

Silver nanoparticles coated with chitosan against *Fusarium oxysporum* causing the tomato wilt

Nanopartículas de plata recubiertas con quitosano contra la marchitez vascular causada por *Fusarium oxysporum* en plántulas de tomate

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ABSTRACT

Fusarium wilt in tomato seedlings is of great economic importance throughout the world due to the significant losses it causes in the crop. Advances in nanotechnology provide alternatives that can be applied for pathogens control. Silver nanoparticles (AgNP) stabilized with chitosan (Cs) are currently in widespread use for the control of plant pathogens. The objective of the present investigation was to determine the effect of the application of Ag-Cs NPs on the tolerance of tomato seedlings to, and control of the vascular wilt caused by, *Fusarium oxysporum*. The results showed that the application of the NPs did not show negative effects in the normal vegetative development of the tomato seedlings (up to 2000 ppm). Nanostructures were significantly effective to inhibit mycelial growth by up to more than 70% and, in addition, the treatment was effective to reduce the severity of the disease in *Fusarium oxysporum* inoculated seedlings after 14 days post-inoculation.

Keywords: silver nanoparticles; chitosan; *Fusarium oxysporum*; tomato wilt.

RESUMEN

El daño por *Fusarium oxysporum* en las plantas de tomate es de gran importancia económica en todo el mundo debido a las importantes pérdidas que genera en el cultivo. Los avances en nanotecnología proporcionan alternativas que pueden aplicarse en el control de patógenos. Las nanopartículas de plata (AgNP) estabilizadas con quitosano (Cs) tienen actualmente un uso generalizado para el control de patógenos de plantas. El objetivo de la presente investigación fue determinar el efecto de la aplicación de AgNP-Cs sobre la tolerancia de plántulas de tomate y control de la marchitez vascular, provocada por el fitopatógeno. Los resultados mostraron que la aplicación de las NPs no mostró efectos negativos en el desarrollo vegetativo normal de las plántulas de tomate (hasta 2000 ppm). Las nanoestructuras fueron significativamente efectivas para inhibir el crecimiento del micelio hasta en más del 70%, además el tratamiento fue eficaz para reducir la gravedad de la enfermedad en plántulas inoculadas con *Fusarium oxysporum* después de 14 días post-inoculación.

Palabras clave: nanopartículas de plata; quitosano; *Fusarium oxysporum*; marchitez de planta de tomate.

INTRODUCTION

The control of plant diseases is the most challenging aspect of crop production. In recent years, the resistance to pesticides available in the market has increased and became a serious problem (Chattopadhyay *et al.*, 2017; Goffeau, 2008). Among the microorganisms related to plant diseases, fungi cause a higher percentage in agricultural systems. *Fusarium* wilt is a fatal vascular wilt syndrome in plants belonging to the Solanaceae family (Omar & Ahmed, 2014).

The fungus *F. oxysporum* comprises more than 120 known strains or special forms, each of which is specific to a single host plant in which it causes disease. *Fusarium oxysporum* f. sp. *Lycopersici* cause wilt in tomato. *Fusarium* wilt is a destructive tomato disease in several countries of the world, and is of great concern to producers due to its large loss of production, fungi prolonged survival in the soil and generation of resistant strains. The disease can be reduced to some extent with the use of resistant cultivars and chemicals. However, the evolution to new pathogenic strains is a continuous problem, and the use of chemical products is expensive and not always effective (Amini & Sidovich, 2010; Malandrakis *et al.*, 2018). Therefore, the control mechanism for this type of diseases, with no harmful effects for crops nor to the environment, has been a major concern for researchers working in the field of disease management.

Therefore, it is of immediate necessity to look for alternative disease control measures that are safe for the environment. In recent years, the use of nanomaterials is expanding and has been considered as an alternative solution to control plant pathogens. Some metallic nanoparticles have been studied and tested for their antifungal properties (Al-Huqail *et al.*, 2018; Janaki *et al.*, 2015; Medda *et al.*, 2015; Shaikh *et al.*, 2019). Several studies have demonstrated the effectiveness of AgNP against phytopathogenic microorganisms. Jo *et al.* (2009) demonstrated that AgNP were effective in reducing the growth of *Bipolaris sorokiniana* and *Magnaporthe grisea* fungi *in vitro* and reducing the severity of the disease in plants by applying the nanomaterial 3 h after the inoculation

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with spores, but their efficacy decreased significantly when applied after 24 h of inoculation. Similarly that AgNP were effective against various phytopathogens such as *Alternaria alternata*, *Botrytis cinerea*, *F. oxysporum* and *Pythium spinosum* (Kim *et al.*, 2012). Mishra *et al.* (2017) showed the antifungal activity of AgNPs against foliar and soil-borne phytopathogens. The inhibitory impact of different silver concentrations (2, 4, 10 µg/mL) on conidial germination was recorded under *in vitro* conditions. In another study, Karimi and Sadeghi (2019) isolated 3 different phytopathogens (*Alternaria alternata*, *Alternaria citri* and *Penicillium digitatum*) from citrus samples. *In vitro* assay was carry out on PDA media treated with 50, 100 and 150 ppm of silver nanoparticles and two conventional fungicides. Results revealed that Ag-NPs showed stronger antifungal activity against the isolated fungi that conventional fungicides.

