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Effect of Nano-silver Fertilization on the Tolerance of Salt Stress by Irrigation with Saline Water in Faba Bean (*Vicia faba* L.)

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ABSTRACT

Background: The problem of salinity is increasing day by day and requires innovative solutions. Therefore, this study attempts to find an alternative to industrially manufactured fertilizers used for stimulating plant growth and productivity under salt-stress environments, using nano-formulations from plant sources (phytotechnology).

Methods: The study was performed in the Department of Ecology laboratories at the University of Kufa, also in the Harmoush farms located along the Najaf-Karbala road, during the 2023-2024 growing season. The crude aqueous extract of *Schanginia aegyptiaca* was used for the green synthesis of silver oxide nanoparticles (S-AgONPs). A factorial field trial was adopted in a completely randomized block design, the first factor, with three concentrations of foliar fertilizer containing S-AgONPs (0, 3, 6) ml/L and the second factor with three concentrations of salt water: 0 (tap water), 50 and 100 mM NaCl.

Result: The results presented that foliar application with S-AgONPs at a concentration of 6 ml/L improved vegetative growth, including plant height, number of leaves, shoot fresh weight, shoot dry weight and total chlorophyll in leaves. The use of saline water at a concentration of 50 mM NaCl had a significant impact on the plant growth parameters against the unsprayed plants. Interaction between both factors revealed significant impacts on the vegetative growth characteristics. S-AgONPs from the aqueous extract of *S. aegyptiaca*, at very low concentrations, have proven effective in stimulating the studied parameters under salt stress conditions, opening the horizon to their potential use in agriculture and fertilizer production.

Key words: Broad beans, S-AgONPs, Salt stress, *Schanginia aegyptiaca*, Vegetative growth.

INTRODUCTION

Salinity causes osmotic stress and ionic toxicity in plants, leading to oxidative stress and negatively impacting crop physiology, including nutrient uptake, growth and productivity (Guda *et al.,* 2017; Sabbagh Tazeh *et al.,* 2021). Salinity affects plants by reducing their capability to absorb water from the soil and accumulating various toxic ions, for instance, sodium (Na^+) and chloride (Cl^-) (Otlewska *et al.,* 2020). Early seedling development characteristic germination rates decrease when the quantity of NaCl rises (Kusvuran, 2015); hence, salinity reduces the dry weight, soluble sugars and chlorophyll content of the entire seedling as well as different plant parts-all of which are thought to be significant physiological markers of salt tolerance (Pal and Pal, 2022). One of the most predominant forms of nonbiotic stress is salinity, causing severe crop losses, particularly in arid and semi-arid regions (Hernandez, 2019). Salt stress exerts two important constraints on plants: the hyperosmotic effect resulting from reduced soil water potential and the hypertonic effect resulting from direct toxicity and ion repulsion, causing nutritional imbalance (Mahdi, 2016). High accumulation of salt can adversely affect cellular metabolic processes, for example, membrane integrity, photosynthesis rate and protein production (Ruiz-Lozano *et al.,* 2012). Plants use diverse mechanisms to tolerate and acclimate to salt stress, including regulation of sodium transport through plastid membranes, plasma and osmoregulation.

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Broad bean (*Vicia faba* L.), an important leguminous crop, is grown in varying environmental circumstances (Singh *et al.,* 2013). Broad beans are widely cultivated globally and used as fresh or dried pods due to their nutritional value, for instance carbohydrates (65.02±1.42 g/100 g), protein (29.11±3.20 g/100 g), lipids (1.2±0.05 g/100 g) and dietary fiber (Kalili *et al.,* 2022) in addition to minerals including zinc, iron, copper, phosphorus, potassium, calcium, magnesium and vitamins (Dhull *et al.,* 2022). Broad beans are also highly efficient at fixing atmospheric nitrogen (Youseif *et al.,* 2017).

S. aegyptiaca is a common halophytic plant related to the Chenopodiaceae family and distributed in lowland Iraq. This plant is well adapted to the extreme environmental conditions of various arid regions and is among the most widespread on saline land, occupying the spaces between the plants (Guest and Al-Rawi, 1966). The side effects of chemical fertilizers prompted researchers to search for alternatives to these fertilizers. The research was directed toward studying *Schanginia aegyptiaca* plant extracts and identifying their active components, as plants contain low-concentration compounds called secondary metabolites and the most important of these compounds are alkaloids, phenols and terpenes (Guda *et al.,* 2020a).

