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Silver nitrate and bio-silver nanoparticles on some morphological, physiological, and anatomical variations of tomato plants

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ABSTRACT: An experiment in a greenhouse was conducted to study the influence of silver nitrate and silver nanoparticles on morphological, physiological, and anatomical characteristics of tomato plants. Silver nanoparticles were synthesized using green tea extract as capping and reducing agents. As observed in FE-SEM images, the biosynthesized silver nanoparticles were circular with a diameter of 61 ± 6 nm. The following effects were observed: i) Morphology: Silver nitrate positively influenced the character of plant length, root length, root dry weight and root fresh weight. ii) Physiology: When tomato plants were treated with Bio-AgNPs and silver nitrate, antioxidant enzymes (SOD and Catalase) and chlorophyll were increased, while total sugar content in tomato leaves was decreased. Silver in silver nitrate or silver nanoparticles did not significantly affect proline content. iii) Anatomy: Exposure to Bio-AgNPs resulted in decreased cortex thickness, vascular bundle length, and the appearance of dark regions in the intercellular space in tomato stem pith and cortex tissues. In contrast, exposure to silver nitrate resulted in decreased stomatal length on both leaf surfaces, decreased number of stomata on the upper surface, and increased number of stomata on the lower leaf surface, compared to other treatments.
Keywords: Bio-AgNPs; phytotoxicity; heavy metals; plant growth; chlorophyll; catalase; plant anatomy.

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RESUMO: Um experimento em uma estufa de produção vegetal foi conduzido para estudar a influência do nitrato de prata e de nanopartículas de prata em caracteristicas morfológicas, fisiológicas e anatomicas de plantas de tomate. Nanopartículas de prata foram sintetizadas usando extrato de chá verde como agentes de cobertura e redução. Por imagens de FE-SEM, as nanopartículas de prata biossintetizadas apresentavam formato circular com um diâmetro de 61 ± 6 nm. Foram observados os efeitos: i) morfologia: O nitrato de prata influenciou positivamente o caráter do comprimento da planta, comprimento da raiz, peso seco e fresco da raiz. ii) fisiologia: quando as plantas de tomate tratadas com Bio-AgNPs e nitrato de prata, as enzimas antioxidantes (SOD e catalase) e clorofila foram aumentadas, enquanto o conteúdo de açúcares totais nas folhas de tomate foi diminuído. A prata, seja em nitrato de prata ou nanopartículas de prata, não afetou significativamente o conteúdo de prolina. iii) Anatomia: a exposição a Bio-AgNPs resultou na diminuição da espessura do córtex, no comprimento do feixe vascular e no aparecimento de regiões escuras no espaço intercelular nos tecidos da medula e do córtex do caule do tomateiro. Em contraste, a exposição ao nitrato de prata resultou na diminuição do comprimento dos estômatos em ambas as superfícies foliares, na diminuição do número de estômatos na superfície superior e, ao mesmo tempo, no aumento do número de estômatos na superfície inferior da folha, em comparação com outros tratamentos.
Palavras-chave: Bio-AgNPs; fitotoxicidade; metais pesados; crescimento de plantas; clorofila; catalase; anatomia vegetal.

1. INTRODUCTION

Tomatoes are an annual herbaceous plant (LANNOY et al., 2001), one of the most important vegetable crops worldwide (BROOKIE et al., 2018). It belongs to the Solanaceae family and genus Solanum, which includes seven other wild species, scientifically known as *Solanum lycopersicum* L. (MILLS, 1990). Tomatoes are widely cultivated in many countries worldwide and are considered a staple vegetable due to their high nutritional value for most people (GIOVANNI et al., 2004). The plant has taproot roots, and its stems are round and covered with hairs that contain glands that secrete a greenish-yellow substance with a distinctive odor (FRANCO, 1999). The leaves are compound, pinnate, and mounted on the stem with a long neck. Flowers are found in cluster inflorescences with limited growth, and the number of flowers in one inflorescence ranges from 4 to 8 (NAJJA, 2008). Due to the importance of the tomato plant worldwide, in general, and in Iraq, in particular, it is necessary to use modern technologies to increase production and encourage the increase of cultivated areas to increase productivity and economic resources. Among these technologies is the technology provided by nanotechnology.

Nanotechnology is one of the new technologies that has entered all aspects of our lives and has been used to increase agricultural production and reduce environmental problems. It can be very useful in manufacturing new generations of fertilizers to use high-efficiency nutrients. Within reasonable bounds, nanotechnology is the creative Manipulation of matter at the molecular and atomic levels. Technology of a nanoscale size or below shows promise for enhancing current agricultural operations through better input management, sustainability, and upkeep in field and livestock agricultural output (MONREAL et al., 2015).

Several studies indicated that using nano-materials increases plant productivity by increasing the plant's absorption of nutrients. It also significantly improves root growth and the plant's metabolism (SHAH; BELOZEROVA, 2009; GIRALDO et al.,2014). In a study done before (SAEIDEH; RASHID, 2014), it was found that treating the cowpea plant at a concentration of 50 mL L^{-1} of nanosilver, it increased the height of the plant and the length of the root system, as well as increasing the fresh and dry weight of the shoot and its chlorophyllate content. A study by SALAMA (2012) indicated that using nanosilver enhances growth parameters of *Brassica juncea* plants, such as root length, leaf area, and biochemical properties such as chlorophyll, carbohydrates, protein contents, and antioxidant enzymes. The germination rate, shoot length, root length, and dry weight of shoots and roots were all significantly impacted by treating wheat plants with silver nitrate and silver nanoparticles at concentrations of 10-20-40-80 ppm, according to the results of the Al-Salama et al. (2024) experiment. On the other hand, Farghaly; Nafady (2015) found that applying silver nanoparticles to tomato and wheat plants had a substantial detrimental impact, lowering the plants' dry weight and chlorophyll a content and inhibiting the germination rate, pigment fractions, and dry weight of the wheat.