However, these authors did not evaluate the nanoparticles *in vivo*. In the literature there is a lot of information about the antifungal effect of the AgNP, however, there is a lack of information about the effect on the plant and the development of the disease at field level, as well as the combination with other materials or polymers that make the nanostructure more stable and improve its properties. Therefore, the antifungal effects of Ag-Cs NPs on *Fusarium* wilt in tomato seedlings were investigated in the present study under *in vitro* and greenhouse conditions.

MATERIALS AND METHODS

Materials

All the reagents were of analytical grade and used without any further purification. Silver nitrate (AgNO₃), sodium borohydride and Chitosan of LMW were purchased from Sigma-Aldrich with a ≥99.5% purity. The wild fungi *Fusarium oxysporum* was isolated from infected plants in the Costa de Hermosillo, the strain was subcultured in potato dextrose agar (ThermoFisher scientific) for morphological identification.

Synthesis and characterization of silver nanoparticles

The silver nanoparticles coated with chitosan (Ag-Cs NPs) were synthesized according to the methodology proposed by Bin Ahmad *et al.* (2011). The chitosan solution was prepared by solubilizing 1.0 g of chitosan in 50 mL of acetic acid. Immediately afterwards, AgNO₃ (50 mL, 0.01 M) was added to the chitosan suspension under constant stirring for 2 h. Subsequently, 20 mL of NaBH₄ (0.04 M) were added to the AgNO₃-chitosan suspension, a color change from pale yellow to brown was immediately observed indicating the formation of the nanoparticles. The nanoparticles were characterized by evaluating the surface charge by means of a zetaseizer (Malvern Instruments) and the morphology and size were observed by transmission electron microscopy.

In vitro evaluation of the antifungal capacity of Ag-Cs NPs against *Fusarium oxysporum*

The antifungal effect of Ag-Cs NPs against the fungus *F. oxysporum* was evaluated. For this, the nanoparticles were dissolved at different concentrations (1000, 1500 and 2000 ppm) in sterile PDA medium at 45 °C and then, the medium was added to Petri dishes to solidification. The medium without addition of the nanoparticles was considered as the control treatment. Subsequently, *F. oxysporum* was inoculated from a culture of 7 days old in the center of the Petri dish. They were incubated at 25 °C for 7 days. Each treatment was performed in triplicate. The diameter of mycelial growth *in vitro* was determined, which was measured at 48, 96 and 168 h after the start of the experiment using a vernier and the results were expressed in cm.

Evaluation of Ag-Cs NPs in development of tomato seedlings

To determine if the of Ag-Cs NPs exerted any toxic effect, different concentrations of the treatment on tomato plants were evaluated. Tomato seedlings used in this experiment were 20 days old after germinated. On the substrate without or with 3% earthworm humus, 50 mL of the nanoparticles were applied at the concentrations of 1000, 1500 and 2000 ppm every 7 days for 30 days. The height and diameter of the plants were measured, as well as dry and fresh weight. Each treatment was carried out in triplicate.

Effect of NPs of Ag-Cs on the development of vascular wilt in tomato seedlings inoculated with *Fusarium oxysporum*

The bioassay was developed by preparing a spore suspension of the fungus at a concentration of 1 x 10⁷ spores per mL with a 30% glucose solution. Subsequently, a small wound was made at the neck of the root to facilitate the infection, and the seedlings of 20 days after germination, were immersed in the spores suspension for a period of 4 h. Seedlings were placed in Petri dishes filled with Peatmost® and incubated in a growth chamber for 14 days with 90% relative humidity at 25 °C. The nanoparticles were applied on the soil at a concentration of 2000 ppm five days after the inoculation. In the experiment, there was control of the disease, treatment control without disease and treatment with nanoparticles. Each treatment was performed with 10 repetitions. After 14 days the severity of the lesions caused by the disease, the height and diameter of the plants and the activity of the catalase enzyme were evaluated.

Statistical analysis

The experiments were carried out under a completely randomized design. To determine the effect of the treatment on the response variables, an analysis of variance of one or two routes was carried out, depending on the case, with a confidence index of 95%. The difference between the means of the treatments was compared using the Tukey-Kramer test. Differences at P <0.05 were considered significant. All this using the NCSS 2007 statistical package.

