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International Journal of Plant Biology & Research

Research Article

Interaction between Silver Nanoparticles and Environment

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Submitted: 27 April 2017

Accepted: 08 May 2017

Published: 10 May 2017

ISSN: 2333-6668

Keywords
- Silver nanoparticles
- Barley
- Germination
- Root elongation
- Nucleic acids
- Proteins

Abstract

Silver nanoparticles (AgNPs) are one of the most widely used nanoparticles involved in ecosystem. Silver nanoparticles were used in the preparation of new pesticide and insecticide formulations. However, the effect of AgNPs is on vascular plants is still unclear. In this paper the effect of Ag NPs (100nm) on germination, root elongation as well as nucleic acids and proteins contents of barley plant was evaluated. Our findings obviously indicate that germination percent was not affected after all treatments whereas seedling length, nucleic acids and proteins contents were highly affected after treatment with the higher concentration of nano particles (100 ppm) as compared to the control (untreated). Our investigations suggest that plant cells as an important marker of the ecosystems need to be included when evaluating the overall toxicological impact of the engineered nano-particles in the environment.

INTRODUCTION

Nanoparticles have a major effect on the physical and chemical properties of the compound, like adsorption on the surface of other solids (i.e. biomaterials), solubility and reactivity. Each of this property reflects on the interaction between different nanomaterial and biomolecule, resulting in toxicological effects [1,2]. However, with the accelerating production of AgNPs into commercial products, there is likelihood of release into the environment, which raises health and environmental concerns [3]. For all those reasons, studying the environmental effect of the nano-matters is a necessary in order to keep up with the increasing usage of Nano-matters in our daily consumed products. Nanoparticles can lead to a wide variety of toxicological effects on human [4], environment [5], bacteria [6] and aquatic organisms [7]. Only few studies on vascular plants showed that AgNPs have detrimental effects on plant growth [8]. Reported that 100nmAgNPs at 100 and 500mg/L resulted in 41% and 57% decreases in the biomass and respiration rates respectively of *Cucurbita pepo* as compared to control plants [9]. Showed that AgNPs could inhibit the growth of *Lemna minor Liyan.* [10] found that silver nanoparticles, in general, had no effect on germination rate whereas significantly reduced root growth at (6nm) than for (20nm). It has been found that nanoparticles have different levels of toxicities which may be size and shape dependent and have the ability of penetrate the cell walls [11,12]. Particles having less than (50 nm) diameter proved to be highly toxic [13,14]. Phytotoxicity studies reported both positive and negative effects of nanoparticles on higher plants as seed germination, root elongation, cell division, growth and metabolic processes [15,16]. Thus, this work aiming to understanding the different effects of silver nanoparticles on one of the most important economic plants e.g. barley plant.

MATERIALS AND METHODS

Nanoparticles

Silver nanoparticles powder was purchased from Nano-structured & Amorphous Materials, Inc. Houston, TX, USA. The physical characteristics of the Ag nanoparticles according to the manufacturer's data are: purity, (93%), size (100 nm) has been investigated under Transmission Electron Microscope (TEM) (Figure 1).

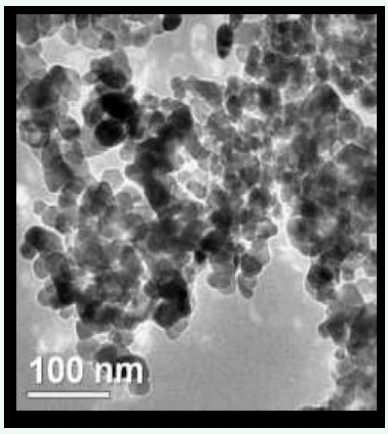

Figure 1 (TEM) of AgNPS.

Plant material

A defined strain of *Hordeum vulgare L* (var Giza-133) was purchased from The Agricultural Research Center, Giza, barley grains were washed and soaked in distilled water for 2 hrs. At room temperature range (23-25°C) before every carried out experiment.

Germination percent (%)

The tested barley grains were dipped in the different concentration nanoparticles (25,50 and 100 ppm) for 24 hrs. Three replicates of each treatment were grown in Petri dishes of equal sizes with one piece of filter paper and 5 ml of tested particles and allowed to germinate in room temperature for 72 hrs. Germination percent (%) of three replicates of each treatment was calculated as the proportion of the seeds that germinated to total number of seeds multiply by 100. Standard deviation (±) was calculated for each mean value for all treatments with respective controls (untreated).

