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The toxicity potential of Ag nanoparticles synthesized from *Cordia myxa* L.

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Kew words: antioxidant capacity, chemical AgNPs, germination value, green AgNPs, lipid peroxidation, phenol, protein.

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Abstract: Plant-mediated nanoparticles synthesis is considered as one of the appealing options in bio-nanoparticles synthesis, however, there is contradictory information about the positive or negative impacts of nanoparticles on plants. Investigating the toxic effects of Ag NPs on model plants, such as onion, can reveal the probability of damage. Thus, the present study was conducted to compare the germination indices and biochemical parameters of edible onion seeds treated with different concentrations of two types of silver nanoparticles (green synthesized and chemical synthesized) to examine the oxidative stress. Based on our results, the interaction between leaf extract and silver salt resulted in a color change from pale yellow to dark brown, the first sign of the AgNPs formation. The green AgNPs treatment improved the onion germination indices. The green AgNPs-exposed seeds displayed a no-significant reduction in protein content and same protease activity as the control treatment, but chemical AgNPs-treated displayed a significant rise of protease and reduction in protein content in concentration more than 0.06 gL^{-1}. In chemical AgNPs-treated seeds both peroxidase and catalase displayed an ascending linear trend and the most activities belonged to chemical AgNPs at 0.05 g L^{-1} (315.62 and 51.45 µmol $min^{-1}g^{-1}$ FW, respectively). Both nanoparticle types made an increase in MDA, but green AgNPs did not significantly differ with control treatment. It can be concluded that the green synthesis of nanoparticles is a safe and suitable alternative for chemical-synthesized metal nanoparticles.

1. Introduction

The production, manipulation, and use of nanoparticles (NPs), due to their distinctive and definite capabilities, has become one of the most attractive research topic in different area of science, including biology, chemistry, engineering, medicine, physics, agriculture and food. Nano-dimension structures involved in many aspects of plant biology such as ionic transport and molecular transmissions. The diameter of plant cell wall pores are in the range of 5 to 20 nm. Furthermore, plasmodesmata, the intracellular channels to facilitate molecular transitions, are also nano-scale (50 to 60 nm in diameter) (Zambryski, 2004).

The nanotechnologies based-products are expected to upturn (Maynard *et al*., 2006; Rejeski and Lekas, 2008). Nanotechnology applications in agricultural sector involve in precision farming, smart feeding, enrichment of food quality, products packaging, products labeling, nano-pesticide, nano-fertilizer, and nano-herbicide (Thiruvengadam *et al*., 2018). The different chemical and physical methods are applied to the synthesis of nanoparticles such as sol-gel, electrochemical, hydrothermal, sono-chemical, and microwaves techniques. Chemical methods had low efficiency, need high temperature, high pressure and high energies during the reaction process. Besides they need the expensive reagent and complex equipment. Furthermore, chemical methods require some non-degradable chemical components, as reducing or stabilizing agents, which finally caused environmental pollution (Senapati *et al*., 2012).

The potential of biological materials to synthesize metal nanoparticles has provided a low-cost and eco-friendly route. Biological synthesis or green synthesis of nanoparticles, uses biological material as the reducing and capping agents (Veerasamy *et al*., 2011). The green synthesis uses microorganisms such as fungus, bacteria (Ahmad *et al*., 2005), yeast and actinomycetes (Kowshik *et al*., 2003) or macro-organisms e.g., plants and algae, as intermediate agents (Niemeyer and Mirkin, 2004; Dubey *et al*., 2010; Prasad *et al*., 2012).

Plant-mediated nanoparticles synthesis is considered as one of the appealing options in bio-nanoparticles synthesis, due to high variety and abundance, no need for a complicated growth conditions, bulk-biomass production, no need for special nutrient media, cost-effectiveness, richness of various effective metabolites, simple single-step synthesis process and suitable for the large-scale synthesis of nanoparticles. Polyphenolic contents of the plant act as reducing agents and eventually act as coating and stabilizing agent of nanoparticles (Dubey *et al*., 2010).

Cordia myxa (L.) or sapistan-tree, a member of Boraginaceae family, is native to tropical areas of Asia. It well documented that *C. myxa* contained flavonoids, glycosides, sterols, terpenoids, saponins, phenolic acids, alkaloids, tannins, coumarins, resins ; and mucilage (Inas *et al*., 2011; Rashed *et al*., 2014): a suitable candidate for green NPs synthesis.

However, there is contradictory information about the positive or negative impacts of nanoparticles on plants. Titanium dioxide nanoparticles increased the dry and fresh weight of spinach by increasing the light absorption, boosting rubisco enzyme activity (Lin and Xing, 2007) andrising the nitrogen metabolism (Yang *et al*., 2007). Zinc and copper nanoparticles showed significant differences in growth, and toxicity symptoms in treated plants (Lee *at al*., 2008; Monica and Cremonini, 2009; Musante and White, 2012; Prasad *et al*., 2012). Titanium nanodioxide (40 µg ml^{-1}) improved seed germination, dry and fresh weight of onion seedling; however, the higher concentrations (50 µg ml^{-1} and more) had a reverse result (Raskar and Laware, 2013). Conversely, this nanoparticle did not affect the length and quantity of the onion root (Klancnik *et al*., 2011). The symptoms have depended on the form and coating type of nanoparticles (Barrena *et al*., 2009).

Due to the growing rate of production and release of nanoparticles in nature (as Nano-fertilizers, Nano-toxins, and Nano-carriers), there are increasing concerns about the possibility of toxicity and oxidative damage (whether chemical or green synthesis) of these particles on the ecosystem. The WHO (World Health Organization) and FAO (The Food and Agriculture Organization) at the meeting on the application of nanotechnologies in the food and agriculture sectors, in Rome in 2010, recognized the potential concern of nanotechnology in agriculture and food sectors. The main concern was about the exponential developing global knowledge on nanotechnology and its applications, which may affect the balance of advantage to risk (FAO/WHO expert meeting report, 2010).

The Ag NPs is known to have antioxidant, antibacterial and antifungal properties and may be used in food, medicine and cosmetics products (El-Nour *et al*., 2010; Abdel-Aziz *et al*., 2014). So, investigating the toxic effects of these particles on model plants, such as onion, can reveal the probability of damage. Thus, the present study was conducted to compare the germination indices and biochemical parameters of edible onion seeds who treated with different concentrations of two types of silver nanoparticles (green synthesized and chemical synthesized) to examine the oxidative stress.

2. Materials and Methods

Research site and plant material collection

The current study was conducted at the Horticultural Laboratory of Agriculture and Natural Resources Faculty, Chemical Laboratory of Science

Faculty, Central Laboratory (University of Hormozgan, Bandar Abbas, Iran) and Molecular Research Center (Hormozgan University of Medical Sciences, Iran), during 2018.

Preparation of Cordia myxa leaf extract

Leaves of *Cordia myxa* L. (a member of Boraginaceae family), were collected from Rooydar city, Hormozgan Province, Iran (57° 6′ E, 60° 3′ N, Elevation: 50 m, RH: 70%, Mean temperature: 28±1°C) during winter, 2018. The healthy leaves were choosing, then washing in tap water followed by rinsing in distilled water and air drying (6 days under the shade conditions at 24±1°C). The dried leaves were powdered using an electric grinder. The leaf extract was prepared by mixing 10 g of dried powder along with 150 mL of deionized water and then heating the mixture at 80°C for 30 min. The solution was pre-filtered through Whatman No. 42 filter paper and re-filtered through Whatman No. 1 filter paper. The obtained extract was collected and stored in the refrigerator (Samari *et al*., 2018).

