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Accumulation of silver nanoparticles and its effect on the antioxidant capacity in *Allium cepa* L.

Acumulación de nanopartículas de plata y su efecto en la capacidad antioxidante en *Allium cepa* L.

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Abstract. Nanotechnology is currently an important worldwide study field because it provides control on matter at a nanometric scale. In food and agricultural fields, the applications of this technology are in early stages. Onion is one of the main vegetables grown in the world. Onion is a low calorie food which contains a large amount of flavonoids. In this work, silver nitrate application in onion was evaluated. An onion crop was developed at the University Antonio Narro in Saltillo, Mexico. Two methods of silver nitrate application were used (a nutritive solution: 0, 20, 40 and 80 mg/L of $AgNO_3$; and foliar spray: 0, 20 and 40 mg/L of $AgNO_3$). The samples were taken at 30, 60 and 90 days after transplanting. Total antioxidant capacity, silver accumulation and silver nanoparticles formation were determined. The results showed that silver nitrate had a positive effect on total antioxidant capacity when it was applied as a nutritive solution. Silver accumulation in different tissues depended on the silver nitrate application method. The silver nanoparticles formation was positive; furthermore, these nanoparticles were found at a greater extent on samples where the $AgNO_3$ foliar spray application method was used. The average of silver nitrate nanoparticles size was from ~300 nm to ~1000 nm.

Keywords: Nanotechnology; Onion; Silver nitrate; Nanoparticles.

Resumen. Hoy en día la nanotecnología es un área de estudio muy importante en el mundo ya que proporciona un control sobre la materia a escala nanométrica. En el área alimenticia y agrícola las aplicaciones de esta tecnología se encuentran en sus etapas iniciales. La cebolla es una de las principales hortalizas cultivadas en el mundo. Es un alimento con escaso aporte calórico y con una gran cantidad de flavonoides. En este trabajo se evaluó la aplicación de nitrato de plata en cebolla. Se desarrolló un cultivo de cebolla en las instalaciones de la UAAAN en Saltillo, México. Se utilizaron dos métodos de aplicación de nitrato de plata con diferentes dosis (solución nutritiva: 0, 20, 40 y 80 mg/L de $AgNO_3$, y nebulización al follaje: 0, 20 y 40 mg/L de $AgNO_3$). Se realizaron muestreos a los 30, 60 y 90 días después del trasplante. Se determinaron la capacidad antioxidante total, acumulación de plata y formación de nanopartículas de plata. Los resultados mostraron que la aplicación de Nitrato de plata tuvo un efecto positivo en la capacidad antioxidante total cuando se aplicó en solución nutritiva. La acumulación de plata en los diferentes tejidos fue dependiente del método de aplicación del nitrato de plata. La formación de nanopartículas de plata fue positiva. Estas nanopartículas se encontraron en mayor medida en muestras en las que se utilizó el método de aplicación de $AgNO_3$ por nebulización al follaje. El tamaño promedio de las nanopartículas de plata fue desde ~300 nm hasta ~1000 nm.

Palabras clave: Nanotecnología; Cebolla; Nitrato de plata; Nanopartículas.

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INTRODUCTION

Nanotechnology is currently an important worldwide study field. Proof of this are the development programs by the European Union, including Japan (CSTP Strategic S&T Priorities in Nanotech. & Materials), USA ["Nanoscale Science and Engineering for Agriculture and Food Systems", a part of the National Research Initiative (NRI)], and Nano4Vitality in the Netherlands (Heugens et al., 2010). The arrival of nanotechnology provides a control at a nanometric scale creating a new class of materials in a diversity of domains (Granda et al., 2009). In the agricultural and food fields, nanotechnology applications are in their initial stages (Kumar-Mishra & Kumar, 2009); at this point, the use of plant parts for nanoparticle biosynthesis is an unexplored and unexploited area (Shankar et al., 2003).

