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Article

Silver nanoparticles in strawberry quality

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Abstract

Silver nanoparticles, being inorganic biostimulants in strawberry crops, can serve as food preservative compounds. The present research was conducted with the aim of evaluating the effect of applying silver nanoparticles via leaves and roots at increasing doses on the quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivar Festival. The experiment was established in a greenhouse in the experimental agricultural field of the Chapingo Autonomous University, Texcoco, State of Mexico, in 2022 and 2023 (latitude 19.4661, longitude -98.8538). Strawberry plants of the Festival cultivar were used as plant material and placed in an open hydroponic system. The treatments of 0, 40, 80, 120, 160 and 0, 5, 10, 15, 20 mg L^{-1} silver nanoparticles were applied via leaves and roots, respectively. At 70 days after the start of treatments, fresh weight, firmness, pH, degrees brix, colorimetry, vitamin C, total soluble proteins, total phenols and anthocyanins were determined. The results showed that the foliar application of silver nanoparticles increased fresh weight, firmness, pH, degrees brix, colorimetry, total phenols, and anthocyanins, and the root application of silver nanoparticles increased firmness, pH, degrees brix, colorimetry, vitamin C, total soluble proteins, total phenols, and anthocyanins. The applications of silver nanoparticles via leaves and roots conclusively increased the quality indicators of strawberry fruits of the Festival cultivar; this makes silver nanoparticles a viable alternative in Mexico's food sovereignty.

Keywords:

Fragaria x ananassa Duch., conservation, indicators, nanotechnology.

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Introduction

Nanotechnology is a technology, science and engineering that stands out because nanoscience is the study of structures at nanometer scales ranging from 1 to 100 nm and nanotechnology uses it in practical, innovative and promising applications in various sciences, such as agriculture. Worldwide, the production of metal nanoparticles is increasing, and they are a productivity factor in higher plants of food interest due to the improvement of biochemical, physiological and agronomic processes (Phogat *et al*., 2018).

Silver nanoparticles (AgNPs) have the ability to improve the quality indicators of different fruits because they behave as inorganic biostimulants, improving nutritional efficiency, tolerance to abiotic stress and crop quality in plants.

To obtain a positive effect of AgNPs on the development of higher plants and consequently on fruit quality indicators, the processes of access, absorption, translocation and assimilation of AgNPs must occur, which depend on their concentration, dimension and coating (Wang *et al*., 2023). On the other hand, the bioactive compounds present in fruits, such as strawberries, are important because they generate a functional food for humans.

Among the bioactive compounds, flavonoids stand out, which include anthocyanins and flavonols, followed by hydrolysable tannins, which include ellagitannins and gallotannins, and finally, phenolic acids, which include hydroxybenzoic acids and hydroxycinnamic acids. Bioactive compounds tend to increase their content in strawberry fruits due to the activation of the enzyme phenylalanine ammonia lyase (PAL) by AgNPs (Shahzad *et al*., 2024).

In Mexico, the area allocated to strawberry production in 2022 was 12 913 ha; that is, approximately 1% of the national area, with a production of 578 141 t (SIAP, 2024), showing that it is very important to increase the production and quality of this crop. Finally, this research aimed to evaluate the effect of applying AgNPs via leaves and roots at increasing doses on the quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivar Festival.

Materials and methods

Study site selection

The experiment was conducted in the experimental agricultural field of the Chapingo Autonomous University, Texcoco, State of Mexico, from August 2022 to June 2023, and it is located at 19.4661° north latitude and -98.8538° west longitude at an altitude of 2 250 m. The determination of the quality indicators was carried out at the Multipurpose Laboratory of the Chapingo Autonomous University, Texcoco, State of Mexico, from January to July 2024, and it is located at 19.3614° north latitude and -98.8867° west longitude at an altitude of 2 250 m.

