monitored using a scanning double beam UV-vis spectrophotometer. Transmission electron microscopy (TEM) was used to characterize the morphology of AgNPs obtained from plant extracts. X-ray diffraction (XRD) patterns of AgNPs indicate that the structure of AgNPs is the face centered cubic structure of metallic silver. The surface morphology and topography of the AgNPs were examined by scanning electron microscopy and the energy dispersive spectrum revealed the presence of elemental silver in the sample. The silver phyto nanoparticles were collected from plant extract and tested growth potential and metabolic pattern in (*Lupinus termis* L.) seedlings upon exposure to different concentrations of AgNPs. The seedlings were exposed to various concentrations of (0, 0.1, 0.3 and 0.5 mg L<sup>-1</sup>) AgNPs for ten days. Significant reduction in shoot and root elongation, shoot and root fresh weights, total chlorophyll and total protein contents were observed under the higher concentrations of AgNPs. Exposure to 0.5 mg L<sup>-1</sup> of AgNPs decreased sugar contents and caused significant foliar proline accumulation which considered as an indicator of the stressful effect of AgNPs on seedlings. AgNPs exposure resulted in a dose dependent decrease in different growth parameters and also caused metabolic disorders as evidenced by decreased carbohydrates and protein contents. Further studies needed to find out the efficacy, longevity and toxicity of AgNPs toward photosynthetic system and antioxidant parameters to improve the current investigation.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Recently nanotechnology progress and silver nanoparticles (AgNPs) have been connected in different industries, including daily and medical products. Present production of AgNPs, worldwide, is estimated to be around 500 tons per year and increase over the next few years as expected. (Mueller and Nowack, 2008). By

several routes, silver nanoparticles may be released into the environment during their synthesis, incorporation of the AgNPs into other goods, and recycling or disposal of these goods and nanoparticles corresponding to their bulk materials have been shown high and unique toxicity (Shi et al., 2011). Consequently, is necessary to understanding nanomaterial mode of interaction, uptake, accumulation and impact on the biosystems. It is of concern and increasing importance to control measures to avoid nano pollution in ecological systems (Navarro et al., 2008).

Several pathways exist or are predicted for NP association and uptake in plants (Dietz and Herth, 2012). Prakash et al. (2014) have been examined the impact of various types of NPs on higher plants. Toxicity studies of NPs are still emerging and have shown negative effects on the growth and plants development (Qian et al., 2013).

Lin and Xing (2007) analyzed the phytotoxicity of five types of multiwalled NPs (MWCNT, Al, Al<sub>2</sub>O<sub>3</sub>, Zn and ZnO) on seed germination and seedling root growth in six higher plant species

\* Corresponding author at: Alexandria University, Faculty of Science, Botany and Microbiology Department, 21511 Alexandria, Egypt.

E-mail address: [mibramim@gmail.com](mailto:mibramim@gmail.com) (M.M. Ibrahim).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

(*Raphanussativus*, *Lolium perenne*, *Lactuca sativa*, *Brassica napus*, *Zea mays* and *Cucumis sativus*). The biosystems impact at various levels is expected to device and implement proper mitigation or control measures to avoid nano pollution turning out to be a serious ecological apprehension (Navarro et al., 2008). To indicate most of the toxicological studies of AgNPs it have been conducted on microbial and animal cells; however studies which focused on plant cells was restricted (Navarro, et al., 2008). Recently, Ma et al. (2010) had shown that different sizes of AgNPs (20–80 nm) were toxic to *Arabidopsis thaliana* seedlings and they caused at very low concentration a stunted growth, indicating an increase in toxicity after increasing AgNPs concentration.

Most of the phytotoxicity studies reported so far have used chemically synthesized nanoparticles, whose synthesis and fabrications utilize toxic chemicals (Lin and Xing, 2007). Henceforth, it is accepted that the harmful impressions initiated by AgNPs to the plant systems may be because of the toxic compounds adsorbed on the nanomaterials surface.

From this point forward, an effort was made, in the present study, to investigate the possible effects of green AgNPs on growth and metabolism of *Lupinus termis* L plant.

Through green chemistry approach, silver nanoparticles were synthesized using *Coriandrum sativum* shoot extract which is an annual important spice annual crop of the *Apiaceae* and is one of valuable medicinal plants which grown all over the world and comes from the Mediterranean region. (Renata, 2013).

Bitter lupine (*Lupinus termis* L.) is considered one of the family Fabaceae (Leguminosae), it is a valuable ancient legume which has a high adaptation to poor soils and dry climates and contains high amount of protein, dietary fiber, oil, minerals and different functional components (Ismail et al., 2014). Earlier in Egypt and other countries for thousands of years, Lupine seeds have been used for human feed and as a medication (ARC, 1994). Across Egypt, *Lupinus termis* is cultivated in a wide range of environments and its seed have a nutritional quality superior to other legumes seed and similar to soybean seed (Raza and Jmsgard, 2005).

This research aims to characterize green silver nanoparticles through high resolution transmission electron microscopy (HRTEM) and X-ray diffraction analysis. Germination, morphological and anatomical changes in leaves of *Lupinus termis* were studied in response to different concentration of green AgNPs. Biochemical responses were emphasized as chlorophyll content, florescence, protein, total soluble sugar, total phenol contents and activities of total antioxidant enzymes in AgNPs treated seedlings.

