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Article

Application of silver nanoparticles and their relationship with the growth of strawberry

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Abstract

The use of silver nanoparticles has increased recently due to their application in different areas of science. In plants, they have shown different effects depending on the species and concentration. The present research aimed to evaluate the effect of applying silver nanoparticles via the leaves and roots at increasing doses on the growth of plants of strawberry (*Fragaria* x *ananassa* Duch.) cultivar Festival. The experiment was established in a greenhouse in the experimental agricultural field of the UACH, Texcoco, State of Mexico, in 2022 and 2023. Strawberry plants of the Festival cultivar were used as plant material and placed in an open hydroponic system. Subsequently, silver nanoparticles were applied via the leaves (0, 40, 80, 120 and 160 mg L^{-1}) and roots (0, 5, 10, 15 and 20 mg L^{-1}), respectively. At 56 days after the start of treatments, plant height, crown diameter, number of leaves, number of runners, number of flowers and number of fruits were recorded, showing as results that foliar dosing of silver nanoparticles increased crown diameter (25.8%), number of leaves (19.8%), number of flowers (18.3%), number of fruits (28.3%) and number of runners (50%), and root dosing increased plant height (11.9%), number of leaves (14.9%), number of flowers (21.1%), number of fruits (31.8%), and number of runners (31.8%). In conclusion, the application of silver nanoparticles via the leaves and roots increases the growth variables measured in strawberry plants of the Festival cultivar; therefore, silver nanoparticles are an alternative for food sovereignty.

Palabras clave:

Fragaria x ananassa Duch., berries, development, nanotechnology.

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Introduction

The development of agricultural inputs based on nanomaterials, such as nanofertilizers and nanopesticides, offers great potential to improve the application of agricultural inputs by suppressing pathogenic activity and improving crop yield and quality. AgNPs are currently incorporated into various consumer products, such as textiles, cosmetics, paints, and food packaging (Zhang *et al*., 2019). Currently, the agricultural sector must produce in a more economically feasible way and with the least environmental impact, with nanotechnology being an alternative in sustainable agriculture (Singh *et al*., 2020).

Certain reports highlight that biosynthesized nanoparticles have a lower level of toxicity than nanoparticles synthesized by chemical methods due to the stability they show with organic compounds, thus reducing toxic residues both in their synthesis and in their agronomic application (Sadak, 2019). Higher plants use the chemical properties of AgNPs for the catalysis of key reactions, such as active sites of many enzymes, or to maintain protein structure, so they are required in minimal quantities for good cell metabolism (Adisa *et al*., 2019).

In aspects of plant nutrition, the access process occurs when AgNPs are potentially bioavailable at the root level, in turn, absorption occurs when AgNPs cross the cell membrane and thus, the transport of these silver nanoparticles occurs in the vascular systems, that is, at the level of xylem and phloem, and finally, assimilation occurs when AgNPs are incorporated into plant metabolism, generating a certain effect (Ur Rahim *et al*., 2021).

A study on the crops of cowpeas (*Vigna sinensis*) and wheat (*Triticum aestivum*) showed that the application of 50 mg L^{-1} and 70 mg L^{-1} AgNPs, respectively, increased growth and biomass by stimulating physiological, biochemical, and agronomic activity; hence, AgNPs stimulate growth in different crops (Yan and Chen, 2019).

Recently, the cytotoxic and genotoxic activity in the onion (*Allium cepa*) crop was investigated through the use of Bionag Argovit™ AgNPs, where it was evaluated in a concentration range from 5 to 100 µg ml^{-1}; that is, 10 to 17 times higher than that used for other silver nanoparticle formulations with polyvinylpyrrolidone AgNPs-PVP in other crops.

As a result, there was no cytotoxic or genotoxic damage in the concentrations evaluated, highlighting that the metal-coating agent ratio plays a fundamental role in this response and should be considered within the key physicochemical parameters for the design and manufacture of safer nanomaterials in agriculture (Casillas-Figueroa *et al*., 2020).