Obtaining silver nanoparticles

The AgNPs used were those of the commercial formulation Bionag Argovit™, which contains 12 mg ml^{-1} metallic silver and 188 mg ml^{-1} polyvinylpyrrolidone (PVP) of 15-30 kD in water, an average content of 20% AgNPs (200 mg ml^{-1} AgNPs) with an average hydrodynamic diameter of metallic silver with PVP of 70 nm (Juárez-Moreno *et al*., 2016).

Obtaining plant material

Strawberry (*Fragaria* x *ananassa* Duch.) plants of the Festival cultivar were used.

Experiment management

Polyethylene bags of 30 x 30 cm were used, employing tezontle with a granulometry of 4 mm as a substrate. The nutrition of the plants was based on Steiner's universal nutrient solution, which

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is completely constituted by (in mol m^{-3}) 12 of NO_3^{-}, 1 of $H_2PO_4^{-}$, 7 of SO_4^{-2}, 7 of K^{+}, 9 of Ca^{+2} and 4 of Mg^{+2}. The drip irrigation systems were established to continue with the transplantation of strawberry plants of the Festival cultivar.

The distance between the plants was 30 cm and between blocks 1 m. Strawberry plants were irrigated at an interval of 2 to 3 times per day throughout the biological cycle of the strawberry crop.

Application of silver nanoparticles

For the experiment on the foliar application of silver nanoparticles and for the experiment on the root application of silver nanoparticles, different plants were used to perform five treatments in each one, which were to apply concentrations of 0, 40, 80, 120, 160 mg ml^{-1} and 0, 5, 10, 15, 20 mg ml^{-1} of AgNPs, respectively, performing the application at eight different intervals; that is, at 7, 14, 21, 28, 35, 42, 49 and 56 days after transplantation. The applications were made in the early hours of the day when there was a relative humidity greater than 60% and temperatures below 18 °C in a time interval of 1 to 3 min per plant.

Measuring fruit characteristics

For both the experiment on the root application of silver nanoparticles and the experiment on the root application of silver nanoparticles, 25 plants were randomly selected from each experiment, highlighting that each experiment was made up of 100 experimental units, totaling 200 experimental units for the two experiments.

Of the 25 plants selected, five plants were marked for each treatment carried out. At 70 days after the first application of AgNPs, the harvest was carried out considering the maturity index criterion that the fruits had a minimum of 50% of their surface with a faint red or pink coloration for the determination of the quality indicators.

Evaluation of quality indicators

The quality indicators evaluated in the strawberry fruits were fresh fruit weight (g) with an Ohaus Explorer® H-4738 scale-China, firmness (kilogram-force) with a Chatillon® MT500 mechanical force tester-China, pH with a Conductronic® PC45 potentiometer-USA, degrees brix (%) with an Atago® PAL^{-1} 3810 refractometer-Japan, colorimetry (lightness, HUE angle, and Chroma saturation index) with a MeterTo® JZ-300 general colorimeter-China.

Vitamin C in milligrams per gram per fresh matter weight (mg g^{-1} FMW) using the technique described by Roe and Kuether (1943), total soluble proteins (mg g^{-1} FMW) using the technique described by Bradford (1976), total phenols (mg g^{-1} FMW) using the technique described by Singleton and Rossi (1965), and anthocyanins (mg C3GE g^{-1} of FMW) through the technique described by Abdel and Hucl (1999); it should be noted that, for each quality indicator, 15 fruits were selected per treatment and each treatment had four replications per block for both the experiment of foliar application and the experiment of root application of silver nanoparticles.

Experimental design

A completely randomized block experimental design (CRBD) was used, establishing five blocks with five treatments, where each treatment was tested four times for both the experiment of foliar application of silver nanoparticles and the experiment of root application of silver nanoparticles, where the respective applications were carried out independently of each other, giving a total of 100 experimental units per experiment and 200 experimental units in total for the two experiments. The experimental unit was a 30 x 30 cm black polyethylene bag containing a strawberry plant of the Festival cultivar.

