Hindawi
Journal of Nanomaterials
Volume 2024, Article ID 7524774, 13 pages
https://doi.org/10.1155/2024/7524774

Research Article

Evaluating Impacts of Biosynthetic Silver Nanoparticles on Morphophysiological Responses in Barley (*Hordeum vulgare* L.)

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Received 15 June 2023; Revised 28 August 2023; Accepted 11 January 2024; Published 27 January 2024

Academic Editor: Rajeshkumar Shanmugam

In recent years, nanotechnology has shown promising potential to enhance sustainable agriculture. Besides their use as antifungal and antimicrobial agents, silver nanoparticles (AgNPs) are the most widespread nanomaterials and are found in a capacious range of agrocommercial products. This study was designed to investigate the responses of morphophysiological characteristics in barley (*Hordeum vulgare* L.) to biologically synthesized silver nanoparticles. Spherical shapes with 8–20 nm size AgNPs at different concentrations (0, 50, 100, 150, 200, and 250 mg/L) were applied to barley plants in a hydroponic system. Following 7 days of sowing, the growth performance, chlorophyll contents, oxidative damage, and the activity level of antioxidant enzymes were quantified in different parts of the plant. The results indicated a remarkable boost in the growth performance and chlorophyll contents of barley plants up to a concentration of 150 mg/L. Interestingly, the levels of proline, lipid peroxidation, enzymes; superoxide dismutase (SOD), catalase (CAT), (APX), and (GR) activities were enhanced significantly in response to all AgNPs treatments. In general, the application of AgNPs substantially improved the growth and related morphophysiological attributes in barley. Our results provide new insights with respect to the effects of AgNPs on barley growth and their potential applications in increasing the performance of other crop species.

1. Introduction

At present, nanotechnology is getting increasing attention as an area of science dealing with the production of nanoparticles (NPs) [1]. NPs distinguished with their small size (1–100 nm), large surface-to-volume ratio in compared to larger particles of the normal metals, these make to possesses unequaled physicochemical properties [2, 3]. The novel features of NPs are fetching the interest of numerous research scientists for their application in a diverse fields such as food, agricultural, biomedical, energy harvesting, and the environment [4]. The application of NPs in the agricultural field is considered essential as it is directly associated with the life of humans and animals [5]. NPs find application in agriculture for various purposes, such as enhancing plant growth as nanofertilizers, controlling pest-related issues as pesticides, and occasionally serving as sensors to assess soil quality and monitor the plants health [6, 7]. Giving attention to nanofertilizers, earlier reports documented that some beneficial nutrients are transferred to the plants at the nanoscale level for supporting plant development and enhancing its yield [8–11]. Where nanofertilizers might have characteristics that are effective for plants; release nutrients in need, control the release of chemical fertilizers that regulate plant development, and enhance target activity [12, 13]. Pradhan and Mailapalli [14] reported that the newly engineered nanomaterials were able to boost plant growth and increase crop yields through the regulation of metabolic processes. Notably, among various types of nanoparticles composed of noble metal oxides, silver nanoparticles (AgNPs) are by far the most widely exchanged engineered nanomaterials, present in a diverse array of commercially available products [15]. Their synthesis and applications continue to be reported and discussed. Although many studies have reported three

common methods for the preparation of AgNPs, these include physical, chemical, and biological methods [16]. The biological methods which use nontoxic substances have gained more importance because they are benign and ecofriendly [17]. Plant extracts stand out as one of the foremost biological approaches for producing silver nanoparticles; this is primarily owing to the ready availability, cost-effectiveness, stability, and compatibility of plants, making them a highly favorable choice [18]. The medicinal plants which rich in phytochemicals act effectively as reducing, stabilizing, and capping agents for nanomaterials synthesizing [19]. *Ochradenus arabicus* is a medicinal plant found in Saudi Arabia, and it belongs to the *Resedaceae* family. Because it possesses a multitudes phytochemical compound that can severe as reducing agents for nanoparticle synthesis such as phenols, flavonoids, and alkaloids [20]. The plant can be used effectively for biofabrication of different types of nanoparticles including AgNPs.

Presently, there is a significant surge in the utilization of AgNPs across various agricultural and industrial applications. Their distinctive characteristics have garnered significant attention for their possible applications in agriculture, including the enhancement of crop growth, the management of various microbial phytopathogens, and other protective measures for crops [21–23].

Numerous contradictory findings have been documented, showcasing both negative and positive effects of AgNPs on plant growth and development. These discrepancies are observed irrespective of the method used for their synthesis, whether it is through chemical or biological routes [24]. Stimulatory effects of AgNPs on plant growth were observed in different plant species such as *Betula pendula* [25], *Cucumis sativus* [26], and *Zea mays* [27]. Conversely, the negative impacts of AgNPs on plants have been evident, particularly in terms of their ability to hinder plant growth [28, 29]. The concentration and size of the AgNPs are limiting factors in the effect that occurs in the plant, in addition to dosage, duration of exposure, and type of plant species. There are many studies indicating that appropriate concentrations of AgNPs play an important role in boosting growth, photosynthetic efficiency, and notable secondary metabolite production [30–32]. For instance, recent studies showed a positive response to plant growth indices after exposure to optimal levels of AgNPs. While in a study reported by El-Temsah and Joner [33], exposing 10 mg/L AgNPs to barley seeds resulted in a total inhibition of germination; and a significant reduction in the shoot of *Linum usitatissimum* exposed to the same concentration. Furthermore, in numerous studies, exposure of plants to an optimal concentration of nanoparticles has been shown to trigger the activation of antioxidant enzymes and mitigate the impact of reactive oxygen species (ROS) [34].

Barley (*Hordeum vulgare* L.) holds the fourth position globally among cereal grains in terms of production, spanning approximately 7 million hectares of cultivation. Its annual production totals 147.4 Mt, achieving a productivity rate of 3.13 t/ha [35]. This plant has been a significant food grain crop since ancient times and is recognized for its richness in trace minerals and dietary fiber [36]. In addition, it is an important forage for livestock in most arid and semiarid regions [37]. Unfortunately, very limited studies about nano were focused on the applications of AgNPs in hydroponic farming to enhance crop growth [38–40]. This study examined the morphophysiological responses of barley under AgNPs exposure.

2. Materials and Methods

2.1. Synthesis of Ag Nanoparticles. The medicinal plant *Ochradenus arabicus* was obtained from the tissue culture laboratory of King Saud University, the plant was multiplied *in vitro* on MS medium, and its shoot was extracted with Milli Q water, and these extracts were employed in the bioreduction process of silver nitrate to produce AgNPs following the method as outlined by Shaikhaldein et al. [41]. The size, morphology, and elemental analysis of AgNPs were determined by using transmission electron microscopy (TEM) (JEM-1011; JEOL Ltd., Tokyo, Japan). Ultraviolet–visible (UV–vis) spectrophotometer 1800 (Shimadzu, Japan) was applied to verify the reduction method used for AgNPs synthesis. For crystallographic, the structure determination of the synthesized AgNPs; X-ray diffraction (Rigaku Ultima IV, Neu-Isenburg, Germany, XRD) was performed. The data were collected in the 2θ range, and the size of the crystallite domains was determined using D. Scherrer's equation.