Hasan et al. (2021) observed an increase in shoot length, chlorophyll, carbohydrate, and protein content in lettuce plants in response to silver nanoparticles, while lettuce plant growth decreased by 40% when plants were treated with silver nitrate ($AgNO_3$). Given the importance of the tomato from a nutritional standpoint, limited studies are available on the effect of silver nanoparticles. The research aims to synthesize silver nanoparticles using green tea extracts and to study the effect of Bio-AgNPs and silver nitrate on morphological, physiological, and anatomical variations of tomato plants.

2. MATERIAL AND METHODS

2.1. Preparation of Bio-silver nanoparticles and their characterization

The Bio-AgNPs preparation procedure is conducted as outlined by Ridha et al. (2021). Dry green leaf tea was utilized to prepare colloidal silver nanoparticles due to the existence of natural compounds that act as reducing agents and capping agents. Dried leaves (5g) were soaked in one hundred milliliters of boiled deionized water and heated for ten minutes. The obtained extract was chilled and drained of debris. The deionized water was transferred to the filtered extract (15 ml) to reach the required volume of 98 ml. The pH of the final volume was adjusted to alkaline with a pH value of 9 by adding a few drops of NaOH (0.01M). A volume of silver nitrate solution (1 mL) with a concentration of 0.01M is dropped into the combination and agitated employing a magnetic stirrer at 500 rpm for one day. During this stage, the color of the solution was converted to brown. The prepared solution, which involved silver nanoparticles, was washed three ones using deionized water and centrifuged at 12000 RCF for ten minutes to separate AgNP. The deposition was collected and added to deionized water. The colloidal silver nanoparticles were preserved in a foiled bottle at 25 °C for the following investigations. The synthesized bio-silver nanoparticles were characterized using the following analysis: the UV-spectrophotometer, ultraviolet spectrophotometer, Fourier-transform infrared spectroscopy (FT-IR), Field emission scanning electron microscopy (FE-SEM), Energy-dispersive X-ray analysis (EDAX).

2.2. Determination of the effect of silver nanoparticles and silver salt on tomato plants

The study was conducted in the greenhouse in the winter season of 2023-2024 in the College of Sciences, Babylon, Iraq. The study included the effect of irrigation on tomato plants with silver nitrate at a concentration of 50 mg L^{-1} and Bio-silver nanoparticles at a concentration of 10 mg L^{-1}, on some morphological, physiological, and anatomical indicators of tomato plants. The seeds were planted in pots with a diameter of 17 cm containing 3 kg of soil and animal manure in a 1:1 ratio, in 9 pots with three replicates, with irrigation twice a week for each treatment.

2.2.1. Morphological study

Data were taken 60 days after randomly selecting three plants from each experimental unit for each replicate and then extracting their average. It included measuring the plant height with a metric tape from the area of contact with the soil to the end of the plant height (SING; STOCKOPF, 1971). The root length of each plant was measured using a metric ruler, according to the fresh weight of the shoot group after separating it from the root group after washing it from soil, then weighed using a sensitive electronic balance, Metler HK 160, and the average fresh weight was extracted by dividing the weights of the shoot plant by their numbers to calculate the fresh weight. Then, the same plants were placed in a Hirayama electric oven at 65 °C for 72 hours until the weight stabilized. Then, the plants were weighed using a sensitive balance to calculate the dry weight (AL-JUBOORY; SHAKIR, 2019). The same method was followed to calculate the fresh and dry weight of the root group.

2.2.2. Physiological study

Measurement of catalase (CAT) activity: The method outlined by AEIBI (1984) was used to assess CAT activity. This method is based on the change in absorbance at 240 nm. Using 0.5 g of fresh leaves powdered in 10 ml of sulfosalicylic acid aqueous solution, the proline content was calculated using the Bates et al. (1973) method. GSH (reduced glutathione) in the leaves was measured using the MORON et al. (1979) technique. Dithionitrobenzene (DTNB) and acid-soluble sulfhydryl groups (non-protein thiols, of which reduced glutathione makes up over 93%) combine to form a yellow complex. At 412 nm, the colored complex's absorbance was measured. The approach of (Marklund; Marklund, 1974) was used to measure SOD activity based on the enzyme's capacity to stop pyrrocalol from oxidizing at pH (8.2). The quantity of soluble sugars in the leaves was measured using the DUBIOS et al., 1956 method. The standard curve was used to calculate the total amount of sugars. A field chlorophyll measurement device (SPAD) was used to measure the amount of chlorophyll.

2.2.3. Anatomical study

2.2.3.1. Preparation of stem cross sections

The sections were prepared manually using a sharp blade for fresh samples from potted plants. The thin sections of the stems were placed on glass slides, and safranin stain mixed with 70% glycerin was added. They were covered with a cover slip and stored until examination.

2.2.3.2. Preparation of leaf epidermis

Ahmad et al. (2010) prepared the upper and lower epidermis by placing the fresh leaf on a glass slide. The epidermis was peeled off using a dissecting scalpel, washed with distilled water, immersed in a solution of artificial bleach until the chlorophyll pigment disappeared, and washed with distilled water again. After being moved to a different glass slide and stained with a solution of glycerin and safranin, the peeled epidermis was prepared for study by placing a cover slip on the slide. Using an Ocular micrometer and a Zeiss compound microscope, the samples were inspected, their stomata and cells measured, and they were photographed using an Infinix mobile lens. One-way analysis of variance (ANOVA) was used to analyze the data. The statistical program Minitab v17 was utilized to compare treatment group differences.

3. RESULTS

3.1. Physical features of biosynthesized silver nanoparticles

Several analysis including an ultraviolet spectroscopy, The analysis of Fourier-transform infrared spectroscopy (FT-IR) is written in section of the materials and methods, Field emission scanning electron microscopy (FE-SEM), Energy-dispersive X-ray analysis (EDX)" were performed to characterize the physical features of biosynthesized silver nanoparticles and were applied and to evaluate its potential on morphological, physiological and anatomical factors of the tomato plant.