RESULTS AND DISCUSSION

Synthesis and Characterization of Nanoparticles

Nanotechnology has generated great interest in the development of agricultural products, since it represents an excellent opportunity to reduce the use of synthetic agrochemicals, with the possibility of reducing the environmental impact, and enhance the uptake of nutrients from the soil. Figure 1 shows the TEM micrographs of the NPs stabilized with chitosan and the size distribution of elaborated nanoparticles, since the morphology and size are extremely important to predict if the plant will be able to capture and biodistribute the NPs. The TEM images of the sample show spherical morphologies with less than 2% polydispersity, with a mean diameter of 33.26 ± 6.15 nm (Figure 1b). The functionalization and stabilization with chitosan can be clearly seen in Figure 1a with the contrast difference.

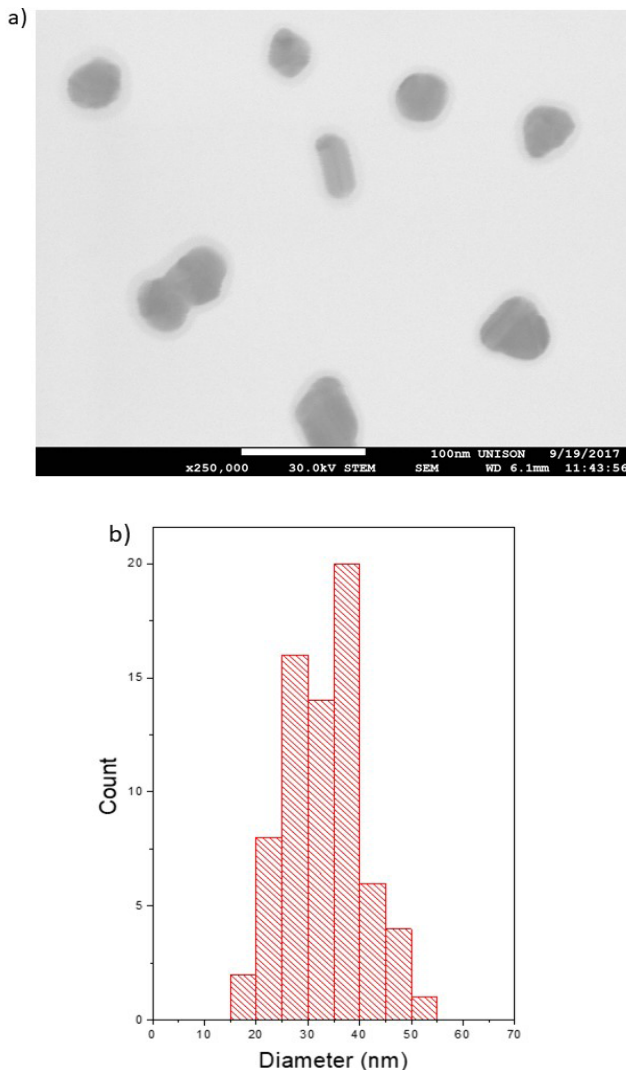


Figure 1. TEM micrographs of the Ag-Cs NPs. a) approach 250,000 x of the nanoparticles, b) size distribution of AgNPs-Cs.
 Figura 1. Micrografías TEM de las AgNPs-Cs. a) acercamiento 250,000 x de las nanopartículas, b) distribución de tamaño de AgNPs-Cs.

A number of variables, such as particle size, AgNP surface charge and the physicochemical features of the soil, largely govern the chemistry, fate and transport of the AgNPs in the soil system and, therefore, its bioavailability and its subsequent bioaccumulation in plant tissue (Grün *et al.*, 2019). A retention of AgNP has been evidenced in soil suspensions that correlated mainly with the clay content of the soil, and the authors advocated the role of the heterocoagulation of AgNP with natural colloids to explain this correlation (Grün *et al.*, 2019; Tolaymat *et al.*, 2010). Torrent *et al.* (2019) evidenced that cation exchange capacity and electrical conductivity are the main parameters controlling the adsorption processes in soil of AgNP with different sizes (45, 75, 200 and 200 nm) and coated with polymers of different charge (citrate, polyvinylpyrrolidone and polyethyleneglycol (PEG))

It has been reported that several stabilizing agents modify the surface properties of AgNP where their mobility can be altered by the electrostatic interaction of different types of soil. For example, a positively charged soil can prevent long-distance mobility of citrated AgNP negatively charged. Conversely, a negatively charged soil can make the AgNP more mobile in such soils. In this study we used Cs as a stabilizing agent and its surface charge was evaluated resulting in a potential ζ of around 50 (+), as a result of the polymer chains amino groups ionization. This promotes the absorption by clays for later incorporation by the plant, by cation exchange (Thio *et al.*, 2011).