In Mexico, in 2022, the area allocated to the production of strawberry was 12 913 ha; that is, approximately 1% of the national territory, with a production of 578 141 t (SIAP, 2024). Therefore, it is of utmost importance to increase the yield and nutritional characteristics of the strawberry fruits. Limited research has been done on the effects of AgNPs on strawberry growth. This study aimed to evaluate the effect of applying AgNPs via the leaves and roots at increasing doses on the growth of plants of strawberry (*Fragaria* x *ananassa* Duch.) cultivar Festival.

Materials and methods

The experiment was carried out in the experimental agricultural field of the Chapingo Autonomous University, Texcoco, State of Mexico, in 2022 and 2023. The experimental agricultural field is located at 19.4661° north latitude and -98.8538° west longitude at an altitude of 2 250 masl.

Silver nanoparticles

The commercial AgNPs formulation Bionag Argovit™ (Vector-Vita Research and Production Center, Novosibirsk, Russia) was used, which contains 12 mg ml^{-1} metallic silver and 188 mg ml^{-1} PVP of 15-30 kD in water, an average content of 20% AgNPs (200 mg L^{-1} AgNPs) with an average hydrodynamic diameter of metallic silver with PVP of 70 nm (Juárez-Moreno *et al*., 2016).

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Plant material

The strawberry seedlings used were from the Festival cultivar from Zamora, Michoacán, with initial morphological characteristics of leaves (5), plant height (11.2 cm) and crown diameter (1.3 cm).

Experiment management

Polyethylene bags of 30 x 30 cm were used, where tezontle with a granulometry of 4 mm was utilized as a substrate. The nutrition of the plants was carried out with Steiner's (1984) universal nutrient solution, which in complete form is constituted by (in mol cm^{-3}): 12 of NO_3^-, 1 of $H_2PO_4^-$, 7 of SO_4^{-2}, 7 K^+, 9 of Ca^{+2} and 4 of Mg^+. The process of establishing the experiments for the AgNPs application via the leaves and roots began with the disinfection of the tezontle substrate with sodium hypochlorite at a dose of 40 ml L^{-1}, immersing the tezontle in the disinfectant solution for 60 minutes and then extracting and rinsing it with water for three times, ending with its solarization for three days.

Subsequently, drip irrigation systems were established to continue with the transplantation of Festival strawberry plants, which took place on September 2, 2022. The distance between the plants was 30 cm and between blocks 1 m, placing a shade cloth during the first 15 days to reach temperature (18 °C) and relative humidity (70%) levels. At seven days after transplanting, Steiner's universal nutrient solution was applied at a concentration of 30% to help standardize the strawberry plants.

At 45 days after transplantation, Steiner's nutrient solution was applied at a concentration of 60%. At 90 days after transplantation, in the phenological stage of fruiting, Steiner's nutrient solution was applied at a concentration of 100%. Strawberry plants were irrigated three times per day for 7 min for each irrigation throughout the crop's biological cycle.

Application of silver nanoparticles

The experiments for the application of silver nanoparticles via the leaves and roots consisted of cinco treatments each, which were 0, 40, 80, 120 and 160 mg L^{-1} and 0, 5, 10, 15 and 20 mg L^{-1} AgNPs, respectively, applied at ocho different intervals; that is, at 7, 14, 21, 28, 35, 42, 49 and 56 days after transplantation. The doses applied were selected after a comprehensive literature review of previous research on the application of AgNPs in plants.

The treatments with the highest doses of the experiments for silver nanoparticle application through the leaves and roots were 160 mg L^{-1} and 20 mg L^{-1}, respectively, and were selected considering the toxicity of metals in higher plants (Rezvani *et al*., 2012).

Experimental design

The experimental design used was the completely randomized block design (CRBD), establishing five blocks with five treatments, where each treatment was tested four times per experiment, where they were differentiated by the way of applying AgNPs, which was through the leaves and roots, resulting in a total of 100 experimental units per experiment. The experimental unit was a 30 x 30 cm black polyethylene bag containing a Festival strawberry plant.