Onion (*Allium cepa* L.) is one of the main vegetables grown worldwide. It is estimated that 2 million hectares are sown, yielding 32.5 million tons of production. Mexico is one of the ten main producers with 1.2 million tons approximately (SAGARPA, 2009). It is known that onion is a low calorie food with a high amount of flavonoids, which are powerful antioxidant compounds (Casanoves, 2007). It is also known that metabolism activation of cellular antioxidants and accumulation of these, belong to responses induced by heavy metals (Dietz et al., 1999).

Plants have a natural capacity of absorbing heavy metals from soil (Islam et al., 2007). Their accumulation depends on concentration and chemical species (Máthé & Anton, 2002). It is known that plants growing around silver (Ag) mines accumulate this metal, mainly in their radical system (Ratte, 1999). Hirsch et al. (1993) and Hirsch (1998) observed that most plants grew normally in soils with high Ag concentration. However, for some plants, such as Chinese cabbage and lettuce, growth was adversely affected in soils with over 14 mg/kg Ag. It is known that silver in the ionic form has bactericidal properties and it functions as an ethylene inhibitor (Uda et al., 1995); silver nitrate ($AgNO_3$) also increments fresh weight of crops such as cut rose (Son et al., 2003). Gardea-Torresdey et al. (2003) reported silver nanoparticles formation for the first time using plants as biotransformation tools. These authors showed that the silver cation (Ag^+) is reduced on solid medium, absorbed by roots and transported to outbreaks as nanoparticles (Ag^0). Harris & Bali (2008), tested $AgNO_3$ on *Brassica juncea* and *Medicago sativa,* and reported high silver accumulation and discrete formation of nanoparticles with an average size of ~50 nm. This was the first report of silver hyperaccumulation on plants.

Haverkamp & Marshall (2009) described the application of different silver sources [$AgNO_3$, $Na_3Ag(S_2O_3)_2$, and $Ag(NH_3)_2NO_3$] in nutritive solutions on *Brassica juncea.* They determined that not all the accumulated silver was transformed into Ag^0, partly remaining as Ag^+. The fact that nanoparticles can be toxic for some organisms such as bacteria, algae, invertebrates, fish and mammals, particularly humans, should be considered (Handy & Owen, 2008).

The aims of this work were to (1) assess the formation of silver nanoparticles in *A. cepa,* (2) determine silver accumulation in different plant parts, and (3) know the silver effects on the total antioxidant capacity of onion tissues.

MATERIALS AND METHODS

An experiment was carried out in a tunnel with a polyethylene plastic cover (20 m long, 5 m wide, 2.5 m high) at the University Antonio Narro in Saltillo, Mexico. Onion plants (*A. cepa* L.) var. "Crystal White Wax" were placed in 10 L plastic bags filled with a perlite substrate. Weekly silver nitrate applications began at the time of transplantation. Two methods were used: application of a (1) Douglas nutritive solution (Douglas, 1976) at four concentrations (0, 20, 40 y 80 mg/L of $AgNO_3$); and (2) foliar spray at three concentrations (0, 20 y 40 mg/L of $AgNO_3$). Onion plants were sampled 30, 60, and 90 days after transplanting, and silver content was determined in different plant organs. At the end of the experiment, root and leaf length; bulb diameter (polar and equatorial); and root, leaf and bulb fresh matter were recorded. In addition, the total antioxidant content, refractive index and pH from the fresh bulb extracts were determined.

A completely randomized experimental design was used. The Statistical Analysis System (SAS) 7.0 was used for data analysis, and a LSD (α=0.05) mean comparison test was performed to identify statistical differences between treatments.

Determination of the total antioxidant capicity. In the final sample, the collected bulbs were cut and chilled to avoid dehydration. At the laboratory, 5 g samples were taken from a bulb core, and placed in previously frozen mortars. Ten mL of phosphate buffer pH 7 were added, and it was ground all together. The resultant extract was centrifuged during 10 minutes at 3000 rpm and the liquid obtained was placed in clean test tubes.

