International Research Journal of Biotechnology (ISSN: 2141-5153) Vol. 3(10) pp. 190-197, December, 2012
Available online http://www.interesjournals.org/IRJOB

Full Length Research Paper

Effects of silver nanoparticles in some crop plants, Common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.)

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Accepted December 13, 2012

Effects of silver nanoparticles on plant growth parameters such as shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of economic important pulses, common bean (*phaseolus vulgaris* L.) and corn (*Zea mays* L.) was probed in the present research. The study was carried out in a randomized block design with three replications. Five levels of silver nanoparticles (20, 40, 60, 80 and 100 ppm) were used. After germination, daily supply with 15 ml from each concentration was carried out for 12 days during plant growth. The results showed that small concentrations of silver nanoparticles had a stimulating effect on the growth of the plantlets, while the enhanced concentrations induced an inhibitory effect. However, increasing concentration of silver nanoparticles from 20 to 60 ppm has led to an increase in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of the two tested crop plants. Additionally, the lowest amount of these parameters was found with control plants, but the enhancing level of silver nanoparticles resulting in the reduction of these compounds.

Keywords: Ag nanoparticles, chlorophyll, carbohydrate, protein, plants.

INTRODUCTION

Nanoparticles (nano scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002; Roco, 2003), that can drastically modify their physico-chemical properties compared to the bulk material (Nel et al., 2006). It is worth noting that nanoparticles can be made from a fully variety of bulk materials and that they can explicate their actions depending on both the chemical composition and on the size and/or shape of the particles (Brunner et al., 2006).

Depending on the origin, a further distinction is made between three types of NSPs: natural, incidental and engineered. Natural nanoparticles have existed from the beginning of the earth history and still occur in the environment (volcanic dust, lunar dust, mineral compositions, etc.). Incidental nanoparticles, also defined as waste or anthropogenic particles, take place as the result of manmade industrial processes (diesel exhaust, coal combustion, welding fumes, etc.). Engineered nanoparticles can be grouped into four type: 1- carbon based materials, usually including fullerene, single walled carbon nanotube (SWCNT) and multiwalled carbon nanotubes (MWCNT); 2 – metal based materials such as quantum dots, nanogold, nanosilver, nanozinc, nanoaluminum and nanoscales metal oxides like TiO_2, ZnO and Al_2O_3; 3 – dendrimers which are nano-sized polymers built from branched units, capable of being tailored to perform specific chemical function; 4 – composites which combine nanoparticles with other nanoparticles or with larger bulk-type materials (Lin and Xing, 2007) and present different morphologies such as spheres, tubes, rods and prisms (Yu-Nam and Lead, 2008).

Engineered nanoparticles have three different unique characteristics, size, structure and properties. These nanoparticles received a particular attention for their positive impact in improving many sectors of economy, including consumer products, pharmaceutics, cosmetics, transportation, energy and agriculture etc., and are being increasingly produced for a wide range of applications within industry (Novack and Bucheli, 2007; Roco, 2003).

Higher plants strongly interact with their atmospheric and terrestrial environments and are expected to be affected as a result of their exposure of NSPs. Only a few studies are available on the effects of nanoparticles on higher plants. The majority of the reported studies point

