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Colloidal Silver Nanoparticles Enhance Bulb Yield and Alleviate the Adverse Effect of Saline Stress on Lily Plants

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ABSTRACT

Salinity occurring in intensively used agricultural, industrialized, and urbanized areas is one of the main factors in soil degradation. The effect of silver nanoparticles (AgNPs) on plant growth under environmental stresses is still not fully understood. Two experiments were conducted on the response of Asiatic lilies to treatment with colloidal AgNPs. In Experiment I, the study aimed to evaluate the effect of treating 'Osasco' lily bulbs with colloidal AgNPs (0, 25, 50, 100, and 150 ppm) on growth, flowering, and bulb yield, as well as the production of bulblets. Compared with the control, the applied colloidal AgNPs at all concentrations caused an acceleration of flowering and an increase in bulb diameter and the fresh weight of the aboveground part of the plants and bulbs. In addition, treatment with colloidal AgNPs at concentrations of 100 and 150 ppm increased bulblets' number and fresh weight. In Experiment II, the effects of colloidal AgNPs (100 ppm) and NaCl stress (600 mM) on the growth parameters, assimilation pigment content, and chemical composition of 'Bright Pixi' lily leaves were evaluated. As a result of the application of colloidal AgNPs, plants flowered faster and had increased height, petal width, fresh bulb weight, bulb diameter, and several scales in the bulb. Under NaCl stress, plants had reduced fresh weight of the aboveground part and bulb, bulb diameter, number of scales in a bulb, and contents of assimilation pigments, N, K, Ca, Cu, Mn and Zn. Colloidal AgNPs offset the adverse effects of salinity on bulb yield by increasing fresh bulb, bulb diameter, and the number of scales in lily bulbs. In conclusion, using colloidal AgNPs can contribute to developing new methods of bulbous plants production and an effective strategy to protect plants from ever-increasing land salinization.

Keywords: nanomaterials, environmental stress, soil salinity, plant growth, colloidal silver.

INTRODUCTION

Nanotechnology deals with the fabrication, design, and modification of nanoscale structures having at least one dimension smaller than 100 nm (Fincheira et al., 2021). Nanoparticles and nanomaterials are distinguished by unique physical, chemical, and biological properties due to their relatively low mass, large surface area relative to volume, quantum effect, enhanced adsorption and absorption capacity, and tendency to agglomerate rapidly (Wang et al., 2017). According to the Global Market Trajectory & Analytics Research and Markets Ltd. 2021 report, market demand for nanotechnology products is worth $42.2 billion and is estimated to reach $70.7 billion by the end of 2026 (GMTAR 2023). Currently, approximately 1,300 nanoscale materials are available in a free state or as an aggregate or agglomerate. In agriculture and horticulture, nanomaterials are used in plant biotechnology, in producing fertilizers and plant protection products, and in storing and marketing agricultural products and food (Wang et al., 2016; Yashwant et al., 2022). However, the use of nanoparticles in agronomy compared to other branches is still small. Of the approximately 29,000 patents in nanotechnology granted worldwide, only 500 of them relate to agriculture and nutrition. Therefore, it is expedient to conduct research and development on a larger scale using nanoparticles in crop production (Yadav et al., 2019).

Silver nanoparticles (AgNPs) are various industries' most widely used nanomaterials. AgNPs are clusters of metallic silver Ag atoms, generally smaller than 100 nm and containing 20,000 to 15,000 silver atoms. It is estimated that AgNPs contain almost a quarter of all nanotechnology products (Yaqoob et al., 2020). AgNPs have well-documented bactericidal and fungicidal properties (Crisan et al., 2021). AgNPs are used in in vitro propagation, protection, fertilization, flower shelf life extension, and cut greens in horticulture. AgNPs exhibit a broad spectrum of biological activity and can affect plant growth and development (Byczyńska et al., 2019; Rana et al., 2021). However, the effect of AgNPs on plants is uneven and can be positive or negative (Cvjetko et al., 2017; Guzmán-Báez et al., 2021). AgNPs have been shown to have stimulatory or inhibitory effects on seed germination, plant organ development, flowering, water management, chlorophyll content, biomass, and plant yield. Individual species and even plant varieties differ in their response to AgNPs (Anjum et al., 2013; Byczyńska et al., 2019; Rana et al., 2021; Chen et al., 2023). Most experiments on the effects of AgNPs on plants are conducted under laboratory conditions, often in vitro, in phytotrons, on a small sample of plants, unaffected by environmental factors (Siddiqi and Husen, 2022). Among the few studies, there are such in which the effects of AgNPs on plant growth are evaluated under production conditions (Salachna et al., 2019). Additionally, plant responses' long-term effects to AgNPs are poorly understood.