2.2. Plant Growth Conditions. The experiment was performed in a controlled condition of the hydroponic system at King Saud University. The composition of the Hoagland elements was prepared as previously explained by Zoufan et al. [42]. The pH of the nutrient solution was adjusted to 5.5. Seeds of barley (*Hordeum vulgare*) which were purchased from the market in Riyadh, Saudi Arabia, with 90%–95% viability were used. Initially, the seeds were sterilized with 20% (v/v) sodium hypochlorite, then after, they were rinsed with distilled water. The barley seeds were soaked in distilled water. After 24 hr, the seeds were transferred to a plastic pot supplemented with a complete nutrient solution and different concentrations of AgNPs including 0 (control), 50, 100, 150, 200, and 250 mg/L of AgNPs. Each plastic pot included 30 seeds. The nutrient solutions underwent regular aeration using an air pump, and they were changed every 2–3 days. The pots were arranged on shelves in a completely randomized design with three replicates. The barley plants were harvested after 7 days of sowing for further studies.

2.3. Measurement of Plant Morphological Parameters. Plants were harvested after 7 days of sowing in a hydroponic system, and phenotypic indices were determined by measuring the leaf and root length using a meter scale. Following the recording of fresh weight by using the electronic balance, leaves and roots were dehydrated in an oven at 105°C for 24 hr to estimate their dry weight.

2.4. Estimation of Photosynthetic Pigments. Contents of chlorophyll in barley leaves were determined using the method described by Arnon [43]. Barley leaf samples (0.1 g) were drenched in 0.2 mL of chilled 80% acetone for 24 hr. The samples underwent centrifugation at 10,000 rpm for a duration of 5 min. The supernatant's absorbance was determined

using an 80% acetone solution as a blank at wavelengths of 645 and 663 nm, utilizing a UV-1800 spectrophotometer (Shimadzu, Japan). The chlorophyll levels were subsequently determined using the following equations:

$$\text{Chlorophyll } a(ch\,a) = 12.7 \times \text{A663} - 2.69 \times \text{A645}, \quad (1)$$

$$\text{Chlorophyll } b(ch\,b) = 22.9 \times \text{A645} - 4.68 \times \text{A663}, \quad (2)$$

where A663 and A645, and A470 are the absorbance values read at 663 and 645 nm, respectively.

2.5. Quantitation of the Proline Content. To assess the proline content, the methodology reported by Bates et al. [44] was used. Initially, 0.4 g of fresh barley leaves were homogenized in 10 mL of 3% aqueous sulfosalicylic acid. Subsequently, the homogenate underwent centrifugation at 10,000 rpm at a temperature of 4°C for a duration of 10 min. Following this, 2 mL of the obtained supernatant was combined with 2 mL of ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was heated to 60°C and boiled for 2 hr, and then it was allowed to cool by immersing the tubes in an ice bath for 15 min. Following this cooling step, 6 mL of toluene was introduced into each tube and vigorously mixed for 20 s. The absorbance of the upper layer was then quantified at 520 nm using a spectrophotometer. The proline content was subsequently reported in micrograms per gram (μg/g) of fresh weight.

2.6. Lipid Peroxidation Content. Lipid peroxidation was evaluated to assess membrane damage by measuring the production of TBARS (thiobarbituric acid reactive substance) content as described by De Vos et al. [45]. The plant tissues were first homogenized in 5 mL of 0.1% trichloroacetic acid (TCA), followed by centrifugation at 10,000 rpm for 10 min. Following centrifugation, the resulting clear supernatant, amounting to 1 mL, was combined with 4 mL of a solution containing 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA (trichloroacetic acid). This mixture was then placed in a hot water bath at a temperature of 95°C for a duration of 30 min, followed by rapid cooling in an ice bath. The mixture was centrifuged at 5,000 rpm for 10 min at 4°C. The absorbance of the supernatant containing malondialdehyde (MDA) was measured by spectrophotometry at 440, 532, and 600 nm via a UV-1800 spectrophotometer (Shimadzu, Japan).

2.7. Antioxidant Enzyme Activity Assays. The activity of antioxidant enzymes was assessed by preparing extracts from barley leaf samples; this was done by homogenizing the leaves in a solution containing100 mM phosphate buffer (pH 7.0), 1 mM ethylenediaminetetraacetic acid, 1% (w/v) PVP (polyvinylpyrrolidone) and 0.2% Triton X-100. Following that the blend was subjected to centrifugation at a speed of 10,000 rpm for a duration of 10 min to acquire the supernatant. The supernatant was later used as the enzyme extract. The evaluation of superoxide dismutase (SOD; EC 1.15.1.1) activity was conducted following the procedure outlined by Marklund and Marklund [46]. Catalase (CAT EC 1.11.1.6) activity was measured following the method of Claiborne [47]. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assessed according to the procedure reported by Nakano and Asada [48]. The activity of glutathione reductase (GR; EC 1.6.4.2) was determined as a method described by Schaedle and Bassham [49].

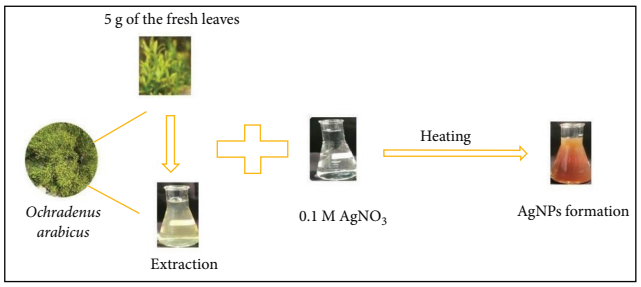

FIGURE 1: Biosynthesized AgNPs from regenerated shoots of *O. arabicus*.

2.8. Statistical Analysis. Statistical analysis of the data was performed utilizing one-way analysis of variance (ANOVA) through SPSS software (version 20). The outcomes were presented as the mean of three replicates ± standard deviation (SD) and subjected to comparison using Duncan's new multiple range test at a significance level of ($P \leq 0.05$). Principal component analysis (PCA) and Pearson's correlation analysis were conducted using Origin Pro software, version 2023.

3. Results

3.1. Characterization of the AgNPs. The aqueous solution of the silver nitrate ($AgNO_{3)}$ turned from light yellow to brown color after 10 min with the addition of *Ochradenus arabicus* leaves extract, giving the incipient indicator of the successful formation of AgNPs (Figure 1). This successful initial production of AgNPs was validated by identifying the surface plasmon resonance (SPR) using a UV–vis spectrophotometer. As depicted in Figure 2(a), the SPR of biosynthesized AgNPs was observed at 400 nm.