Antifungal Activity of Nanoparticles

Figure 2 shows the *in vitro* antifungal capacity of Ag-Cs NPs against the phytopathogenic fungus *F. oxysporum*. The results show a larger diameter of the fungus colony at longer incubation times, which was expected since the conditions are ideal for the fungus growth. The highest fungal growth occurred in the control treatment with a final diam-

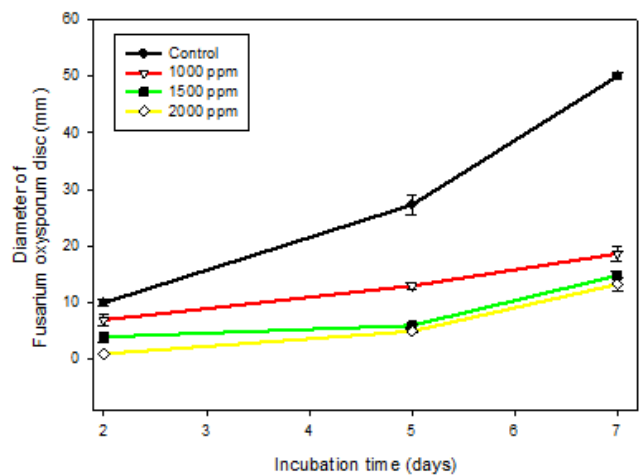


Figure 2. Antifungal capacity in vitro of silver nanoparticles coated with chitosan against *Fusarium oxysporum* in an incubation period of 7 days at 25 °C ($p > 0.05$).

Figura 2. Capacidad antifúngica in vitro de nanopartículas de plata recubiertas con quitosano contra *Fusarium oxysporum* en un periodo de incubación de 7 días a 25 °C.

eter of 50 mm. On the other hand, the nanostructures were significantly effective to inhibit mycelial growth, comparing it with the growth of the untreated fungus (control) ($p < 0.05$). Among the treatments, the nanoparticles at the 1000 ppm concentration managed to reduce the growth of the fungus by 64%, while the concentrations of 1500 and 2000 ppm were more effective ($p < 0.05$) since they managed to reduce fungal growth, 70% and 74% respectively, without statistical differences between them.

For many decades, silver (Ag^+) has been studied in its use in disinfection processes against several pathogenic microorganisms, since it has environmentally friendly characteristics and a powerful antimicrobial capacity. Kim *et al.* (2012) evaluated the use of AgNP at concentrations of 10, 25, 50 and 100 ppm against 18 phytopathogenic fungi on potato dextrose agar. The authors found a dose-dependent antifungal effect, observing 100% growth inhibition of the fungi *Alternaria brassicicola*, *Cylindrocarpo destrutans*, *Fusarium sp.*, *Pythium aphanidermatum* and *Pythium spinosum* at the highest concentration (Kim *et al.*, 2012).

In another study, the antifungal activity of Ag, copper and the mixture of these NPs against two phytopathogenic fungi, *A. alternata* and *Botrytis cinerea* was investigated (Ouda, 2014). In this study the metallic nanoparticles were applied at different concentrations, finding that at 15 ppm produced the maximum inhibition of the fungal hyphae growth. At this concentration the AgNP inhibited the growth by 59.3 and 52% of *A. alternata* and *B. cinerea*, respectively, being more effective than the copper nanoparticles and their mixture. It has been proposed that the antimicrobial effect of AgNP is different according to the microbial species.

The AgNP mode of antifungal action can be through morphological, structural and physiological changes, altering the cell membrane, affecting the fungus hyphae and conidia, producing reactive oxygen species (ROS) that cause damage to proteins, lipids and nucleic acids (Ouda, 2014). Dasgupta and Ramalingam (2016) showed that the main mechanism of nano-silver formulations is ROS generation and increase in membrane permeabilization. In addition, they can have a detrimental effect on sugar, proteins, n-acetyl glucosamine and lipids of the cellular filtrate and cell wall components of phytopathogens. They have also been associated with the union and penetration in the cell membrane to kill spores, although this mechanism is not completely known (Pietrzak *et al.*, 2015). Kumari *et al.* (2019) demonstrated that after membrane disintegrity, the cells lead to death by loss of membrane structure and osmotic balance in AgNP treated fungus; additionally, they observed collapse of redox homeostasis, antioxidant machinery, cellular virulence and damage of cell wall and membrane by disrupting osmotic balance as major mechanisms.

Evaluation of Ag-Cs NPs in development of tomato seedlings

In order to ensure that the nanoparticles did not have any harmful effect on the tomato plants, we evaluated their

effect, at different concentrations, on the size of the plant in height and diameter of the stem and dry weight of leaves, stem and root. In addition, this was done in the absence or presence of earthworm humus in order to improve the cation exchange of the substrate (Berilli *et al.*, 2018).