Morphological trait measurement

For the experiments on the application of silver nanoparticles via the leaves and roots, 25 plants from each experiment were randomly selected for data collection. Five plants per treatment of each respective experiment were marked to estimate growth. At 56 days after the first application of AgNPs, data were collected for the following growth variables.

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Growth

For each experiment, plants randomly selected were used to evaluate growth variables. Plant height (cm), crown diameter (cm), number of leaves, number of flowers, number of fruits, and number of runners were recorded and the data obtained were averaged.

Statistical analysis

The data obtained were used to perform an analysis of variance and Tukey's means comparison test, with a significance level of 0.05, using the Sas 9.4 statistical package.

Results and discussion

Growth variables by applying silver nanoparticles via the leaves and roots

The foliar and root application of silver nanoparticles influenced strawberry growth variables, so the results of the means comparison tests are presented below (Table 1 and 2).

Table 1. Plant height (cm), crown diameter (cm), number of leaves, number of flowers, number of fruits and number of runners of strawberry cultivar Festival, sprayed with 0, 40, 80, 120 and 160 mg L^{-1} of Bionag Argovit^{TM} AgNPs, at 56 days after the start of foliar treatments.

Treat. of AgNPs (mg L^{-1})	Plant height (cm)	Crown diameter (cm)	Num. of leaves	Num. of flowers	Num. of fruits	Num. of runners
0	17.5 ±0.8a	1.8 ±0.1c	10.8 ±0.6b	6.9 ±1c	6.7 ±0.7c	1.6 ±0.3b
40	18.4 ±0.1a	1.9 ±0.1bc	10.9 ±0.6b	7.7 ±0.6ab	8.6 ±0.4a	2.1 ±0.3a
80	18.2 ±1a	1.9 ±0.2b	12.6 ±1.1a	7.3 ±0.6bc	7.6 ±0.5b	2.4 ±0.3a
120	18.1 ±0.3a	2.2 ±0.1a	13 ±1.1	8.1 ±0.9a	7.9 ±0.9ab	2.2 ±0.4a
160	18.1 ±0.8a	2.0 ±0.1b	11.6 ±0.6b	8.2 ±0.4a	7.8 ±0.4b	2.1 ±0.4a
HMSD	0.939	0.174	0.921	0.774	0.752	0.449
R^2	0.228	0.3	0.443	0.385	0.292	0.184
CV	8.255	13.997	12.447	16.139	15.502	34.04

Means with different letters in each column indicate statistical differences between treatments (Tukey, $p \leq 0.05$). AgNPs= silver nanoparticles.

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Table 2. Plant height (cm), crown diameter (cm), number of leaves, number of flowers, number of fruits and number of runners of strawberry cultivar Festival, sprayed with 0, 5, 10, 15, and 20 mg L^{-1} Bionag Argovit™ AgNPs, at 56 days after the start of root treatments.

Treat. of AgNPs (mg L^{-1})	Plant height (cm)	Crown diameter (cm)	Num. of leaves	Num. of flowers	Num. of fruits	Num. of runners
0	17.3 ±0.8c	1.9 ±0.2a	10.3 ±0.6b	7.3 ±0.9b	5.5 ±0.9b	2 ±0.7b
5	18.3 ±0.9b	2.1 ±0.2a	10.6 ±1.1b	8.6 ±0.6a	7.2 ±1.3a	2.7 ±0.4a
10	18.5 ±0.9b	2.1 ±0.2a	11.9 ±0.9a	8.1 ±0.8ab	7.1 ±1.1a	3 ±0.3a
15	19.4 ±0.5a	2 ±0.2a	11.1 ±1.2ab	8.9 ±1.4a	7 ±0.6a	2.6 ±0.2a
20	19.1 ±0.5ab	2 ±0.1a	11.1 ±0.6ab	8 ±0.7ab	7.2 ±1.3a	2.8 ±0.2a
HMSD	0.769	0.199	1.116	0.851	1.312	0.483
R^2	0.506	0.333	0.226	0.512	0.182	0.32
CV	6.611	15.538	16.13	16.506	30.796	29.26

Means with different letters in each column indicate statistical differences between treatments (Tukey, $p\leq 0.05$). AgNPs= silver nanoparticles.