Synthesis and purification of silver nanoparticles (AgNPs) using C. myxa leaf extract

For AgNPs synthesis, 2.0 ml of *C. mixa* leaf extract was added to 25 ml of 7.0 mM aqueous solution of $AgNO_3$ (Merck, Germany) and the reaction pH was adjusted to 11.0 using NaOH. The mixture was continuously stirred at room temperature (24±1°C) for 3 h. The yellow-mixture turned to dark-brown (Samari *et al.*, 2018). The green synthesized AgNPs were centrifuged at 10000 rpm for 30 min and rinsed with deionized water. Then, the obtained sediments were re-dispersed in deionized water to get rid of any free phyto-molecules. This process (centrifugation and re-dispersion) was repeated three times to ensure the purification of nanoparticles and separation of unbonded compounds.

Characterization of silver nanoparticles

Preliminary characterization of the green synthesized AgNPs was carried out using a S-3100 UV-Vis spectrophotometer (Scinco, Korea, which is operated at a resolution of 2 nm and is equipped with a 10 mm quartz cuvette).

An aliquot of the dried purified pellets, obtained from centrifugation, was used for X-ray diffraction (XRD). The XRD pattern was obtained using a powder X-ray diffractometer (Bruker D8 Advance powder diffractometer) with Cu-Kα radiations (λ=1.5406 nm, in a 2θ range from 20° to 80°). Fine configuration of the green synthesized AgNPs was measured using a transmission electron microscopic examination (TEM). For this, the colloidal AgNPs were allowed for sonication and then a thin film was prepared on the carbon coated grid (Cu Mesh 300). TEM observations were made with (Zeiss-EM10C) operated at an accelerating voltage of 80 kV.

Preparation of colloidal solution of AgNPs (green and chemical synthesized)

The chemical AgNPs wasprovided from Iranian Nano-biotechnology company. Chemical and green nanoparticles were dispersed separately in distilled water and placed in an ultrasonic apparatus (SONICA® Ultrasonic Cleaners) for 30 min for homogenization. Then, colloidal solutions containing each nanoparticle were prepared at the concentrations of 0, 0.03, 0.06, 0.012, 0.025 and 0.05 g L^{-1}.

For germination assay, red onion seeds (*Allium cepa* L.), disinfected (0.04% of sodium hypochlorite solution, 3 min), rinsed in distilled water (3 times, each for 1 min), dipped in Nano-solutions for 30 min and finally rinsed in distilled water. Seeds were then cultured in sterilized glass Petri dishes (80 mm in diameter, covered with filter papers). Each Petri dish contained 100 seeds.

Germination indices

Germination percentage and germination value. Germination test was conducted under a laboratory condition (24±1°C). The seeds were placed on 80 mm petri dishes and were covered with filter papers. The seeds were irrigated daily with double distilled water. Counting was done by the emerge of the roots (the seed with root length equal or more than seed diameter were assumed as germinated). The counting continued until 3 days fixed germination. The germination indices including germination percentage and germination value were calculated using the equations (1) and (2) (Okoro, 1976).

$$\text{Germination (\%)} = \text{The total number of germinated seeds / total seed)} \times 100 \quad (1)$$

$$GV = MDG \times PV \quad (2)$$

where GV is germination value and MDG is mean daily germination and calculated by the dividing of germination percentage by the number of days to the end of the test. PV or peak value, derived from all the cumulative germination percentages on any day divided by the number of days to reach these percentages.

The length of radicle and plumule. Seedling root and stem length was measured using a ruler (express in mm).

Biochemical analysis

Preparation of enzyme and protein extract. About 0.5 g of the root was homogenized in liquid nitrogen. Then, 1.0 ml of extraction buffer (in 100 ml: containing of 50 mM potassium phosphate buffer with pH: 7.0, 0.0372 g EDTA and 1.0 g PVP) was added and centrifuged (15 min, 12000 rpm, 4°C). At last, the supernatant was used to assay protein content, and the activity of catalase, peroxidase, and protease (Dhindsa *et al*., 1981).

Protein content assay. The Bradford method (1976) was used to determine protein content. Briefly, 1.0 ml of Bradford reaction solution (containing 0.01 of Coomassie Brilliant Blue G250, 5.0 ml of 96% ethanol and 10.0 ml of 85% phosphoric acid) was added to 50 µl of protein extract. The optical absorption of the extracts was determined by a spectrophotometer (Cecil CE2501 model) at 595 nm with a plastic cuvette (Bradford, 1976).

Peroxidase activity assay. After adding 33 µl of the enzyme extract to 1.0 ml of the peroxidase reaction solution (containing 13 mM guaiacol, 5 mM hydrogen peroxide and 50 mM phosphate potassium buffer at pH=7.0), the absorption of the extracts was recorded at 470 nm (Chance and Maehly, 1995).

Catalase activity assay. For catalase assay 50 µl of the enzyme extract was mixed with 1.0 ml catalase reaction solution (containing 50 mM phosphate potassium buffer at pH=7.0 and 15 mM hydrogen peroxide). The absorption was recorded at 240 nm (Dhindsa *et al*., 1981).

Protease activity assay. After adding 350 µl of 50 mM sodium phosphate buffer (pH=7.5) and 800 µl of 1% casein (W/V) to 50 µl of the enzyme extract, the mixture was incubated under laboratory condition (24±1°C) for 10 min. Then, 400 µl of 10% trichloroacetic acid (w/v) was added and the mixture re-incubated for 20 more min. Finally, after centrifugation (10000 rpm, 5 min), the optical absorption of extracts was determined at 280 nm with a quartz cuvette (Kwmbhavi *et al*., 1993).

Malondialdehyde assay (MDA). About 0.1 g of the root was homogenized with 5.0 ml of 1% trichloroacetic acid. The extract was then centrifuged (10000 rpm, 5 min) and the supernatant (250 µl) was placed in a water bath (95°C for 30 min), after adding one ml of MDA (containing 20% trichloroacetic acid and 5% thiobarbituric acid). The samples were placed on ice and then re-centrifuged (1000 rpm, 5 min). Finally, the optical absorption of the extracts was recorded at 532 and 600 nm (Alexieva *et al*., 2001).

Total phenol assay. The total phenol was assayed using the Spanos and Wrolstad (1990) technique; about 0.1 g of root sample was homogenized with 15.0 ml of 80% methanol. The extract was then centrifuged (10000 rpm, 10 min). Then, 490 µl of distilled water and 500 µl of Folin reagent were added to 10 µl of the supernatant and were placed under dark condition (24±1°C, for 3 min). Subsequently, 500 µl of 1% sodium carbonate (1.0 g sodium carbonate - 100 ml distilled water) was added to each sample and re-placed under dark condition (24±1°C) for 30 more min. Finally, the absorbance of extracts was determined at 765 nm.