The crystallinity structure of the synthesized AgNPs was performed using XRD analysis. The biofabricated AgNPs demonstrated five intense planes at 2θ values corresponding to the lattice planes (111), (200), (331), (241), and (311) evidence that the formed AgNPs manifested in the form of nanocrystals (Figure 2(b)). The presence of a distinct diffraction peak at the (111) lattice plane confirmed the successful synthesis of AgNPs, as determined by the Scherrer equation. The measured crystallite size of the silver nanoparticles was 15 nm.

The morphological characteristics of the biosynthesized AgNPs were investigated by TEM. The TEM image (Figure 2(c)) illustrated that the formed AgNPs were spherical, with sizes ranging from 8 to 20 nm with a mean of 15 nm approximately (Figure 2(d)).

3.2. Effect of AgNPs on Barley Growth. The application of various concentrations of AgNPs (0, 50, 100, 150, 200, and 250 mg/L) significantly influenced on the morphometric developmental attributes of *H. vulgare* including the length, fresh, and dry weight of both leaves and roots (Figure 3). The concentration of 100 mg/L provoked the highest increase in the all-developmental index, where shoot fresh and dry weight increased by 253% and 442%. While the same growth

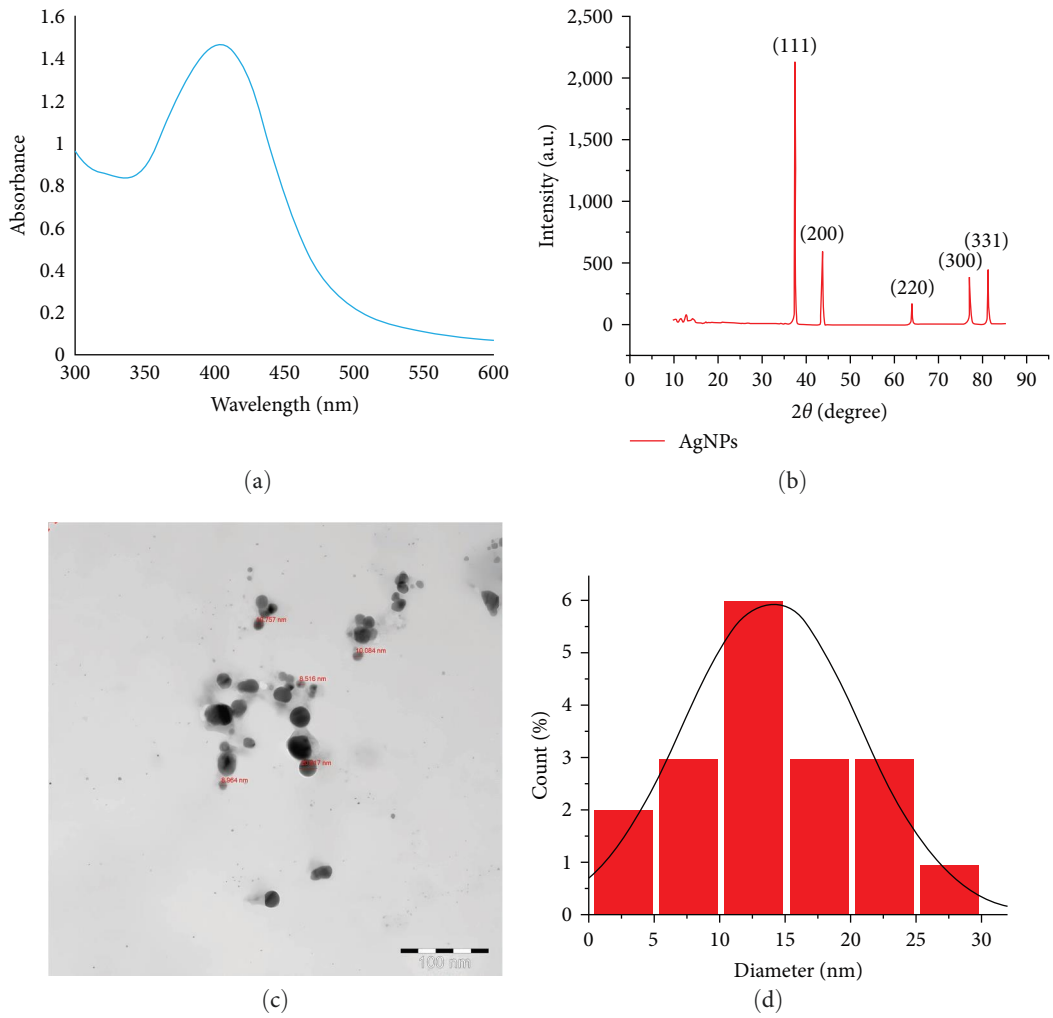

Figure 2: UV–visible absorbance spectrum of AgNPs (a). XRD diffraction pattern of AgNPs (b). TEM photograph of synthesized AgNPs (c). The distribution of particle sizes in the synthesized AgNPs (d).

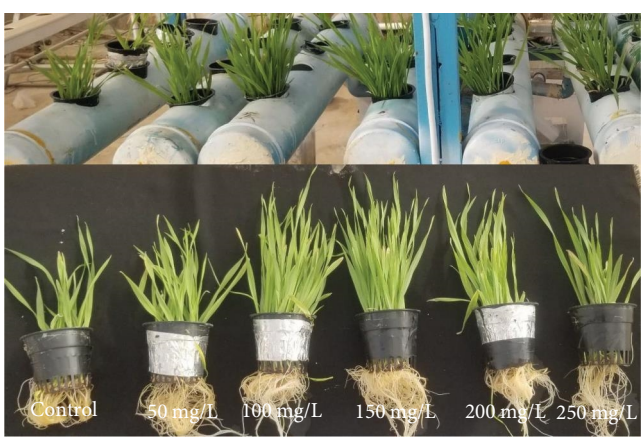

Figure 3: Impact of different AgNPs concentrations on barley (*Hordeum vulgare*) plants after 7 days in hydroponic culture.

characteristics were increased by 169% and 150% in root, respectively, and plant length increased by 28.6% in comparison to the control plants. A similar increase was also observed in the concentration (150 mg/L) which enhanced the shoot fresh and dry weight by 252% and 411%, root fresh and dry weight by 160% and 131%, and length of the plant by 25% over the control plants (Table 1). The highest concentration (250 mg/L) resulted in the least number of all morphological parameters compared to other treatments except the control groups.