As shown in Figure 1, the UV-Vis spectrum exhibited a strong absorption of biosynthesized silver nanoparticles at 422 nm, in contrast to the green tea extract employed as reducing and capping agents, which is characterized by the absence of a specific peak related to the existence of AgNPs. FT-IR analysis obtained between 4000 cm^{-1} and 400 cm^{-1} is used to determine functional groups absorbed on the surface of biosynthesized silver nanoparticles. The FT-IR analysis obtained between 4000nm and 400 nm determines functional groups absorbed on the surface of biosynthesized silver nanoparticles. The FT-IR spectrum of biosynthesized silver nanoparticles revealed the existence of four functional groups, which matched with activity groups observed in the spectrum of green tea extract. The broad long curve at 3436 cm^{-1} represented an O–H stretching of alcohol in polyphenols, and the peak at 2072 cm^{-1} demonstrated an N–H stretching in amines (WIDATALLA et al., 2022). Moreover, the narrow, cute carve at 1636 cm^{-1} was recognized as C=O bonds, referring to the existence of ketones, quinones, carboxylic acids, and esters (ROLIM et al. 2019). Finally, the curve situated at 678 cm^{-1} was indicated as an aliphatic chain (HAMOUDA et al., 2019). The FE-SEM images illustrated the Bio-AgNPs at different magnifications, exhibiting well-distributed Bio-AgNPs with spherical morphology, varying between 54 nm and 67nm (Figure 2). The EDAX is used to analyze the chemical structure of the nanoparticles created. The EDAX diagram of Bio-AgNPs demonstrated the existence of silver at a high ratio compared to low proportions of carbon, oxygen, and chlorine. The extra small curve may be associated with organic molecules found in plant extracts.

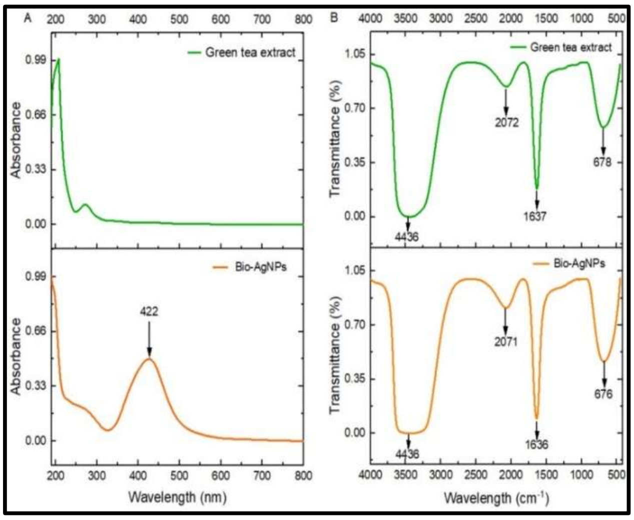

Figure 1. Ultraviolet spectrum of biosynthesized silver nanoparticles (Bio-AgNPs) and green tea extract (A), FT-IR of biosynthesized silver nanoparticles (Bio-AgNPs) and green tea extract (B).
Figura 1. Espectro ultravioleta de nanopartículas de prata biossintetizadas (Bio-AgNPs) e extrato de chá verde (A), FT-IR de nanopartículas de prata biossintetizadas (Bio-AgNPs) e extrato de chá verde (B).

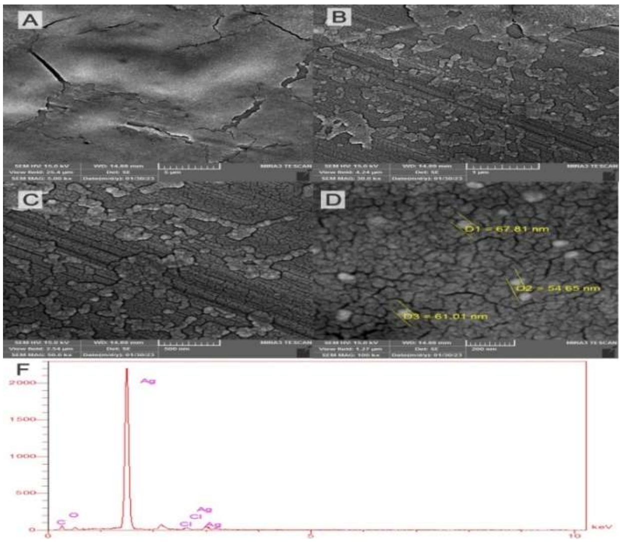

Figure 2. FE-SEM images of Bio-AgNPs at various magnifications. 5 µm (A), 1 µm (B), 500 nm (C), and 200 nm (D). EDAX diagram of Bio-AgNPs (F).
Figura 2. Imagens FE-SEM de Bio-AgNPs em várias ampliações. 5 µm (A), 1 µm (B), 500 nm (C) e 200 nm (D). Diagrama EDAX de Bio-AgNPs (F).

3.2.Morphological study:

Significant differences were observed between the treatments in plant length (Table 1), as Bio-AgNPs treatment decreased to the lowest average plant length compared to other treatments. In contrast, silver nitrate treatment recorded the highest value of 15.27 cm. Significant differences were found between values of tomato root length, as root length increased when plant was treated with silver nitrate to highest average for this trait, reaching 18.67 cm, compared to the control and nano silver treatments, which recorded a convergence or similarity of 12.73 and 12.17 cm, respectively, with a non-significant difference between them. Both silver nitrate and Bio-AgNPs caused a significant increase in fresh weight of tomato plant shoots compared to the control treatment, reaching 4.24 and 4.21 grams, respectively.

Table 1. Effect of silver nitrate and Bio-AgNPs on tomato plant morphology.
Tabela 1. Efeito do nitrato de prata e Bio-AgNPs na morfologia da planta de tomate.

Treatments / Parameters	Control	$AgNO_3$	AgNPs
Plant length (cm)	12.80 b	15.27 c	10.97 a
Root length (cm)	12.73 a	18.67 b	12.17 a
Fresh weight of stem (g)	3.25 b	4.24 a	4.21 a
Fresh weight of root (g)	1.05 b	1.54 c	0.63 a
Dry weight of stem (g)	1.50 b	1.54 b	1.27 a
Dry weight of root (g)	0.36 b	0.48 c	0.24 a

Different letters refer to Sig. at $p \leq 0.05$.
Letras diferentes referem-se a Sig. em $p \leq 0{,}05$.