Antioxidant capacity (DPPH assay). The DPPH assay was followed by Brand-Williams *et al.* (1995) procedure. Briefly, about 0.1 g of root sample was homogenized with 15.0 ml of 80% methanol and incubated under dark laboratory condition (24±1°C, for 24 h). Then, the extracts were centrifuged (10000 rpm, 10 min). After adding 40 µl of methanol, 350 µl of DPPH and 1550 µl of 80% methanol to 600 µl of the supernatant, the samples were kept under the dark condition at 4°C, for 20 min. Finally, the optical absorption was recorded at 517 nm and the antioxidant capacity was calculated using the equation (3):

$$\text{Antioxidant capacity\%} = [(A_{cont} - A_{samp})/A_{cont})] \times 100 \quad (3)$$

where A_{cont} and A_{samp} are the standard and sample optical absorption.

Data analysis

The research was conducted as a factorial experiment based on complete random design, with six replications (each replicon contains 100 seed). The statistical analysis was done using SAS Version 9.1.3 (SAS Institute Inc. Cary, NC, USA, 1990). The factors were AgNPs type (green AgNPs and chemical AgNPs) and AgNPs levels (0, 0.03, 0.06, 0.012, 0.025 and 0.05 g L^{-1}). The Shapiro-Wilks test confirmed the data normality (procedure: Proc Univariate, SAS). The Multivariate Analysis of variance, considering the AgNPs type and AgNPs levels as independent variables were done (procedure: Proc Glm, SAS). Pillai's trace test confirmed the variance homogeneity (procedure: Proc Glm, SAS). Tukey's test was performed for mean comparison (procedure: Files, Sedit, Factor, Range, P<0.01, MSTATC). Excel 2013 was used to draw the figures.

3. Results

Synthesis and characterization of C. myxa synthesized AgNPs

The interaction between leaf extract and silver salt resulted in a color change from pale yellow to dark brown, the first sign of the AgNPs formation. This process occurred under room temperature (24±1°C) and lasted 3 hours (Fig. 1).

UV-Vis Spectroscopy is one of the most extensive techniques to confirm the production of nanoparticles. The UV-Vis spectrum of synthesized AgNPs using *C. myxa* leaf extract (Fig. 2A) showed a significant peak with λ_{max}around 410 nm due to SPR (Surface Plasmon Resonance) of AgNPs.

TEM images were used to detect the surface morphology and size distribution of synthesized AgNPs by *C. myxa* leaf extract. TEM micrographs of the AgNPs confirmed the spherical shape of particles, 3-10 nmsize, well-distributed and little aggregation in solution (Fig. 2B); however, the average size of particles was found to be 5.8 nm.

The XRD patterns of synthesized AgNPs showed prominent Bragg reflections at 2θ values of 38.1, 44.25, 64.55 and 77.2 (Fig. 3A), which is related to the (111), (200), (220) and (311) Bragg reflections of face-centered cubic (FCC) AgNPs. SEM analysis confirmed the nano dimension of chemical AgNPs (Fig. 3B).

Germination indices

Germination percentage and germination value. The germination percentage was influenced by the type and concentration of nanoparticles (Table 1).

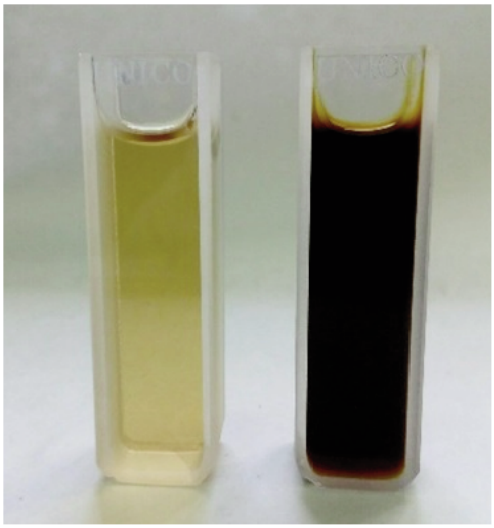

Fig. 1 - The mixture of *C. myx*a leaf extract and $AgNO_3$ (Left), green-synthetized AgNPs colloidal solution (Right).

The green AgNPs treatment improved the onion germination. Most germination percentage belonged to green AgNPs (100% in 0.05 g L^{-1}), and the least value

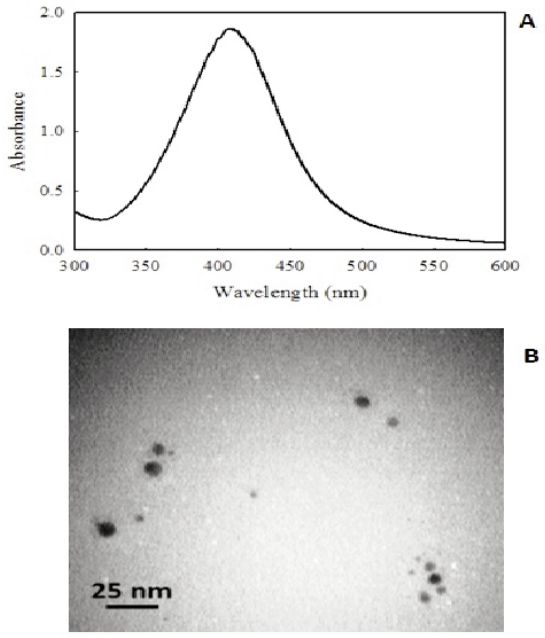

Fig. 2 - The SPR spectrum of *C. myxa* synthesized with the AgNPs (A), and TEM image of AgNPs synthetized by *C. myxa* leaf extract (B).

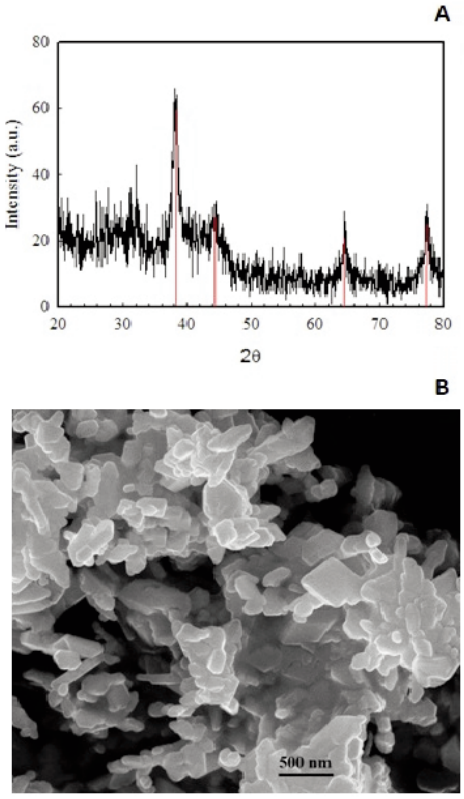

Fig. 3 - The XRD patterns of the *C. myxa* synthesized AgNPs (A) and electron microscopic scanning image of chemical AgNPs (B).

(70%) belonged to chemical AgNPs treatment at concentrations more than 0.06 g L^{-1} (Fig. 4A).

The germination value also influenced by the interaction of treatments (Table 1). Green AgNPs treatment did not differ with control, while treatment with the chemical AgNPs caused a significant reduction in the germination value (from 1.88 in controlreached to 0.12 in the 0.05 g L^{-1}). Most and least germination values (1.90 and 0.12) were observed in onion seeds treated with the high concentration of green AgNPs and chemical AgNPs, respectively (Fig. 4B).