Plant height

Plant height was not affected by foliar application of 40, 80, 120, and 160 mg L^{-1} AgNPs compared to the 0 mg L^{-1} control treatment (Table 1). On the other hand, plant height (19.38 cm) was affected through the root application of 15 mg L^{-1} AgNPs. There were no statistically significant differences in plant height when applying 5, 10, and 20 mg L^{-1} via the roots (Table 2).

In this regard, Rezvani *et al*. (2012) in saffron (*Crocus sativus*) and Sadak (2019) in fenugreek (*Trigonella foenum-graecum*) found that applications of silver nanoparticles of 40 mg L^{-1} increased plant height and they mention that AgNPs act by stimulating the increase in the levels of indole-3-acetic acid, which is a precursor of auxins, and the decrease in ethylene levels.

Crown diameter

The treatment that generated the largest crown diameter (2.24 cm) was the foliar application of 120 mg L^{-1} AgNPs. There were no statistically significant differences in crown diameter when applying 0, 40, 80, and 160 mg L^{-1} foliarly (Table 1). On the other hand, crown diameter was not affected by the root application of 5, 10, 15, and 20 mg L^{-1} AgNPs compared to the 0 mg L^{-1} control treatment (Table 2).

Increases in stem diameter (cm) were reported by Gupta *et al*. (2018) in rice (*Oryza sativa* L.), Das *et al*. (2018) in beans (*Phaseolus vulgaris*), Byczy#ska *et al*. (2019) in tulip (*Tulipa gesneriana* L.) and Alkaç *et al*. (2022) in lilies; they found that applications of silver nanoparticles of 40, 50, 100 and 50 mg L^{-1}, respectively, increased stem diameter.

It is highlighted that AgNPs intervene through multiple hormonal signaling pathways, optimizing the process of synthesis and translocation of carbohydrates and proteins and in turn, making the absorption process of the macroelement nitrogen more efficient, which optimizes the synthesis of proteins, specifically glycoproteins, which are essential components of the cell wall in higher plants.

Number of leaves

The plants that had the highest number of leaves (12.6 and 13) were those treated with the foliar application of 80 and 120 mg L^{-1} AgNPs. There were no statistically significant differences in the number of leaves when applying 0, 40, and 160 mg L^{-1} through the leaves (Table 1). However, the treatment that also generated the highest number of leaves (11.9) was the root application of 10 mg

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L^{-1} AgNPs. There were no statistically significant differences in the number of leaves when applying 0, 5, 15, and 20 mg L^{-1} via the roots (Table 2).

Increases in leaf numbers were reported by El-Batal *et al*. (2016) in beans (*Phaseolus vulgaris* L.), Vinkovik *et al*. (2017) in chili pepper (*Capsicum annuum* L.), and Sarmast and Salehi (2021) in tobacco (*Nicotiana tabacum*); they discovered that applications of silver nanoparticles of 10, 0.01, and 50 mg L^{-1}, respectively, increased the number of leaves, emphasizing that the applied AgNPs act as suppressors of ethylene genes and signaling pathways, inhibiting leaf abscission and consequently, biostimulating the genes and signaling pathways of auxins and cytokinins that stimulate cell division.

Number of flowers

The treatment that generated the highest number of flowers (8.1 and 8.16) was the foliar application of 120 and 160 mg L^{-1} AgNPs. There were no statistically significant differences in the number of flowers when applying 0, 40 and 80 mg L^{-1} via the leaves (Table 1). On the other hand, the number of flowers (8.65 and 8.9) was also affected by the root application of 5 and 15 mg L^{-1} AgNPs. There were no statistically significant differences in the number of flowers when applying 0, 10 and 20 mg L^{-1} via the roots (Table 2).