The nano treatment gave the lowest values of fresh weight of root, dry weight of shoots, and root, which were 0.63, 1.27, and 0.24 g, respectively, compared to other treatments, which recorded a convergence or similarity of 12.73 and 12.17 cm, respectively, with a non-significant difference between them. Both silver nitrate and Bio-AgNPs caused a significant increase in fresh weight of tomato plant shoots compared to the control treatment, reaching 4.24 and 4.21 grams, respectively. The nano treatment gave the lowest values of fresh weight of root, dry weight of shoots, and root, which were 0.63, 1.27, and 0.24 g, respectively, compared to other treatments.

3.3. Physiological study

Tomato leaves treated with silver and nano-silver showed high levels of antioxidant enzymes activity, both SOD and Catalase, with a significant difference from control treatment. Silver nitrate did not show any significant effect on non-enzymatic antioxidant GSH compared to control treatment, unlike silver nanoparticles which caused a significant decrease of 43.05 $\mu g\ g^{-1}$ of weight The results of the statistical analysis did not show any significant difference between treatments in proline content of tomato leaves, as shown in (Table 2). The concentration of total soluble sugars in tomato leaves decreased after plant exposure to 10 ppm of nanosilver and 50 ppm of silver nitrate during irrigation for 60 days. It was noted from the results of the study that the treatment of tomato plants with nanosilver and silver-nitrate had a positive effect in increasing the content of chlorophyll pigment in leaves, which reached 48.87 and 47.63 µg/g.f.w., respectively, compared to the control treatment.

3.4. Anatomical study

3.4.1. Stem

Table 3 and Plate 1 show the tomato stem's cross-sectional characteristics. The stem's overall cross-section tends to be round and consists of a single layer of oval-shaped cells on the outside.

Table 2. Effect of silver nitrate and Bio-AgNPs on tomato plant physiology.
Tabela 2. Efeito do nitrato de prata e Bio-AgNPs na fisiologia da planta de tomate.

Treatments / Parameters	Control	$AgNO_3$	AgNPs
SOD (U/g.f.w)	32.97 a	43.47 **b**	44.71 **b**
Catalase (Ku/g.f.w)	15.43 a	19.29 **b**	19.60 **b**
GSH (µg/g.d.w)	52.41 b	51.25 **b**	43.05 **a**
Chlorophyll (µg/g.f.w)	40.53 a	47.63 **b**	48.87 **b**
Proline (µmole/g.d.w)	0.56 a	0.53 **a**	0.61 **a**
Total sugars (gm/g.d.w)	5.09 b	3.59 **a**	3.41 **a**

Different letters refer to Sig. at $p \leq 0.05$.
Letras diferentes referem-se a Sig. em $p \leq 0{,}05$.

Table 3. Effect of silver nitrate and Bio-AgNPs on the quantitative properties of tomato stem.
Tabela 3. Efeito do nitrato de prata e Bio-AgNPs nas propriedades quantitativas do caule do tomateiro.

Parameters / Treatments	Control	$AgNO_3$	AgNPs
Epidermis thickness	21.9 a	20.0 a	28.8 b
Cortex thickness	234.4 b	285.6 c	149.9 a
No. of collenchyma cells	12.5 b	4.8 a	4.3 a
No. of parenchyma cells	13.1 b	2.5 a	2.3 a
No. of bundles	8.0 a	8.0 a	9.3 a
No. of vessels	6.0 a	6.0 a	7.0 a
Length of bundle	290.0 b	391.3 c	250.0 a
Thickness of pith	1800.0 a	2087.5 b	1837.5 a

Different letters refer to Sig. at $p \leq 0.05$.
Letras diferentes referem-se a Sig. em $p \leq 0{,}05$.

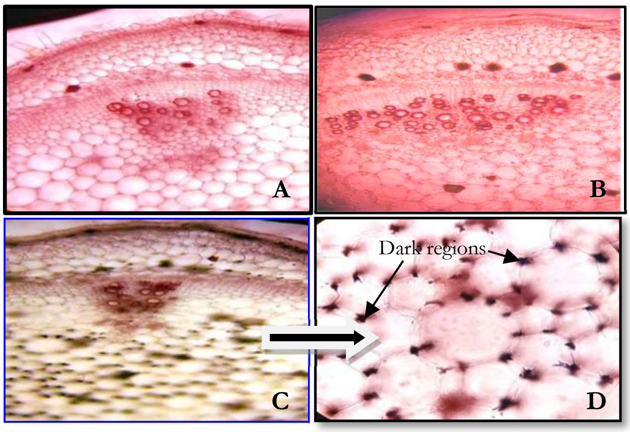

Plate 1. Stem anatomical response of a tomato in the different treatments: control (A), silver nitrate (B), silver nanoparticles (C), dark regions in the intercellular spaces between parenchyma cells (D) in the Pith stem (10X).
Placa 1. Resposta anatômica do caule de um tomateiro nos diferentes tratamentos; controle (A), nitrato de prata (B), nanopartículas de prata (C), regiões escuras nos espaços intercelulares entre as células do parênquima (D) no caule Pith (10X).

The thickness of the epidermis varies, with the highest average thickness reaching 28.8 µm when treated with silver nanoparticles, compared to silver nitrate treatment, which recorded the lowest average thickness reaching 20 µm. The next layer is the cortex layer, which is divided into several distinct layers with different shapes and sizes. The outer cortex layer is characterized by smaller collenchyma (angular) cells than the spherical parenchyma cells of the inner cortex. The highest mean cortex thickness was recorded in the silver nitrate treatment at 285.6 µm, while the lowest mean thickness was recorded at 149.9 µm when treated with silver nanoparticles.