The length of radicle and plumule. The effect of type and concentration of AgNPs was significant on the length of plumule (Fig. 5). There was no significant difference between the green AgNPs and the control. Even, the chemical AgNPs was not different from the control up to the concentration of 0.06 g L^{-1}. The higher amount of chemical AgNPs, reduced the

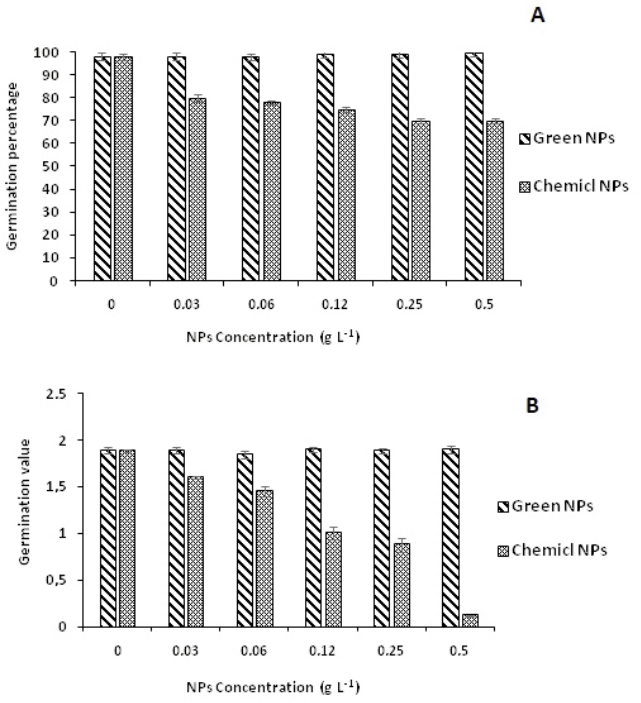

Fig. 4 - The influence of AgNPs type and concentration on germination percentage (A) and germination value (B) in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

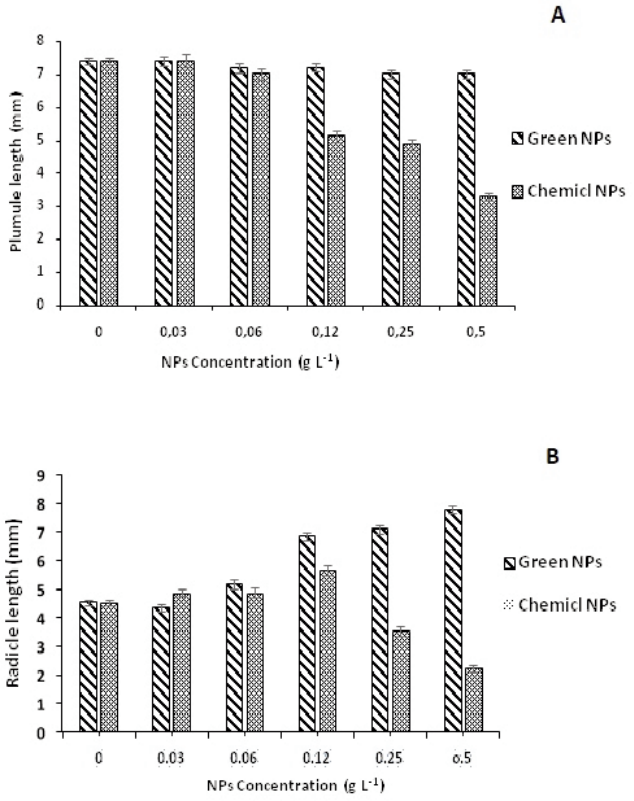

Fig. 5 - The influence of AgNPs type and concentration on plumule length (A) and radicle length (B) in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

Table 1 - The interaction of AgNPs type and concentration on *Allium cepa* biochemical parameters

SOV	Germination percentage	Germination value	Radicle length	Plumule length	Protein	Peroxidase	Catalase	Protease	MDA	Phenol	IC_{50}
NPs type	4408.33 ** (z)	89.89 **	284.21 **	926.64 **	0.11	540853.35 **	3600.65 **	9151.33 **	0.112 *	886.31 **	14.57
NPs concentration	3.80	0.07	9.98 **	5.11 **	0.27 *	40965.25 **	13476.80 **	1.40 *	0.030 **	17.84	34.52 *
NPs type × NPs concentration	15.53*	0.31 **	4.90 **	1.70	0.15*	38719.77 **	7566.10 **	1.87 *	0.020 **	17.89 **	21.90 **
Error	13.10	0.26	1.17	1.92	0.16	5852.71	683.03	1.14	0.020	55.16	24.61

(z) The mean square values are given.
* and **: state significant at 5 and 1% respectively
AgNPs type ×AgNPs concentration: states the interaction of AgNPs type and AgNPs concentration

plumule length (from 7.40 mm in the control treatment reached to 3.28 mm in the 0.05 g L^{-1} of chemical AgNPs) (P≤0.01) (Fig. 5A). The radicle length had a different pattern in response to the type and concentration of the nanoparticles. Green AgNPs improved radicle length (from 4.53 mm in control treatment reached to 7.76 mm in high concentration) (P<0.01). The chemical AgNPs made a decreasing trend in this parameter and reached it to 2.22 mm in the concentration of 0.05 g L^{-1} (Fig. 5B).

Biochemical indices

Protein content and protease activity. Despite the significant effect of two AgNPs types on protein content and protease activity (Table 1, P≤0.01), the interaction between AgNPs type and concentration had a diverse pattern (Fig. 6A and B). The green AgNPs-exposed seeds displayed a no-significant reduction in protein content (from 1.1 to 1 mg g^{-1}). The reduction of protein content in chemical AgNPs-treated seeds was dose-dependent (Fig. 6A). Green AgNPs-treated seeds had the same protease activity as the control treatment. Chemical AgNPs displayed a significant rise in concentration more than 0.06 gL^{-1} (from 1.77 µmol min^{-1} g^{-1} FW in control treatment to 6.21 µmol min^{-1} g^{-1} FW at the high concentration) (Fig. 6B).

Antioxidant enzymes (peroxidase and catalase activities). By the results, the activity of both antioxidant enzymes was influenced by the type and concentration of the AgNPs (Table 1). Green AgNPs-treated seeds indicated no significant difference with control. In chemical AgNPs-treated seeds both enzymes displayed the linear trend, so most peroxidase and catalase activities belonged to chemical AgNPs at 0.05 g L^{-1} (315.62 and 51.45 µmol $min^{-1}g^{-1}$ FW, respectively) (Fig. 7A and B).