3.3. Effect of AgNPs on Photosynthetic Pigments. The photosynthetic pigments: chlorophyll *a* (*ch a*) and chlorophyll *b* (*ch b*) content of the plants differed significantly as per the AgNPs concentrations (0, 50, 100, 150, 200, and 250 mg/L) to which *H. vulgare* were treated (Figure 4). The pigments *ch a* and *ch b* were enhanced by (17%, 34%), (22%, 194%), (20%, 165%), (18%, 140%), and (6%, 78%), respectively, in comparison to control plants. An AgNPs concentration of 100 mg/L resulted led to the highest concentrations of both photosynthetic pigments, in contrast, the control group exhibited the lowest concentrations of all photosynthetic pigments.

3.4. Effect of AgNPs on Proline Content and Lipid Peroxidation. The application of AgNPs treatments resulted in a significant increase in proline content in both leaves and roots, where proline levels were increased as per the increasing of NPs concentrations by 66%, 106%, 110%, 168%, and 209% in leaves and 80%,

Table 1: Morphological indices of barley (*Hordeum vulgare*) after application of different AgNPs treatments.

Treatment (mg/L)	Fresh weight (g)		Dry weight (g)		Plant length (cm)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	6.67 ± 0.20^{d}	2.26 ± 0.15^{d}	0.43 ± 0.02^{e}	0.05 ± 0.005^{c}	20.6 ± 0.57^{d}	8.2 ± 0.57^{d}
50	15.30 ± 0.40^{b}	4.11 ± 0.10^{c}	0.86 ± 0.02^{c}	0.08 ± 0.005^{b}	24.3 ± 0.57^{c}	9.1 ± 0.57^{c}
100	23.50 ± 0.26^{a}	5.60 ± 0.15^{b}	2.3 ± 0.15^{a}	0.12 ± 0.005^{a}	28.6 ± 0.57^{a}	11.6 ± 0.57^{b}
150	23.43 ± 0.37^{a}	6.10 ± 0.10^{a}	2.2 ± 0.15^{a}	0.13 ± 0.005^{a}	28.3 ± 0.57^{a}	13.3 ± 0.57^{a}
200	14.87 ± 0.21^{b}	5.93 ± 0.10^{ab}	1.82 ± 0.02^{b}	0.12 ± 0.005^{a}	25.6 ± 0.57^{b}	9.3 ± 0.57^{c}
250	10.37 ± 0.24^{c}	2.31 ± 0.10^{d}	0.64 ± 0.03^{d}	0.04 ± 0.005^{c}	21.6 ± 0.57^{d}	8.5 ± 0.57^{d}

The data represent the mean of three replicates ± standard deviation (SD). The letters "a"–"e" denote significant differences between the treatments at a significant level of $P \leq 0.05$, as determined by Duncan's test.

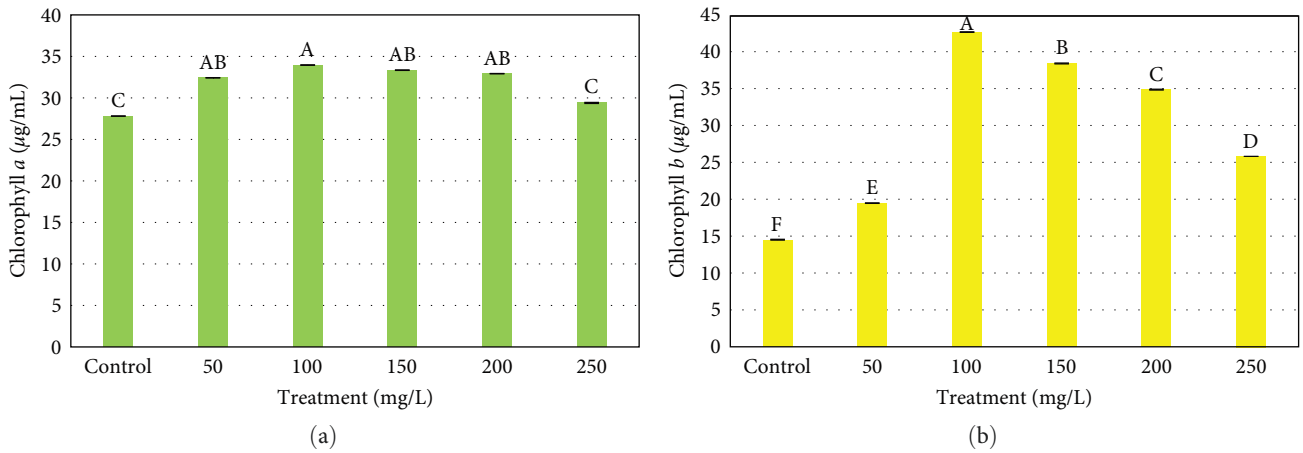

Figure 4: Impact of different AgNPs treatments on chlorophyll *a* (a) and chlorophyll *b* (b) in the leaf of barley after 7 days of treatment in hydroponic system. Error bars represent the standard deviation obtained from three technical repetitions of samples. The data represent the mean of three replicates ± standard deviation (SD). The letters "A"– "F" denote significant differences between the treatments at a significance level of $P \leq 0.05$, as determined by Duncan's test.

103%, 201%, 218%, and 395% in roots over the controls. The highest level of proline was measured in plants exposed to 250 mg/L AgNPs in both leaves and roots. While controls recorded the minimum proline levels, as shown in Figures 5(a) and 5(b).

Lipid peroxidation expressed in terms of MDA content was increased in both shoots and roots of hydroponically grown barley exposed to AgNPs treatments by (11%, 7%), (57%, 48%), (57%, 50%), (87%, 117%), and (105%, 126%), respectively, compared to that of the control, and the concentration 250 mg/L of AgNPs registered the maximum values of MDA, while the minimum levels were recorded control plants (Figures 5(c) and 5(d)).

3.5. Effect of AgNPs on SOD and CAT Activities. Activities of enzymatic antioxidants positively responded to AgNPs application in barley plants. SOD and CAT activities significantly increased due to the increase in the levels of AgNPs concentration. The highest level of antioxidant enzymes' activity was observed when plants were exposed to 200 mg/L of AgNPs. A significant increase in leaf and root by (136%, 147%) and (341%, 173%) was recorded in SOD and CAT activities in comparison to control plants (Figure 6).

3.6. Effect of AgNPs on APX and GR Activities. As shown in Figure 7, the activities of ascorbate peroxidase (APX) and glutathione reductase (GR) were increased in the roots and shoots by the presence of AgNPs in all concentrations compared with the control. The maximum level of both enzymes was monitored when barley plants were treated with 200 mg/L of AgNPs. The APX was increased by (136%, 340%), while GR enhanced by (189%, 197%) in shoot and roots, respectively.

3.7. Correlation Study. The PCA and Pearson's correlation were performed to understand the relationships between the different AgNPs treatments and the various morphophysiological parameters in barley. The principal components explained 94.82% (62.73% and 32.09%) of the total variance (Figure 8). Pearson's correlation analysis presented in Figure 9 shows a positive correlation and negative correlation among different morphophysiological characteristics, as illustrated in Figure 9. Briefly, morphological parameters were correlated positively with fresh weight, dry weight, plant height, and chlorophyll content. In contrast, they were correlated negatively with the contents of lipid peroxidation and proline.