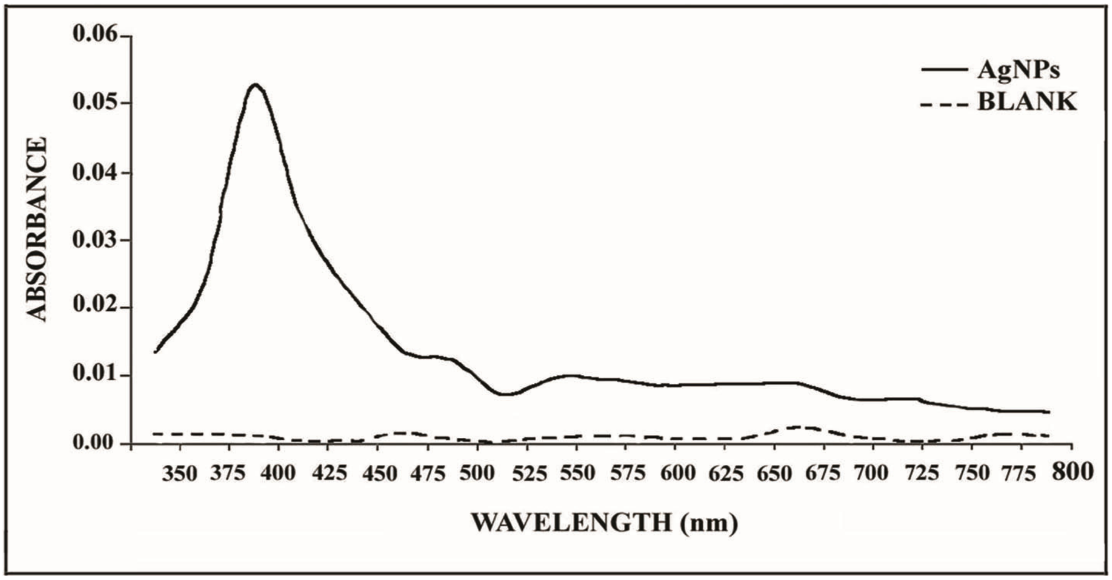


Figure 1. UV-Visible absorption spectrum of silver nanoparticles immediately after preparations.

to the positive impacts of nanoparticles on plant growth with a few isolated studies pertaining to negative effect. Numerous studies have demonstrated that TiO_2 nanoparticles promoted photosynthesis and nitrogen metabolism and thus greatly improved growth of spinach at a concentration as low as 20 mg/l (Hong et al., 2005a,b; Liu et al., 2005; Yang et al., 2006). Another study by Lin and Xing (2007, 2008) investigated phytotoxicology of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc and zinc oxide) on seed germination and root growth of six higher plant species (radish, rape, rye grass, lettuce, corn and cucumber). Seed germination was not affected except for the inhibition of nanoscale zinc (nano-Zn) on rye grass and zinc oxide (nano-ZnO) on corn at 2000 mg/l. Inhibition on root growth varied greatly among nanoparticles and plants. Oprisan et al. (2011) investigated the chlorophyll contents in the sunflower seedlings supplied with magnetic nanoparticles.

Another study by Mahajan et al. (2011) studied the effect of nano-ZnO particles on the growth of plant seedlings of mung (*Vigna radiate*) and gram (*Cicer arietinum*). They found that at certain optimum concentration, the seedlings displayed good growth over control and beyond that retardation in growth was observed. Similar results were reported with the application of nano-iron oxide on soybean yield and quality. The results showed that nano-iron oxide at the concentration of 0.75 g/l was increased leaf + pod dry weight and pod dry weight. The highest grain yield was observed with using 0.5 g/l nano-iron oxide that showed 48% increase in grain yield in comparison with control (Sheykhbaglou et al., 2010).

Silver nanoparticles (AgNPs) are currently one of the most widely commercially used nanomaterials (Chen and Schluesener, 2008). AgNPs toxicity has been reported in bacteria (Chio et al., 2008; Chio and Hu, 2008). Meyer et al. (2010) investigated the intracellular uptake and associated toxicity of three silver nanoparticles with different sizes in *caenorhabdities elegans*. They observed growth inhibition by all AgNPs at concentrations in the low mg/l levels.

Seif et al. (2011) study the effect of nanosilver and silver nitrate on abscission and yield of seed in borage. They showed that increasing the concentration of silver nitrate from 100 to 300 ppm caused a decrease in seed yield. On contrast, a raise in the concentration of nanosilver from 20 to 60 ppm has led to an improvement in the seed yield. Additionally, the lowest amount of seed yield was found with control plants.