Malondialdehyde (MDA), phenol and DPPH (antioxidant capacity). The MDA influenced by AgNPs treatment (Table 1) and the less value was observed in control treatment (0.03 mg g^{-1} Fw). Both nanoparticle types made an increase in MDA, but green AgNPs did not significantly differ with control treatment. Also, the content of MDA had a linear relation

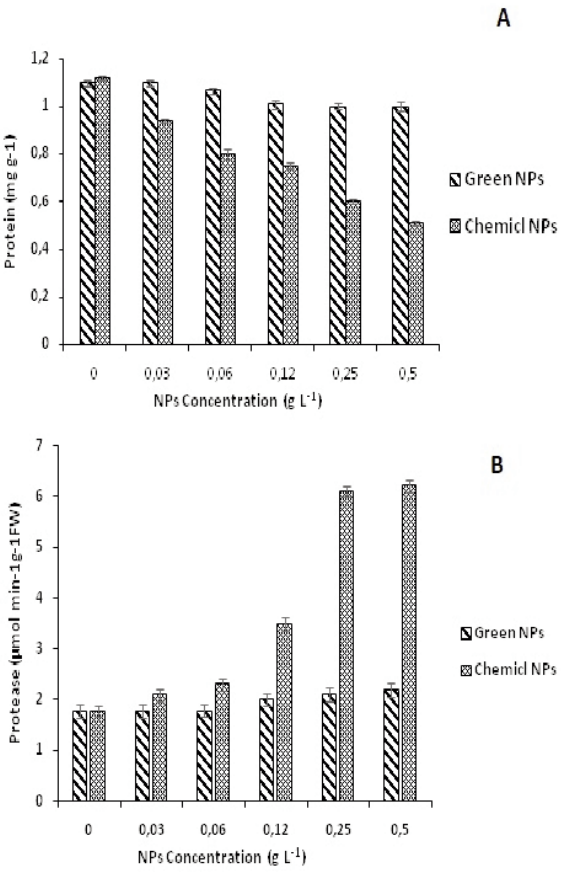

Fig. 6 - The influence of AgNPs type and concentration on protein content (A) and activity of protease (B) in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

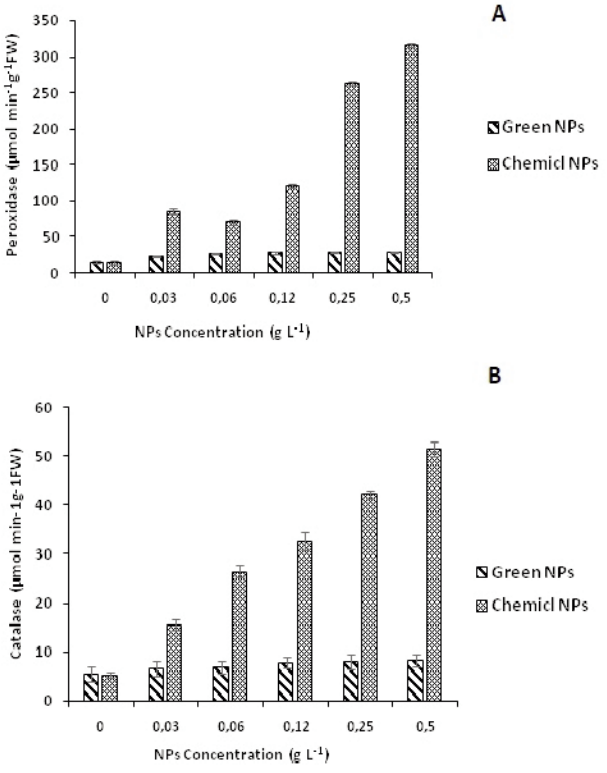

Fig. 7 - The influence of AgNPs type and concentration on activities of peroxidase (A) and catalase (B) in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

with chemical AgNPs concentration (from 0.03 mg g^{-1} Fw in the control reached to 0.25 mg g^{-1} FW at high concentration of chemical AgNPs) (Fig. 8A).

The type and concentration of AgNPs influenced phenol content. The most value was assigned to chemical AgNPs (15.16 mg Gallic g^{-1} FW at 0.05 g L^{-1}), and the least value to control treatment (4.10 mg Gallic g^{-1} FW) (Fig. 8B).

The amount of antioxidant capacity was also affected by NPs treatments (Table 1). This parameter was expressed in mg g^{-1} fresh weight based on the-half-maximal inhibitory concentration (IC_{50}). The control value (10.67 mg g^{-1} FW) followed a descending trend in both AgNPs types. It reached to 8.97 mg g^{-1} FW in green AgNPs while to 1.01 in chemical AgNPs (Fig. 9).

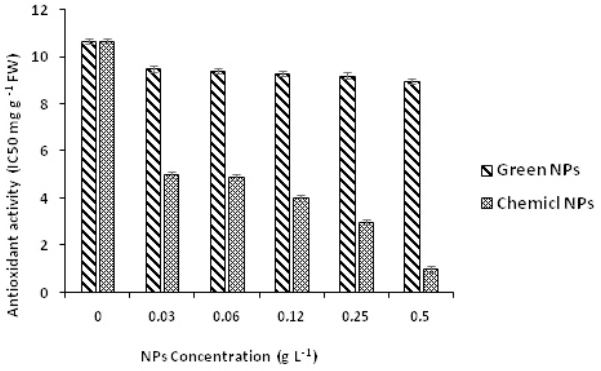

Fig. 9 - The influence of AgNPs type and concentration on antioxidant capacity in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

4. Discussion and Conclusions

Plants contain flavonoids, phenols, aroma, latex and alcohols, and some of these compounds are responsible for the reduction of metal ions and production of metal NPs from the metal salts. It has been stated that the leaf extract of *Cordia* species possesses phenolic and flavonoids derived compounds such as robinin, rutin, datiscoside, hesperidin, dihydrorobinetin, chlorogenic and caffeic acid (Al-Ati, 2011). The hydroxyl and ketonic groups of such compounds, construct chelate structures by binding to metal ions (Issaabadi *et al*., 2016). In present work, the leaf extract of *C. myxa* was used as a reducing and stabilizing agent for phyto-synthesis of AgNPs. This eco-friendly procedure was free-hazard and non-toxic. The successful synthesis of green AgNPs from plant extracts has already reported in *Chenopodium album*, *Camellia sinensis* and *Rhus coriaria* (Song and Kim, 2009; Dwivedi and Gopol, 2010).

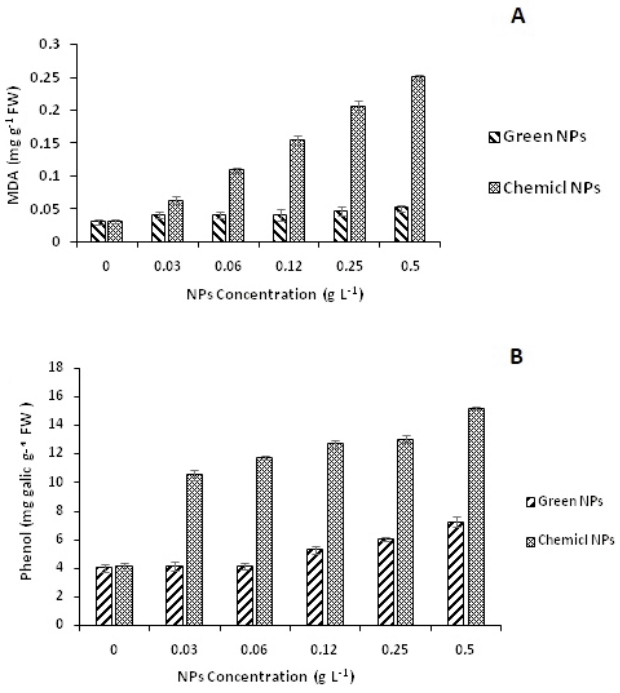

Fig. 8 - The influence of AgNPs type and concentration on MDA (A) and total phenol (B) in *Allium cepa*. Means ± SD of six replicates. The same letter denotes lack of a statistically significant difference (Tukey, p<0.01).