A Total Antioxidant Status Kit Assay of Calbiochem was used for antioxidant determination. This kit contains a saline phosphate buffer solution, chromogen (Metmyoglobin and ABTS [radical cation 2,2-Azinobis-(3-etilbenzotiazolin-6-sulfonate)]), stabilized substrate (hydrogen peroxide) and "Trolox" (-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxílic acid) with 1.5 mM of concentration as standard.

An Espectronic 21 Loush & Lamb spectrophotometer with a maximum absorbance capacity of 0-2 and a resolution of 0.01, capable of measuring absorbance at 600 nm, cells of 1 cm length, was used. A Sol-bat J-12 centrifuged with a maximum capacity of 5000 rpm and a 500 resolution was also used. In order to maintain the temperature at 37 °C, a 184-Model Precision thermal bath with a maximum capacity of 250 °C, and 0.25 °C of resolution, was used. Further an Ohaus Scout Scale with a maximum capacity of 600 g, and 0.1 g resolution, was used.

In order to analyze the samples, the three reagents included in the kit were prepared as follows: 10 mL and 7.5 mL of buffer pH 7 were added to the chromogen and to the substrate, respectively; 1 mL of distilled water was added to the standard.

The spectrophotometer was adjusted to 600 nm against air. The stabilized hydrogen peroxide and the chromogen were equilibrated at 37 °C during five minutes before use.

First, a blank was prepared adding 20 µL of distilled water in one cell plus 1 mL of chromogen. In another cell, the standard was prepared to add 20 µL of Trolox plus 1 mL of chromogen. The initial absorbance reading was taken (A_0) in both cells. Immediately afterwards, the onion sample extracts were analyzed. Two hundred µL of centrifuged extracts plus 1 mL of chromogen were placed for each sample. After mixing them, the initial absorbance was taken. Afterwards, 200 µL of diluted stabilized hydrogen peroxide was added to each cell, then mixed and the starting time was taken. The final absorbance (A) was measured after three minutes of color development. The temperature was maintained at 37 °C throughout the test. For assessing the levels of antioxidants in the samples, the Trolox standard concentration was used accordingly to its kit lot number (1.5 mM).

The absorbance gradient (ΔA) was determined for the samples (ΔA_m), the standard (ΔA_e) and the blank (ΔA_b) with the following general equation:

$$\Delta A = A - A_0 \quad (1)$$

The Trolox Equivalent Antioxidant Capacity (TEAC) was calculated for each sample using the following formula:

$$\text{TEAC (mM)} = \frac{\textit{standard Trolox concentration}\ (15\text{mM}) \times [\Delta A_b - \Delta A_m]}{\Delta A_b - \Delta A_e} \quad (2)$$

The result of each sample was expressed as mM of Trolox equivalent by mg (TEAC) of sample fresh weight.

Silver content determination using the Inductively Couple Plasma (ICP). The determination of silver content was carried out in the Laboratory of Plastics in Agriculture in CIQA Saltillo, Mexico. An Irish Advantage 14034000 ICP Thermo Jarrel Ash was used.

A sample was dried at 65 °C during 72 hours and 1 g was taken. A 20 mL of concentrated nitric acid was mixed with the sample in a 100 mL beaker, then covered with a watch glass and was set to digest in the acid, boiling on a warming rack at 125 °C until the sample organic matter was completely consumed, namely, when no solids scattered in the sample were observed and the solution was completely clear.

Once the sample was digested, the obtained solution was cooled down at room temperature and filtered through a No. 42 Wathman paper. This solution was then diluted using deionized water to 50 mL. Next, a silver standard was placed on the ICP and the calibration curve was generated to determine the silver content in the sample.

Determination of silver nanoparticles in *A. cepa*. After the silver content in the examined samples was obtained, those samples with higher silver content were selected to determine the formation of silver nanoparticles. This procedure was done in the Laboratory of Food Quality Management at the Uruapan Technological Institute of Higher Learning in Michoacan, Mexico, using a Jeol JSM 6480 LV Scanner Electronic Microscopy (SEM). The technique used was zooming in the samples with the SEM, focusing on parts that showed the highest silver contents. This process was repeated several times to find the silver nanoparticles and to obtain the pictures containing their dimensions.