Figure 3 shows the results of tomato seedling height after being exposed to Ag-Cs NPs with and without 3% humus. Three different doses of the treatment were compared (1000, 1500, and 2000 ppm) and it can be observed that the height of the control and treated seedlings with the doses of 1000 and 1500 ppm with and without humus and the 2000 ppm without humus did not show significant difference between them ($p < 0.05$). On the other hand, the seedlings to which the highest concentration with humus was applied showed greater development after 30 days of the application, presenting a significant difference with the control and the rest of the treatments with and without humus ($p < 0.05$). These results indicate that silver nanostructures have no toxic effect on plants and on the contrary it seems to benefit their development.

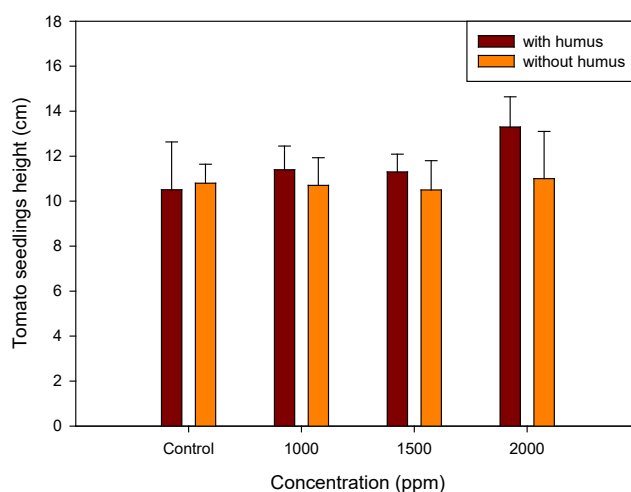


Figure 3. Height of tomato seedlings exposed to different concentrations of silver nanoparticles coated with chitosan at 30 days after application.

Figura 3. Altura de plántulas de tomate expuestas a diferentes concentraciones de nanopartículas de plata recubiertas de quitosano a los 30 días después de la aplicación.

Figure 4 shows the results of dry weight and fresh weight of root, stem and leaf of tomato seedlings after 30 days of the first application of Ag-Cs NPs in the presence and absence of earthworm humus. It can be observed that the control treatment had the lowest dry and fresh weight while there is a significant difference due to the effect of the nanoparticles; although there is no clear trend in the concentrations of the treatments. Comparing the effect of earthworm humus, the presence of humus significantly favors the increase in tomato seedlings biomass with the three nanoparticles concentrations. This can be attributed to the fact that earthworm humus allows a greater occurrence of cation exchange reactions and this improves the absorption

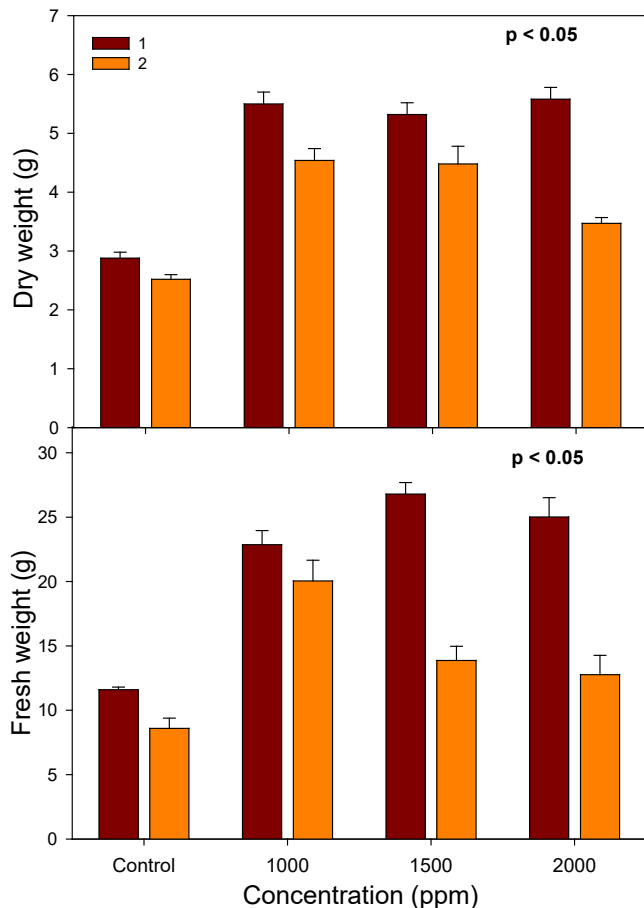


Figure 4. A) Dry weight and B) Fresh weight of tomato seedlings (stem, root and leaf) exposed to different concentrations of silver nanoparticles coated with chitosan at 30 days after application. Treatment 1 is with earthworm humus and Treatment 2 is without earthworm humus. $p < 0.05$ indicates significant differences between all treatments.