## 2. Materials and methods

### 2.1. Materials

The healthy leaves of *Coriandrum sativum* were collected from the botanical garden of faculty of science, Alexandria University, Alexandria, Egypt. Seeds of *Lupinus termis* (Var. Giza 2) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Ultra purified water was used for experiment and all the chemicals were obtained from Sigma Aldrich, Alexandria, Egypt.

### 2.2. Preparation of plant extract

Fresh leaves of *Coriandrum sativum* (100 g) were chopped into fine pieces and transferred to sterile 250 mL conical flask. Aqueous leaf extract of *Coriandrum sativum* was prepared by heating the chopped fresh leaves in 100 mL of distilled water on sand bath for 30 min to facilitate the formation of aqueous extract and the extract was filtered through Whatmann No.1 filter paper to remove insol-

uble fractions and macromolecules. The filtrate was mixed with equal volume of acetone and make centrifugation for the resultant precipitate at 1500 rpm for 10 min and keep it in refrigerator at 4 °C. Use this extract as stabilizing and reducing agent.

### 2.3. Synthesis of silver nanoparticles

In the typical synthesis of silver nanoparticles, 12 mL of the aqueous extract of *Coriandrum sativum* was added to 88 mL of 1 mM ( $10^{-3}$  M) solution of silver nitrate in 250 mL Erlenmeyer flask. The resulting greenish mixture was incubated for 24 h in a rotary shaker (200 rpm) at 26 °C. The reaction was started at room temperature and in absence of light to minimize photo-activation of silver nitrate. The appearance of brown color indicate the completion of Ag reduction and the synthesis of CSL-AgNPs. The solution obtained was transferred to an amber colored bottle to prevent autoxidation of silver. Distilled water was used as controls throughout the experiment.

### 2.4. Characterization of silver nanoparticles

Indication of the bio-reduction of AgNPs in the aqueous *C. sativum* extract was evidenced by monitoring the absorption spectra in the range between 200 and 800 nm using double beam UV-vis spectrophotometer (T70/T80 series UV/Vis spectrophotometer PG Instrument Ltd., England). A spectrum of silver nanoparticles was plotted with wavelength nm on x-axis and absorbance on y-axis. XRD was recorded in the 2 $\theta$  range of 30–80° using XRD Brukerd 8 Advance CuKa Target, Germany, the energy of which was 8.04 keV and wavelength was 1.54 Å. The applied voltage was 40 kV and current was 25 mA. Scherer equation was used to estimate the crystallite size of the AgNPs. The morphology of silver nanoparticles was confirmed by Transmission Electron Microscopy (TEM). Analysis of silver nanoparticles were performed using a TEM (Joel JEM CX100, Japan) with an acceleration voltage of 80 kV. Silver nanoparticles were imaged after air drying of a droplet on a carbon-coated 200-mesh copper grid.

### 2.5. Germination experiments

For the study, *Lupinus termis* L (Giza 2) seeds were surface sterilized using 1% mercuric chloride and 70% ethanol for 5 min and were thoroughly washed with deionized water then soaked in sterilized distilled water for 24 h. Triplicate experiments were conducted in which each plate contained 10 seeds and placed equidistantly into 100 mm × 15 mm Petri dish containing 10 ml (2%) agar supplemented with different concentrations (0–900 ppm) AgNPs. Dishes were maintained under regulated conditions for 3 more days. After the completion of incubation period the germination was recorded 3 days after treatment and the effect of AgNPs on seed germination were evaluated according to the parameters described by Vashisth et al. (2010). The following equations were used to calculate germination percentage (%G) and relative germination (RG),

$$\% G = \text{Number of Germinated seeds} / \text{Total number of seeds} \times 100 \quad (1)$$

$$\% RG = \%G \text{ in treatment} / \%G \text{ in control} \times 100 \quad (2)$$

### 2.6. Influence of CSL-AgNPs on seedling growth

In another experiment with a certain selected concentrations of AgNPs (0, 100, 300 and 500 ppm) for the study of germination were

selected considering that the worst case situation at which plants could be exposed is that 500 ppm. Seeds were allowed to germinate in each pot (15 cm diameter × 20 cm height), filled with pre acid washed sand for 5 days. All pots were placed in a growth chamber and seedlings were further grown under the following conditions: 16/8-h day/night period, controlled temperature 25/20 °C day/night and 80% relative humidity under photon flux density of 340  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity. Aliquots of fifty milliliters of AgNPs suspension were administered to each pot day after day. After 10 days of plant growth, seedlings were harvested and separated into root and shoot and used immediately for root length, shoot length, number of leaves, fresh and dry weight analysis and biochemical parameters.

### 2.7. Germination indices

The seedling vigor indices (I&II) were calculated based on Vashisth and Nagarajan (2010) equations

$$\text{Vigor index I} = \text{germination \%} \times \text{seedling length (root + shoot)} \quad (3)$$

$$\text{Vigor index II} = \text{germination \%} \times \text{seedling weight (root + shoot)} \quad (4)$$

Other parameters such as Plant height stress tolerance index (PHSI), Root length stress tolerance index (RLSI) and Dry matter stress tolerance index DMSI) are calculated by using the following formula

Plant height stress tolerance index was calculated by using the following formula

$$(\text{PHSI}) = \text{Plant height of stressed plant} / \text{Plant height of control plant} \times 100$$

Root length stress tolerance index was calculated by using the following formula

$$(\text{RLSI}) = \text{Root length of stressed plant} / \text{Root length of control plant} \times 100$$