The number of collenchyma and parenchyma cells was similar in silver nitrate and silver nanoparticle treatments, with a significant difference from the control treatment, which recorded the lowest mean values. The vascular tissue in the tomato stem appeared as a ring of vascular bundles of alternating sizes, i.e., small and large, connected by vascular elements. The vascular bundles were oval in shape, open collaterals, and varied in thickness. There were no significant differences between the numbers of vascular bundles and the number of xylem vessels per bundle in all treatments. Treatments differed in the lengths of the vascular bundles. The silver nitrate treatment recorded the highest average bundle length of 391.3 µm, with a significant difference from the nano treatment, which reached 250.0 µm as the minimum average bundle length. As for the Pith (composed of parenchyma cells of equal diameter and spaces between them), its average diameter increased when treated with silver nitrate by 2087.5 µm, compared to the control and nano treatments. Dark areas were also observed in the intercellular spaces under the epidermis, cortex, or pith.

3.4.1. Leaf

An anatomical study of tomato leaf epidermis (Plate 2) showed that it contains irregularly shaped undulate walls on both the upper and lower surfaces. The shape of the walls changed from undulate to straight due to the nano-treatment in some upper epidermal cells (Tables 4 and 5).

Table 4. Effect of silver nitrate and Bio-AgNPs on the quantitative properties of tomato upper epidermis.
Tabela 4. Efeito do nitrato de prata e Bio-AgNPs nas propriedades quantitativas da epiderme superior do tomateiro.

Treatments / Parameters	Control	$AgNO_3$	AgNPs
Length of epidermis	87.5 b	70.6 a	63.1 a
Width of epidermis	51.3 b	43.1 a	43.1 a
Length of stomata	30.0 b	25.0 a	31.9 b
Width of stomata	20.9 a	18.1 a	20.0 a
No. of stomata	2.0 b	1.3 a	2.5 c
No. of cells	69.8 a	69.5 a	73.5 a

Different letters refer to Sig. at p≤0.05.
Letras diferentes referem-se a Sig. em p≤0,05.

Table 5. Effect of silver nitrate and Bio-AgNPs on the quantitative properties of tomato lower epidermis.
Tabela 5. Efeito do nitrato de prata e Bio-AgNPs nas propriedades quantitativas da epiderme inferior do tomateiro.

Treatments / Parameters	Control	$AgNO_3$	AgNPs
Length of epidermis	68.1 a	75.6 a	96.9 b
Width of epidermis	49.5 b	43.8 a	42.5 a
Length of stomata	30.6 b	22.5 a	31.3 b
Width of stomata	21.9 a	20.9 a	21.3 a
No. of stomata	10.8 a	14.5 b	11.5 a
No. of cells	49.5 a	82.5 c	59.8 b

Different letters refer to Sig. at p≤0.05.
Letras diferentes referem-se a Sig. em p≤0,05.

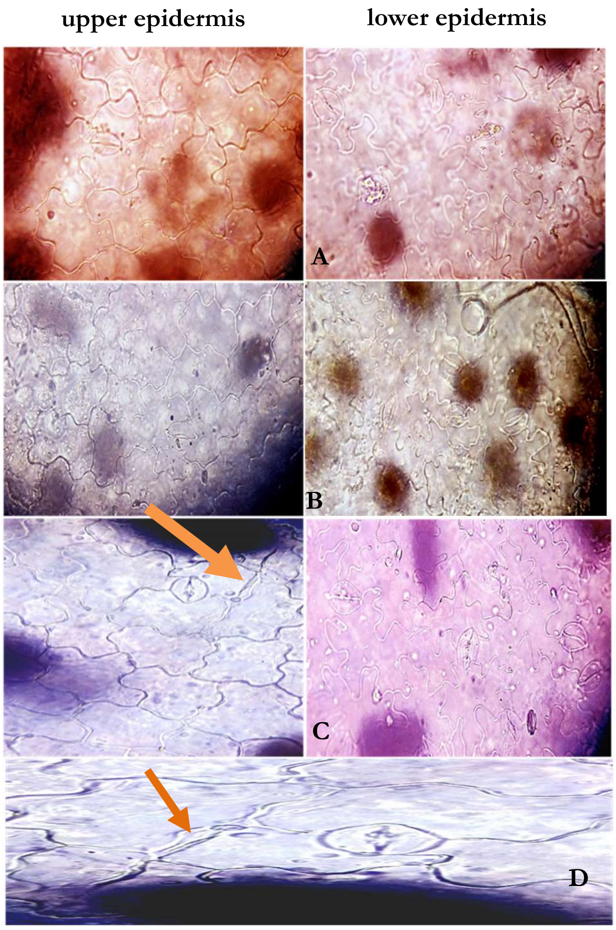

Plate 2. Leaf epidermis anatomical response of a tomato in the different treatments: control (A), silver nitrate (B), Bio-AgNPs (40X) (C), shape of walls changed from undulate to straight (D) as a result of nano-treatments.
Placa 2. Resposta anatômica da epiderme foliar de um tomateiro nos diferentes tratamentos: controle (A), nitrato de prata (B), Bio-AgNPs (40X) (C), formato das paredes alterado de ondulado para reto (D) como resultado dos nanotratamentos.

Silver nitrate and nano treatments negatively affected the length and width of the upper epidermal cells, reaching 70.6 and 63.1 µm, respectively, compared to the control treatment, which recorded the lowest average for these two traits, with a significant difference. As for the lower surface, it was observed that the nano-silver treatment gave the highest average length of epidermal cells compared to the other treatments, reaching 96.9 µm. In contrast, the width of the epidermal cells in the control treatment gave the highest average width, reaching 49.5 µm compared to the nano-silver and silver nitrate treatments. Stomata were found on tomato plants' upper and lower epidermal surfaces. Thus, the leaf is of the amphistomatous type. Silver nitrate caused a decrease in the length of all treatments. Nano-silver positively affected the number of stomata on the upper surface by 2.5 times compared to the rest of the other treatments, while the number of stomata on the lower surface reached 14.5 when treated with silver salts. There was no difference in the number of epidermal cells on the upper surface in all treatments. In contrast, the treatment with silver salts recorded the highest number of epidermal cells on the lower surface.