The NPs synthesis basis is oxidation of hydroxyl, carbonyl, and aldehyde and subsequently, reduction of metal ions during the neutralization of the electric charge (Sivaraman *et al*., 2009). In present research the *C. myxa* extract turned to dark brown, due to the surface plasmon resonance of AgNPs and confirmed the successful synthesis of the AgNPs (Kasthuri *et al*., 2009; Roopan *et al*., 2013). In this study, the synthesis process lasted 3 hours at room temperature condition. Other reports have also mentioned that this rapid NPs synthesis dose not require high temperatures (Sivaraman *et al*., 2009). The FCC crystal structure of green synthesized AgNPs was found by comparing the obtained XRD data with JCPDS File No. 04-0783 (Sutradhar and Saha, 2016), clearly indicate the highly crystalline structure of green AgNPs.

Nanoparticles play an important role in many areas of biology, chemistry and agriculture (Navarro *et al*., 2008), but their adverse potential on the environment is being subjected to extreme discussions. NPs penetrate to various parts of the plants, some stored within the cell and some in extracellular space (Lee *et al*., 2008). High AgNPs concentrations pass through the cell by diffusion, cause mitochondrial

mal-function, generation of ROS (He *et al*., 2012; Roh *et al*., 2012). On the other hand, easy penetration of nanoparticles into the seed shell develops water absorption and increases the activity of rubisco enzyme, which finally simplifies germination (Gao *et al*., 2006). In the present study, *Cordia myxa* AgNPs increased onion germination percentage and value. The improvement of seed germination has reported in nano-metal treated spinach (Gao *et al*., 2006), maize (Lin and Xing, 2007) and peanut (Prasad *et al*., 2012).

The AgNPs have to penetrate plant cell walls, the natural sieves, and roots plasma membranes to enter the xylem tissues and then dislocated to stems and finally leaves (Dietz and Herth, 2011). In fact, the roots are the first target for lethal materials (Sresty and Rao, 1999). There are disagreeing reports regarding the effects of nanoparticles on the plant root and system. The NPs treatment made a decrease in radish root length (Wang *et al*., 2015), but an increase in the shoot and root length of rice (Hao *et al*., 2016). Also, ZnO NPs caused root elongation in radish, lettuce, corn, and cucumber (Lin and Xing, 2007). ZnO NPs (500 mg L^{-1}) increased soybean's root length, while higher concentrations resulted in a significant reduction (Lopez-Moreno *et al*., 2010). The root cap cells of AgNPs-exposed *Lolium multiflorum* were damaged and malformed, which lastly reduced root growth and dry matter (Yin *et al*., 2011).

Plant root inter-connect with physical and chemical factors of the root zone. The elongation zone of onion root may act as a sensitive receiver for external signals. The length and morphology of onion roots is an important parameter that reflects the toxicity of the chemical compounds (Odeigah *et al*., 1997). It has reported that AgNPs lessened root length of onion whichrelatedto a reduction in water absorption and cell division (Kumari *et al*., 2009). In this study, an inhibition in radicle and plumule development in chemical AgNPs-treated seeds indicate its toxicity potential. Moreover, the root length reduced more than the shoot length in AgNPs-treated samples.

The toxicity of AgNPs in biological systems is closely related to its surface oxidation, releasing the Ag ions, and interaction with macromolecules (Reidy *et al*., 2013), especially with sulfur-containing molecules e.g. proteins, due to silver- sulfur strong tendency (Liu *et al*., 2011). The nano-dimension plant pores, the surface charge of NPs (more negative AgNPs limit the cell-particle interactions and made lower toxicity) and coating type (chemical or biological) influence the intensity of AgNPs' toxicity (Choi and Hu, 2008; El Badawy *et al*., 2011).

In the present study, protein content and protease activity of green AgNPs treated seeds did not significantly differ from the control. However, the high concentration of chemical AgNPs obviously decreased protein content and boosted protease activity. This alignment related to the role of protease in protein catabolism. High concentrations of AgNPs denature the membrane and release LPS (lipopolysaccharide) and purines. Then limit the proton mobility, react with thiol groups of some enzymes, deactivate enzymes, bind to protein groups, denature proteins, produce hydrogen peroxide, which finally causes oxidative stress (Hwang *et al*., 2008; Zhu *et al*., 2008).

The regulated production of free radicals in organisms maintains the oxidation and reduction homeostasis cycle; however, there is a group of antioxidant enzymes who prevent and deactivate ROS. Also, extracellular antioxidant molecules, such as ascorbate, scavenge free radical molecules (Shams *et al*., 2011). Increasing the activity of antioxidant enzymes, catalase and peroxidase, inplant's exposed to the chemical metal NPs treated plants (Krishnaraj *et al*., 2012; Singh *et al*., 2013; Wang *et al*., 2015; Cvjetko *et al*., 2017) confirms our results.

Nanoparticles interact with the cell membrane (Khan *et al*., 2011), then depending on their nature and concentration, reduce the destructive effects of oxidative stress, cause programmed cell death (Lei *et al*., 2008). Silver nanoparticles could damage cell division, cause chromatin bridge, disturbed metaphase, make multiple chromosomal breaks and final cell disintegration (Kumari *et al*., 2009). According to Burman *et al.* (2013), zinc oxide NPs linearly increased ROS and MDA content. The MDA content of tomato and tobacco plants who exposed to AgNPs was higher than control plants (Cvjetko *et al*., 2017). Also, an increase in phenol content has already been reported in metal NPs treated plants (Singh *et al*., 2013). IC_{50} is a measure of the potency of a substance in inhibiting the biological function and indicates how much of this substance is required to inhibit the biological process. In our research, the chemical AgNPshad IC_{50}around 8 times more than green AgNPs, which confirms the more toxicity risk of chemical AgNPs. The same trend was observed in green AgNPs synthesized from Aegle marmelos extract (Patil *et al*., 2015). The results of the present work displayed that green AgNPs significantly enhanced antioxidant abilities by stimulating polyphenols and ascorbic acid in onion.

Globally, incredible changes in agricultural production patterns have taken place, through the application of modern labor-saving technologies, mechanization, and improved crop varieties. In sustainable agriculture, the application of nano-fertilizers is a talented option to provide the food needed for the growing population worldwide. Green synthesis of nanoparticle as an environmental-friendly technique, by minimizing and reducing hazardous material, gradually has introduced itself in the commercial production of nanoparticles. The present research used an eco-friendly and low-cost methodology, without the use of any danger or lethal chemicals for AgNPs synthesis from leaf extract of *C. myxa* under room temperature conditions. The phenolic and flavonoid contents of leaf extract acted as reducing and stabilizing agent in nanoparticles synthesis and AgNPs with a good quantity and stability were synthesized.

Various bioassays are available to assess the relative toxicity of chemicals in different organisms. However, there is no single test that can detect the damage of classes of chemical compounds. A germination test is a sensitive tool used in physiological and cytogenetic studies. According to our findings, the green nanoparticle synthesis, not only had no oxidative effect on onion germination parameters, but also some stimulant effect was observed. The chemical nanoparticle motivated the plant's defense responses, by inducing oxidative stress and limited germination in a dose-dependent manner. As a final conclusion, it can be noted that the low-cost and easy synthesis of nanoparticles from plant sources, is a safe and suitable alternative for chemical-synthesized metal nanoparticles.

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References

ABDEL-AZIZ M.S., SHAHEEN M.S., EL-NEKEETY A.A., ABDEL-WAHHAB M.A., 2014 - *Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using* Chenopodium murale *leaf extract.* - J. Saudi Chem. Soc., 18: 356-363.