Lilies (*Lilium* spp.) are ornamental, edible, and medicinal plants that have accompanied humans since ancient times (Zhou et al., 2021). Asiatic hybrids are the most crucial group of lilies in large-scale production for cut flowers and potted plants. These lilies have versatile uses in horticulture, so there is a high demand for their bulbs and new cultivation technologies. On a mass scale, lilies are propagated vegetatively from bulb scales (Tang et al., 2020). The formation process and yield of bulblets are mainly influenced by environmental factors, growth regulators, and biostimulants (Sochacki et al., 2018; Gioi et al., 2019, Li et al., 2023).

In cultivating lilies and other crops, soil salinity is an increasingly severe problem. Currently, up to 20% of all cultivated land is saline, and global climate change means the areas affected by excessive salinity are increasing (Butcher et al., 2016; Isayenkov and Maathuis 2019). Few studies on lilies indicate that excessive salinity can lead to stunted growth, decreased biomass, and a marked reduction in flower quality (Ayad et al., 2019; Kang et al., 2021). The effect of salinity on lily bulb yield is far less well understood.

Recently, there has been emerging evidence that nanoparticles, due to their unique properties, can increase the tolerance of plants under abiotic stress conditions (Zhao et al., 2020; Zulfiqar and Ashraf, 2021). To date, no studies have described nanoparticles' effect on lily growth under extreme salinity conditions. This research evaluated colloidal AgNPs as a potential source of biostimulatory factors in cultivating Asian lily cultivars under both normal and saline conditions. The study aimed to determine the effects of colloidal AgNPs on (i) the growth, flowering, and bulb yield; (ii) the regeneration potential and yield of bulblets; and (iii) the growth and yield of lily bulbs grown under saline stress. It was hypothesized that colloidal AgNPs have long-term effects on the growth and yield of lily bulbs and alleviate salinity stress.

MATERIALS AND METHODS

Two experiments were conducted at the West Pomeranian University of Technology in Szczecin (lat. 53°26′17″ N, long. 14°32′32″ E). Commercially available colloidal AgNPs (Sigma–Aldrich) of size 7 to 25 nm were used. Bulbs of two Asiatic hybrid lily varieties recommended for potting were used in the study: 'Osasco' with an average circumference of 14.6 cm from Ogrodnictwo Wiśniewski Jacek Junior (Poland) and 'Bright Pixi' with an average circumference of 12.9 cm from Benex (Poland). The bulbs were planted into plastic pots of 16 cm diameter and 2 dm^3 capacity into TS1 medium with pH 6.4, an electrical conductivity (EC) of 0.54 mS cm^{-1} and the following composition (mg dm^{-3}): N -NO_3 - 182; P - 131, K - 402, Ca - 1646, Mg - 172, Cl - 18. Plants were grown in 2015. (experiment 1) and 2016. (Experiment 2) from mid-March to mid-July under natural photoperiod conditions in an unheated plastic tunnel, where the vents opened when the air temperature exceeded 20 °C.

Experiment 1

'Osasco' lily bulbs were soaked for 1 h in AgNP solutions of 0 (control), 25, 50, 100, and 150 ppm before planting. Deionized water was used to prepare the solutions. After 24 h, the dried bulbs were planted individually into pots. There were 20 bulbs in each variant, 5 in repetition. The number of days from planting the bulbs to the beginning and end of the flowering of the plants was calculated. The beginning of flowering was set as the development of the first flower on the plant. The moment when the petals fell off at the last flower on the plant was taken as the end of flowering. Plant height and petal length were determined when the first flower was developed. Leaves and flowers were counted, and the fresh weight of plants and bulbs was determined.