Dry matter stress tolerance index was calculated by using the following formula

$$(\text{DMSI}) = \text{Dry matter of stressed plant} / \text{Dry matter of control plant} \times 100$$

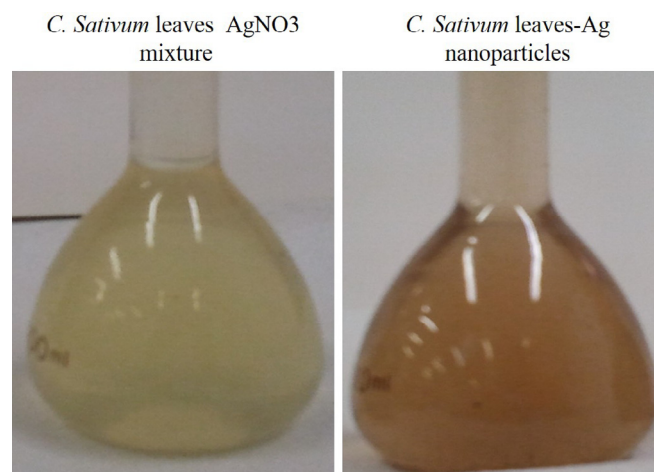
### 2.8. Statistical method

Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 20) software. The statistical test used as follow: Arthematic mean, standard deviation, for compare between more than two groups ANOVA-test was used for parametric data, followed by post hoc test by Duncan method Sokal and Rohlf (1995).

## 3. Results

### 3.1. Characterization of silver nanoparticles

The leaf extract of *Coriandrum sativum* was used as a reducing and stabilizing agents for the synthesis of *C. sativum* leaves silver nanoparticles (CSL-AgNPs). Rapid appearance of a yellowish-brown color in the reaction mixture as a result of surface plasmon, suggested the formation of colloidal AgNPs. The color noted by visual observation increased in intensity giving brown color after



**Fig. 1.** *C. sativum* leaves extract with  $\text{AgNO}_3$  before and after the synthesis of CSL-AgNPs.

24 h of incubation and hence confirmed the completion of reaction between leaf extract and  $\text{AgNO}_3$  (Fig. 1).

The analysis of silver nanoparticles by using UV-Visible spectrometric analysis showed absorption spectra at 450 nm and this suggesting the occurrence of bio-reduction for the silver nitrate forming silver nanoparticles (Fig. 2).

The examination of particle size of CSL-AgNPs by TEM showed that, the particle size ranged between 1 and 16 nm silver nanoparticles and the average size of the CSL-AgNPs ranged between 5 and 10 nm (Fig. 3A). The size and shape of CSL-AgNPs were uniform and are with varying shapes (Fig. 3). A significant content of AgNPs was recognized after quantitative analysis for GSL-AgNPs by energy dispersive X-ray analysis EDX (Fig. 3B).

X-ray Diffraction Spectroscopy data showed the broad diffraction peaks of the CSL-AgNPs and are marked by their Bragg indices. Peaks indicating the presence of face centered unit cells of CSL-AgNPs. The dots mark the measured diffraction data, the continuous red and blue lines represent the calculated profile and difference between the observed and calculated intensities, respectively. The diffracted peaks at  $2\theta$  38.2, 44.3, 64.7 and 77.4 positions were indexed to 111, 200, 220 and 311 planes of pure silver and that indicated the synthesis of CSL-AgNPs (Fig. 4).

### 3.2. Effect of CSL-AgNPs on *Lupinus termis* L. seed germination and seedling growth parameter

The data presented in Table 1 and showed in Fig. 5 showed that treatment of *Lupinus termis* L. seeds with different concentrations of AgNPs ranged from 100 ppm to 900 ppm acquired varied germination percentages and their germination percentage decreased with increasing the AgNPs concentration over 100 ppm. Also, the relative growth (RG) decreased to one third of the control by 900 ppm. The produced radicles were sensitive to higher concentrations of AgNPs as they are coiled and appeared brown from 600 ppm to 900 ppm as shown in Fig. 5 and Table 1. The relative germination decreased gradually with increasing AgNPs concentration more than 100 ppm (Table 1).

### 3.3. Effect of CSL-AgNPs on seedling growth of *Lupinus termis* L

Tables 2 and 3 showed the diverse seedling growth parameters including fresh weight and dry weight, as well as, root and shoot elongation in *L. termis* seedlings exposed to various concentrations of CSL-AgNPs at 0, 100, 300 and 500 ppm. The seedling growth

