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SILVER NANOPARTICLES AND NPK FERTILIZER EFFECTS ON THE PROLINE, PEROXIDASE, AND CATALASE ENZYMES IN WHEAT

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SUMMARY

This research investigated the effects of bio-silver nanoparticles (AgNPs) on proline content, peroxidase, and catalase enzyme activity of two Iraqi wheat (*Triticum aestivum* L.) cultivars (Ibaa 99 and Al-Rasheed) compared with NPK fertilizers. The biosynthesis of AgNPs from *A. graveolens* aqueous extract, and their characterization occurred through the alteration in color of the reaction blend, as an unambiguous proof for AgNPs' formation. Determining the size and shape of AgNPs used a scanning microscope and an atomic force microscope to characterize them. Uv-spectrophotometer described the AgNPs, revealing the peak of highest absorption at (λmax) 408 nm. The X-Ray Diffraction device application diagnosed the AgNP properties. The research transpired at the AL-Nahrain Laboratories, where cultivated cultivars in September 2022 had three replications for each concentration of biosynthesized AgNPs and NPK treatments (0.1, 1.5, and 2.0 mg/ml), and a control for comparison. A significant decrease in proline was evident for Al-Rasheed cultivar, while a significant increase appeared in Ibaa 99 cultivar. A notable decrease in proline resulted from NPK fertilizer treatments. Peroxidase and catalase enzyme activity significantly rose in both cultivars, while nonsignificant differences were visible when using NPK between them.

Keywords: Wheat (*Triticum aestivum* L.), biofertilizers, silver nanoparticles, antioxidant enzymes activity, *Apium graveolens* L.

Key findings: In wheat (*Triticum aestivum* L.) crops, the silver nanoparticles can be safe for use to improve the physiological and biochemical traits and replace the chemical fertilizers negatively affecting the soil and human health.

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INTRODUCTION

The cultivation of crop plants, especially cereals, plays a significant role in economies of developed and developing nations, also helping to feed the world's expanding population. In the community, the food security mainly depends on increased grain production. Wheat (*Triticum aestivum* L.) belongs to the family Poaceae, used as an essential food worldwide. Over 50% of the global population primarily depends on wheat to meet their daily nutritional requirements. Therefore, it is important to enhance the wheat grain production and its nutritional values to meet the needs of the growing population (Ikram *et al*., 2020).

Population growth, exhaustion of natural resources, and underground problems are major elements exerting stress on the environment in using chemo-fertilizers, which may cause considerable damage to the ecology, soil, and human health. However, the demand for environmentally safe agriculture products is increasing, including biotechnological progress. Furthermore, mineral fertilizers and pesticides application instigates substantial environmental disruption and inevitably contributes to the proliferation of nutritionally compromised food items. For instance, nitrous oxide, a greenhouse gas, results from nitrogen fertilizers' use (Solomon *et al*., 2012; Pandiselvi *et al*., 2017).

It is noteworthy that the prolonged use of mineral fertilizers has considerably reduced organic matter in the cultivated soil, causing soil acidification and serious threats to plant survival. Therefore, injudicious use of chemical fertilizers to increase production may also have negative consequences, such as, leaching, pollution, and friendly microorganisms and insects' destruction, ultimately reducing soil fertility (Khan *et al*., 2019). Nanotechnology gains traction in the field of agriculture; hence, scientists work tediously to develop nanodevices to control agriculture on nanoplatforms. As a result, fungicides containing nanoparticles emerged, aiding in the destruction of fungal species without causing environmental pollution. Hazardous chemical forces and reactions are functional in the physical and chemical processes of nanomaterial synthesis, which degrade the environment (Javed *et al*., 2021). Given that plant-mediated synthesis is biocompatible and contributes significantly to the improvement in crop plants, it also provides advantages over conventional physical and chemical methods for material synthesis (Sultana *et al*., 2021).

Nanofertilizers' use can improve the elemental efficiency, lessens soil toxicity from excessive mineral fertilizer consumption, and decrease frequent fertilizer application. Foliar nanotechnology liquid fertilizer enhances overall plant growth and fruit yield of cucumber, by providing the nutrients to plants, improving photosynthesis rate, chlorophyll formation, overall plant growth, and dry matter production (El-Shawa *et al*., 2022). The application of biosynthetic metal nanoparticles reduces the deterioration of carotenoids and chlorophyll in the leaves, which also keeps high activities of antioxidant enzymes.