Phytotoxicity of *Oryza sativa* was studied by directly exposing it to silver nanoparticles solutions. Transmission Electron Microscope (TEM) revealed that various particle sizes deposited inside the root cells. It was found that during penetrations of particles inside the cell of root, they damaged the cell wall as well as vacuoles to enter. It may be due to the penetrations of large particles entering through small pores of cell walls (Harajyoti and Ahmed, 2011).

The aim of the present study, displayed the effect of silver nanoparticles in some crop plants common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) was probed. The plant growth parameters (shoot and root lengths, leaf surface area, chlorophyll, carbohydrate, and protein contents) were investigated in this research.

MATERIALS AND METHODS

The AgNPs were obtained from King Abd Alla Institute for

Table 1. Effect of silver nanoparticles on shoot ant root lengths of *Phaseolus vulgaris* and *Zea mays*.

AgNPs concentration ppm	*Phaseolus vulgaris*				*Zea mays*			
	Shoot length (cm)	LSD (0.05)	Root length (cm)	LSD (0.05)	Shoot length (cm)	LSD (0.05)	Root length (cm)	LSD (0.05)
Control	18.3±2.3		10.7±1.4		28.3±1.5		11.5±1.2	
20	19.5±1.6	1.2*	11.6±1.7	0.9*	32.1±2.1	3.8*	12.3±1.4	0.8*
40	22.1±3.2	3.8*	12.1±2.8	1.4*	34.5±1.7	6.2*	14.7±1.6	3.2*
60	26.9±1.4	8.6*	16.7±1.6	6.0*	36.2±2.3	7.9*	16.8±2.5	5.3*
80	15.2±2.6	3.1*	8.6±3.0	2.1*	24.4±3.1	3.9*	10.5±2.3	1.0*
100	12.5±3.1	5.8*	5.3±2.1	5.4*	20.1±1.8	8.2*	6.8±1.7	4.7*

Note: The mean difference is significant with $P < 0.05$.

nanotechnology. According to the manufacturer, the particle size ranged from 10 to 30 nm (average 20 nm) and spherical in shape. Particles size and morphology were characterized by UV spectral analysis, as shown in Figure 1.

The experimental design was randomized complete block design with three replications. The test species were common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) and chosen as biological material considering its economic importance for agriculture and foods. Seeds of plant materials of *Phaseolus vulgaris* and *Zea mays* were obtained from the Agriculture Research Centre Giza, Cairo, Egypt.

Seeds were sterilized in a 5% sodium hypochlorite solution for 10 minutes (USEPA, 1996), rinsed through with deionized water several times. Their germination was conducted on water porous paper support in petri dishes (25 seed per dish) at controlled temperature of 25 ± 1 ºC, and stored in a dark container. After 24 h. the seeds were checked for germination and the sprouted seeds were used in the tests. Healthy and uniform seedlings were allowed to grow in pots. The pots of 14 cm diameter and 18 cm in height were filled with fertile loam soil up to ¾ the height of the pot. Daily supply with 15 ml silver nanoparticles per every test plantlets was carried out for 12 days along with control. Plant growth being conducted in controlled conditions of temperature (25 ± 1 ºC) illumination (dark/light cycle: 14/10 h.) and 80% humidity into a green house of Botany Department, Faculty of Science, King Saud University.

Silver nanoparticles were added daily in different concentrations (20, 40, 60, 80 and 100 ppm) for each test plants. Each concentration was prepared in three replicates. After 12 days of growth, the shoot and root lengths were long enough to measure using a ruler. Fresh and dry weights were measured, leaf surface area were measured using portable area meter Model L1 - 3000. Chlorophyll A, chlorophyll B and carotenoid pigments were accomplished based on method of Stirban (1985), carbohydrate content was measured according to Nelson (1944) and Somogyi (1952). Protein content was measured according to Lowry et al. (1951).