The dug bulbs 4 weeks after flowering were used for propagation by scales. For this purpose, 4 mother bulbs from each variant (0, 25, 50, 100, and 150 ppm colloidal AgNPs) were divided into scales. All scales from a single bulb were repeated (an average of 52.1 scales per bulb). Scales from individual bulbs were placed separately in 2 l plastic bags filled with the moist substrate (50% acid peat and 50% perlite). The bags with the substrate mixed with the scales were stored in the dark at 20-22 °C for 12 weeks and then at 3-5 °C for 16 weeks under high relative humidity (RH > 90%). After this period, the number of bulblets formed on the scales from one bulb and the fresh weight of total bulblets was determined using a balance with a reading accuracy of 0.001 g. Their percentage yield structure was calculated based on the results obtained for the fresh weight of individual bulblets.

Experiment 2

The following treatments were applied to ‘Bright Pixi’ lilies: (i) control - untreated plants, (ii) bulbs soaked before planting in a solution of 100 ppm colloidal AgNPs, (iii) plants watered with 600 mM NaCl solution, and (iv) bulbs soaked before planting in a solution of 100 ppm colloidal AgNPs and plants watered with 600 mM NaCl solution. In each experimental variant, 20 bulbs were planted, 5 in each repetition. Bulbs in the colloidal AgNP solution were soaked for 1 h. Watering the plants with 600 mM NaCl solution with electrolytic conductivity (EC) = 2.32 mS cm^{-1} was started 35 days after planting the bulbs during the vegetative stage. Salting was carried out thrice, every 5 days, using 100 ml of NaCl solution for each plant. Nonsalted plants were watered with tap water with an EC of 0.25 mS cm^{-1}. The concentration of 100 ppm colloidal AgNPs was chosen based on the results of experiment 1, where bulb quality was best at this concentration. The concentration of 600 mM NaCl was chosen based on the preliminary experiment, where an apparent adverse effect on the biomass of lily plants was shown at this concentration. Measurements of morphological characteristics were carried out analogously to experiment 1. Fresh leaves collected from the middle part of the plants at the flowering stage were used for analysis. The assimilation pigment content was determined using a SPEKOL 11 spectrophotometer (Carl Zeiss Jena, Jena, Germany) in fresh leaves according to the method described in an earlier paper (Salachna et al., 2019). Macro (P, K, Ca, Mg, Na) and micronutrient (B, Cu, Fe, Mn, Zn) contents were determined in dried (48 hours, 60 °C) and ground leaves using inductively coupled plasma–optical emission spectrometry (ICP–OES) with the Optima 2000 DV sequential spectrometer (Perkin-Elmer, Boston, MA, USA). Nitrogen content was determined by the Kjeldahl method (the Kjeldahl apparatus Vapodest, Königswinter, Germany). Analyses were performed according to the methods (Zawadzińska et al., 2022). The assimilation pigment and mineral contents were determined in 3 replicates.

The experiments were set up in a random subblock design. The results were verified by analysis of variance (ANOVA) for a single classification. The classification factor for experiment 1 was the level of colloidal AgNPs (0, 25, 50, 100, and 150 ppm), and for the second experiment, four treatments (control, AgNPs, NaCl stress, and AgNPs + NaCl stress) were used. Before proceeding to the variance analysis, the variances’ homogeneity and normality of the distributions were checked. The Tukey HSD test was used to compare means. The Excel spreadsheet and TIBCO Statistica™ Professional 13.3.0 software package (TIBCO Software, USA) were used for statistical analysis.

RESULTS

Table 1 shows the effect of soaking bulbs before planting in a solution of colloidal AgNPs on the growth and morphological parameters of ‘Osasco’ lilies. The application of colloidal AgNPs at concentrations of 25, 50, 100, and 150 ppm accelerated the flowering of plants by 3.1, 4.8, 5.5, and 5.8 days, respectively, compared to control plants. It was shown that plants from bulbs treated with colloidal AgNPs flowered slightly longer. The most extended flowering period (21.8 days) was characterized by lilies treated with 150 ppm AgNPs. None of the four colloidal AgNP concentrations caused a significant difference compared to the control for plant height, number of flowers, petal length, and width. Treatment with colloidal AgNPs at all concentrations increased the fresh weight of the aboveground parts of the plants from 21.5% to 32.0% and the fresh weight of the bulbs from 36.3% to 64.0% compared to the control. Applying colloidal AgNPs also significantly increased the bulb diameter from 19.9% to 22.0%. Plants treated with colloidal AgNPs at a concentration of 100 ppm had the highest fresh weight of aboveground and bulb parts, bulb diameter, and the number of scales in the bulb. No signs of colloidal AgNP-induced phytotoxicity were observed on leaves, flowers, and bulbs (Figure 1).