RESULTS

Only the total content of antioxidants showed differences between treatments (Tables 1 and 2).

The total antioxidant capacity found in onion bulbs is shown in Figures 1 and 2. Statistically significant differences were found between treatments as well as between application methods.

The results regarding the silver content on different tissues of the onion plant, and its variations throughout the experiment are shown in Tables 3 and 4. As was expected, Ag con-

Table 1. Mean comparison of evaluated variables at the end of *A. cepa* crop development when an Ag nutritive solution application method was used (LSD, α=0.05).
Tabla 1. Comparación de medias de las variables evaluadas al final del desarrollo del cultivo de *A. cepa* por el método de aplicación de Ag en solución nutritiva (LSD, α=0,05).

Treatment	Length (cm)		Bulb diameter (cm)		Fresh weight (g)			pH	Brix degrees
	Root	Leaf	Polar	Equatorial	Root	Leaf	Bulb		
0 mg/L	15.26a	29.16a	3.33a	4.70a	1.35a	3.71a	33.17a	5.4a	6.70a
20 mg/L	20.33a	33.33a	3.73a	5.20a	2.24a	8.12a	56.37a	5.4a	7.77a
40 mg/L	17.16a	33.83a	3.26a	4.53a	3.43a	13.22a	37.19a	5.6a	8.30a
80 mg/L	15.66a	25.66a	3.43a	4.71a	1.33a	5.32a	51.02a	5.3a	6.13a

Identical letters indicate no statistically significant differences among treatments.
Letras iguales indican que no existen diferencias estadísticamente significativas.

Table 2. Mean comparison of evaluated variables at the end of *A. cepa* crop development when foliar spray Ag application method was used (LSD, α=0.05).
Tabla 2. Comparación de medias de las diferentes variables evaluadas al final del desarrollo del cultivo de *A. cepa* por el método de aplicación de Ag por nebulización al follaje (LSD, α=0,05).

Treatment	Length (cm)		Bulb diameter (cm)		Fresh weight (g)			pH	Brix degrees
	Root	Leaf	Polar	Equatorial	Root	Root	Leaf		
0 mg/L	18.67a	24.50a	3.47a	5.37a	1.77a	2.57a	57.82a	6.0a	5.67a
20 mg/L	20.00a	36.50a	3.67a	5.60a	3.37a	8.40a	67.76a	6.2a	6.37a
40 mg/L	23.67a	22.67a	3.43a	5.47a	1.86a	5.83a	60.30a	5.9a	6.83a

Identical letters indicate no statistically significant differences among treatments.
Letras iguales indican que no existen diferencias estadísticamente significativas.

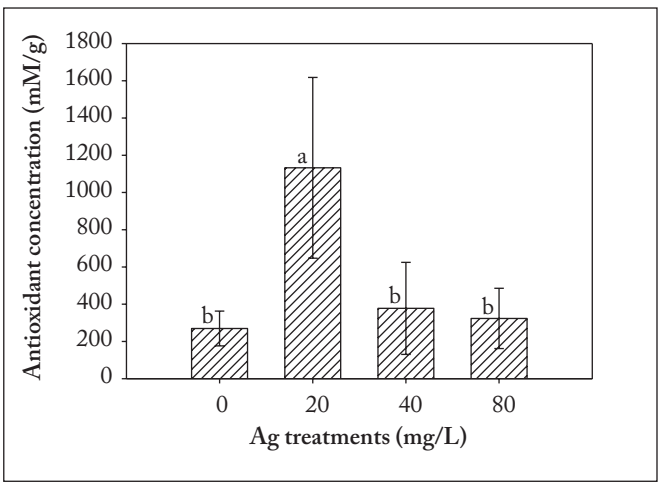

Fig. 1. Total antioxidant content on *A. cepa* by Ag application method in the nutritive solution.
Fig. 1. Contenido total de antioxidantes en *A. cepa* por el método de aplicación de Ag en solución nutritiva.