Currently, the use of chemical fertilizers declines by using bio-organic fertilizer, eventually decreasing environmental pollution and the cost of producing numerous crops, especially grain crops (Mohamed *et al*., 2022). Increasing needs for further development of non-toxic, inexpensive, high-yielding, and eco-friendly synthesis methods of nanoparticles are certain. Using bionanotechnology can improve food sufficiency by enhancing crops' yield, and nanoparticles can boost the productivity and growth of various crops, especially cereal crops, as an alternative to traditional fertilizers (Oraibi *et al*., 2022). Therefore, the presented study sought to determine the effects of foliar application of vegetable-made silver nanoparticles and NPK fertilizer on the content of proline, peroxidase, and catalase enzymes in two Iraqi wheat cultivars.

MATERIALS AND METHODS

Plant samples and aqueous extract preparation

Acquiring *Apium graveolens* L. plants locally commenced in June 2022, with their classification undergoing verification by the National Herbal Commission, General Commission for Agricultural Research, Baghdad, Iraq. The aqueous extract of the *A. graveolens* plants proceeded a conventional preparation. The said process involved thoroughly drying the green plant parts with dry air after washing them with water to remove impurities from their surface. Combining the 50 g ground powder of *A. graveolens* along with 250 ml of sterilized deionized water continued in a glass beaker with a size of 1000 ml. The said combination sustained boiling in a water bath at 45 °C for 30 min. The extract's filtering used a Whatman No. Filter Paper-1, then reached storage for later use at 4 °C (Talib *et al*., 2023).

Preparation of the silver nitrate solution

For preparing a 1 mM concentration of silver nitrate solution, the study used the molarity law described below:

$$M = W/(M.wt) \times 1000/V$$
$$0.001 = W/169.87 \times 1000/100,\ W = 0.016987\ g$$

Thus, the 0.016987 g of silver nitrate dissolved in 100 ml of deionized water obtained a 1 mM silver nitrate solution ready for use.

Green-manufucture of silver nanoparticles

The *A. graveolens* extract was a prime ingredient to develop silver nanoparticles (Al-Othman *et al*., 2017). Mixing the 100 ml of a 1 Mm silver nitrate solution continued with 10 ml of *A. graveolens* aqueous extract. The mixture's heating on a magnetic vibrating plate progressed at a temperature of 45 °C for 20 min. Then, an observation of the mixture's color change ensued as preliminary evidence for the construction of silver nanoparticles. Then, retaining 100 ml of silver nitrate solution of 1 mmol concentration earlier prepared ensued for use as a control in certain measurements.

Biosynthesized silver nanoparticles

The solution of biosynthesized nanoparticles incurred drying, afterward, preparing three different concentrations of silver nanoparticles (1.0, 1.5, and 2.0 mg/ml) continued sequentially. Similarly, the preparation of the same concentrations of NPK occurred for comparative study.

Characterization of biosynthesized silver nanoparticles

Biosynthesized silver nanoparticles' diagnosis used: Field Emission Scanning Electron Microscopy (FE-SEM), French MIRA3 FE-SEM Scanner, to know the shape and size of silver nanoparticles. It also used a gold-carbon electron microscope holder clipped with about 5 µl of ready-to-examine solutions to ascertain the size and shape of biosynthesized particles in prepared samples, drying at room temperature, and then tested in magnifying powers. The Atomic Force Microscopy (AFM) determined the roughness and surface morphology of the synthesized silver nanoparticles. The Angstrom Advanced AA2000. X-Ray Diffraction (XRD) analysis used in the diagnosis process included centrifugation of biosynthesized silver nanoparticles for 15 min at 10,000 r/m. Later, re-dispersion ensued in a sterile D.W. with another centrifugation for 10 min at 10,000 r/m. The samples' drying followed in an oven at 50 °C, with an analysis by XRD, XRD measurement of biosynthesized silver nanoparticles. The Ultraviolet-Visible spectrometer employment had a wavelength of 190–1100 nm.