‘Osasco’ lily scales formed bulblets with adventitious roots at the base of the adaxial and abaxial parts of the scales (Figure 2). There was a significant effect of colloidal AgNPs on the number and total fresh weight of bulblets (Figures 2, 3). Scales from parent bulbs treated with colloidal AgNPs at 100 and 150 ppm yielded more bulblets by 15.1 and 23.6%, respectively, which at the

Table 1. Effect of AgNPs on days to anthesis (DA), flower longevity (FL), flower number (FN), tepal length (TL), tepal width (TW), plant height (PH), above-ground part fresh weight (AGPFW), bulb fresh weight (BFW), scales number (SN), and bulb diameter (BD) of lily ‘Osasco’ plants. Values for each parameter followed by differing letters are significantly different at $p \leq 0.05$ (ANOVA and Tukey’s test)

Traits	AgNPs concentration				
	Control	25 ppm	50 ppm	100 ppm	150 ppm
DA (days)	90.3 ± 2.00 a	87.2 ± 1.15 b	85.5 ± 1.08 b	84.8 ± 0.50 b	84.5 ± 2.25 b
FL (days)	17.6 ± 1.39 b	19.2 ± 1.71 ab	20.0 ± 1.34 ab	18.9 ± 0.39 ab	21.8 ± 1.52 a
FN	7.63 ± 0.48 a	7.50 ± 1.08 a	8.50 ± 1.78 a	8.50 ± 0.41 a	8.13 ± 0.25 a
TL (cm)	7.48 ± 0.09 a	7.65 ± 0.24 a	7.53 ± 0.17 a	7.49 ± 0.17 a	7.64 ± 0.49 a
TW (cm)	4.79 ± 0.14 a	4.84 ± 0.24 a	4.65 ± 0.34 a	4.69 ± 0.14 a	4.88 ±0.25 a
PH (cm)	33.5 ± 0.76 a	34.4 ± 0.80 a	33.9 ± 0.78 a	33.8 ± 0.88 a	34.7 ± 1.75 a
AGPFW (g)	78.7 ± 5.19 c	95.6 ± 4.66 b	101 ± 7.95 ab	104 ± 11.6 a	103 ± 3.99 a
BFW (g)	30.0 ± 1.10 c	41.4 ± 5.03 ab	42.8 ± 2.44 ab	49.2 ± 6.90 a	40.9 ± 3.16 b
SN	47.2 ± 1.26 b	54.0 ± 3.46 ab	51.2 ± 2.02 ab	58.0 ± 4.54 a	50.2 ± 5.57 b
BD (mm)	46.3 ± 1.40 b	56.3 ± 3.44 a	56.5 ± 4.47 a	57.1 ± 4.24 a	55.5 ± 2.45 a

Figure 1. Appearance of flowering plants (A) and parent bulb (B) of lily ‘Osasco’ treated with colloidal AgNPs: from left 0 (control), 25, 50, 100, and 150 ppm AgNPs

same time had increased fresh weight by 39.6 and 36.5% compared to the control. Analysis of the yield structure of bulblets (Figure 4) shows that bulblets with a weight of 0.01-0.20 g accounted for the largest share in each variant. The highest proportion of bulblets weighing 0.01-0.20 g was observed in the control (79.3%), and the lowest proportion was observed in the 25 ppm colloidal AgNP variant (54.8%).

The application of colloidal AgNPs for bulb soaking positively affected most of the evaluated morphological traits of 'Bright Pixi' lilies (Table 2). As a result of the application of colloidal AgNPs, plants flowered faster by 3.3 days

Figure 2. Formation of bulblets on 'Osasco' lily scales (A); Yield of bulblets obtained from bulb scales soaked in a solution of colloidal AgNPs: from left 0 (control), 25, 50, 100 and 150 ppm AgNPs (B)