Nanotechnology has recently been used for its multiple uses in various fields. Due to their elevated surface area to size ratio, nanomaterials differ from the basic materials that make them up in mechanical, thermal, chemical, biological and other ways (Guda *et al.,* 2021). There are multiple methods for producing nanomaterials mechanically, chemically and physically, but they are considered unsafe for the atmosphere, so researchers resorted to finding secure methods for producing nanomaterials, such as synthesizing them from plants, called green synthesis (Husayn and Guda, 2023b). AgNPs are known as nanoparticles of silver metal whose size ranges from 1 to 100 nm (Prabhu and Poulose, 2012). Among the applications of silver nanoparticles (AgNPs) is that they have stimulating effects on plants. Therefore, nanoscience technology has opened the horizon in the field of nanofertilizers and the preparation and manufacture of nanoparticles, which are an alternative. One of the methods used to regulate plant growth to obtain plants with a good shoot and root system and good specifications is to use foliar fertilization (Glotra *et al.,* 2023). Supplying fertilizers through foliar spraying increases the absorption of nutrients to the target region and reduces the harmful effects of stress (Das *et al.,* 2025). Although it is a contemporary technique of fertilization, it works best in conjunction with ground fertilization rather than as a replacement for it. Fertilizers containing the macronutrients nitrogen, phosphorus and potassium led to improvements in vegetative growth indicators, yield and root quality (Amin *et al.,* 2013). This was confirmed by the experiment implemented on the Jerusalem artichoke plants, which were treated with organic fertilizer extract, potassium sulfate and zinc sulfate, as it gave the highest rates of plant height, dry weight of the shoots and total chlorophyll content of the leaves (Husayn and Guda, 2023a).

Despite the importance of this research and its many uses, there is still little research conducted. Hence, this experiment was carried out to manufacture AgNPs from the aqueous extract and identify them *via* physical and chemical techniques compared with the raw aqueous extract. Moreover, this extract was used as a foliar application in various doses alongside saline water irrigation to investigate its significance on certain aspects of faba bean plant vegetative growth.

MATERIALS AND METHODS

Collecting plant samples and producing an extract

In September 2023, an *S. aegyptiaca* plant was collected from the wild in the Najaf Governorate. The National Herbal Authority/General Authority for Agricultural Research in Iraq verified the plant's categorization. Using the Guda *et al.* (2016) approach, the crude extract was made by thoroughly washing the plant's vegetative portion with water to eliminate impurities from its surface and then thoroughly desiccating it. Afterward, grounding of 50 g of plant leaves was combined with 250 ml of deionized water and then heated. After the combination was in a water bath set at 45°C for 30 minutes, the extract was filtered through Whatman filter paper and kept for later use at 4°C. All lab work was done in the facilities of the Department of Ecology, Faculty of Science, University of Kufa.

$AgNO_3$ Solution Preparation and AgNPs Green Biosynthesis

A ready-to-use 1 mM solution of silver nitrate was provided by dissolving 0.016 g of $AgNO_3$ in 100 mL of deionized water. The method (Owied *et al.,* 2023) was used to synthesize AgNPs from the *S. aegyptiaca* extract. Subsequently, 10 ml of the plant extract was mingled with 100 ml of $AgNO_3$ solution at a concentration of 1 mM, heating the mixture on a magnetic vibrating plate at 45°C for 20 minutes and then observing the color alteration of the admixture as an initial sign of AgNP formation.

Characterization of silver nanoparticles

A French scanning electron microscope MIRA3 FE-SEM was used to characterize the prepared samples in order to ascertain the size and form of the particles within them. (Rahi *et al.,* 2023) by placing around five microliters of the prepared solution for inspection on a carbon and gold clip electron microscope holder, allowing it to dry at room temperature and testing it under various magnifications. Utilizing an Angstrom Advanced AA2000 atomic force microscope, the surface morphology, roughness and diameter of the produced AgNPs were assessed. According to Qader *et al.* (2019), a droplet of the test solution was deposited on a 1 × 1 cm glass slide and allowed to desiccate at ambient temperature before testing. X-ray diffraction spectroscopy revealed the phase fluctuation and grain size of AgNPs (Thajeel *et al.,* 2020). 48 hours after the green nanoparticles were prepared, a sample was obtained and subjected to a UV spectrometer examination at 190-1100 nm wavelength. The water used was distilled and deionized. At room temperature, the product's optical characteristics were investigated (Guda *et al.,* 2021).