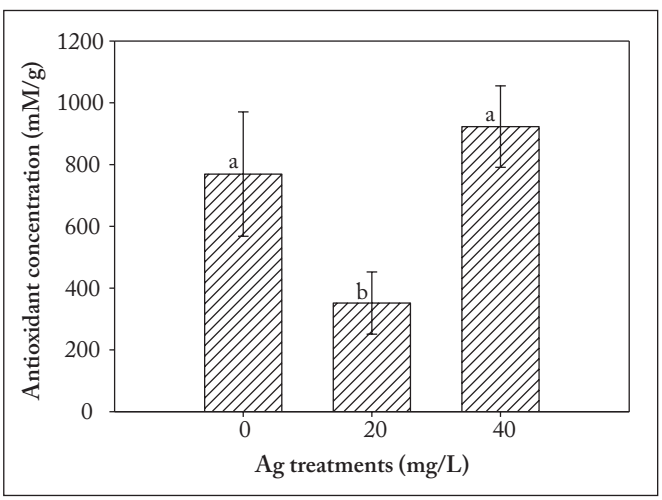

Fig. 2. Total antioxidant content on *A. cepa* by foliar spraying Ag application method.
Fig. 2. Contenido total de antioxidantes en *A. cepa* por el método de aplicación de Ag por nebulización al follaje.

Table 3. Ag content (mg/kg) in different *A. cepa* organs when an Ag application method to the nutritive solution was used.
Tabla 3. Contenidos de Ag (mg/kg) en diferentes órganos de *A. cepa* por el método de aplicación de Ag en solución nutritiva.

Treatment		Sample 1		Sample 2		Sample 3		
	Rep.	Bulb	Leaf	Bulb	Leaf	Bulb	Leaf	Root
0 mg/L	1	1	1	1	1	0	0	0
	2	0	2	0	0	0	0	1
	3	0	0	0	0	0	0	5
20 mg/L	1	18	2	1	15	0	1	30
	2	43	1	4	2	0	0	21
	3	0	5	0	9	1	0	37
40 mg/L	1	1	3	0	1	0	2	48
	2	2	3	1	0	0	1	122
	3	0	6	0	1	0	0	75
80 mg/L	1	2	2	8	2	4	10	80
	2	0	7	3	5	6	25	119
	3	0	1	0	-	0	5	29

Table 4. Ag content (mg/kg) on different *A. cepa* organs when foliar spray Ag application method was used.
Tabla 4. Contenido de Ag (mg/kg) en diferentes órganos de cebolla mediante el método de aplicación de Ag por nebulización al follaje.

Treatment		Sample 1		Sample 2		Sample 3		
	Rep.	Bulb	Leaf	Bulb	Leaf	Bulb	Leaf	Root
0 mg/L	1	0	0	0	0	0	0	0
	2	0	1	0	0	0	0	1
	3	0	0	0	0	0	0	5
20 mg/L	1	0	3	1	11	3	3	11
	2	0	1	0	8	0	11	7
	3	0	2	0	11	0	26	0
40 mg/L	1	1	12	6	123	0	72	161
	2	3	9	1	196	0	266	25
	3	1	17	3	175	0	91	3

tent increased with solution concentration for both methods. In the Ag application method to the nutritive solution, the highest contents were found in the roots in the last sampling (Table 3). Whereas for foliar spray, highest values were recorded in the second and third sampling, in the onion plant leaves and roots (Table 4).

Moreover, Table 5 shows the mean comparisons. For both Ag application methods existed statistically significant differences between treatments. The highest Ag application corresponded to the highest Ag accumulation in onion leaves for both application methods.

Table 5. Mean comparisons (LSD α=0.05) within each of the different Ag application methods considering plant parts, treatments and time factors.
Tabla 5. Comparación de medias (LSD α=0,05) dentro de los diferentes métodos de aplicación de Ag considerando las partes de la planta y los factores tratamiento y tiempo.