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Statistical analysis

An analysis of variance and Tukey comparison of means test were performed, with a significance level of 0.05, using the SAS 9.4 statistical package.

Results and discussion

The effect of the foliar and root applications of silver nanoparticles significantly influenced the quality indicators measured in strawberries cultivar Festival, highlighting that, in the colorimetry quality indicator, lightness, HUE angle, and saturation index (Chroma) were determined, as can be seen in Table 1, 2, 3 and 4.

Table 1. Quality indicators of fresh weight (g), firmness (kilogram-force), pH, degrees brix (%), lightness and HUE angle of strawberries cultivar Festival sprayed with 0, 40, 80, 120 and 160 mg L^{-1} of Bionag Argovit^{TM} AgNPs at 70 days after the start of foliar treatments.

Treat. of AgNPs (mg L^{-1})	Fresh weight (g)	Firmness (kilogram-force)	pH	Degrees Brix (%)	Lightness	HUE angle
0	15.88 ±1.29b	0.392 ±0.021d	3.63 ±0.014e	5.17 ±0.27b	27.01 ±0.49d	27.08 ±1.25c
40	19.73 ±1.65a	0.51533 ±0.049c	3.75 ±0.015d	6.22 ±0.61a	30.69 ±0.31c	31.61 ±0.85b
80	17.97 ±1.71ab	0.87933 ±0.044a	3.84 ±0.017c	5.64 ±0.58a	33.06 ±0.23b	32.29 ±0.91b
120	18.23 ±1.51ab	0.64133 ±0.016b	4.11 ±0.054a	6.03 ±0.49a	34.14 ±0.29b	32.04 ±1b
160	17 ±1.71b	0.54733 ±0.01c	3.92 ±0.011b	6.15 ±0.59a	37.23 ±1.18a	36.73 ±2.8a

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

Table 2. Quality indicators of saturation index (Chroma), vitamin C (mg g^{-1} FMW), total soluble proteins (mg g^{-1} FMW), total phenols (mg g^{-1} FMW) and anthocyanins (mg C3GE g^{-1} FMW) of strawberries cultivar Festival sprayed with 0, 40, 80, 120, and 160 mg L^{-1} of Bionag Argovit^{TM} AgNPs at 70 days after the start of foliar treatments.

Treat. of AgNPs (mg L^{-1})	Saturation index (Chroma)	Vitamin C (mg g^{-1} FMW)	Total soluble proteins (mg g^{-1} FMW)	Total phenols (mg g^{-1} FMW)	Anthocyanins (mg C3GE g^{-1} FMW)
0	16.69 ±0.85e	0.651 ±0.22a	2.77 ±0.28a	1.37 ±0.12d	0.154 ±0.02d
40	22.25 ±0.47d	0.6512 ±0.23a	2.78 ±0.29a	1.53 ±0.21b	0.1757 ±0.04c
80	26.05 ±0.45c	0.6518 ±0.28a	2.78 ±0.52a	1.45 ±0.1c	0.1974 ±0.02b
120	29.88 ±0.94b	0.6513 ±0.17a	2.78 ±0.51a	1.92 ±0.11a	0.2083 ±0.02ab
160	35.84 ±1.52a	0.6516 ±0.25a	2.78 ±0.45a	1.47 ±0.16c	0.2167 ±0.04a

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

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Table 3. Quality indicators of fresh weight (g), firmness (kilogram-force), pH, degrees brix (%), lightness and HUE angle of strawberries cultivar Festival sprayed with 0, 5, 10, 15 and 20 mg L^{-1} of Bionag Argovit™ AgNPs at 70 days a fter the start of root treatments.