Statistical analysis

Each treatment was conducted with three replicates and the results were presented as mean ± SD (standard deviation). Each of the experimental values was compared to its corresponding control. The results were analyzed by one way Anova with used Statistical Package for Social Sciences (SPSS) Version 11.5.

RESULTS AND DISCUSSION

The silver nanoparticles were characterized by UV – Visible Spectrum, astrong silver nanoparticles absorptions spectra at 400 nm (Figure 1).

According to the manufacturer the particle sizes ranged from 10 to 30 nm (average 20 nm) and spherical in shape. The absorptions spectra are due to Plasmon excitations of particles (Bae et al., 2002). Distribution and particle sizes were mainly depending upon spectral analysis (Khanna et al., 2007). The concentrations of silver nanoparticles were chosen in the range 20, 40, 60, 80 and 100 ppm according to other studies (Racuciu and Creanagae, 2009).

The effect of silver nanoparticles on shoot and root lengths of common bean and corn are shown in Table 1. It was observed that with increase in Ag NPs concentration, the shoot and root lengths also increase. However, after certain concentration (60 ppm) the shoot and root was found to decline. For common bean and corn the best growth is response for shoot (19 and 12 %) and root (21 and 18 %) was observed at concentration of 60 ppm over control. At highest concentration, 100 ppm the retardation in shoots length (19 and 17 %) and root length (33 and 26%) for common bean and corn respectively. The reduction in shoot and root lengths at higher doses may be attributed to toxic level of nanoparticles. It was reported that the AgNPs concentration of 20, 40 and 60 ppm showed statistically significant stimulation on shoot and root elongation of the tested plants (common bean and corn). Concentra-tion of 80 and 100 ppm of silver nanoparticles showed

Table 2. Effect of silver nanoparticles on fresh ant dry weights of *Phaseolus vulgaris* and *Zea mays*.

AgNPs concentration ppm	*Phaseolus vulgaris*				*Zea mays*			
	Fresh weight (gm)	**LSD (0.05)**	**Dry weight (gm)**	**LSD (0.05)**	**Fresh weight (gm)**	**LSD (0.05)**	**Dry weight (gm)**	**LSD (0.05)**
Control	7.8±2.1		3.9±0.15		11.9±2.5		4.1±0.5	
20	9.1±3.1	1.3*	4.1±0.91	0.2*	15.6±3.4	3.7*	5.2±0.1	0.8*
40	10.3±1.5	2.5*	5.3±1.1	1.4*	22.9±1.9	11.0*	7.6±1.1	3.2*
60	14.5±1.8	6.7*	6.8±0.8	2.7*	24.9±2.1	13.0*	9.1±0.6	*4.7
80	6.8±2.3	1.0*	2.9±0.7	1.0*	10.3±0.3	1.6*	3.8±0.4	0.6*
100	5.5±1.3	2.3*	1.2±0.5	2.7*	8.9±1.8	3.0*	2.7±0.8	1.7*

Note: The mean difference is significant with P < 0.05.

Table 3. Effect of silver nanoparticles on leaf surface area of *Phaseolus vulgaris* and *Zea mays*.

AgNPs concentration ppm	*Phaseolus vulgaris*		*Zea mays*	
	Leaf surface area (cm^2)	**LSD (0.05)**	**Leaf surface area (cm^2)**	**LSD (0.05)**
Control	4.6±0.4		6.0±1.5	
20	4.9±0.05	0.3	8.4±2.5	2.4*
40	5.1±1.1	0.5*	9.5±3.1	3.5*
60	7.2±0.8	2.6*	10.2±1.9	4.2*
80	4.01±0.08	0.59*	5.6±3.5	0.4
100	2.3±0.3	0.3	3.7±2.8	0.3

Note: The mean difference is significant with P < 0.05.

statistically significant inhibition on shoot and root elongation. Thus silver nanoparticles can be reported with minimal toxicity on the tested plants, this is a good evidence for demonstrating that common bean and corn plants respond to add Ag NPs in a limited range, above which toxic levels are reached causing subsequent declines in growth. These results agree with Mahajan et al. (2011); Mihaela and Dorina (2007) and Seif et al. (2011).