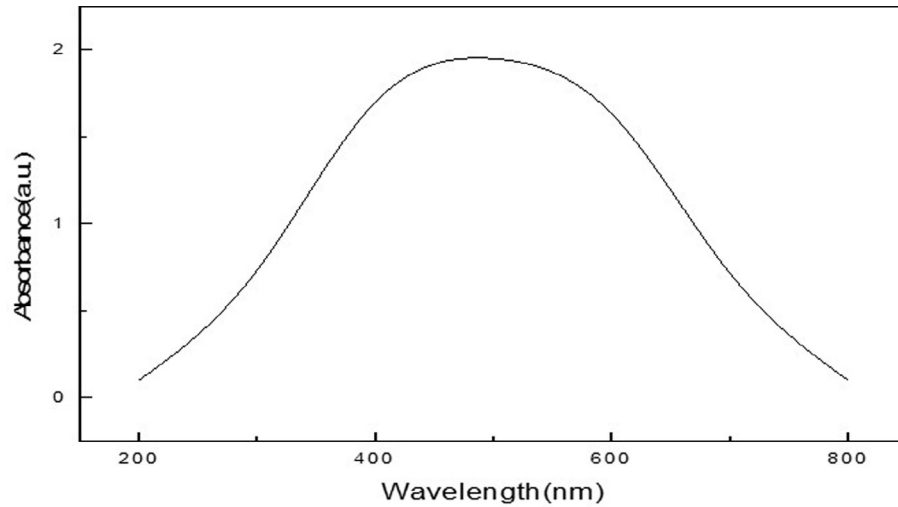


Fig. 2. Absorbance peak of *C. sativum* leaf AgNPs at 450 nm of silver nanoparticles recorded by UV-visible spectra.

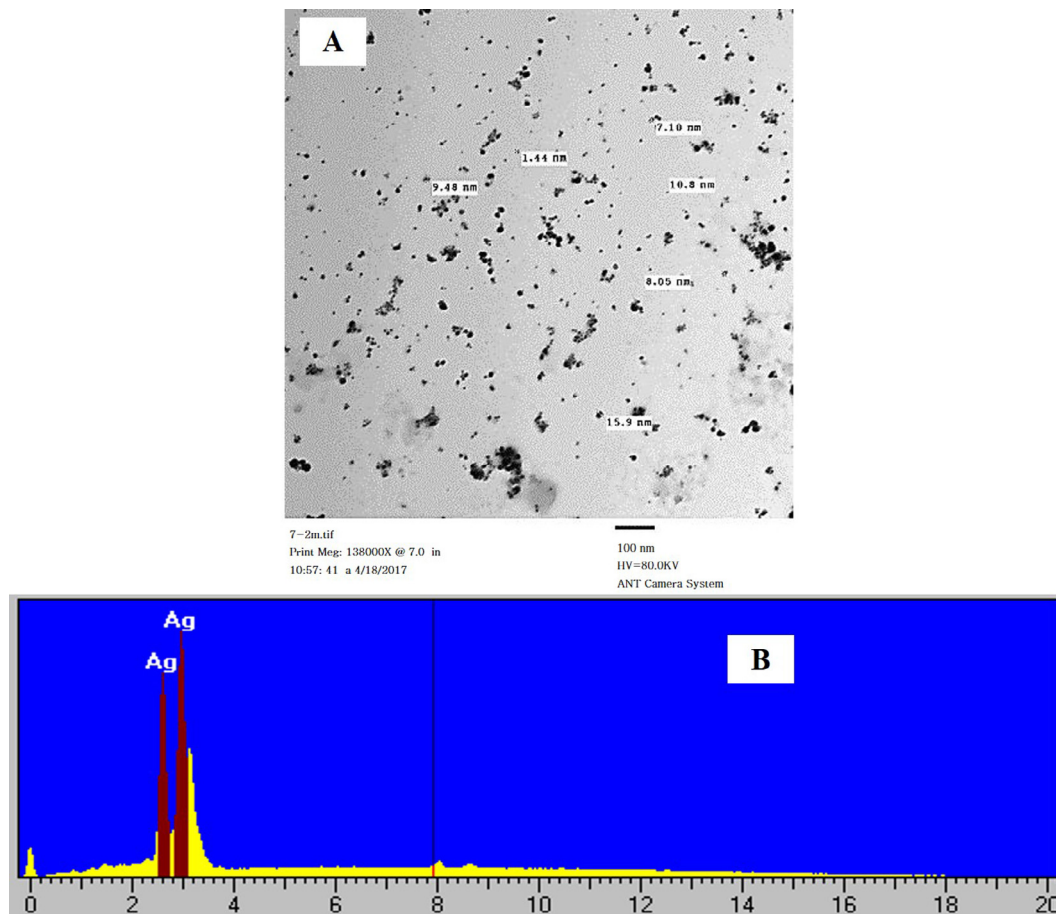


Fig. 3. Transmission electron microscope (TEM) micrograph of CSL-AgNPs spread in cooper grid (A) and Energy dispersive X-ray characterization spectrum of CSL-AgNPs. Visible peaks approve the presence of silver in the sample (B).

parameters have been considered as primary indicator for the phytotoxicity of CSL-AgNPs, especially with higher concentrations. Significant decrease in the fresh and dry weight of both shoot and root was observed upon exposure to higher concentrations of CSL-AgNPs (300 and 500 ppm) (Tables 2 and 3). On the other hand

lower dose concentration of CSL-AgNPs (100 ppm) caused a noticeable increase in growth parameters, including fresh and dry weight of both shoot and root.

Similar pattern was observed for the effect of CSL-AgNPs in root and shoot length under different concentration of CSL-AgNPs. It



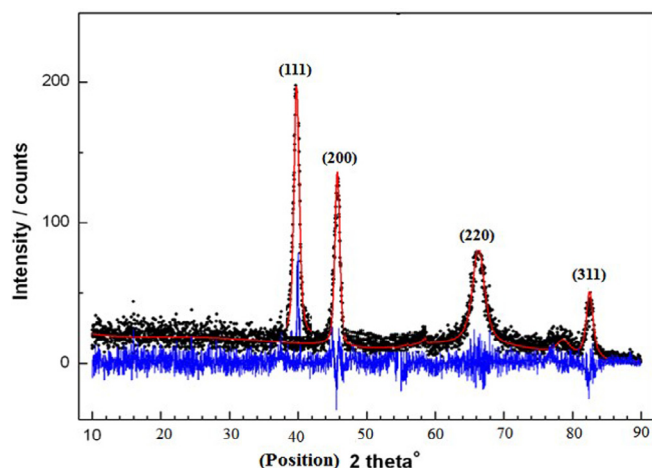


Fig. 4. X-ray diffraction pattern of the synthesized *C. sativum* leaf extract -AgNPs.