Wheat cultivars treatment with silver nanoparticles and NPK

Iraqi wheat cultivars Ibaa 99 and Al-Rasheed seeds, cultured in September 2022, commenced in pots containing 50/50 w/w soil and peat moss in a growth chamber. (Photoperiods 16/8hrs:light/dark, light intensity: 1000 Lux, temperature: 20 °C ± 2 °C, and relative humidity: 60%, watered ensuring pots do not dry out, with three replications for each concentration of biosynthesized AgNPs and the NPK treatments [0.0, 0.1, 1.5 and 2.0 mg/ml]). Obtaining dry weights of treated seedlings (drying in an oven at 45 °C) used a sensitive balance. Proline concentrations, peroxidase, and catalase enzymes' activities were notable about eight weeks after seed germination with twice-a-week foliar spraying of AgNPs and the NPK concentrations under controlled conditions (Naliwajski and Skłodowska, 2021).

Statistical analysis

The statistical program Genstat was the application analyzing the resulted data according to the factorial experiment. The experiment had a randomized complete block design (RCBD) with three replications per every treatment.

RESULTS

The green synthesis of AgNPs by the water extract of *A. graveolens* leaves exhibited a color change occurring in the plant extract from light brown to dark brown when adding silver nitrate solution at room temperature for 12 h (Figure 1). The change in color was the proof for the synthesis of AgNPs. The appearance of silver particles of this color was visible and not silver, as a result of the occurrence of the phenomenon of surface plasmon resonance.

Using a scanning electron microscope, characterization of shape and size of AgNPs revealed these particles were non-clumpy and almost spherical (Figure 2). The sizes of silver nanoparticles of the *A. graveolens* aqueous extracted from leaves were between 37.18–19.58 nm, and the average size was 28.45 nm, falling within the limits of known nanoparticle sizes.

By examining through the atomic force microscope to detect the silver nanoparticles of *A. graveolens* extract showed the size of these particles was 17.90 nm. The diameter of the silver nanoparticles ranges between 45–90 nm, and the average diameter of the nanoparticles was 69.44 nm. The atomic force microscope provided two- and three-dimensional images to clarify the shape and dimensions of the nanoparticles (Figure 3). The silver nanoparticles of *A. graveolens* plant extract's detection used a UV spectrophotometer, and the results revealed the highest absorption peak was at (λmax) 408 nm (Figure 4). The XRD device employed characterized the silver nanoparticles, with the obtained peaks were at 100, 111, 200, 220, and 311, which corresponded with Bragg reflections at the value of 2θ for angles 31.70°, 38.32°, 44.5°, 64.65°,and 77.5°, respectively (Figure 4).

The biosynthesized AgNPs affect the concentration of proline and the activity of catalase and peroxidase enzymes (Table 1). The results revealed a significant decrease in proline content for the wheat cultivar Al-Rasheed at concentrations of 1.0 and 1.5 mg/ml of biosynthesized AgNPs, observed to be at 8.06 and 15.59 U gm-1, respectively. But, it was nonsignificant at 2 mg/ml (24.64 U gm-1) compared with the control (23.85 U gm-1). In contrast, for Ibaa 99 cultivar, the difference was not significant at a concentration of 1.5 mg/ml (6.91 U gm-1), with a marked increase only at the treatments of two AgNP concentrations, 1.0 and 2.0 mg/ml (23.8 and 17.10 U gm-1, respectively), compared with the control (5.54 U gm-1). The lowest value appeared at a concentration of 1.0 mg/ml (8.06 U gm-1) in the Al-Rasheed cultivar. Furthermore, a substantial increase in proline arose in the Al-Rasheed cultivar (18.0 U gm-1) compared with the cultivar Ibaa 99 (13.3 U gm-1). The NPK fertilizer treatments showed the proline concentration notably decreased with an increased NPK concentration. However, no significant difference surfaced between the two cultivars in -their proline content.