The effect of silver nanoparticles on fresh and dry weight of common bean and corn is shown in Table 2. Fresh and dry weights were found to be in accordance with shoot and root lengths for corresponding silver nanoparticles treatment. For Ag NPs at 60 ppm treatment, common bean showed increase in fresh weight 30 % and 27 % increase in dry weight over control. In case of corn at the same AgNPs concentration fresh weight increased 35 % and dry weight 33 % over control was observed. At the highest concentration of 100 ppm, 17 % decrease in fresh weight and 52 % in dry weight for common bean was observed over control. While for corn, 14 % decrease in fresh weight and 20 % decrease in dry weight were observed.

The fresh and dry weights were both significantly higher than those of the untreated plants (controls). The best results were found at 60 ppm silver nanoparticles, the fresh weight and dry weight per plant were higher than those of the control by 30 % and 27 % respectively for common bean. For corn fresh weight and dry weight per plant were higher than those of the control by 35 % and 33 % respectively. The same results were obtained by Zheng et al. (2005).

In general, the leaf surface area of the tested common bean and corn was significantly (P < 0.05) increased as the silver nanoparticles increased till certain level (60 ppm), the leaf surface area was found to be decline as shown in Table 3. For common bean and corn the high leaf surface area was observed at concentration of 60 ppm (22 and 26 % respectively) over control. At highest concentration 100 ppm the decline in leaf surface area was observed in the two tested plants (33 and 23 % respectively). These results are confirmed by results obtained from other studies (Karthick and Chitrakala, 2011). At the same time, the use of low concentrations of nanocalcium carbonate caused increasing number of leaf and leaf area (Liu et al., 2005).

Effect of silver nanoparticles on chlorophyll content (chlorophyll A and B, total chlorophyll and carotenoids) of common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) showed significantly (P < 0.05) increase above control as shown in Figure 2. In concentration 60 ppm of silver nanoparticles chlorophyll A and chlorophyll B increases by 49% and 33% compared to the control in common bean (*Phaseolus vulgaris*). In corn (*Zea mays*) treated crop, the chlorophyll A and B increases by 46% and 26% compared to control respectively. Above 60 ppm