Table 1

Effect of different concentrations of CSL-AgNPs (0–900 ppm) on germination percentage (%G) and relative germination (RG) of *Lupinus termis* L. after 5 days of treatment.

1507807527940Conc. CSL-AgNPs (ppm)	Germination percentage (%G)	Relative germination (RG)
0	90%	100%
100	100%	111%
150	95%	106%
200	90%	100%
400	70%	78%
600	60%	66.6%
800	50%	56%
900	30%	33%

was observed that, as the concentrations of AgNPs over 100 ppm caused also a statistically significant decline in root and shoot length as shown in Table 2.

#### 3.4. Effect of CSL-AgNPs on growth indices

As shown in Table 4, there was a concentration-dependent reduction in different growth indices including plant height stress

tolerance index (PHSI), root length stress tolerance index (RLSI), dry mass stress index (DMSI), vigor indexes I and II especially, upon exposure to the higher concentrations of CSL-AgNPs (Table 4). Seedlings of *L. termis* exposed to higher concentrations of CSL-AgNPs showed a parallel reduction in all measured growth indices and reached highly significance reduction in seedlings exposed to 300 and 500 ppm in comparison with control. Significant decrease in PHSI, RSLI and DMSI values (74.78, 62.65 and 58.43) was observed in 500 ppm concentrations of CSL-AgNPs. Conversely significant increase in PHSI, RSLI and DMSI values i.e. 112.3, 118.07 and 138.20 were noted in 100 ppm concentrations of CSL-AgNPs respectively (Table 4). Data with respect to the vigor indexes I and II clearly show that both increased up to 100 ppm concentration of CSL-AGNPs and decreased significantly both in 300 and 500 ppm concentrations of CSL-AgNPs (Table 4).

#### 4. Discussion

Nanotechnology applications are currently generally appropriated all through our life, and particularly in agricultural systems. Moreover, nanoparticles physicochemical characteristics, are among the potential possibility for controlling the redox status and changing the development, growth potential, performance, and plant quality. Using plant materials for synthesis of different kinds of nanoparticles has been revolutionized in the last few years (Palanisamy et al., 2014). In the present study, the particle size of the synthesized CSL-AgNPs ranging in between 1 and 16 nm, which may explain the capability of these particles to penetrate the cells. The mechanism of action of different kinds of nanoparticles is unclear in different organisms including plants (Kim et al., 2007; Kim et al., 2011; Mohamed et al., 2014).

Characterization of our synthesized CSL-AgNPs was done using different procedures such as UV-visible spectroscopy, electron microscope equipped with Energy dispersion X-ray diffraction and X-ray diffraction. Absorption spectrum of the prepared CSL-AgNPs showed one sharp peak with surface plasmon transition absorption indicating the synthesis of spherical AgNPs as reported by Forough and Farhadi (2010). This peak is well documented for various metal nanoparticles with particle size ranging from 2 to 100 nm (Suing and Seng, 2013).

The obtained X-ray diffraction peaks corresponded to cubic structure with face centered for Ag ions in its purified form and the size of silver nanoparticles ranging between 1 and 16 nm

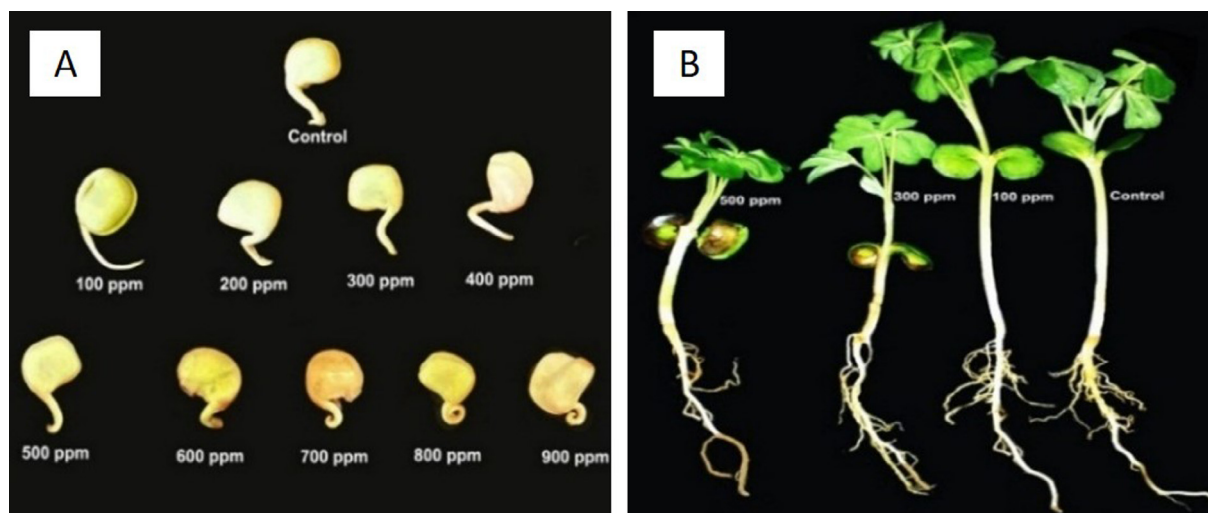


Fig. 5. (A) Effect of different concentrations of CSL-AgNPs (0–900 ppm) on *Lupinus termis* L seed germination. (B) Effect of different concentrations of CSL-AgNPs (0, 100, 300 and 500 ppm) on growth parameters of *Lupinus termis* L.