Root growth (cm)

After germination, the lengths of the different roots for all treatments and related controls (untreated) were measured for three replicates. Standard deviation (±) was calculated for each mean value for treatments and related controls.

Nucleic acids determination

Extraction of nucleic acids (DNA&RNA) was carried out according to Schneider technique [17]. Estimation of total DNA was done using Diphenylamine reaction according to [18] and the optical density was measured at 595 nm, whereas total RNA was determined using Orcinol reaction according to method of [19] and the optical density at 660 nm. Three replicates were measured for each treatment and untreated. Also, standard deviation (±) was calculated for each treatment and respective control.

Protein determination

Total soluble proteins were determined according to [20]. Optical density was read at 595 nm.

RESULTS AND DISCUSSION

Germination percent and root growth

The effect of silver nanoparticles AgNPs (100 nm) on germination rate of barley at 25, 50 and 100 ppm was examined. The obtained data (Table 1) and (Figure 2) clearly revealed slightly effects on germination percent as compared to the control (untreated). These results indicating that silver nanoparticles have no any toxicological effect on seed germination. This is consistent with other studies that reported that AgNPs had less effect on germination process [21-23]. This may be explained by the protective effect of the grain coats which can have selective permeability [24]. AgNPs may aggregate or be complexed by ligands which can cause a decreased in toxicity and would lead to lower exposure to seeds and seedlings [25-27], reported that the seed coats of tobacco were most likely not permeable to the NPs of Al oxide ,therefore the germination process was not affected. Regarding root growth, Exposure to Ag NPs has a

Table 1: Germination percent (%) of barley plant after exposure to Ag NPs (100nm) at (25,50 and 100ppm).

Treatment	Germination (%)
Control	91.3 ± 0.21
25ppm	90.8 ± 0.73
50ppm	90.0 ± 0.73
100ppm	90.0 ± 0.63

Mean value (±) standard deviation

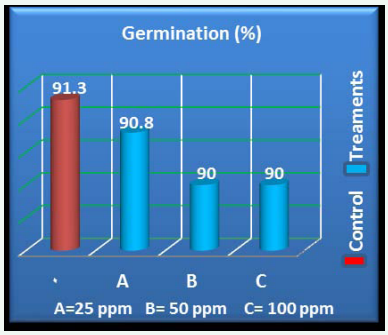

Figure 2 Germination percent after treatment with AgNPs.

marked reduction in root lengths where the average root growth was 0.93 cm after treatment with the higher concentration (100ppm) as compared to the control of the average root length 2.95 cm, (Table 2) and (Figure 3). Our findings were consistent with some studies which reported that exposure of some seeds to several nanomaterials significantly reduced root growth [10]. It has also been reported that NPs may have to penetrate cell wall and plasma membranes of epidermal layers in roots to enter vascular tissues explaining why the root response was strong [28]. The process of seed germination and root growth is a rapid and widely used acute phytotoxicity test owing to sensitivity, simplicity, low cost and suitability for unstable chemicals. Seed coats, which can have selective permeability, play a very important role in protecting the embryo from harmful external factors. Pollutants as nano-metals could penetrate root system causing obviously root growth inhibition, may not affect seed germination if they cannot pass through seed coats. This may explain that seed germination in our study was not affected by exposure to nanoparticles suspension.

Nucleic acids contents

The results of the present investigation clearly revealed that treatment of barley seedlings with the higher dose (100ppm) led to highly reductions in nucleic acids as well as protein contents as compared with their respective controls (Table 3). These reductions were concentration-dependent. Regarding DNA (Figure 4), the lowest value (0.87mg) was recorded after treatment with (100ppm) as compared to the control (2.03mg). The effects of the tested nanoparticles on RNA contents (Figure 5), were similar to that of DNA where the decreases, also, were concentration dependent. The minimum content (1.36mg) was recorded after treatment with (100ppm) comparing with the control (2.83mg). This work concluded that silver nanoparticles have a mechanism of action that dose-dependent. A possible

Table 2: Root length (cm) of barley plant after exposure to Ag NPs (100nm) at (25, 50 and100ppm).

Treatment	Root length(cm)
Control	2.95 ± 0.18
25ppm	2.05 ± 0.33
50ppm	1.87 ± 0.23
100ppm	0.93 ± 0.36

Mean value (±) standard deviation

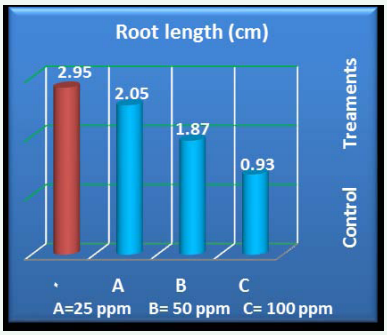

Figure 3 Root length of barley plant after treatment with AgNPs.