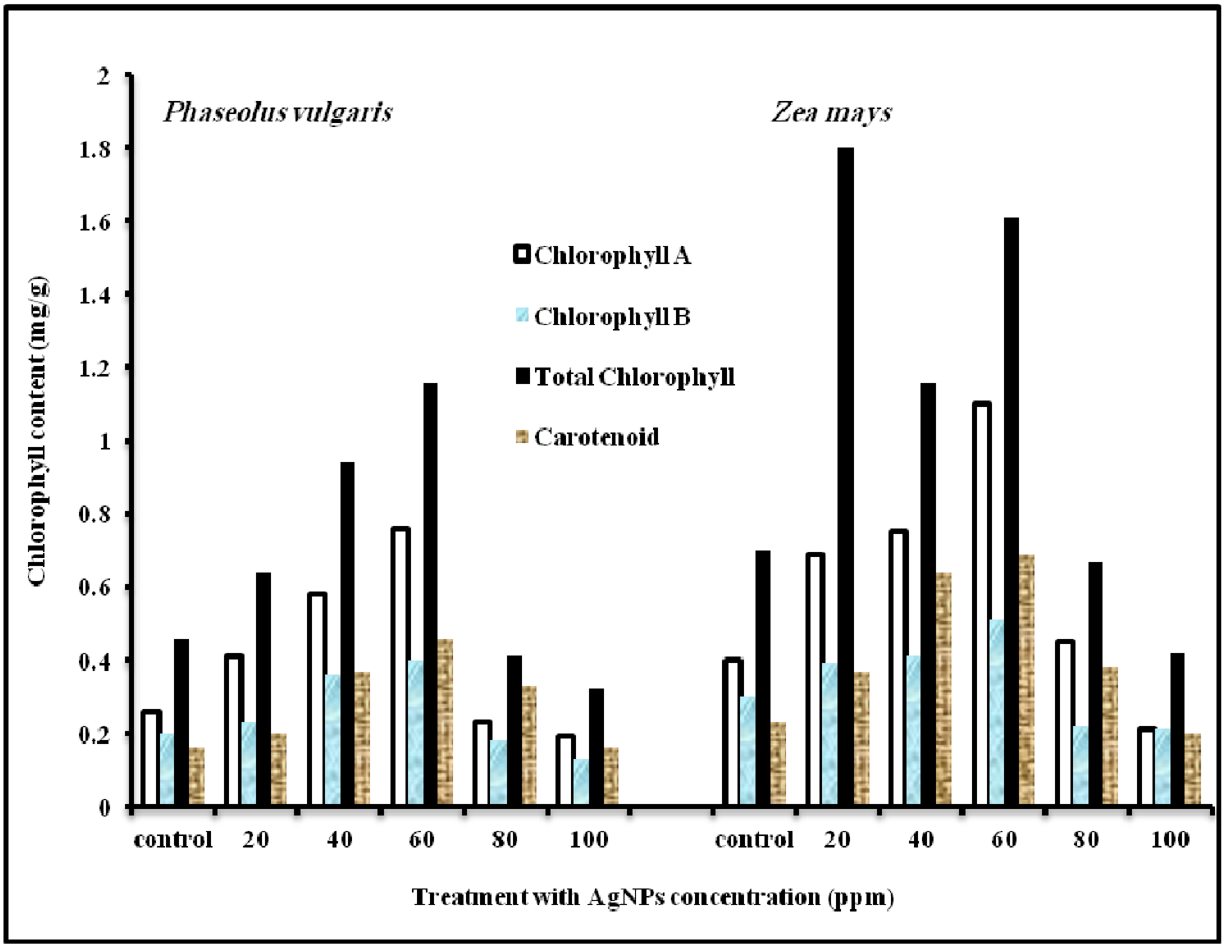


Figure 2. Effect of silver nanoparticles on chlorophyll content (mg/g) of Phaseolus vulgaris and Zea mays.

concentration of AgNPs, the chlorophyll contents of the tested crop plants decreased significantly ($P < 0.05$). Similar response was getting for the other two pigments analyzed (total chlorophyll and carotenoids). These results are confirmed by results obtained from other studies (Karthick and Chitrakala, 2011), they demonstrated that chlorophyll A content was significantly ($P < 0.05$) increased by Ag nanoparticles in green gram and sorghum. In other studies, response of corn (*Zea mays*) seedlings to the administration of 20, 40, 60, 80 and 100 µl/l of Fe_3O_4 NP suspension concentration resulted in the diminution of chlorophyll ratio while *Cucurbita pepo* seedlings exhibited a slight increase of chlorophyll contents (Racuciu et al., 2009). Numerous studies have demonstrated that TiO_2 nanoparticles promoted photosynthesis and nitrogen metabolism, and thus greatly improved growth of spinach at a concentration as low as 20 mg/l (Hong et al., 2005a, b; Zheng et al., 2005; Yang et al., 2006; Gao et al., 2008). It was also pointed out that a mixture of nanoscale SiO_2 (nano - SiO_2) and TiO_2 (nano - TiO_2) could increase nitrate reductase in soybean (*Glycine max*), enhanced its abilities of absorption and utilization of water and fertilizer, stimulated its antioxidant system and apparently hastened its germination and growth (Lu et al., 2002).