4. DISCUSSION

Some research in recent years has pointed towards the production of nanoparticles using plants due to their availability, ease of handling, and safety, in addition to the plant containing many vital molecules such as phenols, terpenes, alkaloids, flavonoids, and others, which are known to be a medium for the creation of nanoparticles (KAVITHA et al., 2013). Silver nitrate positively affected plant length, unlike nano silver, which decreased plant length. This study agrees with El-Timsah; Gunner (2010) that nano silver at a concentration of ten mg L^{-1} reduced plant length in *Linum usitatissimum* and *Hordeum vulgare*. There was no significant effect of nano silver on root length, which was almost similar to the control treatment. This is what Abdel-Azimb; Al-Sayed (2013) indicated that nano silver particles did not affect root length in fava bean plants.

The results of treating tomato plants with silver nanoparticles and silver nitrate led to an increase in the fresh weight of the plant. The results were similar to the study of Hojjat (2015), where treating fenugreek plants with a concentration of 10 ppm of silver nanoparticles increased the fresh weight of the plant. As for the fresh and dry weight of the root as well as the dry weight of the plant, we note that silver nitrate caused a significant increase in the mentioned characteristics compared to the nano-treatment at a concentration of 10 mg L^{-1}, which caused a significant decrease in them. This is consistent with the study of Castro-Gonzalez et al. (2019), who reported that exposure of rice (Oryza sativa) to 0.5 mg L^{-1} of silver particles significantly reduces the biomass of roots and shoots.

Our results show that treatment of tomato plants with nano silver and silver nitrate led to an increase in antioxidants SOD and catalase compared to the control. This is consistent with Gracia et al. (2006). Saleeb et al. (2019) reported that treatment with silver nitrate and nano silver caused an increase in GSH in sunflower plants. This is contrary to our results, in which silver nitrate did not significantly affect GSH, while nano treatment caused a decrease in GSH.

The effect of nano silver or its salt negatively affected the plant's content of total soluble sugars, and these results are consistent with what was mentioned by (KRISHNARAJ et al., 2012). The study showed that the chlorophyll content of the leaves increased when treated with silver and nano salts, and the study results were consistent with the study of other researchers (SALAMA, 2012; RAZZAQ et al., 2015). Nanoparticles of essential and non-essential elements affect physiological processes, and plant growth and development depend on their size, composition, concentration, and method of use (COLBERT et al., 2022).

The stem section of the tomato plant is circular, the epidermal cells appeared in a single row, and reached the highest average thickness in the nano treatment compared to the rest of the other treatments. Our results are consistent with those of Elfeky et al. (2013) in their study on *Ocimum basilicum*, where the epidermal cells in plants treated with nanoparticles became larger and reached their maximum size. The cortex was distinguished into angular collenchyma and parenchyma tissue; the silver nitrate treatment recorded the highest average thickness, compared to the nano treatment, which recorded the lowest average thickness. The vascular bundles were of the open type and lateral. Dark-colored deposits appeared between the intercellular spaces of the parenchyma cells in the cortex and pith region. Our study is consistent with the results of Geisler-Lee et al. (2013) and Castro-Gonzalez et al. (2019), in the presence of accumulations or deposits of nanoparticles in the spaces between cells through the reabsorption pathway that passes through the vascular bundles and moves to the next cells through the cell membrane and reaches the leaves (Table 1). The characteristics of the tissue sections in tomato plants are consistent with what was described by Tuylu (2018).

As for the surface appearance of the epidermal cells, their cell walls appeared undulated in shape on both surfaces. Due to the nano-treatment, some of the shape of the walls changed from undulate to straight in some epidermal cells of the upper surface only, which is consistent with Liu et al. (2022), which indicates that the shape of the walls changes as a result of the plant cell being exposed to different stresses.

Both nano-silver and silver nitrate varied in their effect on the dimensions of epidermal cells and stomata and their numbers for upper and lower surfaces. The effects of silver nanoparticles on plant anatomy, growth and development processes depend on the size of the shape, the concentration used, the duration of treatment, the type of plant, the plant organ used, and the condition of the treated plants, which may be positive, negative or neutral (TRIPATHI et al., 2017b).

5. CONCLUSIONS

Exposure to silver nitrate improved most growth parameters of a tomato plant. In contrast, exposure to Bio-AgNPs showed a negative effect on morphological characters except for fresh weight of the stem.

Silver nitrate and Bio-AgNPs did not affect proline content in tomato leaves, while total sugars decreased to their lowest average in both treatments compared to the control. Bio-AgNPs caused a decrease in the non-enzymatic antioxidant GSH and an increase in the enzymatic activities of SOD, catalase and chlorophyll.

The number of chlorenchyma and parenchyma cells in tomato stems increased when exposed to silver, whether silver nitrate or Bio-AgNPs. In contrast, silver showed no effect on the number of vascular bundles and xylem vessels per vascular bundle.

The effects of bio-silver nanoparticles appeared as dark areas in the intercellular spaces between parenchyma cells in the stem and cortex. At the same time, silver in both forms in the surface view of tomato leaves showed a different effect on increasing or decreasing the number of cells and stomata.

5.1. Recommendations

Repeat the experiment using another plant and concentrations higher than 10 mg L^{-1} biosynthesized silver nanoparticles, paying attention to nanotechnology in agriculture and taking precautions against its negative effects.

6. REFERENCES

ABDEL-AZEEM, E. A.; ELSAYED, B. A. Phytotoxicity of silver nanoparticles on *Vicia faba* seedlings. **New York Science Journal**, v. 6, n. 12, p. 148-155, 2013.

AEIBI, H. Catalase in vitro. **Methods of Enzymology**, v. 105, p. 121-126, 1984. https://doi.org/10.1016/S0076-6879(84)05016-3

AHMAD, K. A. L. I. D.; KHAN, M. A.; AHMAD, M.; SHAHEEN, N. I. G. H. A. T.; NAZIR, A. B. D. U. L. Taxonomic diversity in epidermal cells of some sub-tropical plant species. **International Journal of Agriculture & Biology**, v. 12, n. 1, p. 115-118, 2010.