Figura 4. A) Peso seco y B) Peso fresco de plántulas de tomate (tallo, raíz y hoja) expuestas a diferentes concentraciones de nanopartículas de plata recubiertas de quitosano a los 30 días después de la aplicación. $p < 0.05$ indica diferencias significativas entre todos los tratamientos.

of silver particles in the soil (Anjum *et al.*, 2013; De Farias *et al.*, 2018).

The results of our study showed that the humus increases the height of the plants at high silver concentrations, since it plays a role in the absorption of silver ions by the root, due to the improvement in the capacity of cation exchange in the soil, which allows a greater mobility of Ag^+ ions and retention of these to be taken by the rhizosphere.

Several studies have evaluated the effect of AgNP application on the vegetative development in different crops. Raliya *et al.* (2015) applied zinc oxide and titanium nanoparticles on tomato crops, finding significant differences in vegetative development with a 25% increase in the best treatment. Likewise, AgNP-Cs (0.1% w / v) showed a growth promoting effect on the germination of chickpea seed, seedling length, fresh weight and dry weight (Anusuya & Banu, 2016). In addition, the treatment increased the

chlorophyll content and the activity of the α , β -amylase enzymes, ascorbate peroxidase, peroxidase and catalase. The authors report that the possible reason for the growth rate increase, is a greater absorption of inorganic nutrients that accelerated the decomposition of organic substances during the photosynthetic process, which increased the rate of photosynthesis. In addition, the key to increase the germination rate of the seed is the penetration of nanomaterials in the seed (Anusuya & Banu, 2016).

However, some studies report adverse effects with the application of AgNP. Song *et al.* (2013) found a decrease in the biomass of tomato seedlings at the lowest concentration evaluated. In another study, *Lupinus termis L.* seedlings were exposed to 0.5 mg/L of AgNP for ten days, with significant reductions observed in growth parameters such as sprouting, root lengthening and fresh weight (Al-Huqail *et al.*, 2018). In addition, the treatment caused metabolic disorders such as reduction of total chlorophyll, sugars and proteins content. This can be attributed to the characteristics of the plants, the release of toxic ions, as well as the size and shape of the particles that stimulate stress. However, at lower concentrations these effects were not observed.

In summary, the use of Ag-Cs NPs at the three concentrations evaluated did not present a toxic effect after application every 7 days for 30 days in tomato seedlings. In addition, the earthworm humus with the three concentrations of the nanoparticles favored the height, the diameter of the stem and the dry and fresh weight of the seedlings biomass in comparison with the control seedlings, emphasizing this effect at the concentration of 2000 ppm.

Effect of NPs of Ag-Cs on the development of vascular wilt in tomato seedlings inoculated with *Fusarium oxysporum*

The tomato seedlings were infected with *F. oxysporum*, observing wilt and yellowing symptoms after 14 days of inoculation (Figure 5). On the other hand, control seedlings without inoculation and those treated only with Ag-Cs NPs had a higher number of leaves and a bright green color compared to infected seedlings. The group treated with Ag-Cs NPs, managed to reduce the severity of the disease with aspect very similar to control group, after 5 days of inoculation.

Figure 6 shows the height and diameter of the stem of the tomato seedlings with the different treatments. The development of the Ag-Cs NPs treated seedlings, after 5 days of inoculation, did not affect the height and diameter of the plants stem ($p > 0.05$), since no significant difference was observed against the control seedlings. Therefore, the treatment can be said did not work as a growth promoter, although it was not the objective of our study.

Figure 7 shows the dry weight of tomato seedlings 14 days after inoculation with a *Fusarium oxysporum* spores solution, and it can be seen that there were significant differences at the end of the experiment with respect to the treatment without application of Ag-Cs NPs ($p > 0.05$). On the other hand, it did not show differences regarding the control, demonstrating that the application of Ag-Cs NPs, for the