AHMAD A., SENAPATI S., KHAN M.I., KUMAR R., SASTRY M., 2005 - *Extra-intracellular, biosynthesis of gold nanoparticles by an alkalo tolerant fungus,* Trichothecium. - J. Biom. Nanotechnology, 1: 47-53.

AL-ATI T., 2011 - *Assyrian plum (Cordia myxa L.).* - Postharvest Biol. Technol. Trop. Subtrop. Fruits, 116-126e.

ALEXIEVA V., SERGIEV I., MAPELLI S., KARANOV E., 2001 - *The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat*. - Plant, Cell & Environ., 24: 1337-1344.

BARRENA R., CASALS E., COLÓN J., FONT X., SÁNCHEZ A., PUNTES, V., 2009 - *Evaluation of the ecotoxicity of model nanoparticles.* - Chemosphere, 75: 850-857.

BRADFORD M.M., 1976 - *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.* - Analytical Biochemistry, 72: 248-254.

BRAND-WILLIAMS W., CUVELIER M.E., BERSET C., 1995 - *Use of free radical method to evaluate antioxidant activity.* - Lebensmittel Wissenschaft und Technologie, 28: 25-30.

BURMAN U., SAINI M., KUMAR P., 2013 - *Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings*. - Toxicological and Environmental Chemistry, 95: 605-616.

CHANCE B., MAEHLY A.C., 1995 - *Assay of proxidases.* - Methods Enzymol., 11: 755-764.

CHOI O., HU Z.Q., 2008 - *Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria.* - Environ. Sci. & Techn., 42: 4583-4588.

CVJETKO P., ZOVKO M., ŠTEFANIĆ P.P., BIBA R., TKALEC M., DOMIJAN A.M., VRČEK I.V., LETOFSKY-PAPST I., ŠIKIĆ S., BALEN B., 2017 - *Phytotoxic effects of silver nanoparticles in tobacco plants*. - Environ. Sci. and Pollut. Res., 25: 5590-5602.

DHINDSA R.S., PLUMB-DHINDSA P., THORPE T., 1981 - *Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase.* - J. Exp. Bot., 32: 93-101.

DIETZ K.J., HERTH S., 2011 - *Plant nanotoxicology*. - Trends in Plant Sci., 16: 582-589.

DUBEY S., LAHTINEN M., SILLANPAA M., 2010 - *Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of* Rosa rugose. - Colloids and Surfaces A: Physicochemical and Engineering Aspects, 364: 34-41.

DWIVEDI A.G., GOPOL K., 2010 - *Biosynthesis of silver and gold nanoparticles using* Chenopodium album *leaf extracts*. - Coll. Suf. Phys. Eng. Asp., 360: 27-33.

EL BADAWY A.M., SILVA R.G., MORRIS B., SCHECKEL K.G., SUIDAN M.T., TOLAYMAT T.M., 2011 - *Surface charge-dependent toxicity of silver nanoparticles.* - Environ. Sci. Techn., 45: 283-287.

EL-NOUR K.M.A., EFTAIHA A.A., AL-WARTHAN A., AMMAR R.A., 2010 - *Synthesis and applications of silver*

nanoparticles. - Arab J. Chem., 3: 135-140.

FAO/WHO, 2010 - *Expert meeting on the application of nanotechnologies in the food and agriculture sectors: potential food safety implications*. - FAO/WHO Meeting report, Rome, Italy.

GAO F., HONG F., LIU C., ZHENG L., SU M., WU X., YANG F., WU C., YANG P., 2006 - *Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach: inducing complex of rubisco-rubisco activase.* - Biol. Trace Elem. Res., 111: 239-253.

HAO Y., ZHANG Z., RUI Y., REN J., HOU T., WU S., RUI M., JIANG F., LIU L., 2016 - *Effect of different nanoparticles on seed germination and seedling growth in rice*. - 2nd Annual International Conference on Advanced Material Engineering, pp. 166-173.

HE D., DORANTES-ARANDA J.J., WAITE T.D., 2012 - *Silver nanoparticle-algae interactions: Oxidative dissolution, reactive oxygen species generation and synergistic toxic effects.* - Environ Sci. Technol., 46: 8731-8738.

HWANG E.T., LEE J.H., CHAE Y.J., KIM Y.S., KIM B.C., SANG B., GU M.B., 2008 - *Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria*. - Small, 4: 746-750.

INAS Z.A., HALA A.K., GEHAN H.H., 2011 - *Gastroprotective effect of* Cordia myxa *L. fruit extract against indomethacin-induced gastric ulceration in rats*. - Life Sci. J., 8: 433-445.

ISSAABADI Z., NASROLLAHZADEH M., SAJADI S.M., 2016 - *Green synthesis of the copper nanoparticles supported on bentonite and investigation of its catalytic activity*. - J. Clean. Prod., 142: 3584-3591.

KASTHURI J., KATHIRAVAN K., RAJENDIRAN N., 2009 - *Phyllanthin-assistedsynthesis of silver and gold nanoparticles: a novel biological approach.* - J. Nanopart Res., 11: 1075-1085.

KHAN S.S., MUKHERJEE A., CHANDRASEKARAN N., 2011 - *Studies on interaction of colloidal silver nanoparticles (SNPs) with five different bacterial species*. - Coll. Surf B Biointerfaces, 87: 129-138.

KLANCNIK K., DROBNE D., VALANT J., DOLENC KOCE J., 2011 - *Use of a modified Allium test with nano Tio_2.* - Ecotoxicology Environ. Safety, 74: 85-92.

KOWSHIK M., ASHTAPUTRE S., KHARRAZI S., VOGEL W., URBAN J., KULKARNI S.K., PAKNIKAR K.M., 2003 - *Extracellular synthesis of silver nanoparticles by a silver- tolerant yeast strain MKY3.* - Nanotechnology, 14: 95-100.

KRISHNARAJ C., JAGAN E., RAMACHANDRAN R., ABIRAMI S., MOHAN N., KALAICHELVAN P., 2012 - *Effect of biologically synthesized silver nanoparticles on* Bacopa monnieri *(Linn.) Wettst. plant growth metabolism*. - Process Biochemistry, 47: 651-658.

KUMARI M., MUKHERJEE A., CHANDRASEKARAN N., 2009 - *Genotoxicity of silver nanoparticles in* Allium cepa. - Sci. Total Environ., 407: 5243-5246.

KWMBHAVI A.A., KULKARNI A., PANT A., 1993 - *Salt-tolerant and thermostable alkaline protease from bacillus subtilis NCIM no. 6.4.* - Appl. Biochem. Biotecnol., 38: 83-92.

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 aestrivum*): plant agar test for water-insoluble nanoparticles.* - Environ. Toxic. Chem., 27: 1915-1921.

LEI Z., SU M.Y., WU X.C., QU C.X., CHEN L., HUANG H., LIU X.Q., HONG F.S., 2008 - *Antioxidant stress is promoted by Nano-anatase in spinach chloroplasts under UV-Beta radiation*. - Biol. Trace Element Res., 121: 69-79.

LIN D., XING B., 2007 - *Phytotoxicity of nanoparticles: inhibition of seed germination and root growth*. - Environ. Pollution, 150: 243-250

LIU J., PENNEL K.G., HUR R.H., 2011 - *Kinetics and mechanisms of nanosilver oxysulfidation.* - Environ. Sci. Technol., 45: 7345-7353.