Field experiment and data analysis

During the growing season of 2023-2024, an experiment was conducted on *V. faba* (L.) plants in Al-Harmoush farms along the Najaf-Karbala road, Iraq. The purpose of the experimentation was to determine the effects of foliar fertilizer applications at varying concentrations and a balanced nutrient solution on various aspects of the vegetative growth of *V. faba* L. plants. A soil test was done pre-cultivation in the lab of the Department of Ecology, Faculty of Science, University of Kufa, as shown in (Table 1). A (3 × 3) factorial field trial was executed in a completely randomized block design with three replicates. The first factor was three foliar fertilizer concentrations (0, 3 and 6) ml/L and the other factor was irrigation with water at different salinity levels: Tap water (for the control plants) and two different salinity levels (50 and 100 mM NaCl). Each replicate contained nine treatments, with all service operations performed whenever necessary. The foliar fertilizer was sprayed with two sprays in the early morning (Al-Bayati *et al.,* 2023), with 10 days apart and irrigation with the saline water was twice, 10 days apart. The foliar fertilizer concentrations were prepared by taking 3 ml of the foliar fertilizer and completing the volume to 1 liter with distilled water for the purpose of obtaining concentrations of 3 ml/L and so on. The results were analyzed using the analysis of variance (ANOVA) procedure and the treatment means were compared by the least significant difference (L.S.D.) test at the 0.05 probability level. GenStat v.12 software was used for data analysis.

Vegetative growth characteristics

Plant height (cm)

Three plants of beans were taken per experimental unit, their lengths were measured and then the average height of each plant was taken.

Number of leaves (leaf/plant)

The number of leaves per sampled plant was calculated and then the average was extracted.

Fresh weight of the shoot (g/plant)

It was taken using a scale and the average was calculated.

Dry weight of the shoot (g/plant)

The dry weight of the shoot per sampled plant was calculated and the average was extracted; all above were estimated according to the method of Guda *et al.* (2019).

Total chlorophyll of bean leaves (mg/100 g fresh weight)

Leaf chlorophyll collectively was estimated as reported by the method (Goodwin, 1976).

RESULTS AND DISCUSSION

Green synthesis of nanoparticles AgNPs

The final color of the aqueous extract of the *S. aegyptiaca* powder was yellow. The biosynthesis of AgNPs from the targeted solution of *S. aegyptiaca* showed that the color changed at 30 minutes after adding a solution of silver nitrate $AgNO_3$, at 45°C, as the color altered from yellow to dark brown. This confirms the incident of a reduction reaction among the active secondary compounds in the plant extract with the nitrate salt and the formation of nanoparticles. No change in the color of nanoparticles was observed after different periods. The color alteration is clear initial evidence of AgNPs constitution and a positive green biosynthesis. The color changes that occurred were the result of the reduction process occurring due to the phenomenon of surface plasmon resonance. This property sometimes occurs in some metals, including silver, as a result of the transformation of the diameters of the particles. metals at the nanoscale (Hamady *et al.,* 2017; Guda *et al.,* 2018; Thajeel *et al.,* 2020; Rahi *et al.,* 2023).