Foliar Spray						
Treatment	Leaf	Bulb	Root		Leaf	Bulb
	Mean	Mean	Mean	Sample	Mean	Mean
0 mg/L	0.11b	0.00b	0.00a	1	5.00b	0.56a
20 mg/L	8.44b	0.44b	6.00a	2	58.22a	1.22a
40 mg/L	106.78a	1.67a	63.00a	3	52.11a	0.33a
Nutritive Solution						
Treatment	Leaf	Bulb	Root		Leaf	Bulb
	Mean	Mean	Mean	Sample	Mean	Mean
0 mg/L	0.44b	0.22b	2.00b	1	2.75a	5.58a
20 mg/L	3.89ab	7.44a	29.33ab	2	3.29a	1.50a
40 mg/L	1.89b	0.44b	81.67a	3	3.67a	0.92a
80 mg/L	6.72a	2.56ab	76.00a			

Identical letters indicate no statistically significant differences between treatments.
Letras iguales indican que no existen diferencias estadísticamente significativas.

With the application of Ag with the foliar spray method, it was possible to observe that the highest concentration of the element resulted in the highest Ag concentration in the onion bulb. On the other hand, the application of 20 mg/L of Ag in the nutritive solution resulted in the highest Ag concentration in the onion bulb. Both methods showed statistically significant differences (Table 5).

Concerning the Ag concentration in onion roots, the application through nutritive solution showed statistically significant differences. The highest Ag concentration in roots was found in the treatment with 20 mg/L of Ag. Meanwhile, spraying Ag over the leaves did not determine the existence of statistically significant differences (Table 5).

Respect to sampling times, only the leaves with the foliar spray method showed statistically significant differences. The highest concentration was found in the second and third samples (Table 5).

With respect to silver nanoparticle formation on plant structures, positive results were found in some samples (Table 6). Seven samples with Ag^0 formation belong to the application method by foliar spraying, and two samples belong to the application method by nutritive solution. The silver nanoparticle formation was found in the highest $AgNO_3$ concentration treatments in both methods. Figure 3 shows resulting images from elements scanning by SEM. In these pictures different sizes and forms for silver nanoparticles are shown. They belong to different samples. In Figure 4, a spectral image is shown as a consequence of the elements found in sample number 3. Different mineral elements found, including Ag, are visible in this image.

Table 6. Characteristics of samples with silver nanoparticle formation.
Tabla 6. Características de muestras con formación de nanopartículas de plata.

Method	Treatment	Sample	Tissue	Ag content	
				% Weight	% Atomic
Nutritive solution	80 mg/L	1	Leaf	0.17	0.02
	80 mg/L	2	Root	0.08	0.01
Foliar spray	40 mg/L	3	Bulb	15.80	2.48
	40 mg/L	4	Bulb	5.09	0.72
	40 mg/L	5	Leaf	4.64	0.65
	40 mg/L	6	Leaf	1.00	0.13
	40 mg/L	7	Leaf	17.62	2.64
	40 mg/L	8	Leaf	23.87	3.84
	40 mg/L	9	Leaf	0.14	0.02

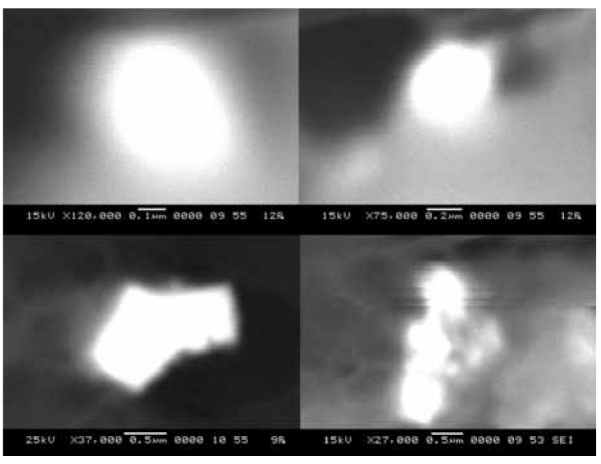

Fig. 3. Different sizes and shapes of silver nanoparticles found using the SEM.
Fig. 3. Nanopartículas de plata con diferentes tamaños y formas encontradas con escaneo de elementos por SEM.