Treat. of AgNPs (mg L^{-1})	Fresh weight (g)	Firmness (kilogram-force)	pH	Degrees Brix (%)	Lightness	HUE angle
0	17.85 ±2.43a	0.40466 ±0.026e	3.68 ±0.021d	5.18 ±0.07c	27.61 ±0.47d	27.1 ±1c
5	18.7 ±1.77a	0.63733 ±0.012b	3.84 ±0.016c	5.88 ±0.59b	31.27 ±0.58c	31.22 ±0.58b
10	19.97 ±1.86a	0.76266 ±0.044a	3.82 ±0.022c	5.86 ±0.45b	31.63 ±0.43bc	31.18 ±1.08b
15	18.47 ±1.75a	0.56733 ±0.013c	3.97 ±0.016b	6.56 ±0.52a	36.36 ±1.06a	36.99 ±2.31a
20	19.71 ±2.24a	0.494 ±0.011d	4.21 ±0.092a	6.67 ±0.43a	32.87 ±0.33b	33.55 ±0.82b

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

Table 4. Quality indicators of saturation index (Chroma), vitamin C (mg g^{-1} FMW), total soluble proteins (mg g^{-1} FMW), total phenols (mg g^{-1} FMW) and anthocyanins (mg C3GE g^{-1} of FMW) of strawberries cultivar Festival sprayed with 0, 5, 10, 15, and 20 mg L^{-1} of Bionag Argovit™ AgNPs at 70 days after the start the treatments via roots.

Treat. of AgNPs (mg L^{-1})	Saturation index (Chroma)	Vitamin C (mg g^{-1} FMW)	Total soluble proteins (mg g^{-1} FMW)	Total phenols (mg g^{-1} FMW)	Anthocyanins (mg C3GE g^{-1} FMW)
0	15.9 ±0.68e	0.6501 ±0.27c	2.76 ±0.27c	1.28 ±0.11d	0.1888 ±0.02d
5	21.55 ±0.41d	0.6521 ±0.32b	2.79 ±0.68ab	1.53 ±0.22b	0.2114 ±0.02c
10	25.77 ±0.43c	0.6512 ±0.31bc	2.8 ±0.76a	1.44 ±0.13c	0.2032 ±0.02c
15	36.29 ±1.76a	0.6525 ±0.36ab	2.78 ±0.54b	1.92 ±0.11a	0.2411 ±0.02b
20	29.24 ±0.52b	0.6536 ±0.4a	2.79 ±0.54ab	1.45 ±0.16bc	0.2587 ±0.04a

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

Fresh weight (g)

The fresh weight (g) of strawberry fruits after the foliar application of AgNPs showed statistically significant differences between treatments. The treatment that obtained the highest fresh fruit weight (g) (19.73) was the one with plants supplied with 40 mg L^{-1} AgNPs via leaves (Table 1). On the other hand, the fresh weight (g) of strawberry fruits after the root application of AgNPs did not show statistically significant differences between treatments (Table 3).

Studies developed by Shahzad *et al*. (2024) in strawberry (*Fragaria* x *ananassa* Duch.) found that applications of silver nanoparticles in amounts of 50 mg L^{-1} increased the fresh weight of the fruit, emphasizing that AgNPs act by blocking ethylene signaling, delaying senescence and optimizing metabolic processes.

Firmness (kilogram-force)

The firmness of the strawberry fruit (kilogram-force) after applying AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that had the highest fruit firmness (kilogram-force) (0.8793) (0.7626) were those with plants supplied with 80 mg L^{-1} AgNPs via leaves and 10 mg L^{-1} AgNPs via roots, respectively (Tables 1 and 3).

In grapes (*Vitis vinifera*), Elatafi and Fang (2022) found that applications of silver nanoparticles at 100 mg L^{-1} increased firmness, highlighting that silver ions (Ag^{+}) AgNPs inhibit the formation of 1-aminocyclopropane-1-carboxylic acid, which, in turn, inhibits the formation of the growth hormone ethylene, which inhibits the ripening process and thus, the degradation of protopectin into pectin is also inhibited.