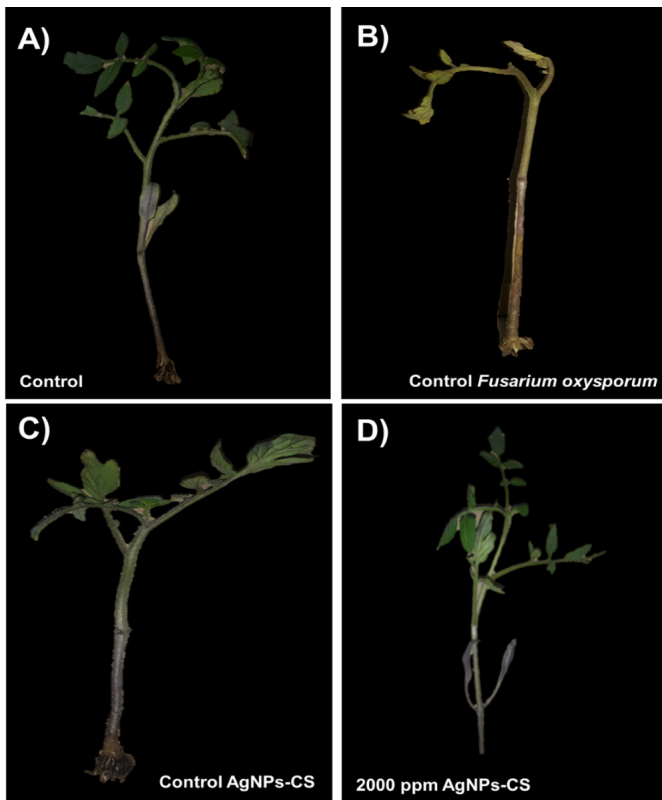


Figure 5. Tomato seedlings after 14 days in an incubation chamber at 25 °C. A) uninoculated seedlings, B) seedlings inoculated with *Fusarium oxysporum*, C) uninoculated seedlings treated with Ags-CS NPs, D) seedlings inoculated and treated with 2000 ppm AgNPs-CS.

Figura 5. Plántulas de tomate durante 14 días en una cámara de incubación a 25°C. A) Plántulas sin infectar, B) Plántulas infectadas con *Fusarium oxysporum*, C) Plántulas sin infectar tratadas con nanopartículas de plata recubiertas de quitosano (AgNPs-CS), D) Plántulas infectadas y tratadas con 2000 ppm de AgNPs-CS.

control of phytopathogens such as *F. oxysporum*, could be an alternative to eradicate vascular wilt in tomato seedlings and it can work as a corrective treatment.

Table 1 shows the enzymatic activity of catalase in tomato seedlings with the different treatments. With respect to time, the catalase activity increases with the days of incubation in most treatments. Compared to control, *F. oxysporum* fungus infected tomato seedlings and treated with Ag-Cs NPs 5 days after inoculation, showed higher catalase activity (130%), followed by tomato seedlings only inoculated (128%). The control seedlings without infection and without exposure to the nanoparticles showed the lowest catalase activity. Catalase is a key antioxidant enzyme in the plant's defense system against pathogens; therefore, these results indicate that both inoculation and the nanoparticles induces the defense system of the plants.

Ag-Cs NPs showed significant antifungal activity in plant inoculation experiments and AgNP can bind and penetrate directly into the cell membrane to kill the fungus spores, although the AgNP penetration into the microbial cells membranes has not been fully understood (Ouda, 2014). Because the plants are of large foliar area and are of a stationary nature, they have a higher probability of exposure

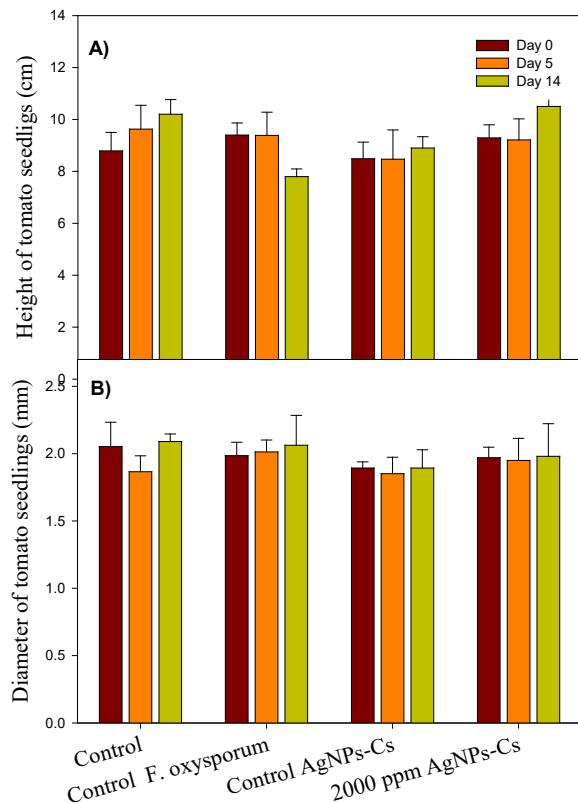


Figure 6. A) Height and B) diameter of tomato seedlings with different treatments during 14 days of incubation at 25 °C 90% relative humidity. **Figura 6.** A) Altura y B) diámetro de plántulas de tomate con diferentes tratamientos durante 14 días de incubación a 25°C 90% de humedad relativa.