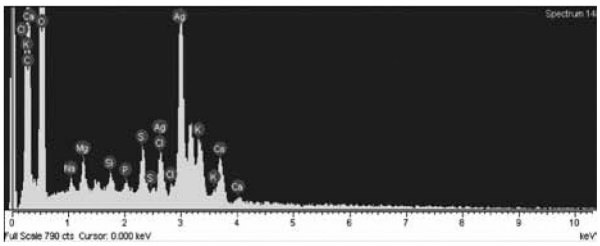

Fig. 4. Spectral image emitted as a result of elements found in sample #3 and foliar spray method on *A. cepa* bulb tissue.
Fig. 4. Imagen espectral emitida como resultado de los elementos encontrados en la muestra #3 y método de nebulización al follaje en el tejido del bulbo de *A. cepa*.

DISCUSSION

Our results are consistent with those reported by Hirsch et al. (1993) and Hirsch (1998), who found that plants grow normally in the different Ag concentration tested (Tables 1 and 2). Particularly, the onion seems to show tolerance to heavy metals (Iannacone & Alvariño, 2005). Ratte (1999) mentioned that $AgNO_3$, albeit being one of the most toxic silver compounds, can be beneficial for plants in small amounts. We assume that this positive response has to do with the activation of the antioxidant metabolism (Dietz et al., 1999), as seen in the onion treated with the silver application method by nutritive solution (Fig. 1). On the other hand, the null effect on onion biomass contrasts with that reported in the cut rose by Son et al. (2003).

In terms of silver accumulation in the different plant organs, it was found that this depended largely on the application method (Table 5). When silver was applied in nutritive solution, it was accumulated more on the plant roots. These results agree with those of Ratte (1999), who studied plants growing near silver mines. Our results on $AgNO_3$ concentrations are in agreement with reports of Máthé & Antón (2002). They claim that heavy metal accumulation on plants depends on substrate concentration and on the chemical species in question.

On the other hand, results of silver application by foliar spraying method indicated that silver cumulative amount depended on its concentration in the solution or medium where it was applied (Table 5).

Harris & Bali (2008) found in roots of *Brassica juncea* and *Medicago sativa* silver cumulative levels of 12.4 and 13.6% of the dry matter weight with concentrations of 1000 and 10000 ppm of $AgNO_3$ in the substrate, respectively. It was recorded for each case that the accumulation was in the form of discrete nanoparticles. In this paper, with lower Ag concentrations, silver nanoparticle formation and accumulation were also recorded in the highest $AgNO_3$ concentration treatments. In contrast with Harris & Bali (2008), the silver nanoparticle average size was about ~550 nm. Nevertheless, recorded sizes varied from ~300 nm to ~1000 nm. The different nanoparticle forms circular, elongated, and irregular shapes (Fig. 3) have to be remarked.

Haverkamp & Marshall (2009) reported a silver accumulation limit of 0.35% of the plant dry weight in nanoparticle form, this value depending on the plant tissue reducing capacity. Moreover, the reduction reactions occur at electrochemical potentials greater than 0 V, referred to a standard hydrogen electrode, as in the case of silver (Vanýsek, 2007). In the present work, the ratio nanosilver/total silver was not evaluated in plant tissues. However, it is important to note that seven out of nine samples with silver nanoparticle formation belonged to foliar spray silver application method, and five out of these seven were found in onion leaves. This leads to the assumption that silver nanoparticle formation was dependent on the differential antioxidant capacity of plant tissues. Based on these results, it is possible to say that there was a greater effect of foliar Ag application on silver nanoparticle formation. Our results add to those of Gardea-Torresdey et al. (2003), who reported for the first time the silver nanoparticle formation using living plant systems.

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REFERENCES

Casanoves, D.M. (2007). Tendencias de consumo de la cebolla. *Revista Horticultura* 201: 32-35.