The results in Table 2 further detailed, with the application of 1.5 mg/ml of AgNPs, the peroxidase enzyme activity remarkably rose in the cultivar Ibaa 99 (Table 2). It had a value at 0.95 U mg^{-1} and nonsignificant differences at 1.0 and 2.0 mg/ml (0.217 and 0.01 U mg^{-1}, respectively) compared with the control (0.071 U mg^{-1}). Cultivar Al-Rasheed provided a significant increase with a value of 1.475 U

mg^{-1} at 1.0 mg/ml of AgNPs. It had no significant differences at 1.5 and 2.0 mg/ml of AgNPs compared with the control treatment (0.176 U mg^{-1}). The cultivars showed significant increase in peroxidase enzyme activity, which occurred in the cultivar Al-Rasheed (0.547 U mg^{-1}) versus cultivar Ibaa 99 (0.312 U mg^{-1}).

The findings enunciated a noteworthy increase in catalase enzyme activity at 1.0 and 1.5 mg/ml AgNPs for wheat cultivars Ibaa 99 and Al-Rasheed, recorded values at 0.031 and 0.03 U mg^{-1} and 0.018 and 0.05 U mg^{-1}, respectively, compared with the control. No significant differences were evident in relation to cultivar effects (Table 3). Likewise, nonsignificant differences occurred for the catalase enzyme activity upon using NPK concentrations of different doses with the tested wheat cultivars, except at 1.5 mg/ml of AgNPs for the wheat cultivar Ibaa 99.

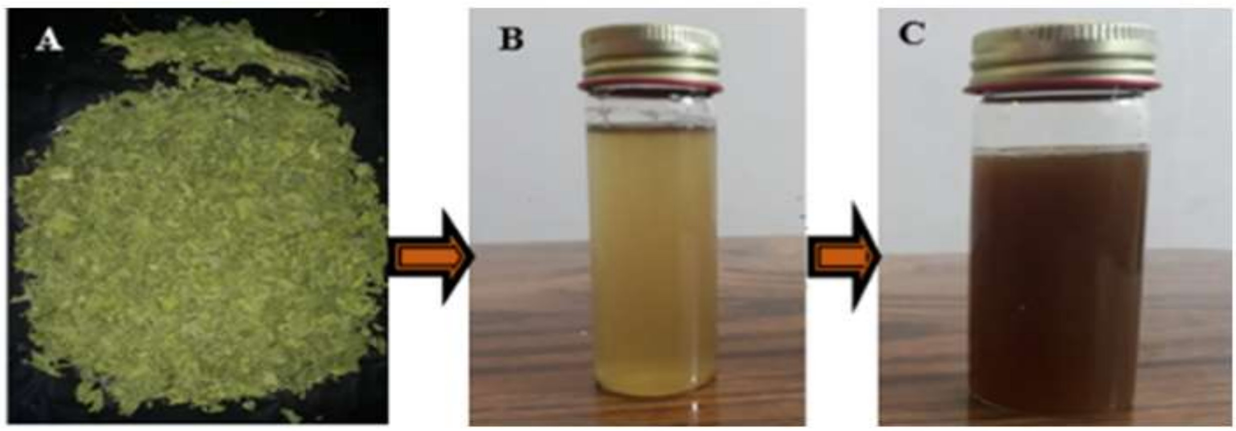


Figure 1. Final color profile of the aqueous extract of *A. graveolens*. A: *A. graveolens* plant leaves, B: *A. graveolens* aqueous extract, C: biosynthesized AgNPs (*A. graveolens* aqueous extract mixed with Silver nitrate after 12 h of stirring under heat).