4. Discussion

Using the leaf extract of *O. arabicus*, Ag^{+} was reduced to synthesize AgNPs via a biological approach, and the initial formation of AgNPs was visually confirmed by the observable

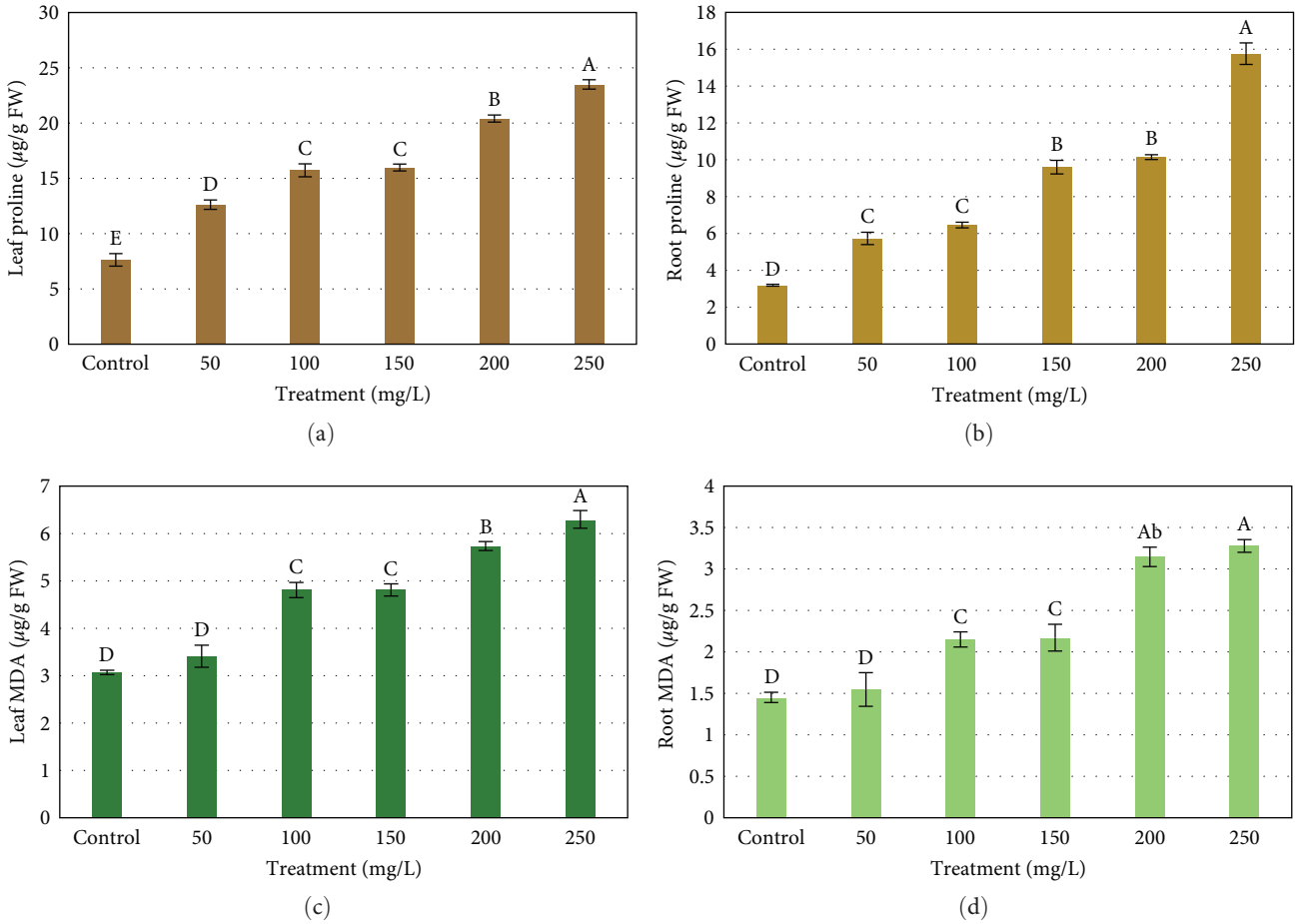

FIGURE 5: Impact of different AgNPs treatments on leaf proline (a), root proline (b), leaf MDA (c), and root MDA (d) of barley after 7 days of treatment in hydroponic system. The data represent the mean of three replicates ± standard deviation (SD). The letters "A"–"D" denote significant differences between the treatments at a significant level of $P \leq 0.05$, as determined by Duncan's test.

change in the color of the reaction solution to brown with heating; this color change can be explained by the efficacious reduction of Ag^+ into metallic nanosilver (Ag^o), which was likely facilitated by the phytochemicals present in the leaf extract of *O. arabicus*. Changing in color from faint yellow to brown after mixing of $AgNO_3$ solution with leaf extract as an indicator of AgNPs formation was reported by Pannerselvam et al. [50]. UV–vis spectroscopy illustrated a strong SPR absorption peak at 400 nm. Consistent with our results, Jebril et al. [51] reported the efficacy of *Melia azedarach* leaf extract to synthesize AgNPs at an SPR value of around 400 nm. This obtained peak (400 nm) is typical for AgNPs and demonstrates that the particles were evenly distributed without clumping together [52]. As indicated in numerous research investigations, the SPR wavelength for silver nanoparticles (AgNPs) produced through biological synthesis falls within the range of 400–450 nm. Any alterations in this measurement can be ascribed to the presence of phytochemicals found in the filtrates, which serve the dual roles of reducing and stabilizing agents [53, 54]. The X-ray diffraction (XRD) analysis of biosynthesized AgNPs confirmed their crystalline nature, displaying a face-centered cubic (FCC) plane (JCPDS File No. 4-0787). This pattern is consistent with the presence of pure silver metal possessing FCC symmetry. The size and morphology of AgNPs were identified using TEM. The TEM examination distinctly revealed that the nanoparticles possess a spherical morphology, and their size falls within the range of 8–20 nanometers. These findings are in accordance with the results reported in earlier studies by Ibrahim et al. [55] and Lashin et al. [56].