**Table 2**  
Effect of CSL-AgNPs on fresh and dry weight of root and shoot; root and shoot length of *Lupinus termis* L. seedlings after 10 days of treatment.

Conc. CSL-AgNPs (PPm)	Root fresh weight (g)	Shoot fresh weight (g)	Seedling fresh weight	Root Length (cm)	Shoot Length (cm)	Seedling length (cm)
C	7.8 ± 0.71 <sup>a</sup>	18.3 ± 1.66 <sup>ab</sup>	26.1 ± 2.37 <sup>a</sup>	8.3 ± 0.75 <sup>ab</sup>	14.3 ± 1.30 <sup>a</sup>	22.6 ± 2.05 <sup>ab</sup>
100	8.2 ± 0.63 <sup>a</sup>	20.1 ± 1.55 <sup>a</sup>	28.3 ± 2.18 <sup>a</sup>	9.8 ± 0.75 <sup>a</sup>	15.6 ± 1.20 <sup>a</sup>	25.4 ± 1.95 <sup>b</sup>
300	6.5 ± 0.72 <sup>ab</sup>	16.1 ± 1.79 <sup>bc</sup>	22.6 ± 2.51 <sup>b</sup>	7.3 ± 0.81 <sup>b</sup>	14.2 ± 1.58 <sup>a</sup>	21.5 ± 2.39 <sup>a</sup>
500	5.8 ± 0.05 <sup>b</sup>	13.0 ± 0.12 <sup>c</sup>	18.8 ± 0.17 <sup>c</sup>	5.2 ± 0.05 <sup>c</sup>	11.7 ± 0.10 <sup>b</sup>	16.9 ± 0.15 <sup>b</sup>
P<	0.011*	0.013*	0.005*	0.016*	0.033*	0.025*

The same small letters indicate that there was no significant difference, while the different letters indicate that there was a significant difference between the two groups.

**Table 3**  
Effect of silver nanoparticles on of 10 days old *Lupinus termis* L. seedlings shoot and root dry mass.

Conc. CSL-AgNPs (ppm)	Root dry Matter(g)	Shoot dry Matter(g)	Seedlings dry matter(g)
Control	0.85 ± 0.08 <sup>a</sup>	1.82 ± 0.17 <sup>a</sup>	2.67 ± 0.24 <sup>a</sup>
100	0.96 ± 0.07 <sup>a</sup>	2.73 ± 0.21 <sup>a</sup>	3.69 ± 0.28 <sup>b</sup>
300	0.63 ± 0.07 <sup>b</sup>	1.94 ± 0.22 <sup>a</sup>	2.57 ± 0.29 <sup>a</sup>
500	0.32 ± 0.00 <sup>c</sup>	1.24 ± 0.01 <sup>b</sup>	1.56 ± 0.01 <sup>c</sup>
P<	0.002*	0.0133*	0.026*

The same small letters indicate that there was no significant difference, while the different letters indicate that there was a significant difference between the two groups.

(Theivasanthi and Alagar, 2012; Arokiyaraj et al., 2014). Transmission electron microscope give us more confirmation, further inputs and more information about the morphology and size of the synthesized AgNPs in presence of *C. sativum* leaf extract ranging between 1 and 16 nm with spherical morphology (Agnihotri et al., 2014). Analysis of Ag ions by EDX equipped with TEM shows the presence of pure silver approving the synthesis of AgNPs (Ali et al., 2011).

From earlier studies, it has been known that one of the properties that determine the phytotoxicity of AgNPs in plants was the size of the particles, and the smaller particles the larger surface area that could result in higher cellular uptake (Geisler-Lee et al., 2013).

In higher plants, phytotoxicity should be investigated in order to progress a comprehensive nanoparticles toxicity profile (USEPA, 2005). As a rapid and widely acute phytotoxicity test seed germination and root elongation is used with several advantages: sensitivity, simplicity, low cost and suitability for unstable samples or chemicals (Munzuroglu and Geckil, 2002). It was well known that germination is considered as a physiological process beginning with water imbibition by seeds and culminating in the radicle emergence (Kordan, 1992).

The desired concentrations (100, 300 and 500 ppm of CSL-AgNPs) used to testify their effect on growth parameters of *Lupinus termis* seedlings were selected based on a preliminary test on seed germination and root growth. Maximum decline to only one fifth in germination percentage was recorded at 900 ppm silver nanoparticle concentration.

All mentioned growth parameters increased significantly with low concentration of CSL-AgNPs (100 ppm) signifying a simultane-

ously effect of this dose, which might direct low dose physiological inductive tolerance in *Lupinus termis* plant.

Silver nanoparticles was found to interfere with the activities of certain hydrolytic enzymes, required during germination process resulting in a reduction of per cent seed germination. Lin and Xing (2007) have also highlighted that seed germination is inhibited at higher concentrations of nanoparticles.