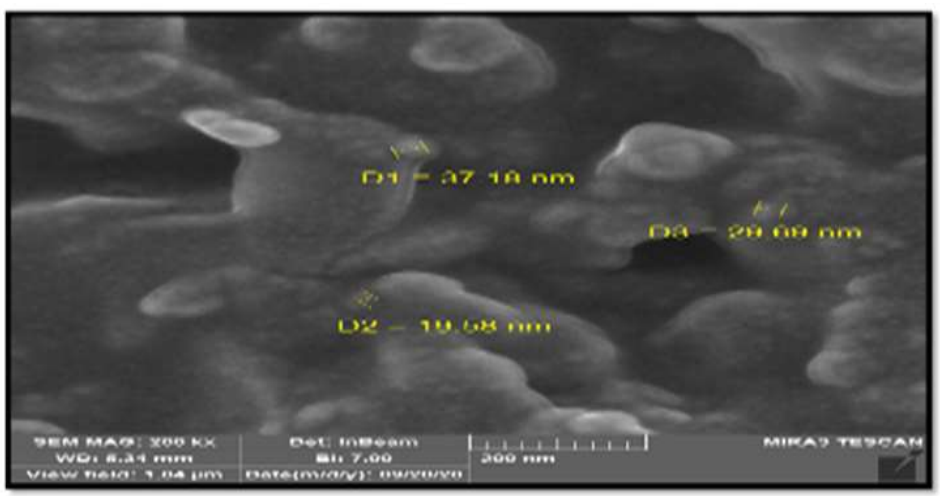
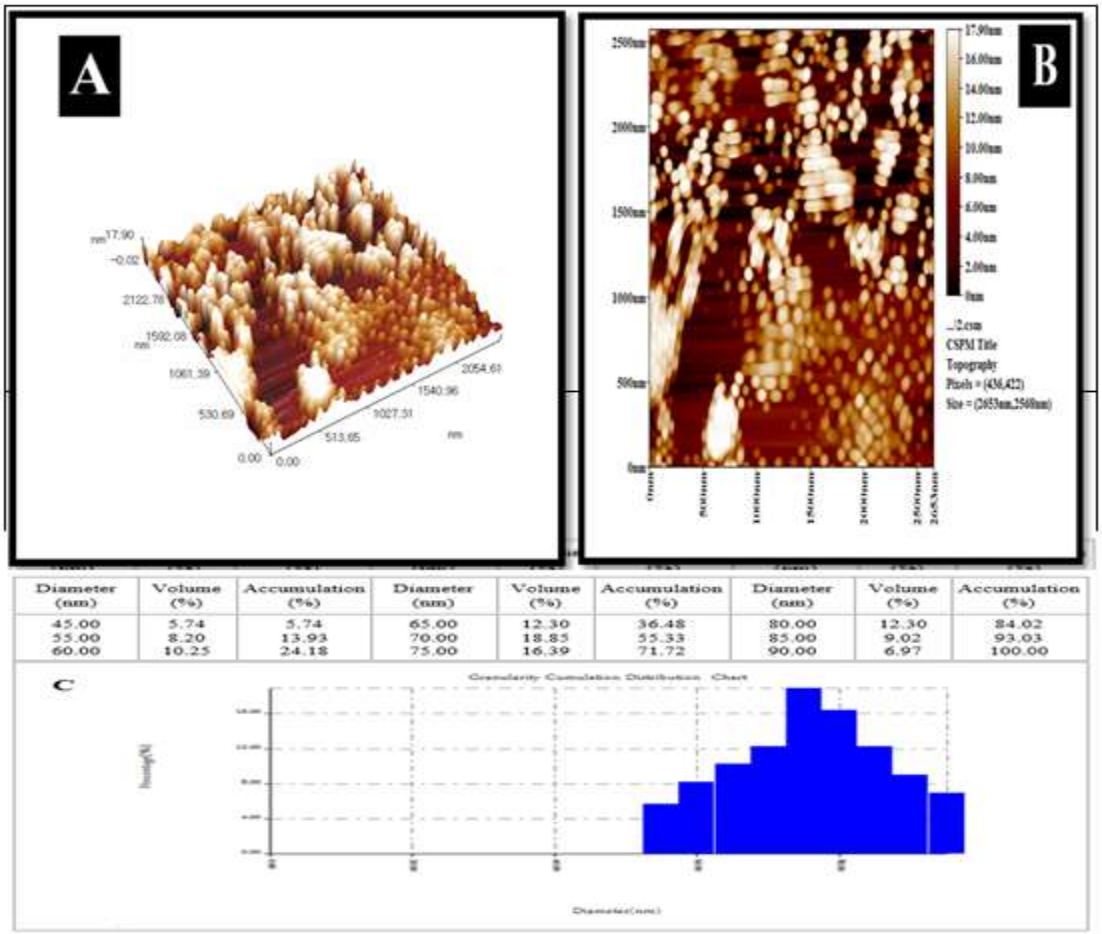


Figure 2. The size and shape of silver nanoparticles manufactured from aqueous extract of *A. graveolens* using SEM.