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pH

The pH of the strawberry fruits after the foliar and root applications of AgNPs showed statistically significant differences between treatments. The treatments that obtained the highest pH in the fruit (4.1) (4.21) were those with plants supplied with 120 mg L^{-1} AgNPs via leaves and 20 mg L^{-1} AgNPs via roots, respectively (Tables 1 and 3).

For their part, in strawberry, Vishal *et al*. (2023) discovered that applications of silver nanoparticles at 2 000 mg L^{-1} increased the pH, mentioning that AgNPs undergo crystallization processes that generate a biostimulation for the conversion of organic acids into sugars, increasing the efficiency of enzymatic reactions of respiration that reduce acidity and result in an increase in the pH of the fruit.

Degrees Brix (%)

The degrees brix (%) of strawberry after applying AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that had the highest degrees brix (%)(6.22, 5.64, 6.03 and 6.15) (6.56 and 6.67) were those with plants supplied with 40, 80, 120 and 160 mg L^{-1} AgNPs via leaves and 15 and 20 mg L^{-1} AgNPs via roots, respectively (Tables 1 and 3).

Studies such as those by Barikloo and Ahmadi (2018) in strawberry found that applications of silver nanoparticles at 3 500 mg L^{-1} increased degrees brix, emphasizing that AgNPs act by optimizing the transformation of carbohydrates into other soluble compounds through the biostimulation of metabolic processes, such as photosynthesis, resulting in the decrease of water in the fruit, which caused an increase in the concentration of soluble solids.

Lightness

The lightness of strawberry fruits after the application of AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that had the highest lightness (37.23) (36.36) were those with plants supplied with 160 mg L^{-1} AgNPs via leaves and 15 mg L^{-1} AgNPs via roots, respectively (Tables 1 and 3).

A study carried out on strawberries and developed by Sogvar *et al*. (2016) found that applications of zinc oxide nanoparticles (ZnONPs) at 3 000 mg L^{-1} increased lightness, underlining that ZnONPs act by making the enzymatic activity that suppresses the release of the growth regulator ethylene more efficient, delaying senescence.

HUE angle (coloration

The HUE angle in strawberry fruits, which indicates either green or reddish coloration, after applying AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that had the highest HUE angle (36.73) (36.99) were those with plants supplied with 160 mg L^{-1} AgNPs via leaves and 15 mg L^{-1} AgNPs via roots, respectively (Tables 1 and 3).

In this regard, in their research on strawberry fruits, Zhang *et al*. (2018) discovered that applications of silver nanoparticles at 5 000 mg L^{-1} increased the HUE angle, highlighting that AgNPs act on enzymatic browning through reactions of polyphenolic compounds with oxygen catalyzed by endogenous polyphenol oxidase.

Saturation index (coloration intensity

The saturation index (Chroma) in strawberry fruits, which indicates how pure, intense or vivid a color is, after the application of AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that had the highest saturation index (35.84) (36.29) were those with plants supplied with 160 mg L-1 AgNPs via leaves and 15 mg L-1 AgNPs via roots, respectively (Tables 2 and 4).

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Taha *et al*.'s (2022) research on strawberries coincided with what was obtained in the present research after the applications of silver nanoparticles at 500 mg L^{-1}, increasing the saturation index, emphasizing that AgNPs modify the biochemical components of flavonoids, anthocyanins, xanthophylls, carotenes, and chlorophylls.

Vitamin C (mg g^{-1} FMW)

Vitamin C from strawberry fruits (mg g^{-1} FMW), essential as an antioxidant compound, after the foliar application of AgNPs did not show statistically significant differences between treatments (Table 2). On the other hand, vitamin C from strawberry fruits (mg g^{-1} FMW) after the root application of AgNPs showed statistically significant differences between treatments. The treatment that obtained the highest amount of vitamin C in the fruit (mg g^{-1} FMW) (.6536) was the one with plants supplied with 20 mg L^{-1} AgNPs via roots (Table 4).