The present study revealed that the roots were sensitive to the increase in AgNPs concentrations. Morphologically, radicals of the germinated seeds were stunted with brown tips when they treated with high concentration of AgNPs. Correspondingly, Geisler-Lee et al. (2013) have demonstrated that AgNPs-treated seedlings had shorter roots and exhibited visible brown root tips as compared with control plants. As observed in this study, the higher sensitivity of roots to AgNPs might be due to the fact that the roots comes in direct contact with AgNPs in the exposure medium and also, the radicle was not adapted to the treatments (Yin et al., 2012).

Pollutants, in low concentration though having obviously inhibitory effect on root growth, may cannot pass through seed coats and not affect germination. This may clarify that seed germination in this study was not significantly modified by low concentration of nanoparticles, even it was induced by 100 ppm AgNPs treatment.

With regard to the root morphology of *Lupinus termis*, the control plants had long straight roots, but the plants exposed to AgNPs had much shorter roots regardless of brown and coiled radicles of the germinated seeds. As the concentration of AgNPs increased the browning phenomenon was more apparent in the root. Earlier it was also reported by Geisler-Lee et al. (2013), that exposure to different concentrations of AgNPs has resulted in dark browning of root tip and the inhibition of root hair development on the main seedling roots. Also, While, Yin et al. (2012) observed the damaged cells and broken root cap of *L. multiflorum* exposed to AgNPs, and wondered that abnormal damaged cells resulted in the loss of root gravitropism which was the reason of radicle coiling of AgNPs treated germinated seeds.

It may be attributed that the toxicity of nanoparticles was due to two different actions, (1) the chemical composition which can caused chemical toxicity. e.g., the release of (toxic) ions and (2) the surface, size and/or shape of the particles which stimuli stress (Bruinink and Stark, 2006). However, shoot fresh weight and shoot and root length increases was recorded at different concentrations of silver nanoparticle treatment, could be mediated via plant

**Table 4**  
Effect of CSL-AgNPs on growth indices, plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), dry mass stress index (DMSI), vigor indexes I and II of *Lupinus termis* L. seedlings after 10 days of treatment growth indices.

Conc. CSL-AgNPs (ppm)	PHSI%	RLSI%	DMSI%	Vigor index I	Vigor index II
Control	100	100	100	2250 ± 204.55 <sup>a</sup>	213.6 ± 19.42 <sup>a</sup>
100	112.3	118.07	138.20	2260 ± 173.85 <sup>a</sup>	369.0 ± 28.38 <sup>b</sup>
300	95.13	87.95	96.25	1720 ± 191.11 <sup>b</sup>	205.6 ± 22.84 <sup>a</sup>
500	74.78	62.65	58.43	1183 ± 10.52 <sup>c</sup>	109.2 ± 0.97 <sup>c</sup>
P<	0.004*	0.0113*	0.022*	0.001*	0.001*

growth regulators (cytokinins and gibberellins) which are involved in cell division and cell elongation (Stampoulis et al., 2009). Mena et al. (2016) have been reported that decrease in shoot and root length of seedlings treated with higher silver nanoparticle and the recorded phenomenon is dose dependent. Vigor index represents the combined effect of various external and internal growth regulating factors. It was recorded an increase in vigor index of the treated seedlings at 100 ppm AgNPs treatment but higher concentration inhibited it.

Higher concentrations of CSL-AgNPs showed negative impact on different growth indices such as plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), dry mass stress index (DMSI), vigor indexes I and II. Our results are consistent with Lee et al., (2008) who examined the impact of Cu-NPs on bean (*Phaseolus radiatus*) and wheat (*T. aestivum*) plants. They found the seedling growth parameters of both plants were significantly decrease due to the presence of copper nanoparticles. On the other hand, another study done by Zheng et al. (2005) found that under exposure of spinach plant to low concentrations of TiO<sub>2</sub>NP a significant improvement in the growth of spinach was exhibited as compared to higher concentrations. According to our results and consistent with others we can concluded that inhibition of plant growth differ significantly among nanoparticles and plants, and it is associated with the nanoparticle concentration.

In conclusion, our results showed that using *C. sativum* leaf extract could be valuable material in a successful preparation of silver nanoparticles which giving an indication that silver nanoparticle treatment might improve the growth profile at 100 ppm. However, our research indicated that exposure of *Lupinus termis* seedlings to high concentrations of CSL-AgNPs (300 and 500 ppm) resulted in a highly significant reduction in all growth parameters and growth indices.

## Acknowledgement

The authors would like to extend their sincere appreciation to the Dean of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-231.