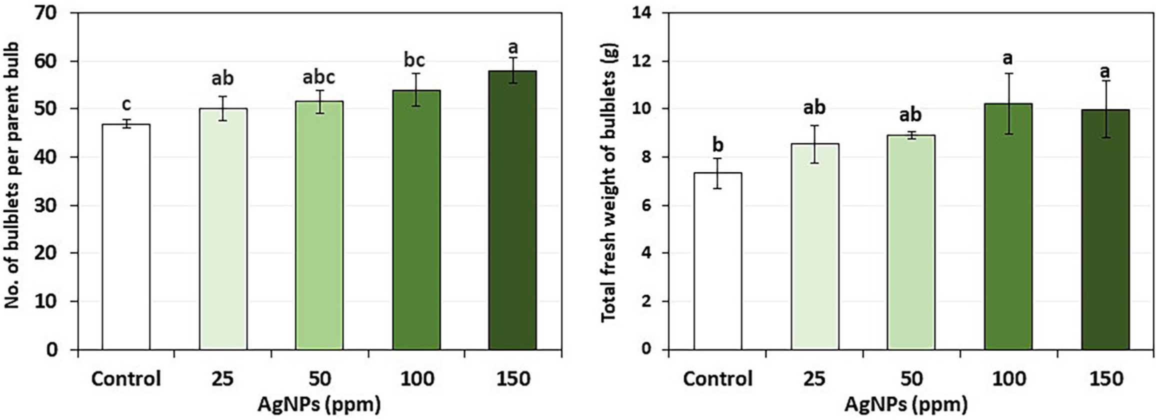

Figure 3. Number of bulblets (A) and fresh weight (FW) of total bulblets produced by scales from one parent bulb (B) treated with colloidal AgNPs. Data are mean ± SD. Different letters above the error bars indicate significant differences for $p < 0.05$

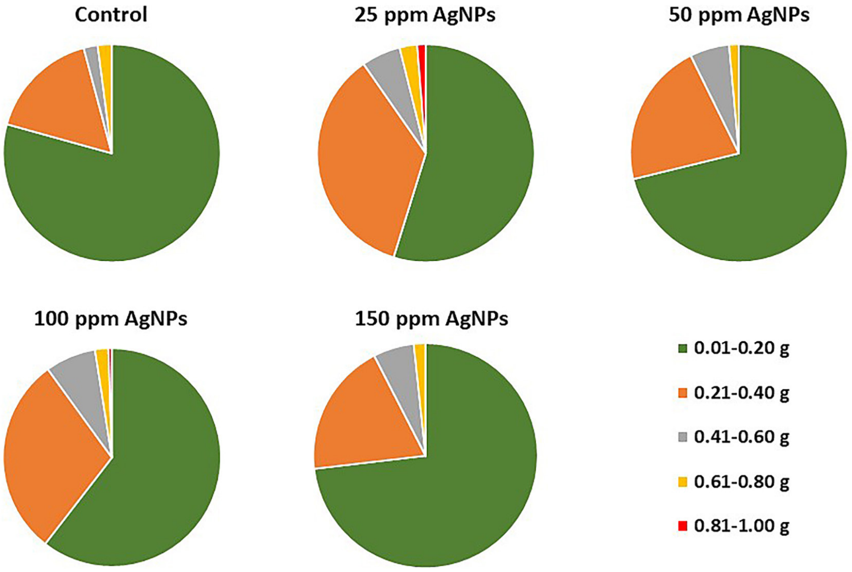

Figure 4. Percentage of bulblets with different fresh weights depending on treatment with colloidal AgNPs

and had increased height (by 8.0%), petal width (13.5%), fresh bulb weight (13.9%), bulb diameter (14.3%) and the number of scales in the bulb (12.3%) compared to plants not treated with colloidal AgNPs. NaCl stress caused a slight delay in flowering and shortened flowering length, as well as a slight reduction in flower petal length and plant height. A much stronger negative effect of NaCl stress was shown by analyzing biomass and bulb yield data. As a result of NaCl stress, plants had reduced fresh weight of the aboveground part (by 36.5%), fresh weight of bulbs (32.5%), bulb diameter (13.0%), and the number of scales in the bulb (40.8%) compared to nonsalted plants. Plants treated with colloidal AgNPs under NaCl stress conditions had a similar number of days to flowering, number of flowers, petal length and width, and fresh weight of the aboveground part compared to NaCl-only plants. Nevertheless, using colloidal AgNPs reduced the harmful effects of NaCl stress on plant height and bulb quality. Plants treated with both colloidal AgNPs and NaCl were characterized by increased height (by 7.6%), fresh bulb weight (42.3%), number of scales per bulb (46.9%), and bulb diameter (13.7%) compared to NaCl-stressed plants.