Mihaela and Dorina (2007) analyzed the influence of magnetic nanoparticles coated with tetramethylammonium hydroxide on the growth of corn (*Zea mays*) plants in early ontogenetic stages. The iron based nanoparticles may have not only a chemical but also a magnetic influence on the enzymatic structures implied in the different stages of photosynthesis. Low concentrations of aqueous ferro fluid solution added in culture medium had a stimulating effect on the growth of the plantlets, while the enhanced concentration of aqueous ferro fluid solution induced an inhibitory effect.

The effect of silver nanoparticles on carbohydrate content of the two tested crop plants was illustrated on Figure 3. The carbohydrate content of common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) were found to be in accordance with the photosynthetic pigments for corresponding AgNPs treatment. For 60 ppm AgNPs treatment common bean plant showed 57% increase and 62% increase in corn plant over control. While at concentration 80 and 100 ppm AgNPs treat-ment significant reduction in carbohydrate (19%, 18% for

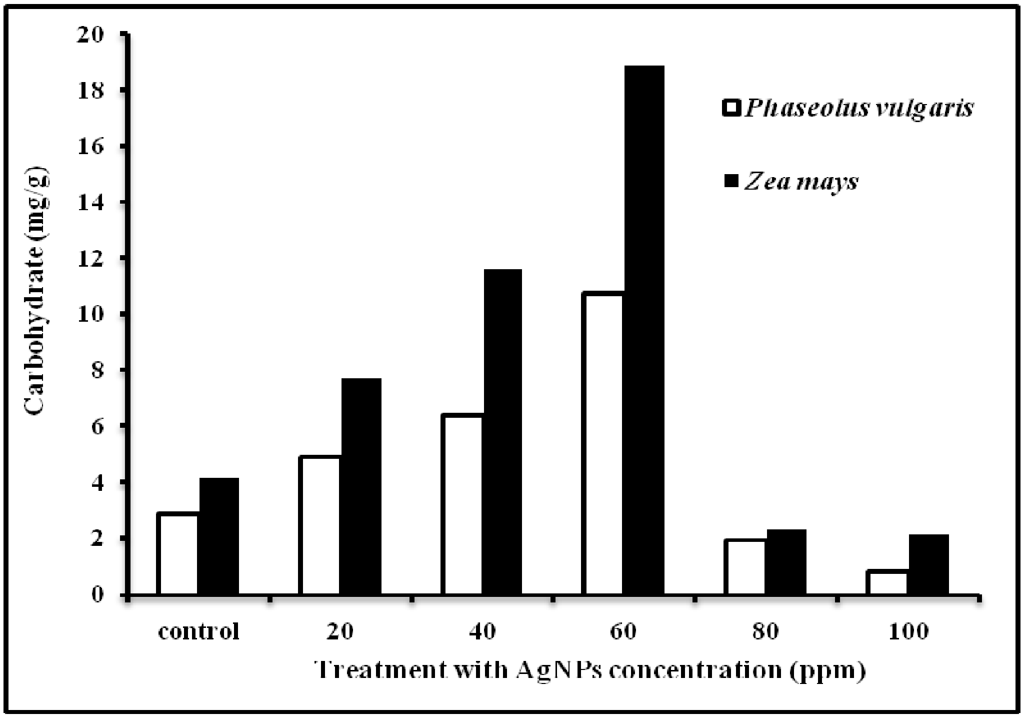
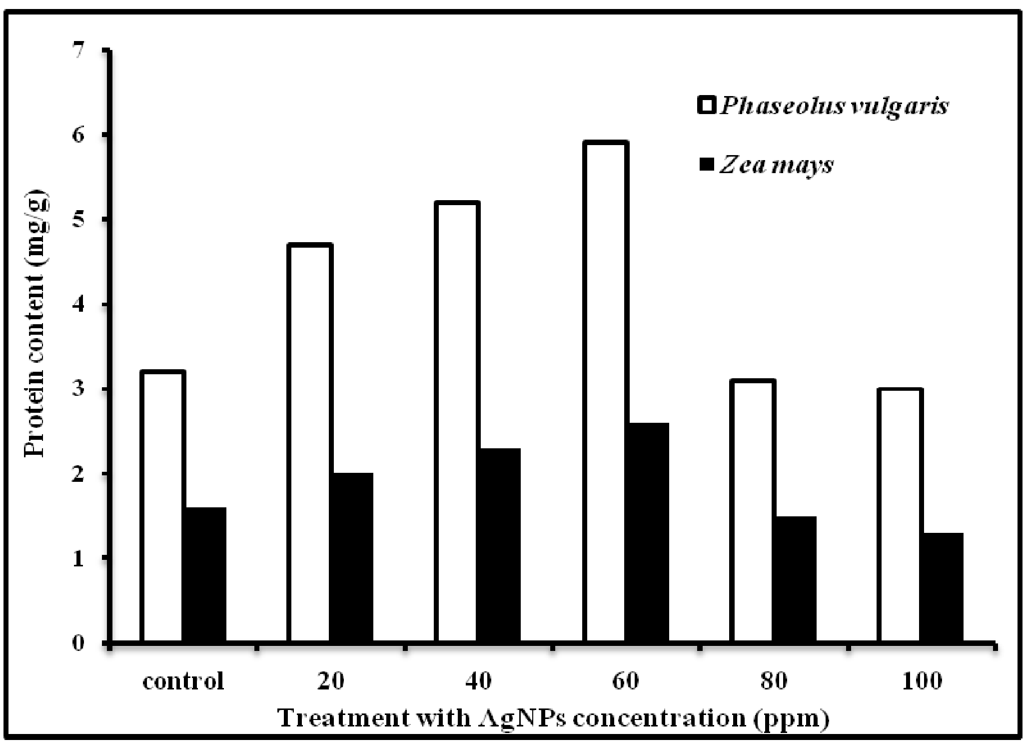