Table 1: Some of the experimental field soil's physical and chemical properties.

pH	EC dS/m	O.M. %	N mg/Kg	P mg/Kg	K mg/Kg	Ca mg/L	Clay g/kg	Silt g/kg	Sand g/kg	Texture
7.58	3.56	3.722	14.4	0.57	2.45	4.30	47	52	789	Sandy soil

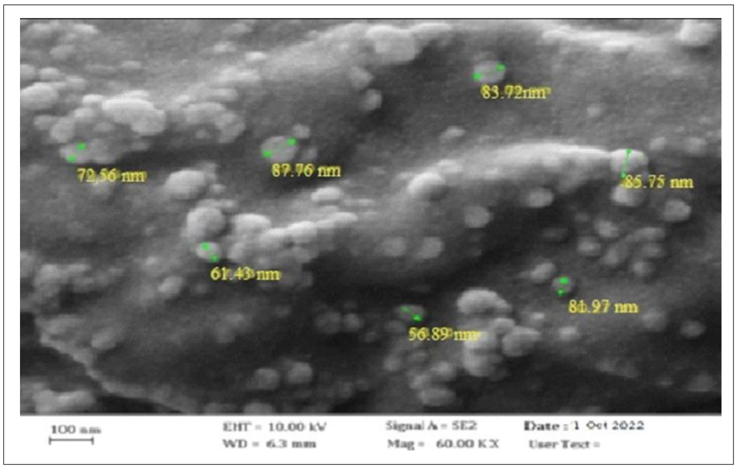

Fig 1: Size and shape of AgNPs synthesized from *S. aegyptiaca* extract by FE-SEM.

Identification of biosynthesized AgNPs by field emission scanning electron microscopy (FE-SEM)

AgNPs that were biosynthesized were examined under an electron microscope to ascertain their size, composition and surface form. The size and form of the biosynthesized AgNPs are shown in Fig 1, where they were spherical and did not aggregate. The nanoparticles were between 56.89 and 85.75 nm in size, which is typically the upper limit of the particle sizes. The produced nanoparticles had a size range of 1 to 100 nm and the findings of this investigation corroborated those of other studies because the AgNP sizes produced for a study implemented by Alwaan *et al.* (2021) for myrtle and celery plants varied from 18.59 to 53.16. Our findings are also consistent with the study of Hamady *et al.* (2017), which produced AgNPs using extracts from fish, lemon and mint. In their investigation, the particle sizes varied from 21.51 to 34.760 nm. However, the diameters of the biosynthetic AgNPs from the ivy plant were between 25.68-141.17 nm and those from the aloe vera plant between 27.2-109.8 nm, according to the study (Qader *et al.,* 2019).

Atomic force microscopy (AFM)

The forms, topography, roughness and protrusions of various particles' and molecules' surfaces-which are represented by surface heights and surface structure-can be understood through the use of atomic force microscopy. This technique describes digital photographs that provide two- and three-dimensional views and their analysis from various angles, as well as quantitative measures of surface properties (Guda and Semysim, 2022). Fig 2 demonstrates that the surface roughness of the bio-synthesized AgNPs reached 75.54 nm, the root mean square value reached 14.08 nm and their size fluctuated between 0-12.7 nm at a rate of 4.96 nm. The increased roughness rate of biosynthesized nanoparticles enhances their anti-biological efficacy (Husayn and Guda, 2023a). 94 produced nanoparticles had a maximum cumulative rate of 51.87 and a presence percentage of 55.03%. The outcomes of the present study recorded a difference from earlier research and this may be due to the difference in plant extracts according to the studied plant species and the secondary metabolic compounds they contain, which are related to the size and roughness of nanoparticles. The size of the nanoparticles produced from ivy and aloe vera plants was 90.07 nm and 53.3 nm, respectively, according to a study (Qader *et al.,* 2019). The corresponding roughness values were 15.9 nm and 7.67 nm. The average size of the nanoparticles ranged between 17.90 and 22.97 nm,

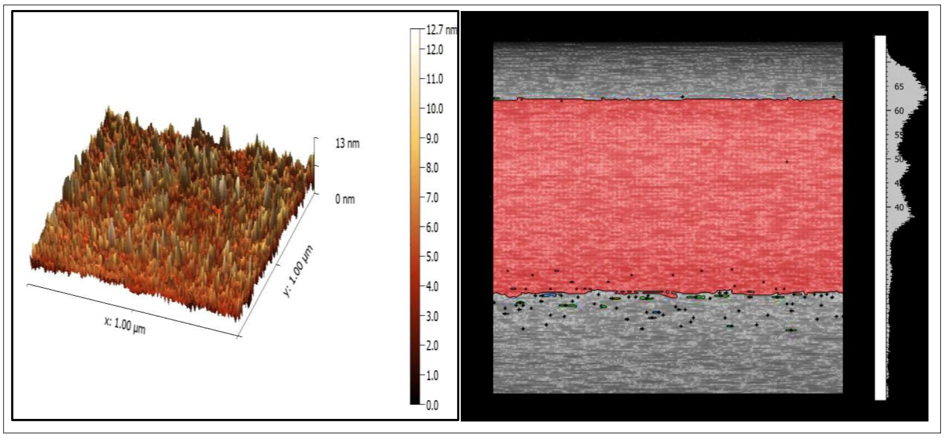

Fig 2: Size and surface roughness of AgNPs synthesized from *S. aegyptiaca* extract.