LOPEZ-MORENO M.L., DE LA ROSA G., HERNANDEZ-VIEZCAS J.A., CASTILLO-MICHEL H., TORRESDEY J.B.C.P.V.J.G., 2010 - *Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants*. - Envir. Sci. Techn., 44: 7315-7320.

MAYNARD A.D., AITKEN R.J., BUTZ T., COLVINV., DONALDSON K., OBERDÖRSTER G., PHILBERT M.A., RYAN J., SEATON A., STONE V., TINKLE S.S., TRAN L., WALKER N.J., WARHEIT D.B., 2006 - *Safe handling of nanotechnology*. - Nature, 444: 267-269.

MONICA R.C., CREMONINI R., 2009 - *Nanoparticles and higher plants*. - Caryologia, 62: 161-165.

MUSANTE C., WHITE J.C., 2012 - *Toxicity of silver and copper to* Cucurbita pepo*: differential effects of nano and bulk-size particles*. - Environ. Toxicology, 27: 510-517.

NAVARRO E., PICCAPIETRA F., WAGNER B., MARCONI F., KAEGI R., ODZAK N., SIGG L., BEHRA R., 2008 - *Toxicity of silver nanoparticles to* Chlamydomonas reinhardtii. - Environ. Sci. Technol., 42: 8959-8964.

NIEMEYER C.M., MIRKIN C.A., 2004 - *Nanobiotechnology: concepts, applications and perspectives.* - John Wiley & Sons, USA, pp. 491.

ODEIGAH P.G.C., MAKINWA J., LAWAL B., OYENIYI R., 1997 - *Genotoxicity screening of leachates from solid industrial waste evaluated* Allium *test.* - ATLA, 25: 311-321.

OKORO O.O., 1976 - *Germination of* Terminalia ivqrensis *seeds source under various conditions of germination.* - In 'Seed problem' Proceedings Second Int. Symp. on Physiology of Seed Germination.

PATIL S., RAJIV P., SIVARAJ R., 2015 - *An investigation of antioxidant and cytotoxic properties of green synthesized silver nanoparticles.* - IAJPS, 2(10): 1453-1459.

PRASAD T.N.V.K.V., KAMBALA V.S.R., NAIDU R., 2012 - *Phyconanotechnology: synthesis of silver nanoparticles using brown marine algae* Cystophora moniliformis *and their characterization*. - J. Appl. Phycology, 25: 177-182.

RASHED K., LUO M.T., ZHANG L.T., ZHENG Y.T., 2014 -

Evaluation of anti-HIV-1 activity of Cordia myxa *L., and phytochemical profile*. - Banat's J. Biotechn., 5(10): 75-82.

RASKAR S.V., LAWARE S.L., 2013 - *Effect of titanium dioxide nano particles on seed germination and germination indic ws in onion.* - Plant Sciences Feed, 3(7): 103-107.

REIDY B., HAASE A., LUCH A., DAWSON K.A., LYNCH I., 2013 - *Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications.* - Materials, 6: 2295-2350.

REJESKI D., LEKAS D., 2008 - *Nanotechnology field observations: scouting the new industrial west.* - J. Cleaner Prod., 16: 1014-1017.

ROH J.Y., EOM H.J., CHOI J., 2012 - *Involvement of* Caenoihabditis elegans *MAPK signaling pathways in oxidative stress response induced by silver nanoparticles exposure.* - Toxicol Res., 28: 19-24.

ROOPAN S.M., MADHUMITHA G., RAHUMAN A., KAMARAJ C., BHARATHI A., SURENDRA T.V., 2013 - *Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using* Cocos nucifera *coir extract and its larvicidal activity.* - Ind. Crop Prod., 43: 631-635.

SAMARI F., SALEHIPOOR H., EFTEKHAR E., YOUSEFINEJAD S., 2018 - *Low-temperature biosynthesis of silver nanoparticles using mango leaf extract: catalytic effect, antioxidant properties, anticancer activity and application for colorimetric sensing.* - New J. Chem., 42: 15905-15916.

SENAPATI S., SYDE A., MOEEZ S., KUMAR A., AHMAH A., 2012 - *Intracellular synthesis of gold nanoparticles using alga* Tetraselmis kochinensis*.* - Materials Letters, 79: 116-118.

SHAMS T., SHAKIL S., UROOJ M., GHAZI A., DAMANHOURI A.M., AHMAD A., 2011 - *Genotoxicity testing and biomarker studies on surface waters: An overview of the techniques and their efficacies.* - J. Environ. Sci. Health, 29: 250-275.

SINGH N.B., AMIST N., YADAV K., SINGH D., PANDEY J.K., SINGH S.C., 2013 - *Zinc oxide nanoparticles as fertilizer for the germination, growth and metabolism of vegetable crops.* - J. Nanoengineering and Nanomanufacturing, 3: 353-364.

SIVARAMAN S.K., ELANGO S.K., SANTHANAM V., 2009 - *A green protocol for room temperature synthesis of silver nanoparticles in seconds.* - Curr Sci, 97: 1055-1059.

SONG J., KIM B.S., 2009 - *Rapid biological synthesis of silver nanoparticles using plant leaf extracts.* - Biopress Biosyst Eng., 39: 79-84.

SPANOS G.A., WROLSTAD R.E., 1990 - *The influence of processing and storage on the phenolic composition of Thompson seedless grape juice*. - J. Agric. Food Chem., 38: 1565-1571.

SRESTY T.V.S., RAO K.V.M., 1999 - *Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea*. - Environ. Exp. Bot., 41: 3-13.

SUTRADHAR P., SAHA M., 2016 - *Silver nanoparticles: Synthesis and its nanocomposites for heterojunction polymer solar cells.* - J. Phys. Chem. C, 120: 8941-8949.

THIRUVENGADAM M., RAJAKUMAR G., CHUNG I.M., 2018 - *Nanotechnology: current uses and future applications in the food industry*. - 3 Biotech, 8(1): 74.

VEERASAMY R., XIN T.Z., GUNASAGARAN S., XIANG T.F.W., YANG E.F.C., JEYAKUMAR N., DHANARAJ S.A., 2011 - *Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities.* - J. Saudi Chem. Soc., 15: 113-120.

WANG L., ZHU J., WU Q., HUANG Y., 2015 - *Effects of silver sanoparticles on seed germination and seedling growth of Radish (*Raphanus *s*ativus *L.).* - Int. Conf. on Civil, Materials and Environmental Sciences, pp. 604-608.

YANG F., LIU C., GAO F., SU M., WU X., ZHENG L., YANG P., 2007 - *The improvement of spinach growth by nanoanatase TiO_2 treatment is related to nitrogen photoreduction.* - Biol. Trace Element Res., 119: 77-88.

YIN L., CHENG Y., ESPINASSE B., COLMAN B.P., AUFFAN M., WIESNER M., ROSE J., LIU J., BERNHARDT E.S., 2011 - *More than the ions: the effects of silver nanoparticles on* Lolium multiflorum. - Environ. Sci. & Technol., 45: 2360-2367.

ZAMBRYSKI P., 2004 - *Cell-to-cell transport of proteins and fluorescent tracers via plasmodesmata during plant development.* - J. Cell Biol., 162: 165-168.

ZHU H., HAN J., XIAO J.Q., JIN Y., 2008 - *Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants*. - J. Environ. Monitoring, 10: 713-717.