Diameter (nm)	Volume (%)	Accumulation (%)	Diameter (nm)	Volume (%)	Accumulation (%)	Diameter (nm)	Volume (%)	Accumulation (%)
45.00	5.74	5.74	65.00	12.30	36.48	80.00	12.30	84.02
55.00	8.20	13.93	70.00	18.85	55.33	85.00	9.02	93.03
60.00	10.25	24.18	75.00	16.39	71.72	90.00	6.97	100.00

Figure 3. Atomic force microscopy AFM showing the size and diameter of silver nanoparticles manufactured from aqueous extract of *A. graveolens* plant. A: 3D shape, B: 2D shape and diameter, C: accumulation analysis.

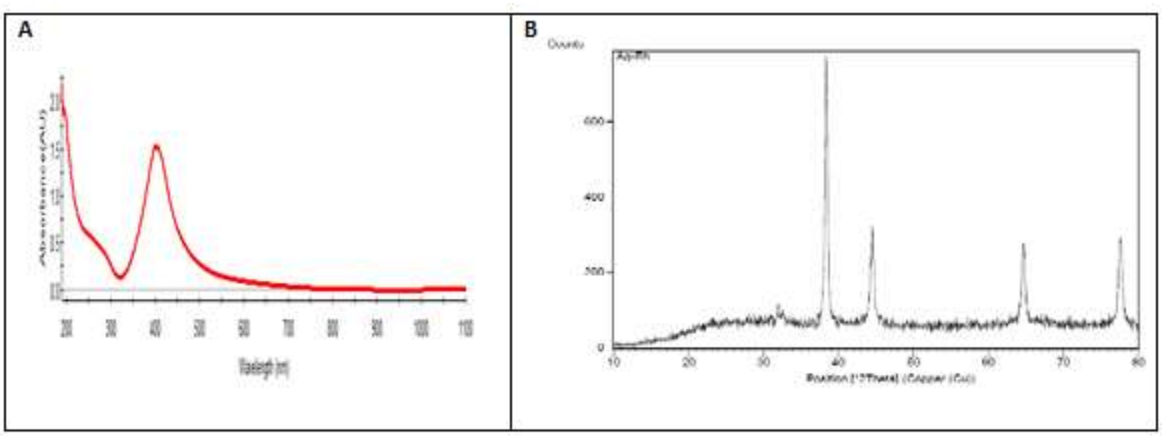


Figure 4. A: UV-vis spectrum of the biosynthetic silver nanoparticles extracted from *A. graveolens* plant. B: X-ray diffraction of silver nanoparticles biosynthesized from *A. graveolens* plant extract.

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Table 1. Effect of foliar application of biosynthesized silver nanoparticles and NPK concentrations on proline concentration of wheat cultivars Ibaa-99 and Al-Rasheed after eight weeks of germination (N = 30).

Wheat cultivars	Ag NPs concentrations (mg/ml)				Means (U mg^{-1})
	0	1	1.5	2	
Ibaa 99	5.54	23.8	6.91	17.1	13.3
Al-Rasheed	23.85	8.06	15.59	24.64	18
$LSD_{0.05}$	Ag NPs concentrations = 6.1, Wheat cultivars = 3.36				

Wheat cultivarS	NPK concentrations (mg/ml)			Means (U mg^{-1})
	1	1.5	2	
Ibaa 99	26.17	16.35	17.12	19.88
Al-Rasheed	26.02	15.59	19.08	20.23
$LSD_{0.05}$	Ag NPs concentrations = 5.31, Wheat cultivars = 1.13			

Table 2. Effect of foliar application of biosynthesized silver nanoparticles and NPK concentrations on peroxidase activity in wheat cultivars Ibaa-99 and Al-Rasheed after eight weeks of germination (n = 30).

Wheat cultivars	Ag NPs concentrations (mg/ml)				Means (U mg^{-1})
	0	1	1.5	2	
Ibaa-99	0.071	0.217	0.95	0.01	0.312
Al-Rasheed	0.176	1.475	0.08	0.46	0.547
$LSD_{0.05}$	Ag NPs concentrations = 0.36, Wheat cultivars = 0.15				

Wheat cultivars	NPK concentrations (mg/ml)			Means (U mg^{-1})
	1	1.5	2	
Ibaa-99	0.237	1.09	1.18	0.83567
Al-Rasheed	0.041	0.25	0.97	0.42033
$LSD_{0.05}$	Ag NPs concentrations = 1.12, Wheat cultivars = 0.34			

Table 3. Effect of foliar application of biosynthesized silver nanoparticles and NPK concentrations on catalase activity of wheat cultivars Ibaa-99 and Al-Rasheed after eight weeks of germination (n = 30).