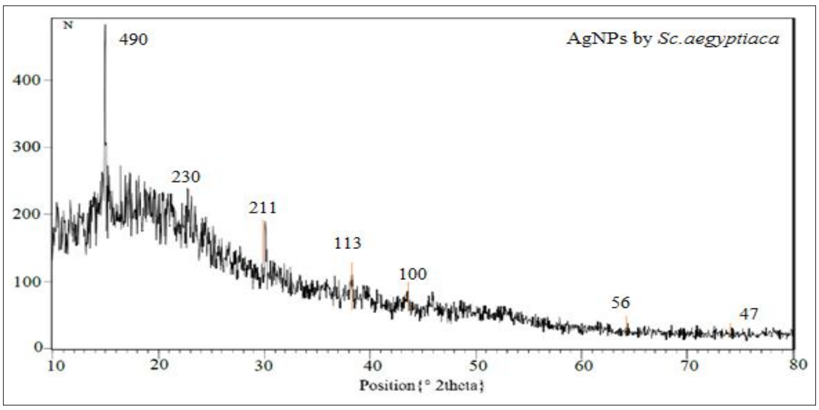

Fig 3: X-ray diffraction of AgNPs synthesized from *S. aegyptiaca* extract.

according to a study by (Alwaan *et al.*, 2021) that focused on the production of AgNPs from celery.

X-ray diffraction (XRD)

The nature and size of biologically active AgNPs using an impurity resulting from the reaction that occurred between the aqueous extract and silver nitrate to manufacture AgNPs, as they were of varying intensity between medium and weak and matched the X-ray diffraction measurements of AgNPs (100, 113, 211, 230 and 410), according to the X-ray diffraction database. X-ray ICDD file JCPDS NO: 04-0783 for silver particles, which validates the synthesis of silver nanoparticle crystals (Fig 3). These results are consistent with Alwaan *et al.* (2021), who showed measurements of the synthesis of AgNPs from myrtle and celery plants in their study. Additionally, the findings align with the study of Hamady *et al.* (2017), where AgNPs were also manufactured from various plant sources and the X-ray diffraction of the synthesized AgNPs was examined, which confirms the silver nanoparticle crystals. These results are in accordance with (Alwaan *et al.,* 2021), which revealed measurements of the synthesis of AgNPs from myrtle and celery plants for their study, which were identical to the X-ray diffraction database for AgNPs. The reason for the difference in the crystalline size of nanoparticles may be due to a difference in the secondary metabolites present in the extracts of different plant species.

UV-visible absorption spectrum

UV was used to detect AgNPs at wavelengths of 1100-200 nm. The presence of the extracted compound glabridin was confirmed by the UV absorption of the compound, which is shown in Fig 4 in the wavelength range of 250-234 nm. The compound's absorption peaks appear at a wavelength range of 350-200 nm (Guda, 2023). The absorption peak at 390 nm wavelength in Fig 4 indicated the existence of AgNPs. Since surface plasmon absorption keeps the results within the diagnostic range for AgNPs (400-450 nm), the size of the produced particles can be counted within the range of nanomaterial sizes (Alwaan *et al.,* 2021). This outcome is in line with research (Qader *et al.,* 2019), which used ultraviolet-visible spectroscopy to investigate AgNPs made from plant extracts of ivy and *aloe vera* and found that the largest absorption peak was at wavelengths of 419-427, respectively. Using ultraviolet-visible spectroscopy to examine AgNPs made from celery and myrtle extracts, the current investigation by Alwaan *et al.* (2021) found that the largest absorption peak was observed at wavelengths of 408-410, respectively.