## References

- Agnihotri, S., Mukherji, S., Mukherji, S., 2014. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *RSC Adv.* 4, 3974–3983.
- Ali, D.M., Sasikala, M., Gunasekaran, M., Thajuddin, N., 2011. Biosynthesis and characterization of silver nanoparticles using marine *cyanobacterium, oscillatoriawillei* ntdm01. *Digest J. Nanomater. Biostruct.* 6 (2), 385–390.
- Agricultural Research Contres Min. -Agri. of Egypt. *Bull.* 226:1–8.
- Arokiyaraj, S., Arasu, M.V., Vincent, S., Prakash, N.U., Choi, S.H., Oh, Y.K., Choi, K.C., Kim, K.H., 2014. Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum* L and its antibacterial and cytotoxic effects: an in vitro study. *Int. J. Nanomed.* 9, 379–388.
- Dietz, K.J., Herth, S., 2012. Plant nanotoxicology *Trends. Plant Sci.* 17 (3), 180.
- Forough, M., Farhadi, K., 2010. Biological and green synthesis of silver nanoparticles. *J. Eng. Environ. Sci.* 4 (7), 281–287.
- Geisler-Lee, J., Wang, Q., Yao, Y., Zhang, W., Geisler, M., Li, K., Huang, Y., Chen, Y., Kolmakov, A., Ma, X., 2013. Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology* 7, 323–337.
- Ismail, M.A.S., Gamal, M.G., Ghada, S.M., 2014. Effect of Ascorbic acid and Niacin on Protein, Oil Fatty Acids and Antibacterial Activity of *Lupinus termis* Seeds. *Int. J. Ph. Phyto. R.* 15 6 (4), 866–873.
- Kim, J.S., Eunye, K., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y. H., Hwang, C.Y., Kim, Y.K., Lee, Y.S., Jeong, D.H., Cho, M.H., 2007. Antimicrobial effects of silver nanoparticles nanomedicine: nanotechnology. *Biol. Med.* 3 (2007), 95–101.
- Kim, H.S., Lee, D.S., Ryu, S.J., Choi, D.S., Lee, S.H., 2011. Antibacterial activity of silver-nanoparticles against *Staphylococcus aureus* and *Escherichia coli* Korean. *J. Microbiol. Biotechnol.* 39 (1), 77–85.
- Kordan, H.A., 1992. Seed viability and germination: a multi-purpose exp. system. *J. Biol. Educ.* 26, 247–251.
- Lee, W.M., An, Y.J., Yoon, H., Kweon, H.S., 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. *Environ. Toxicol. Chem.* 27, 1915–1921.
- Lin, D., Xing, B., 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ. Poll.* 150, 243–250.
- Ma, X., Geiser-Lee, J., Deng, Y., Kolmakov, A., 2010. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Sci. Total Environ.* 408, 3053–3061.
- Mena, Z., Armendari, R., Jose, R., Videira, P., Gardea, J., 2016. Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. *Front. Plant Sci.* 7, 1–11.
- Mohamed, M.I., Amal, A.H., Afaf, I.S., Gehan, A.E., Abdul-Aziz, A., Farid, M.A., Humaira, H.R., Nadine, M., 2014. Synthesis of eco-friendly silver nanoparticles using plant extracts and assessment of their antimicrobial activity. *Fresenius Environ. Bull.* 23 (1a), 184–189.
- Mueller, N.C., Nowack, B., 2008. Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.* 42, 4447–4453.
- Munzuroglu, O., Geckil, H., 2002. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Arch. Environ. Contam. Toxicol.* 43, 203–213.
- Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants and fungi. *Ecotoxicology* 17, 372–438.
- Palanisamy, N.K., Nas, F., Amirulhusni, A.N., Zaini, M.Z., Hussaini, J., Liew Jian Ping, L. J., Durairaj, R., 2014. Antibiofilm properties of chemically synthesized silver nanoparticles found against *Pseudomonas Aeruginosa*. *J. Nanobiotechnol.* 12, 2.
- Prakash, M., Nair, G., Chung, M., 2014. Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. *Environ. Sci. Pollut. Res.* 21, 8858–8869.
- Qjan, H., Peng, X., Han, X., Ren, J., Sun, L., Fu, Z., 2013. Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. *J. Environ. Sci.* 25, 1947–1956.
- Raza, S., Jrnsgard, B., 2005. screening of white lupin accessions for morphological and yield traits. *Afr. Crop Sci. J.* 13 (2), 135–141.
- Renata, N.W., 2013. Essential oil composition of the coriander (*coriandrum sativum* l.) herb depending on the development stage 66 (1), 53–60.
- Shi, Q., Li, S., Jia, J., Jiang, J., 2011. The Hedgehog-induced Smoothed conformational switch assembles a signaling complex that activates Fused by promoting its dimerization and phosphorylation. *Development* 138 (19), 4219–4231.
- Stampoulis, D., Sinha, S.K., White, J.C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* 43, 9473–9479.
- Suing, K.H., Seng, T., 2013. Rapid and efficient synthesis of silver nanofluid using electrical discharge machining. *J. Nanomat.* 3, 1–7.
- Theivasanthi, T., Alagar, M., 2012. Electrolytic synthesis and characterizations of silver nanopowder. *Nano Biomed. Eng.* 4 (2), 58–65.
- U.S. Environmental Protection Agency, 2005. Nanotechnology White Papere External Review Draft. Available from: <[http://www.epa.gov/osa/pdfs/EPA\\_nanotechnology\\_white\\_paper\\_external\\_review\\_draft\\_12-02-2005](http://www.epa.gov/osa/pdfs/EPA_nanotechnology_white_paper_external_review_draft_12-02-2005)>.
- Vashisth, A., Nagarajan, S., 2010. Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. *J. Plant Physiol.* 167, 149–156.
- Yin, L., Colman, B.P., McGill, B.M., Wright, J.P., Bernhard, E.S., 2012. Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS ONE* 7, 1–7.
- Zheng, L., Hong, F., Lu, S., Liu, C., 2005. Effect of nano-TiO<sub>2</sub> on strength of naturally aged seeds and growth of spinach. *Biol. Trace Elem. Res.* 104, 83–91.