In recent years, a multitude of studies have placed significant emphasis on investigating the impact of applying silver nanoparticles on plant growth and development, revealing a spectrum of effects that include both detrimental and beneficial outcomes [57]. These discrepancies in findings were influenced by several factors, notably the characteristics of the silver nanoparticles, such as their shape and size, the concentrations used, the specific conditions of their synthesis, and the particular plant species being studied [24, 58]. An example for the stimulatory effect of AgNPs on the plants, Sadak [59] reported that application of AgNPs on *Trigonella foenumt* resulted in more plant biomass, higher plant length, increase in number of leaves, and enhance some biochemical parameters such as (IAA) and photosynthetic pigments. On the other hand, the detrimental effect of AgNPs was reported in *T. aestivum*, where the application of AgNPs caused a

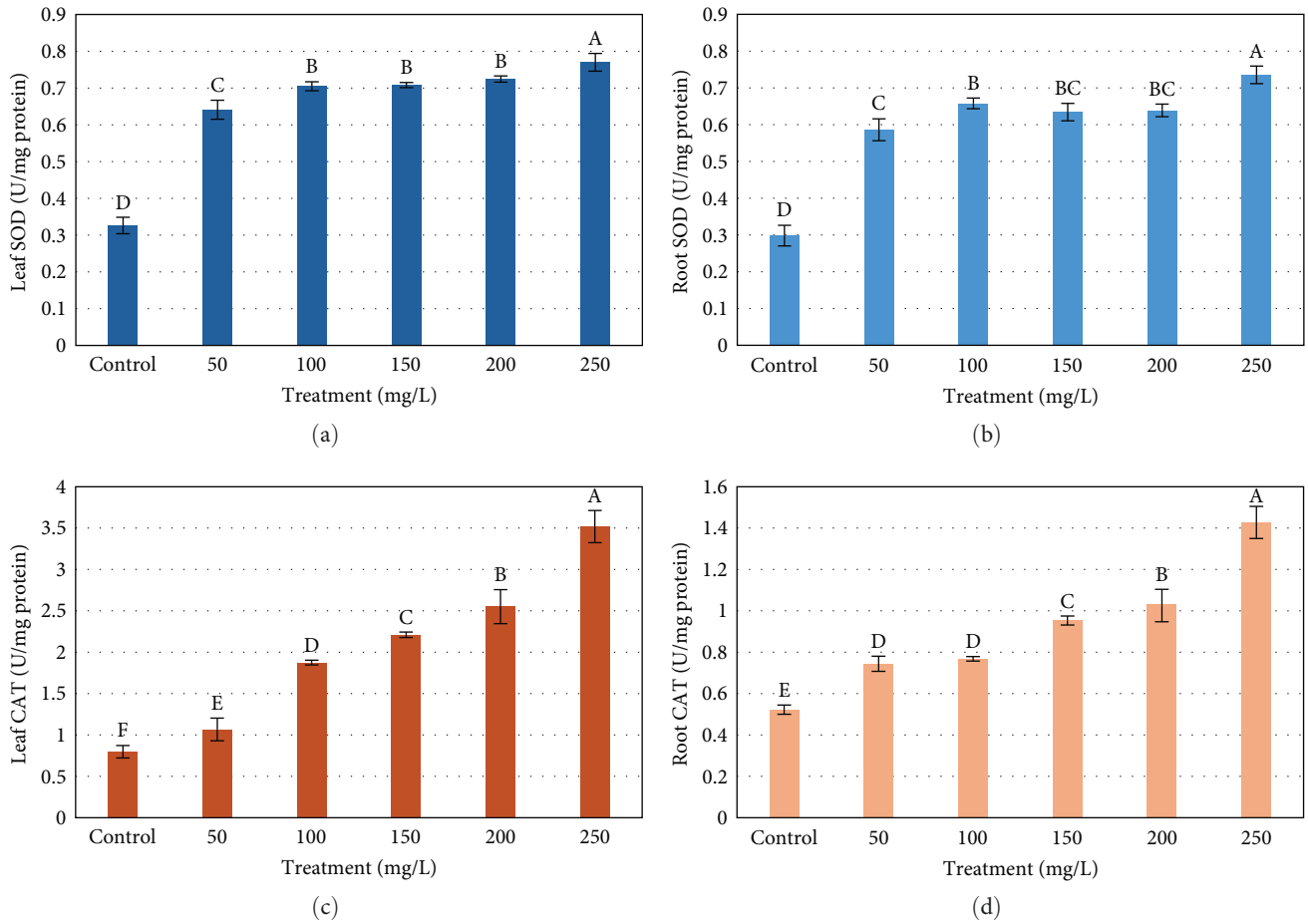

FIGURE 6: Impact of different AgNPs treatments on leaf SOD (a), root SOD (b), leaf CAT (c), and root CAT (d) of barley after 7 days of treatment in hydroponic system. The data represent the mean of three replicates ± standard deviation (SD). The letters "A"–"F" denote significant differences between the treatments at a significance level of $P \leq 0.05$, as determined by Duncan's test.

dramatic reduction on the growth parameters including plant length and biomass [60]. In addition, AgNPs induced accumulation of ROS and caused a severe inhibition of photosynthesis in *Brassica* sp. [61].

The present study reports the advantageous impacts of AgNPs on the morphological and biochemical parameters of *H. vulgare* cultivated in a hydroponic system. Employing AgNPs with an average size of 15 nm as detailed in our study on the barley plant significantly caused phytostimulation by boosting the shoot length, fresh weight, and dry weight for both leaves and roots. The increase in growth parameters is caused by the silver nanoparticles, which are synthesized using $AgNO_3$ as a precursor and *Ochradenus arabicus* leaf extract as reducing agents. $AgNO_3$ served as a source of silver ions that were involved in the reduction process to form AgNPs, while the plant extracts contain various phytochemicals that serve as reducing agents during the synthesis of AgNPs. The formed AgNPs then interacted with the barley plants and enhanced their growth and physiological responses. At certain concentrations of AgNPs (100 and 150 mg/L), barley growth recorded the highest level, while growth parameters were retarded with the application of higher concentrations of AgNPs (200 and 250 mg/L). The induced growth rise caused by different AgNPs treatments, especially at 150 and 200 mg/L, might be due to the role of AgNPs in blocking ethylene signaling in barley plants [62]. In a previous study, a comparable effect on root growth caused by AgNPs was documented in *Arabidopsis* [63], barley [64], and *Pisum sativum* [65]. In agreement with our findings, it has been also recently reported that another type of nanoparticles (iron oxide NPs) improved the germination rate, plant biomass, and plant growth in hydroponically grown barley [66, 67]. This observed improvement could be linked to the interaction of these NPs with various cellular signaling pathways, such as those involved in cell proliferation, ROS scavenging, and hormone signaling, including auxin, abscisic acid, and ethylene [68, 69]. So far, numerous research studies have documented the positive growth-promoting effects of AgNPs when used at their ideal concentrations in plant systems [25, 70].