In this regard, Safa *et al*. (2015) in Gerbera daisy (*Gerbera jamesonii* cv. 'Balance') and Salachna *et al*. (2019) in oriental lilies (*Lilium* cv. Mona Lisa) detected that applications of silver nanoparticles of 10, 50, and 100 mg L^{-1}, respectively, increased the number of flowers per plant, mentioning that AgNPs act as a preventive treatment for the presence of bacteria and fungi.

Likewise, AgNPs correlate with the accumulation of proteins associated with the cell cycle and carbohydrate metabolism and with changes in the expression of genes involved in multiple cellular processes, such as cell proliferation, photosynthesis, and signaling pathways of hormones, such as auxins, abscisic acid, and ethylene.

Number of fruits

The number of fruits (8.6) was affected by foliar application of 40 mg L^{-1} AgNPs. There were no statistically significant differences in the number of fruits when applying 80, 120, and 160 mg L^{-1} foliarly (Table 1). On the other hand, the treatment that also generated the highest number of fruits (7.25, 7.1, 7.05 and 7.16) was the root application of 5, 10, 15 and 20 mg L^{-1} AgNPs compared to the control treatment of 0 mg L^{-1} (Table 2).

Increases in fruit number were reported by Shams *et al*. (2013) in cucumber (*Cucumis sativus* cv. 'Negeen') and Younes and Nassef (2016) in tomato (*Solanum lycopersicum* Mill.); they found that applications of silver nanoparticles of 300 and 10 mg L^{-1} increased the number of fruits per plant, highlighting that AgNPs intervene by activating the enzyme superoxide dismutase (SOD), which decreases stress levels, favoring optimal growth and development.

Likewise, AgNPs act by increasing the concentration of the cysteine complex by activating the enzyme phytochelatin synthase (gamma-glutamylcysteine dipeptidyl transpeptidase), making the protection of living cells more efficient, reducing oxidative stress and resulting in the optimization of photosynthesis and respiration.

Number of runners

The plants that had the highest number of runners (2.1, 2.4, 2.25 and 2.1) were those treated with foliar application of 40, 80, 120 and 160 mg L^{-1} compared to the control treatment of 0 mg L^{-1} (Table 1). On the other hand, the treatment that also generated the highest number of runners (2.75, 3, 2.6, and 2.79) was the root application of 5, 10, 15, and 20 mg L^{-1} AgNPs compared to the control treatment of 0 mg L^{-1} (Table 2).

In this regard, Abbas and Abdulhussein (2021); Tung *et al*. (2021) in strawberry (*Fragaria* x *ananassa* Duch.) found that applications of silver nanoparticles of 10 mg L^{-1} increased the number of

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runners, mentioning that AgNPs, according to their size and shape, influence the ability of the higher plant to access, absorb, translocate and assimilate them to activate various enzymatic mechanisms involved in anabolic and catabolic processes, such as photosynthesis and respiration.

Likewise, AgNPs act by inhibiting the formation and activity of ethylene, delaying senescence by suppressing the activity of hydrolytic enzymes, cellulases and pectinases, and consequently, biostimulating the synthesis of growth regulators, auxins and cytokinins, for the generation of new shoots.

Conclusions

Under the recent reports of the present research work and according to the effect of silver nanoparticles, the foliar applications of AgNPs from 40 to 120 mg L^{-1} considerably improved the growth variables of crown diameter (cm), number of leaves, number of flowers, number of fruits, and number of runners; likewise, the root application of AgNPs from 10 to 15 mg L^{-1} improved the growth variables of plant height (cm), number of leaves, number of flowers, number of fruits, and number of runners. This shows that AgNPs can achieve an increase in yield, decrease the costs and time of production of strawberry cultivar Festival, and be a viable alternative in agriculture and consequently, in Mexico’s food sovereignty.

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