Wheat cultivars	Ag NPs concentrations (mg/ml)				Means (U mg^{-1})
	0	1	1.5	2	
Ibaa-99	0.004	0.031	0.03	0	0.01675
Al-Rasheed	0.007	0.018	0.05	0	0.01875
$LSD_{0.05}$	Ag NPs concentrations = 0.01, Wheat cultivars = 0.013				

Wheat cultivars	NPK concentrations (mg/ml)			Means (U mg^{-1})
	1	1.5	2	
Ibaa-99	0.006	0.02	0.01	0.012
Al-Rasheed	0.004	0.01	0.01	0.00733
$LSD_{0.05}$	Ag NPs concentrations = 0.01,Wheat cultivars = 0.013			

Table 4. Effect of foliar application of biosynthesized silver nanoparticles and NPK concentrations on silver metal conc. (ppm) in wheat cultivars Ibaa-99 and Al-Rasheed after eight weeks of germination (n = 30).

Wheat cultivars	Ag NPs concentrations (mg/ml)				Means
	0.0	1.0	1.5	2.0	
Ibaa-99	0.655	0.385	0.390	0.440	0.468
Al-Rasheed	0.405	0.570	0.370	0.410	0.439
LSD0.05	Ag NPs concentrations = 0. 160, Wheat cultivars = 0. 113				

The results showed a sizable variation in the concentration of silver metal concentrations (ppm) between the cultivars, treated with different concentrations of AgNPs through foliar spray (Table 4). The Ibaa 99 cultivar significantly decreased in Ag concentrations (ppm) in plants, when treated with all concentrations of biosynthesized AgNPs (0.385, 0.390, and 0.440 ppm), compared with the control (0.655 ppm). This may be due to the use of this element in the metabolic processes of the plant. However, a notable increase surfaced in the concentration of silver metal in Al-Rasheed cultivar at 1.0 mg/ml of AgNPs (0.570 ppm). Meanwhile, no significant differences emerged at 1.5 and 2.0 mg/ml of Ag metal concentrations (0.370 and 0.410 ppm) compared with the control (0.405).

DISCUSSION

Results regarding green synthesis of AgNPs were in a greater analogy with findings obtained by Jadoun *et al.* (2021). They mentioned the color of the solution prepared from water extract of the plant begins to change with the adding of mineral salt under controlled conditions. Moreover, it can separate and the reaction mixture's color change can be beneficial to regulate the production of nanoparticles. Following this, the morphological with spectral examinations explained the properties of the manufactured particles. The color of nanoparticles was distinct from light to dark green. Biosynthesized of AgNPs were among the easiest to prepare nanoparticles. In the green synthesis of AgNPs, the solution of silver metal ion with a biological reducing agents were necessary (Singh *et al*., 2021).

The simplest and most affordable method to produce silver nanoparticles, reduced, and stabilized silver ions is by using a combination of active biomolecules, such as, proteins, alkaloids, polysaccharides, vitamins, phenols, amino acids, saponins, and terpenes. Approximately every plant has the potential for use in producing AgNPs (Hano and Abbasi, 2022). The SEM results also agreed with those of Javed *et al.* (2020), who reported the AgNPs were irregular in shape, whereas some nanoparticles had rectangular and cubic shapes. Furthermore, according to Oraibi *et al.* (2022), the biosynthesis of AgNPs from 1 mM of AgNO3 solution using *M. parviflora* extract filtrate can be easy to detect by a change in mixture color from green to yellowish-brown. It resulted from the metal nanoparticle's surface plasmon vibration’s excitement.