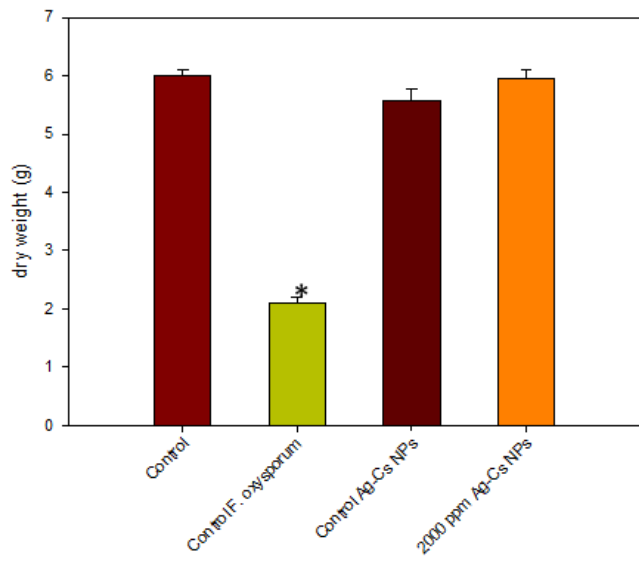


Figure 7. Dry weight of tomato seedlings (stem, root and leaf) exposed to the treatment of silver nanoparticles coated with chitosan (2000 ppm). * indicates significant differences between all treatments ($p < 0.05$). **Figura 7.** Peso seco de plántulas de tomate (tallo, raíz y hoja) expuestas al tratamiento nanopartículas de plata recubiertas de quitosano (2000 ppm). * indica diferencias significativas entre todos los tratamientos ($p < 0.05$).

Table 1. Activity of the catalase enzyme of tomato seedlings 14 days after being exposed to the silver nanoparticles coated with chitosan.**Tabla 1.** Actividad de la enzima catalasa de plantas de tomate durante 14 días después de ser expuestas a las nanopartículas de plata recubiertas de quitosano.

Days	Treatments	Catalase Activity (U/mL)
0	Control	12.22 ± 3.55
7		16.26 ± 2.19
14		16.37 ± 2.05
0	Control Ag-CS NPs	9.24 ± 0.05
7		15.83 ± 0.54
14		16.92 ± 0.94
0	Control <i>F. oxysporum</i>	12.49 ± 0.67
7		21.95 ± 4.10
14		21.09 ± 1.21
0	2000 ppm Ag-CS NPs	13.72 ± 0.82
7		21.32 ± 7.08
14		26.69 ± 3.83

to a wide range of nanoparticles available in their environment (Anjum *et al.*, 2013). The main route of plants exposure to nanoparticles is through the soil through leaching, direct application with deliberate release or from other products (Rajput *et al.*, 2018).

The plants roots have been considered as the main exposure route of plants to NP, which can harbor a significant part of the accumulated nanoparticles, which may or may not produce physical or chemical toxicity in plant (Anjum *et al.*, 2013). A study shows that shoots of *Cucurbita pepo* presented 4.7 more silver concentration in AgNP treated plants that those treated with a bulk solution at the same concentrations, considering a greater silver release from the nanoparticles as the main reason (Stampoulis *et al.*, 2009). Another study has demonstrated the uptake of AgNP through the roots of *Arabidopsis thaliana*, where although most of the nanoparticles adhere to the root boundary, their transport to shoots was also observed (Milewska-Hendel *et al.*, 2019). The possible mechanism of nanoparticles absorption from plants can be through primary roots or lateral roots. Then, they are transported from the root through the stem to the leaf. They could also be adsorbed on the surface of the roots (Tripathi *et al.*, 2017). A study on the uptake of AgNP and Ag⁺ in rice plants, observed a higher silver uptake when it was in NP, compared with that of Ag⁺, this can be explained due the formation of AgCl on the root surface reducing the bioavailability and translocation of Ag and the direct internalization of AgNPs by plant (Yang *et al.*, 2020).

There are few studies that evaluate the effect of metallic nanoparticles in plants. It has been previously reported that AgNP were effective in reducing 50% of the phytopathogens *Bipolaris sorokiniana* and *Magnaporthe grisea* growth on perennial ryegrass (*Lolium perenne*) (Jo *et al.*, 2009). These authors conclude that the effectiveness of nanoparticles is greater with a preventive application due to one that promo-

tes the direct contact of silver with spores and germ tubes to inhibit its viability. In our study, we first inoculated, and then applied the treatment, which would be interesting to evaluate the effect of the nanoparticles in a preventive manner. In a more recent study, Nejad *et al.* (2016) were able to control disease progress of rice plants inoculated with *Rhizotocnia solani* using AgNP in a concentration-dependent manner, this investigation suggests AgNP can replace chemical pesticides in controlling and inhibiting sheath blight, a common disease in rice.

CONCLUSION

In this study silver nanoparticles coated with chitosan were synthesized with the purpose of being used as control for vascular wilt disease caused by *F. oxysporum* in tomato seedlings. The NPs showed antifungal activity *in vitro* and did not affect the normal growth of the plant or show any toxic effect in the tomato seedlings. In addition, the synthesized nanoparticles were effective in eradication of the disease caused by *F. oxysporum*. Our results indicate that nanotechnology is a promising strategy for the control of phytopathogens in agricultural crops.

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