AL-JUBOORY, M. S.; SHAKIR, Walaa M. Effect of spraying potassium on the growth of *Vicia faba* L. **Kirkuk Journal of Science**, v. 14, n. 3, p. 174-187, 2019. https://doi.org/10.32894/KUJSS.2019.14.3.13

Al-SALAMA, Y.; ALGLGHORAIBIL, I.; AlSOUSE, M.; ZEIN, R. The Impact of seed priming silver nanoparticles on germination indicators of durum wheat (*Triticum durum* L., var. Sham 7). **Al-Qadisiyah Journal For Agriculture Sciences**, v. 14, n. 1, p. 23-29, 2024. https://doi.org/10.33794/qjas.2024.145858.1157

BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, v. 39, p. 205-207, 1973. https://doi.org/10.1007/BF00018060

BROOKIE, K. L.; BEST, G. I.; CONNER, T. S. Intake of raw fruits and vegetables is associated with better mental health than intake of processed fruits and vegetables, **Frontiers in Psychology**, v. 9, e487, 2018. https://doi.org/10.3389/fpsyg.2018.00487

CASTRO-GONZALEZ, C. G.; SANCHEZ-SEGURA, L.; GOMEZ-MERINO, F. C.; BELLA-BELLO, J. J. Exposure of stevia (*Stevia rebaudiana* B.) to silver nanoparticles in vitro: transport and accumulation. **Scientific Reports**, v. 9, n. 1, e10372, 2019. https://doi.org/10.1038/s41598-019-46828-y

DUBOIS, M.; GILLES, K. A.; HAMILTON, J. K.; REBERS, P. T.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical chemistry**, v. 28, n. 3, p. 350-356, 1956. https://doi.org/10.1021/ac60111a017

ELFEKY, S. A.; MOHAMMED, M. A.; KHATER, M. S.; OSMAN, Y. A.; ELSHERBINIL, E. Effect of magnetite nano-fertilizer on growth and yield of *Ocimum basilicum* L. **International Journal of Indigenous Medicinal Plants**, v. 46, n. 3, p. 1286-11293, 2013.

EL-TEMSAH, Y. S.; JONER, E. J. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. **Environmental Toxicology**, v. 27, n. 1, p. 42-49, 2011. https://doi.org/10.1002/tox.20610

FARGHALY, F. A.; NAFADY, N. A. Green synthesis of silver nanoparticles using leaf extract of *Rosmarinus Officinalis* and its effect on tomato and wheat plants. **Journal of Agricultural Science**, v.7, n. 11, e277, 2015.

FRANCO, J. A.; PEREZ-SAURA, P. J.; FERNANDEZ, J. A.; PARRA, M.; GARCIA, A. L. Effect of two irrigation rates on yield, incidence of blossom-end rot, mineral content and free amino acid levels in tomato cultivated under drip irrigation using saline water. **The Journal of Horticultural Science and Biotechnology**, v. 74, n. 4, p. 430-435, 1999. https://doi.org/10.1080/14620316.1999.11511132

GALLEGO, S. M.; BENAVIDES, M. P.; TOMARO, M. L. Effect of Heavy Metal Ion Excess on Sunflower Leaves: Evidence for Involvement of Oxidative Stress. **Plant Science**, v. 121, n. 2, p. 151-159, 1996. https://doi.org/10.1016/S0168-9452(96)04528-1

GARCCIA, J. S.; GRATAO, P. L.; AZEVEDO, R. A.; ARRUDA, M. A. Z. Metal Contamination effects on sunflower (*Helianthus annuus* L.) growth and protein expression in leaves during development. **Journal of Agricultural and Food Chemistry**, v. 54, n. 22, p. 8623-8630, 2006. https://doi.org/10.1021/jf061593l

GEISLER-LEE, J.; WANG, Q.; YAO, Y.; ZHANG, W.; GEISLER, M.; LI, K.; HUANG, Y.; CHEN, Y.; KOLMAKOV, A.; MA, X. Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. **Nanotoxicology**, v.7, n.3, p. 323-337, 2013. https://doi.org/10.3109/17435390.2012.658094

GIRALDO, J. P.; LANDRY, M. P.; FALTERMEIER, S. M.; McNICHOLAS, T. P.; IVERSON, N. M.; BOGHOSSIAN, A. A.; REUEL, N. F.; HILMER, A. J.; SEN, F.; BREW, J. A.; STRANO, M. S. Plant Nanobionics Approach to Augment Photosynthesis and Biochemical Sensing. **Nature Materials**, v. 13, n. 4, p. 400-408, 2014. https://doi.org/10.1038/nmat3890

GIOVANNI, C. D.; ORCO, P. D.; BRUNO, A.; CICCARESE, F.; LOTTI, C.; RICCIARDI, L. Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (ol-2) in tomato. **Plant Science**, v. 166, p. 41-48, 2004. https://doi.org/10.1016/j.plantsci.2003.07.005

HAMOUDA, R. A.; HUSSEIN, M. H.; ABO-ELMAGD, R. A.; BAWAZIR, S. S. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. **Scientific Reports**, v. 9, n. 1, e13071, 2019. https://doi.org/10.1038/s41598-019-49444-y

HASAN, M.; MEHMOOD, K.; MUSTAFA, G.; ZAFAR, A.; TARIQ, T.; HASSAN, S. G.; LOOMBA, S.; ZIA, M.; MAZHER, A.; MAHMOOD, N.; SHU, X. Phytotoxic evaluation of phytosynthesized silver nanoparticles on lettuce. **Coatings**, v. 11, n. 2, e225, 2021. https://doi.org/10.3390/coatings11020225

HOJJAT, S. S.; HOJJAT, H. Effect of nano silver on seed germination and seedling growth in fenugreek seed. **International Journal of Food Engineering**, v. 1, n. 2, p. 106-110, 2015.

KAVITHA, K. S.; BAKER, S.; RAKSHITH, D.; KAVITHA, H. U.; YASHWANTHA, R. H. C.; HARINI, B. P.; SATISH, S. Plants as green source towards synthesis of nanoparticles. **International Research Journal of Biological Sciences**, v. 2, n. 6, p. 66-76, 2013.