Table 3: DNA and RNA contents (mg/gm D.W) of barley plant after treatment with Ag NPs (100nm) at (25, 50 and 100ppm).

Treatment	DNA (mg/gmD.W.)	RNA (mg/gm D.W.)
Control	2.03 ± 0.20	2.83 ± 0.12
25ppm	1.66 ± 0.28	2.53 ± 0.12
50ppm	1.37 ± 0.17	1.93 ± 0.28
100ppm	0.87 ± 34	1.36 ± 0.18

Mean value (±) standard deviation

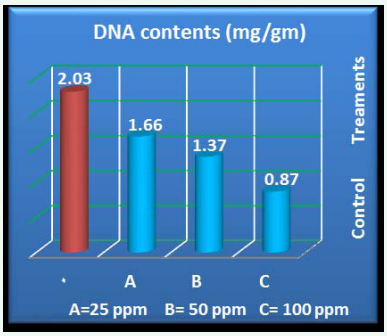

Figure 4 DNA contents after treatment with AgNPs.

mechanism of action of modified silver nanoparticles is their ability to cross the cellular and nuclear membranes, altering their structures and induced ROS which can interfere with the cellular metabolism resulting in DNA damage [29].

Proteins contents

As regard to the effect of silver nanoparticles on the contents of total soluble proteins, the obtained data (Table 4) and (Figure 6) clearly revealed marked depression in total soluble proteins (9.2mg) after treatment with the highest concentration (100ppm) as compared to the control (21.3mg). In this respect, it has been reported that Ag NPs have the ability to damage the genetic materials and able to penetrate cell membranes and reach the cellular nucleus causing DNA damage [29,4]. Showed that the exposure of human cell to Ag NPs caused damage to DNA which was in a dose-dependent manner. Silver nanoparticles are believed to alter the membrane structure by attaching to the sulfur-containing proteins of the cell membrane damaging the cell membrane as well as the DNA of the bacterial cell [30]. Some authors demonstrated that Ag NPs produce reactive oxygen species (ROS) which can alter the metabolism of the cell causing damage of proteins [31]. A number of researchers have shown that silver nanoparticles can destroy the ability of DNA to replicate or can damage DNA and death of the cells [32,33,14]. Recent study on Copper and Zink nanoparticles showed inhibition of some growth parameters in *Pistia stratiotes* after exposure to the tested nanoparticles [34].

Table 4: Proteins contents (mg/gm F.W.) of barley plant after treatment with Ag NPs (100 nm) at (25,50 and 100 ppm).

Treatment	Proteins (mg/ gm F.W.)
Control	21.3 ± 0.16
25ppm	18.3 ± 0.16
50ppm	13.12 ± 0.2
100ppm	9.2 ± 0.18

Mean value (±) standard deviation

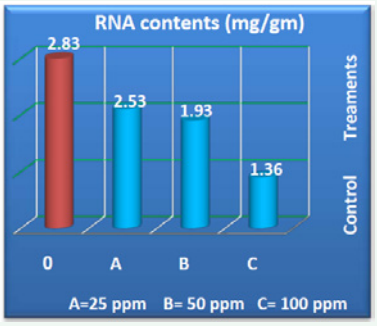

Figure 5 RNA contents after treatment with AgNPs.

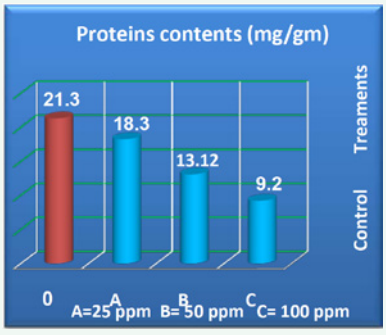

Figure 6 Proteins contents after treatment with Ag NPs.

ACKNOWLEDGMENT

I am grateful and thankful to Prof. Dr. Essam Taher, Biology Department, Faculty of Science and arts, Al- Baha University, for providing me the tested nanoparticles and plant material which have been investigated in this work.

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Cite this article

Alzandi ARA (2017) Interaction between Silver Nanoparticles and Environment. Int J Plant Biol Res 5(2): 1063.