Chlorophyll, the pigment responsible for the green coloration in plants, is a vital and the most abundant pigment for plants. Essentially, chlorophyll acts to absorb light for providing energy for photosynthesis [71]. It considers one of the most essential growth-related factors that significantly contribute to plant productivity [72]. Based on our findings, there was a significant increase in the levels of both chlorophyll

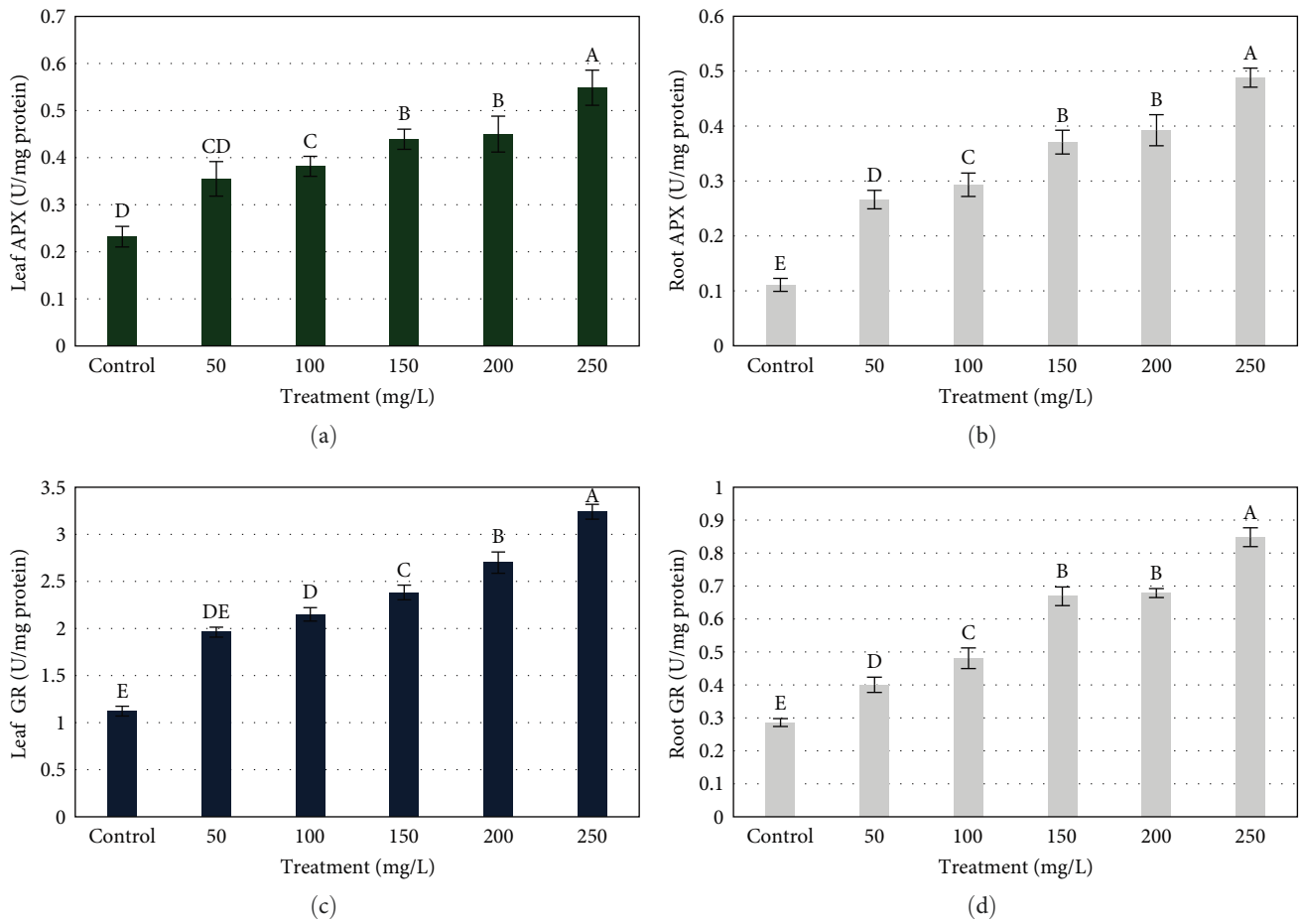

FIGURE 7: Impact of different AgNPs treatments on leaf APX (a), root APX (b), leaf GR (c), and root GR (d) of barley after 7 days of treatment in hydroponic system. The data represent the mean of three replicates ± standard deviation (SD). The letters "A"–"E" denote significant differences between the treatments at a significance level of $P \leq 0.05$, as determined by Duncan's test.

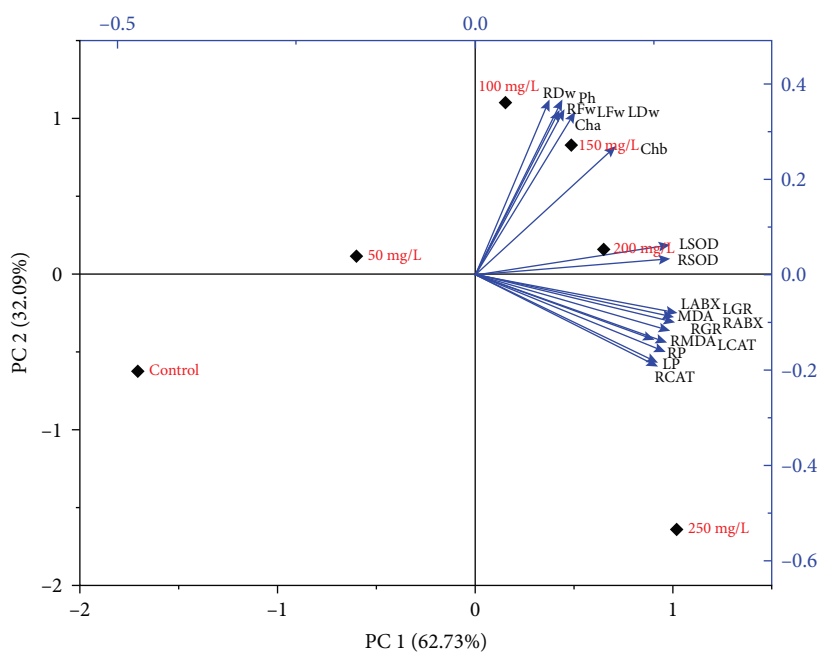

FIGURE 8: Principal component analysis (PCA) of morphophysiological characteristics of barley plants exposed to different concentrations of AgNPs.