The analyses show that applying colloidal AgNPs and NaCl stress significantly affected changes in the pigment content of ‘Bright Pixi’ lily leaves (Table 3). The highest amounts of Chl a, Chl b, Chl a+b, and Car were found in plants treated with AgNPs. Significantly lower amounts of Chl a, Chl b, Chl a+b, and Car were found in plants treated only with NaCl. Compared to the control, there was a marked decrease in nitrogen, potassium, and calcium in the leaves of plants treated only with NaCl (by 19.1%, 36.6%, and 25.1%, respectively) and in the leaves of plants treated with colloidal AgNPs and NaCl (by 21.3%, 29.9% and 20.9%) (Table 4). At the same time, it was shown that under the influence of salinity in the NaCl and colloidal AgNP + NaCl variants, there was a marked increase in Na content (by 4.1 times and 3.6 times, respectively). In the variants of colloidal NaCl stress, and colloidal AgNPs + NaCl stress, there was a decrease in Cu, Mn, and Zn content compared to untreated plants.

Table 2. Effect of AgNPs, NaCl stress and AgNPs + NaCl stress on days to anthesis (DA), flower longevity (FL), flower number (FN), tepal length (TL), tepal width (TW), plant height (PH), above-ground part fresh weight (AGPFW), bulb fresh weight (BFW), scales number (SN), and bulb diameter (BD) of lily ‘Bright Pixi’ plants. Values for each parameter followed by differing letters are significantly different at $p \leq 0.05$ (ANOVA and Tukey’s test)

Traits	Treatments			
	Control	AgNPs	NaCl stress	AgNPs+NaCl stress
DA (days)	71.0 ± 1.00 a	67.7 ± 1.15 b	70.3 ± 1.53 ab	68.3 ± 1.15 ab
FL (days)	11.5 ± 0.50 bc	13.0 ± 0.00 a	10.7 ± 0.58 c	11.7 ± 0.58 b
FN	2.17 ± 0.29 a	2.50 ± 0.50 a	1.83 ± 0.29 a	1.83 ± 0.29 a
TL (cm)	7.18 ± 0.19 ab	7.58 ± 0.13 a	6.66 ± 0.14 b	6.68 ± 0.51 b
TW (cm)	3.92 ± 0.06 b	4.45 ± 0.13 a	3.79 ± 0.12 b	3.78 ± 0.06 b
PH (cm)	36.2 ± 0.29 bc	39.1 ± 1.66 a	35.4 ± 0.51 c	38.1 ± 0.38 ab
AGPFW (g)	31.2 ± 2.60 a	34.4 ± 2.46 a	19.8 ± 1.91 b	20.0 ± 1.65 b
BFW (g)	37.5 ± 1.5 b	42.7 ± 1.8 a	25.3 ± 2.2 c	36.0 ± 0.4 b
SN	65.9 ± 10.0 ab	74.0 ± 5.77 a	39.0 ± 6.25 c	57.3 ± 8.25 b
BD (mm)	53.8 ± 0.4 b	61.5 ± 5.3 a	46.8 ± 2.1 c	53.2 ± 3.0 b

Table 3. Effect of AgNPs, NaCl stress and AgNPs + NaCl stress on photosynthetic pigment concentrations (mg kg-1 fresh weight) in leaves of lily ‘Bright Pixi’ plants. Values for each parameter followed by differing letters are significantly different at $p \leq 0.05$ (ANOVA and Tukey’s test)

Traits	Treatments			
	Control	AgNPs	NaCl stress	AgNPs+NaCl stress
Chl a	718 ± 27.8 ab	850 ± 63.6 a	580 ± 4.10 c	607 ± 11.7 bc
Chl b	337 ± 26.9 ab	379 ± 20.9 a	275 ± 1.60 c	280 ± 2.20 bc
Chl a + b	1055 ± 54.7 ab	1228 ± 84.6 a	855 ± 5.70 c	887 ± 9.60 bc
Car	344 ± 13.4 ab	420 ± 34.3 a	282 ± 6.70 c	293 ± 3.50 bc

Table 4. Effect of AgNPs, NaCl stress and AgNPs + NaCl stress on macronutrient (% dry weight) and micronutrient (mg kg−1 dry weight) concentrations in leaves of lily ‘Bright Pixi’ plants. Values for each parameter followed by differing letters are significantly different at $p \leq 0.05$ (ANOVA and Tukey’s test)