KOLBERT, Z.; SZOOLSI, R.; RONVARI, A.; MOLNAR, Á. Nanoforms of essential metals: from hormetic phytoeffects to agricultural potential. **Journal of Experimental Botany**, v. 73, n. 6, p. 1825-1840, 2022. https://doi.org/10.1093/jxb/erab547

KRISHNARAJ, C.; JAGAN, E. G.; RAMACHANDRAN, R.; ABIRAMI, S. M.; MOHAAN, N.; KALAICHELVAN, P. T. Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. Plant growth metabolism. **Process Biochemistry**, v. 47, n. 4, p. 651-658, 2012. https://doi.org/10.1016/j.procbio.2012.01.006

LANNOY, G. Cultures des légumes. In: RAEMAEKERS, R. H. (Ed.) **Agriculture en Afrique Tropicale**. Bruxelles: DGCI, 2011. p. 429-513.

LIU, J.; SHAO, Y.; FENG, X.; OTIE, V.; MATSUURA, A.; IRHAD, M.; ZHENG, Y.; AN, P. Cell wall components and extensibility regulate root growth in *Suaeda salsa* and *Spinacia oleracea* under salinity. **Plants**, v. 11, n. 7, e900, 2022. https://doi.org/10.3390/plants11070900

LOO, Y. Y.; CHIENG, B. W.; NISHIBUCHI, M.; RADU, S. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. **International Journal of**

Nanomedicine, v. 7, p. 4263-4267, 2012. https://doi.org/10.2147/IJN.S33344

MARKLUND, S.; MARKUND, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase." **European Journal of Biochemistry**, v. 47, n. 3, p. 469-474, 1974.

MILLS, T. C. **Time Series Techniques for Economists**. Cambridge: Cambridge University Press, 1990. 387p.

MONREAL, C. M.; DEROSA, M.; MALLUBHOTIA, S. C.; BINDRABAN, P. S.; DIMKPA, C. The Application of Nanotechnology for Micronutrients in Soil-Plant Systems. **VFRC Report**, v. 3, n. 44, p. 1-53, 2015. https://hub.ifdc.org/handle/20.500.14297/1747

MORON, M. S.; DEPIERRE, J. W.; MANNERVIK, B. Levels of glutathione, glutathione reductase and glutathione s-transferase activities in Rat Lung and Liver. **Biochimica et Biophysica Acta** - **General Subjects**, v. 582, n. 1, p. 67-78, 1979. https://doi.org/10.1016/0304-4165(79)90289-7

NAJJAT, N. R. **Study of some quantitative and qualitative traits in semi-diallel hybrid crosses between some tomato varieties (*Lycopersicum esculentum*)**. 130p. Master's thesis - Tishreen University, Latakia, Syria, 2008.

OBIKWE, C. O.; OBASEKI-EBOR, E. E. Incidence of tomato fungi and their in-vitro inhibition by Honey Distillate (Hy-1). **Nigerian Journal of Microbiology**, v. 7, n. 1, p. 121-127,1987.

RAZZAQ, A.; AMMARA, R.; JHANZAB, H. M.; MAHMOOD, T.; HAFEEZ, A.; HUSSAIN, S. A novel nanomaterial to enhance growth and yield of wheat. **Journal of Nanoscience and Technology**, v. 2, n. 1, p. 55-58, 2016.

RIDHA, D. M.; AL-RAFYAI, H. M.; NAJI, N. S. Bactericidal potency of green synthesized silver nanoparticles against waterborne *Escherichia coli* Isolates. **Nano Biomedicine and Engineering**, v. 13, n. 4, p. 372-379, 2021.

ROLIM, W. R.; PELEGRINO, M. T.; LIMA, B. de A.; FERRAZ, L. S.; COSTA, F. N.; BERNARDES, J. S.; RODRIGUES, T.; BROCCHI, M.; SEABRA, A. B. Green tea extract mediated biogenic synthesis of silver nanoparticles: characterization, cytotoxicity evaluation and antibacterial activity. **Applied Surface Science**, v. 463, p. 66-74, 2019. https://doi.org/10.1016/j.apsusc.2018.08.203

SALAMA, H. M. H. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). **International Research Journal of Biotechnology**, v. 3, n. 10, p. 190-197, 2012.

SALEEB, N.; ROBINSON, B.; CAVANAGH, J.; HOSSAIN, A. K. M.; GOONERATNE, R. Biochemical changes in sunflower plant exposed to silver nanoparticles/silver ions. **Journal of Food Science & Technology**, v. 4, n. 2, p. 629-644, 2019.

SHAH, V.; BELOZEROVA, I. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. **Water, Air, and Soil Pollution**, v. 197, p. 143-148, 2009. https://doi.org/10.1007/s11270-008-9797-6

SINGH, I. D.; STOSKOPF, N. C. Harvest index in cereals. **Agronomy Journal**, v. 63, n. 2, p. 224-226, 1971. https://doi.org/10.2134/agronj1971.00021962006300020008x

TRIPATHI, D. K.; TRIPATHI, A.; SHWETA, S.; SINGH, Y.; SINGH, K.; VISHWAKARMA, G.; YADAV, S.; SHARMA, V. K.; SINGH, R. K.; MISHRA, R. G.; UPADHYAY, N. K.; DUBEY, Y. L.; CHAUHAN, D. K. Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: a concentric review. **Frontiers in Microbiology**, v. 8, eo7, 2017. https://doi.org/10.3389/fmicb.2017.00007

TUYLU, M. Examination of anatomical features of tomato (*Lycopersicon esculentum* Mill.) varieties cultivated under a hydroponic system. **Applied Ecology & Environmental Research**, v. 16, n. 3, p. 3381-3391, 2018.

WIDATALLA, H. A.; YASSIN, L. F.; ALRASHEID, A. A.; AHMED, S. A. R.; WIDDATALLAH, M. O.; ELTILIB, S. H.; MOHAMED, A. A. Green synthesis of silver nanoparticles using green tea leaf extract, characterization and evaluation of antimicrobial activity. **Nanoscale Advances**, v. 4, n. 3, p. 911-915, 2022.

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