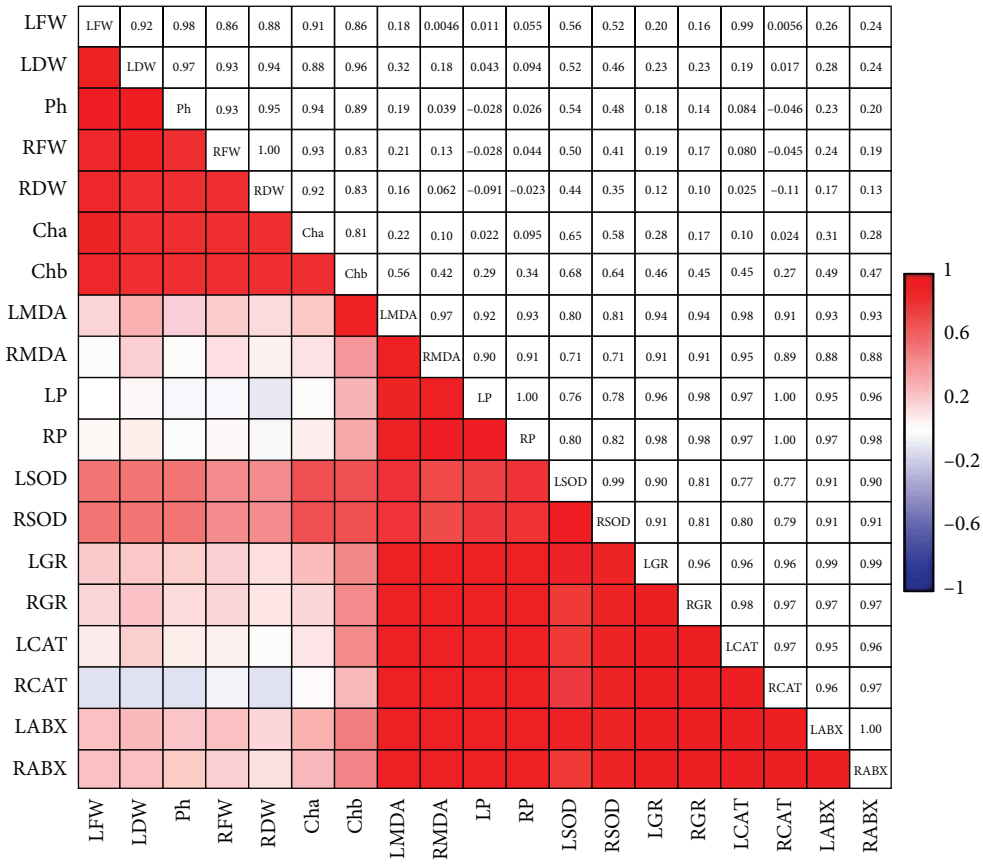

Figure 9: The correlation between different morphophysiological characteristics of barley plants exposed to different concentrations of AgNPs. LFW, leaf fresh weight; LDW, leaf dry weight; Ph, plant height; RFW, root fresh weight; RDW, root dry weight; *Ch a*, chlorophyll *a*; *Ch b*, chlorophyll *b*; LMDA, leaf malondialdehyde; RMDA, root malondialdehyde; LP, leaf proline; RP, root proline; LSOD, leaf superoxide dismutase; RSOD, root superoxide dismutase; LGR, leaf glutathione reductase; RGR, rot glutathione reductase; LCAT, leaf catalase; RCAT, root catalase; LABX, leaf ascorbate peroxidase; and RABX, root ascorbate peroxidase.

a and *b* in barley plants following treatment with AgNPs; however, a reduction of both chlorophyll *a* and *b* was observed when 250 mg/L AgNPs was applied. This finding aligns with the results obtained by Sadak [59] and Latif et al. [73], who also observed that AgNPs have a notable impact on enhancing photosynthesis, and this effect appears to be closely associated with alterations in nitrogen metabolism. A similar increase in chlorophyll has also been reported in barley when treated with Fe_3O_4 nanoparticles [74] and $CoNd_{0.2}Fe_{1.8}O_4$ [75]. Moreover, supporting to our findings Gupta et al. [58] reported that the content of chlorophyll was augmented in rice with low doses of AgNPs treatment while it was inhibited at higher concentrations of AgNPs. The possible reason of decrement in chlorophyll contents in barley under the higher concentrations of AgNPs might be that high doses of AgNPs caused a disruption in chloroplast structure or function which affected on the photosynthetic activity and led to decrease in photosynthesis pigments [76].

The accumulation of proline contents represents a biochemical response of plant cells to stressful conditions [77]. Proline serves a valuable function in mitigating various stresses in plants. Additionally, it serves as a precursor to proteins. In times of stress, proline acts as a metal chelator, contributes to antioxidative defense mechanisms, and serves as a signaling molecule [78]. Reports have shown that AgNPs could enhance the proline content in many plant species including barley. The findings of the current study demonstrated that the use of AgNPs increased the proline level in leaves and roots of barley plants compared to control. Similar results were also reported concerning the increase in the levels of proline content due to the application of AgNPs [30, 79, 80]. This increase is likely a protective response to shield the plants from heightened oxidative stress caused by elevated concentrations of AgNPs [81]. Lipids are major components of cell membranes. Increases in toxic oxygen species lead to the enhancement of free radicals' generation, which results in an increase in the process of lipid peroxidation [82]. Consequently, the accumulation of MDA (malondialdehyde) hinders the development and growth of the plant [83, 84]. Our results in this study showed that MDA was enhanced in AgNPs-exposed plants. The highest MDA content corresponds with the observed inhibition of barley's morphological growth. These results are consistent with the findings reported in *Hordeum vulgare* [85] and *Coriandrum sativum* ([86]), where contents of MDA increased due to NPs application.

Enzymatic antioxidants, such as SOD, CAT, APX, and GR act as major defensive agents, which combat oxidative

damage and are activated in plants under exposure to AgNPs. Our findings showed that all AgNPs treatments caused an observable increase in the activity of SOD, CAT, APX, and GR in both roots and leaves. The maximum activities of antioxidant enzymes were recorded when the plants were treated with the highest dose of AgNPs (250 mg/L), while the minimum level was recorded in the control. After the application of AgNPs, many studies reported increases in the activity of antioxidant enzymes in hydroponically grown plants including *Vigna angularis* [87], *Medicago sativa* [88], and Cabbage [89]. Increased activity of antioxidant enzymes under high concentrations of AgNPs points to the enhanced generation of ROS and instantaneous activation of plant defense mechanisms to counteract oxidative damage stress [58]. Unlike phytotoxicity, the stimulating effect of AgNPs observed in our study, characterized by low ROS production, could be attributed to the effective ROS scavenging mechanism triggered by AgNPs [90].

5. Conclusions

The key findings of this study suggest the positive response of hydroponically grown barley to silver nanoparticles at limited concentrations (100 and 150 mg/L), and such enhancements including the increase in length, biomass production, and chlorophyll contents, and for both leaves and roots. Conversely, higher concentrations of AgNPs (200 and 250 mg/L) caused a reduction in the growth, but enhanced proline and MDA contents, in addition, stimulated the production of SOD, CAT, APX, and GR indicating oxidative stress induced by the excessive doses of AgNPs.

Overall, our findings suggest that the application of AgNPs to barley plants had beneficial effects; thus, the application of 100, 150 mg/L AgNPs could be recommended in hydroponic nutrient solutions to boost barley growth and development. However, future studies should focus on the molecular mechanism of AgNPs in cell developmental processes and secondary metabolism.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors extend their appreciation to the Researchers Supporting Project No. (RSP-2024/73) at King Saud University, Riyadh, Saudi Arabia.

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