The atomic force microscopy results were consistent with those of Urnukhsaikhan *et al.* (2021), who used AFM to characterize the biosynthesized silver nanoparticles. The analysis showed AgNP-S, AgNP-F, and AgNP-W had respective sizes of 131, 33, and 70 nm. Green nanoparticles characterization using an uv-spectrophotometer confirms the presence of silver nanoparticles. Nanoparticles in the prepared solutions, due to surface plasmon absorption (SPR), a phenomenon caused by dipole oscillation, silver nanoparticles have diagnostic qualities coming from absorption peaks that appear at wavelengths between 450 and 400 nm. According to Ashraf *et al.* (2016) in the UV-visible spectrum, a broad, strong peak was visible at 455 nm for nanoparticles made of silver. The peak's widening suggested the particles were polydispersed. With surface plasmon resonance, AgNPs usually display a UV–visible absorption maximum in the 400–500 nm range.

The XRD analysis results also confirmed the examined material was silver nanoparticles after comparing the measurements with the X-ray diffraction database ICDD file JCPDS 04-0783. The average size of the silver nanoparticles’ extraction used the Debye-Scherer's equation, with ranges between 61.5–21 nm. The results also revealed a strong peak (high peak) at the angle of 38.32°, indicating the nanocrystals were more regular, arranged and organized in a certain direction more than the rest of the directions. These results align with findings obtained by Espinosa *et al*. (2020), who reported the XRD pattern for the silver nanoparticle. The reduction reaction of Ag-precursor was incomplete, as evidenced by small diffraction peaks associated with the trigonal structure of AgNO3 (COD entry No. 96-210-5348) and cubic-Ag2O (JCPDS No. 761396). Furthermore, a high peak centering at 2 θ = 38.202, 44.402, 64.602, 77.6, and 81.758° showed to be part of an FCC-Ag structure. All these values correspond to 1 1 1, 2 0 0, 2 2 0, 3 1 1, and 2 2 2, respectively (JCPDS No. 040783).

The study outcomes showed positive effects of biosynthetic silver nanoparticles on crops development and some plant physiological characteristics, such as, proline concentration, peroxidase, and catalase activity. These results were analogous to the observations made by Wahid *et al*. (2020), which confirmed the great interdependence between sustainable agriculture procedures and nanobiology. The compound green AgNPs helped mitigate the harmful effects on the responses and physiological system of different plants. Shibli *et al*. (2022) reported the addition of AgNPs resulted in the development of Q6 plants' mitigation abilities, particularly following the addition of 75 mg/L AgNPs, significantly increasing the viability of microbial growth under 200 mmol NaCl compared with the control group.

The treatment with AgNPs (especially at 75 mg/L) to the media increased the total chlorophyll, protein, and ions while decreasing the proline compared with the control treatment, indicating an enhancement in microshoot tolerance to salt stress circumstances. These findings suggested adding particular concentrations of AgNPs enhanced growth of Q6 and its tolerance under salt stress conditions. Sabir *et al*. (2022) reported spraying leaves with concentrations of AgNPs (25, 50, 75, and 100 ppm) to *Puccinia striiformis*-inoculated wheat plants to assess the disease's incidence in opposition to striped rust. AgNPs within the group of 75 ppm proved to be very successful in preventing wheat bar rust, with improved morphological and physiological properties and decreased enzymatic and non-enzymatic compounds in wheat plant. According to the impacts of green AgNPs at different concentrations with different plants, it indicates the safety of using silver nanoparticles as a natural fertilizer for plants through clear positive effects on various studied phenotypic and physiological characteristics.

Biologically manufactured nanoparticles are friendly to the environment, with no toxic effects on crop plant cells (Ismailova and Azizov, 2022; Bakry *et al*., 2024). The study noted nonsignificant differences in the concentration of silver in the vegetative parts of wheat cultivar Al-Rasheed treated with different doses of silver nanoparticles compared with the control. These results were promising and consistent with those of Ahmed *et al*. (2021), who reported the biosynthetic AgNPs exhibited inhibitory effective at the lowest concentration (10 mg/L). Moreover, these enhanced crop growth and grain yield with the concentration of 30 mg/L, and even at drought conditions, it slightly increased plant growth. These results exhibited promising increases in yield per hectare, reduced the food shortage, and boosted exports.

CONCLUSIONS

Treating the two cultivars with different concentrations of AgNPs resulted in both significant and nonsignificant differences in their proline content, while a decrease in proline occurred with increased NPK concentrations. Silver nanoparticles exhibited a significant effect and enhanced the antioxidant enzymes activities.

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