Effect of foliar fertilizer spraying with S-AgNPs on the bean vegetative growth characteristics

Table 2 findings demonstrated that the following growth characteristics were significantly enhanced by foliar fertilization of AgNPs at a dose of 6 ml/L provided the maximum averages of plant height 19.61 cm, number of leaves 31.54 leaves/plant, fresh weight of the shoot 133.71 g, dry weight of the shoot 9.32 g and total chlorophyll content of the leaves 85.4 mg/100 g fresh weight. In comparison, unfertilized plants produced the minimum averages of (7.23 cm, 18.5 leaves/plant, 65.6 g, 6.53 g and 59.8 mg/100 mg fresh weight (6.53 g), respectively. Nanofertilizers can enhance the quality of agricultural products. For example, Jaafar *et al.* (2022) found that applying a nano-

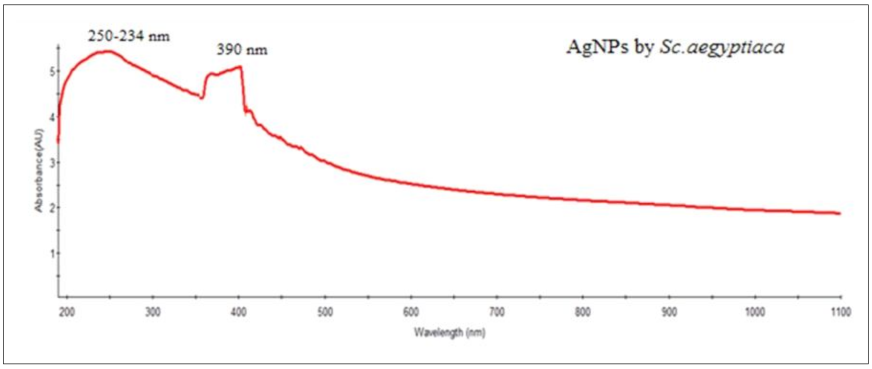

Fig 4: UV-Vis spectrum of AgNPs synthesized from *S. aegyptiaca* extract.

Table 2: Simple effect of spraying with different concentrations of silver nanoparticle foliar fertilizer on vegetative growth characteristics.

Treatments		Plant height (cm)	Number of leaves (leaf/plant)	Shoot fresh weight (g)	Shoot dry weight (g)	Total chlorophyll in leaves (mg/100 g fresh weight)
Silver nanoparticle	0	7.23	18.5	65.6	6.53	59.80
foliar fertilizer	3	16.83	29.44	107.45	8.76	74.20
(ml/L)	6	19.61	31.54	133.71	9.32	85.40
L.S.D. 0.05		1.29	1.07	4.52	0.62	2.68

seaweed extract at a concentration of 2 ml/L yielded the highest values for faba bean vegetative indicators, including plant height, leaf number per plant, branch number per plant and total leaf chlorophyll content. In our research, the substantial improvement in bean plant features is due to the role of the foliar fertilizer of AgNPs, which contains the silver element necessary for the formation of enzymes and vitamins, which increases the size of the leaves and stems. Ag is considered one of the necessary elements for plant nutrition and its crucial role in the process of photosynthesis, protoplasm and the process of respiration. This functions in increasing energy and has a positive effect on increasing the utilization of nitrogen and phosphorus. It also controls the vital activities associated with plant growth and the activation of enzymes. It has a role in transferring sugars from leaves to other parts of the plant and increasing the concentration of carbohydrates Guda *et al.* (2020b) and this is consistent with what was indicated by (Guda *et al.,* 2016) in a study on the potato plant (Sponta variety) conducted at Tishreen University in Syria to study the effect of nano-fertilizers, as it had a significant impact on the height of the plant compared to the control treatment (unsprayed plants), which gave the lowest values. This is consistent with what was stated by Husayn and Guda (2023a) in a study of the effect of some micronutrient fertilizers on the growth and yield of the plant. The four-spray treatment (Mn+Fe+Zn+Cu) was significantly superior to the dry weight of the shoot.