Nutrient	Treatments			
	Control	AgNPs	NaCl stress	AgNPs+NaCl stress
N	2.72 ± 0.09 ab	2.85 ± 0.09 a	2.20 ± 0.06 b	2.14 ± 0.07 b
P	0.19 ± 0.03 a	0.19 ± 0.01 a	0.15 ± 0.02 a	0.17 ± 0.01 a
K	3.17 ± 0.01 a	3.20 ± 0.05 a	2.01 ± 0.03 b	2.22 ± 0.01 b
Ca	1.91 ± 0.08 a	2.18 ± 0.07 a	1.43 ± 0.04 b	1.51 ± 0.02 b
Mg	0.46 ± 0.01 a	0.47 ± 0.01 a	0.48 ± 0.02 a	0.46 ± 0.01 a
Na	0.18 ± 0.03 b	0.23 ± 0.05 b	0.74 ± 0.09 a	0.66 ± 0.02 a
B	89.2 ± 1.36 a	85.9 ± 3.33 a	87.7 ± 3.01 a	81.9 ± 4.66 a
Cu	2.72 ± 0.09 ab	2.85 ± 0.09 a	2.20 ± 0.06 b	2.14 ± 0.07 b
Fe	0.19 ± 0.03 a	0.19 ± 0.01 a	0.15 ± 0.02 a	0.17 ± 0.01 a
Mn	3.17 ± 0.01 a	3.20 ± 0.05 a	2.01 ± 0.03 b	2.22 ± 0.01 b
Zn	1.91 ± 0.08 a	2.18 ± 0.07 a	1.43 ± 0.04 b	1.51 ± 0.02 b

DISCUSSION

Nanoparticles can affect plants’ growth and metabolic processes and, therefore, can be classified as a potential source of biostimulants. In the present study, soaking the bulbs of Asian lilies ‘Osasco’ and ‘Bright Pixi’ before planting in a solution of colloidal AgNPs induced mainly biostimulant effects on bulb yield. As a result of the application of colloidal AgNPs, lily bulbs had increased fresh weight, number of scales, and diameter. The results obtained confirm, in part, our previous studies, where under the influence of AgNPs at concentrations ranging from 25 to 150 ppm, oriental lilies ‘Mona Lisa’ and ‘Littel John’ had increased fresh bulb weight. Similarly, Alcac et al. (2022) showed a positive effect of soaking bulbs in AgNP solutions with concentrations of 50-150 ppm on the bulb yield of ‘Santander’ lilies. The favorable effects of better-quality lily bulbs may be due to the biostimulatory effect of colloidal AgNPs on the biosynthesis of assimilatory pigments, as demonstrated in Experiment 2. It is known that assimilatory pigments are one of the most important chemical compounds in plants affecting the intensity of photosynthesis and, consequently, biomass production (Simkin et al., 2022). The stimulating effect of AgNPs at 100 ppm on chlorophyll and carotenoid content was confirmed by previous studies (Salachna et al., 2019). The explanation for the beneficial effect of colloidal AgNPs on lily bulb yield observed in our study may also be the application method and the protective effect of silver against pathogens (Gupta et al., 2018). In most studies, AgNPs are applied foliar or by watering the plants. Our previous study (Salachna et al., 2019) found that soaking lily bulbs in a solution of AgNPs was a more effective application method than watering or spraying. Therefore, the present study applied AgNPs by soaking lily bulbs. Compared to spraying the leaves or watering the plants, this treatment ensures limited penetration of AgNPs into the ecosystem, which is crucial in discussing the safety of nanoparticle application (Levard et al., 2012; León-Silva et al., 2016). The advantage of using AgNPs to treat seeds or bulbs is that the nanoparticles affect plants from the moment they start growing. Because silver compounds have bactericidal, fungicidal, and virucidal properties, plants treated with AgNPs are less susceptible to pathogens. Wojdyla et al. (2022) showed that treating *Hyacinthus orientalis* ‘Blue Pearl’ bulbs with a formulation containing a solution of hydrogen peroxide enriched with colloidal silver (H_2O_2-Ag^+) significantly reduced fungal growth on the bulbs and in the growing medium. The cited authors also found that H_2O_2-Ag^+ at 2–10% increased the leaf greening index and, depending on the concentration, the width of petals and inflorescences, number of flowers, length and length and width of leaves, and fresh and dry weight of hyacinth plants.