In loquats (*Eriobotrya japonica* Lindl.), Ali *et al*. (2020) found that applications of silver nanoparticles at 0.03 mg L^{-1} increased vitamin C, coinciding with what was obtained after the root application of silver nanoparticles, stressing that AgNPs, acting as an inorganic biostimulant, reduce abiotic stress, favoring the production of primary and secondary metabolites that trigger a higher concentration of vitamin C.

Total soluble proteins (mg g^{-1} FMW)

The total soluble proteins (mg g^{-1} FMW) of strawberries fruits, important for improving organoleptic characteristics, after the foliar application of AgNPs did not show statistically significant differences between treatments (Table 2). In turn, the total soluble proteins (mg g^{-1} FMW) of strawberry fruits after the root application of AgNPs showed statistically significant differences between treatments because the root is the specific organ in higher plants to carry out the processes of access and absorption.

The treatment that obtained the highest amount of total soluble proteins in the fruit (mg g^{-1} FMW) (2.8) was the one with plants supplied with 10 mg L^{-1} AgNPs via roots (Table 4). In their study carried out on tomato (*Solanum lycopersicum*), Girilal *et al*. (2018) found that applications of silver nanoparticles at 100 mg L^{-1} increased total soluble proteins, mentioning that AgNPs act by reducing abiotic stress through antioxidant enzymes, such as catalase, peroxidase, and superoxide dismutase, that protect against damage caused by reactive oxygen species, making protein synthesis more efficient and consequently increasing their concentration in fruits.

Total phenols (mg g^{-1} FMW)

The total phenols in strawberry fruits (mg g^{-1} FMW), indispensable as antioxidant compounds, after applying AgNPs via leaves and roots showed statistically significant differences between treatments. The treatments that obtained the highest total phenols in the fruit (mg g^{-1} FMW) (1.92) (1.92) were those with plants supplied with 120 mg L^{-1} AgNPs via leaves and 15 mg L^{-1} AgNPs via roots, respectively (Tables 2 and 4).

In their work on sage (*Salvia officinalis*), Moazzami-Farida *et al*. (2020) discovered that applications of silver nanoparticles at 100 mg L^{-1} increased total phenols, emphasizing that AgNPs positively induce the activity of the enzyme phenylalanine ammonia lyase (PAL), which catalyzes the conversion of the amino acid phenylalanine into precursors of various phenolic compounds, thus increasing their concentration in fruit.

Anthocyanins (mg C3GE g^{-1} FMW)

Anthocyanins in strawberry fruits (mg C3GE g^{-1} FMW), which are important as antioxidant compounds and which benefit organoleptic characteristics, after the application of AgNPs via leaves

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and roots showed statistically significant differences between treatments. The treatments that obtained the highest anthocyanins in the fruit (mg C3GE g^{-1} of FMW) (0.2167) (0.2587) were those with plants supplied with 160 mg L^{-1} AgNPs via leaves and 20 mg L^{-1} AgNPs via roots (Tables 2 and 4).

In their research on Echium (*Echium amoenum*), Abbasi and Jamei (2018) found that applications of silver nanoparticles at 50 mg L^{-1} increased anthocyanins, mentioning that AgNPs activate a phenolic-type non-enzymatic defense system, thereby increasing the concentration of anthocyanins in the fruit in the presence of high concentrations of metals in order to counteract the effects of toxicity.

Conclusions

Through the analysis of the results, we observed that the application of silver nanoparticles in strawberry cultivar Festival had a positive effect on the quality indicators measured by biostimulating biochemical, physiological and agronomic processes that generated a plant defense mechanism and the production of antioxidant compounds. Finally, in the cultivation of strawberry cultivar Festival, it is recommended to apply AgNPs doses of 80, 120, 160 mg L^{-1} and 10, 15, 20 mg L^{-1} via leaves and roots, respectively, to increase the quality, thereby meaning that Bionag Argovit™ silver nanoparticles are a viable alternative as an inorganic biostimulant in agricultural production for the benefit of Mexico's food sovereignty.

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