Dietz, K.J., U. Krämer & M. Baier (1999). Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad, M.N.V., J. Hagemeyer, (eds). pp. 73-97.Heavy Metal Stress in Plants: From Molecules to Ecosystems. Springer-Verlag, Berlin. 40 p.

Douglas, J.S. (1976). Advanced guide to hydroponics. Drake Publishers. New York.

Gardea-Torresdey, J., E. Gómez, J.R. Peralta-Videa, J.G. Parsons, H. Troiani & M.J. Yacaman (2003). Alfalfa Sportus: A Natural source for the synthesis of silver Nanoparticles. *Langmuir* 19: 1357-1361.

Granda Valdés, M., A.C. Valdés González, J.A. García Calzón & M.E. Díaz-García (2009). Analytical nanotechnology for food analysis. *Microchimica Acta* 166: 1-19.

Handy, R.D., R. Owen & E. Valsami-Jones (2008). The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* 17: 315-325.

Harris, A.T. & R. Bali (2008). On the formation and extent of uptake of silver nanoparticles by live plants. *Journal of Nanoparticle Research* 10: 691-695.

Haverkamp, R.G. & A.T. Marshall (2009). The mechanism of metal nanoparticle formation in plants: limits on accumulation. *Journal of Nanoparticle Research* 11: 1453-1463.

Heugens, E.H.W., S.W.P. Wijnhoven & E.A.J. Bleeker (2010). WP5: Environmental, Health and Safety (EHS) impacts. Technology Sector Evaluation: Agrifood V1.0. ObservatoryNANO. European Commission. 23 p.

Hirsch, M. (1998). Availability of sludge-borne silver to agricultural crops. *Environmental Toxicology and Chemistry* 17: 610-616.

Hirsch, M., M. Ritter, K. Roser, P. Garrisi & S. Forsythe (1993). The effect of silver on plants grown in sludge-amended soils. In: Andren, A.W.; Bober, T.W. (eds.) pp. 69-73. Transport, fate, and effects of silver in the environment. The 1st international conference proceedings. Madison, WI, University of Wisconsin Sea Grant Institute. 121 p.

Iannacone, J. & L. Alvariño (2005). Efecto ecotoxicológico de tres metales pesados sobre el crecimiento radicular de cuatro plantas vasculares. *Agricultura Técnica* 65: 198-203.

Islam, I., X. Yang, Z. He & Q. Mahmood (2007). Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *Journal of Zhejiang University Science B* 8:1-13.

Kumar-Mishra, V. & A. Kumar (2009). Impact of metal nanoparticles on the plant growth promoting rhizobacteria. *Digest Journal of Nanomaterials and Biostructures* 4: 587-592.

Máthé-Gaspar, G. & A. Anton (2002). Heavy metal uptake by two radish varieties. *Acta Biologica Szegediensis* 46: 113-114.

Ratte, H. T. (1999). Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry* 18: 89-108.

SAGARPA (2009). Comportamiento del Mercado, Sistema Producto Cebolla. http://www.tml.sagarpa.gob.mx/intecom/cebolla09.pdf. Consulta 23 de febrero de 2011.

Shankar, S.S., A. Ahmad, R. Pasricha & M. Sastry (2003). Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. *Journal of Materials Chemistry* 13: 1822-1826.

Son, K.C., H.J. Byoun & M.H. Yoo (2003). Effect of pulsing with $AgNO_3$ or STS on the absorption and distribution of silver and the vase life of cut rose "Red Sandra". *Acta Horticulturae* 624: 365-372.

Uda, A., Y. Koyama & K. Fukushima (1995). Effect of silver thiosulfate solution (STS) having different ratios of $AgNO_3$ and $Na_2S_2O_3.5h_2O$ on Ag absorption and distribution, and vase life of cut carnations. *Journal of the Japanese Society for Horticultural Science* 64: 927-933.

Vanýsek, P. (2007). Electrochemical Series. In: Handbook of Chemistry and Physics. 88th Edition (Chemical Rubber Company). 2640 p.