In the present study, colloidal AgNPs in both lily cultivars affected the time from bulb planting to the start of flowering. However, colloidal AgNPs were not observed to have a stimulating effect on flower number and petal length. In earlier studies, the Oriental lilies ‘Mona Lisa’ and ‘Little John’ had increased flower numbers due to AgNP application (Salachna et al., 2019). The uneven effect of AgNPs on lily flower yield is perhaps related to the different responses of the genotype, the size of the nanoparticles, the influence of environmental factors, and the hormesis effect (Guzmán-Báez et al., 2021; Chen et al., 2023). According to many researchers, nanoparticles can stimulate plant growth at low doses and inhibit plant growth at high doses (Salachna et al., 2021; Chen et al., 2023).

Despite numerous studies on the issue of propagation of bulbous plants by scale, few works have addressed the effect of nanoparticles on the yield of bulblets. In our previous study (Byczyńska et al., 2018), we showed that soaking the scales of three lily cultivars (Little John', 'Mona Lisa' and 'Osasco') in a solution of AgNPs at a concentration of 25–100 ppm had a stimulating effect on the number of bulblets. Moreover, treatment of lily scales with AgNPs at a 25-150 ppm concentration increased the fresh weight of bulblets and their roots. In this study, we demonstrated for the first time that colloidal AgNPs can have a concentration-dependent long-term stimulating effect on the formation of bulblets on lily scales. Applying colloidal AgNPs at a concentration of 150 ppm was particularly beneficial, as most bulblets were obtained simultaneously and characterized by increased biomass. The beneficial effect of nanosilver at a concentration of 4 ppm added to the in vitro medium on bulblet formation from bulb scales was also demonstrated by Gioi et al. (2019), who propagated the OT lily 'Sorbonne'. The effect of AgNPs on the process of bulb formation may be related to the effect of AgNPs on plant hormones (Sun et al., 2017). While the biostimulatory effects of AgNPs on plants are increasingly understood, their mechanism remains unexplained.

We investigated the effects of a pre-plant application of colloidal AgNPs of lily bulbs before being grown under salt stress induced by NaCl. In general, NaCl stress negatively affected bulb biomass, yield, pigment, and mineral content. Similar detrimental effects of salinity (3 and 6 dS m^{-1}) on the fresh weight of leaves and shoots were demonstrated in two Asian lilies, 'Fangio' and 'El Divo' (Ayad et al., 2019). Then, a marked reduction in chlorophyll content as a result of salinity (from 35 to 140 mM NaCl) was found in ten lily hybrids (Bai et al., 2021). The deleterious effects of salinity are mainly due to a decrease in water availability for plants, leading to inhibition of cell length growth. In addition, excess Na^+ and Cl^- ions cause disturbances in the ion balance and affect the uptake of ions such as K^+ and Ca^{2+} (Salachna et al., 2016; Salachna et al., 2019). A study showed that AgNPs stimulated the growth of the fresh weight of bulbs and the number of scales in bulbs and positively affected the diameter of lily bulbs when applied under salt-stress conditions. It means that AgNPs effectively mitigated the adverse effects of salinity in lilies. The protective effect of colloidal AgNPs may also have been influenced by the antimicrobial effect of colloidal AgNPs protecting the bulbs from the beginning of growth. The plants grown from bulbs treated with colloidal AgNPs were most likely *a priori* prepared for possible environmental stress. This is because, according to recent discoveries, AgNPs can be a vaccine that induces stress/immune responses in plants, thereby increasing resistance to biotic and abiotic stresses (Yan et al., 2022). The results allow the development of technologies for producing lily bulbs and perhaps other plants on degraded, saline land using saline water for irrigation. Using AgNPs as a biostimulant to improve bulb yield without additional fertilizer can help reduce the use of high fertilizer doses that negatively impact the environment.

CONCLUSIONS

In conclusion, colloidal AgNPs can be used to increase the quality of lily bulb yield and raise the efficiency of plant propagation through scales. Salinity stress in lily cultivation leads to a marked reduction in bulb yield. The application of AgNPs can mitigate its negative impact on the quality of lily bulbs. Given the concern for the environment and the insufficiently understood impact of nanoparticles on ecosystems and human health, further research should be conducted on the safety of AgNPs application.

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