Figure 3. Effect of silver nanoparticles on carbohydrate content (mg/g) of *Phaseolus vulgaris* and *Zea mays.*



common bean and 28%, 31% for corn plant) was observed. The reduction in carbohydrate content of the tested crop plants at higher doses may be attributed to toxic level of nanoparticles causing subsequent declines in growth. The same results were obtained by Liu et al. (2005) who demonstrated that low concentrations of nanocalcium carbonate caused increasing soluble sugar and peanut protein.

The results showed that the effect of silver nanoparticles on protein content of common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) was significant (Figure 4). Application of silver nanoparticles at the concentration of 20, 40 and 60 ppm caused an increase in protein content of the two tested crop plants. At 60 ppm concentration the maximum significantly increase in protein (30% for common bean and 24% for corn) over control. At a dose of 100 ppm, significantly decrease in the protein (32% for common bean and 18% for corn) over control. The increase in protein at certain concentration suggests the optimum dose limit for the growth of common bean and corn plants. However, the decrease in protein beyond this concentration suggests the toxic effect of AgNPs. The same results were obtained by Liu et al. (2005).

CONCLUSION

The present study demonstrated the effect of silver nanoparticles on crop plant species common bean (*Phaseolus vulgaris*) and corn (*Zea mays*). The presence of AgNPs affects growth of common bean and corn at different concentrations. The maximum effect was found at 60 ppm for the two crop plants. Beyond this concentration the growth was inhibited. The effective growth at certain optimum concentration and inhibited growth beyond this concentration may be attributed to the accumulation and uptake of AgNPs by the roots. It was found that the accumulation and uptake of nanoparticles was dependent on the exposure concentration.

ACKNOWLEDGMENTS

The author acknowledges King Abd Alla Institute for nanotechnology (KAIN), and Physics Department, Faculty of Science, King Saud University for advising on physicochemical property measurement nanoparticle.

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