Effect of irrigation with saline water on the bean vegetative growth characteristics

Table 3 shows that irrigation with saline water at a concentration of 50 mM sodium chloride had a significant improvement in the following growth traits (plant height, number of leaves, fresh weight of the shoot, dry weight of the shoot and total chlorophyll content of leaves) which reached (16.82 cm, 37.21 leaves/plant, 126.76 g, 9.31 g, 85.47 mg/100 g fresh weight), respectively. Contrarily, the control plants that were irrigated with tap water showed the lowest rate of growth traits with (10.54 cm, 12.96 leaves/plant, 54.82 g, 5.73 g, 54.21 mg/100 g fresh weight), respectively. Such responses are frequently ascribed to hormetic effects, in which low to moderate salinity levels induce metabolic profile and can improve osmotic adjustment, leading to an increased cell turgor and, in turn, boosting chlorophyll content, elongation of shoots and expansion of leaves (Munns and Tester, 2008; Parihar *et al.,* 2015). Further, the reason for the increment in these traits may be ascribed to the constituent nutrients in sufficient quantities for what the plant needs in the processes of cell division and elongation, especially nitrogen, which affects the increased activity of meristematic tips that work to increase cell division. This elongation occurs as a result of the increase in oxygen concentration and the availability of basic materials that the plant needs for the building processes, with amino acids and some enzyme cofactors, NAD and NADP, which include nitrogen, which leads to an increase in vegetative growth in general (Devasgayam and Jayapaul, 1997).

Table 3: Simple effect of irrigation with saline water at different concentrations on vegetative growth characteristics.

Treatments		Plant height (cm)	Number of leaves (leaf/plant)	Shoot fresh weight (g)	Shoot dry weight (g)	Total chlorophyll in leaves (mg/100 g fresh weight)
Saline water	0	10.54	12.96	54.82	5.73	54.21
concentration	50	16.82	37.21	126.76	9.31	85.47
(mM NaCl)	100	15.98	36.32	123.32	8.93	77.32
L.S.D. 0.05		1.29	1.07	4.52	0.62	2.68

Table 4: Interaction effect of the interaction between spraying with different concentrations of foliar fertilizer containing silver nanoparticles and irrigation with saline water on vegetative growth characteristics.

Treatments			Plant height (cm)	Number of leaves (leaf/plant)	Shoot fresh weight (g)	Shoot dry weight (g)	Total chlorophyll in leaves (mg/100 g fresh weight)
Foliar fertilizer of	0	0	5.43	10.58	46.72	5.69	34.76
AgNPs (ml/L) ×		50	6.78	15.84	51.50	5.89	59.41
Saline water		100	8.94	17.82	53.80	9.42	69.31
concentration	3	0	14.75	19.61	54.75	5.32	48.48
(mM NaCl)		50	15.41	24.74	126.63	7.45	79.65
		100	16.72	34.72	143.45	11.92	82.72
	6	0	16.89	15.21	64.59	6.31	58.54
		50	25.35	45.32	289.51	12.74	89.59
		100	23.61	42.65	212.32	11.89	89.42
L.S.D. 0.05			2.26	1.86	7.84	1.09	4.64

The interaction effect between the foliar fertilizer containing AgNPs and the irrigation with saline water on the vegetative growth characteristics of *V. faba* L. plants

Table 4 showed that the levels of S-AgNPs foliar fertilizer at (6 ml/L) and saline water concentration at (50 mM NaCl) exposed a significant enhancement in the vegetative growth parameters *viz.* plant height 25.35 (cm), number of leaves 45.32 leaves/plant, shoot fresh weight 289.51 (g), shoot dry weight 12.74 (g) and total leaf chlorophyll 89.59 mg/100 g. In contrast, the unsprayed plants irrigated with tap water produced the minimum values of these traits by plant height 5.43 (cm), number of leaves 10.58 leaves/plant, shoot fresh weight 46.72 (g), shoot dry weight 5.69 (g) and total leaf chlorophyll 34.76 mg/100 g.

CONCLUSION

Our research results demonstrate that the application of AgNPs to the leaves of faba bean plants at a concentration of 6 ml/L and irrigation with saline water at 50 mM NaCl showed a synergistic effect on physiological growth characteristics. These observations indicate the potential of using the green nano-fertilizer technology as a complementary strategy for saline water management to improve plant growth and resilience under stress conditions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

All authors declared that there is no conflict of interest.

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