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Article

Dose-Dependent Application of Silver Nanoparticles Modulates Growth, Physiochemicals, and Antioxidants in Chickpeas (*Cicer arietinum*) Exposed to Cadmium Stress

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ABSTRACT: The present study was intended to investigate the effects of silver nanoparticles (Ag NPs) on chickpea plants grown in cadmium (Cd)-contaminated soil. Chickpea seeds sown in earthen pots (filled with soil) were subjected to Cd stress (100 μM) in the form of $CdCl_2$ (10 mL) 10 days after sowing (DAS). Exogenous applications with Ag NP concentrations 50, 100, and 200 μM were used to observe their effects on Cd-stressed plants. Growth, biochemical, and stress parameters were studied. Results showed that Ag NPs positively affected plant growth and ameliorated the toxic effects of Cd stress. Plant height, fresh weight, dry weight, total carotenoid content, rubisco activity, and net photosynthetic rate (P_N) were significantly decreased by Cd stress but enhanced by 28, 29, 31, 30, 33, and 35%, respectively, by foliar application of Ag NPs. Similarly, Ag NPs increased the activity of superoxide dismutase (61%), catalase (58%), and peroxidase (68%) and reduced the malondialdehyde (28%) and hydrogen peroxide (23%) in chickpea plants. Protein content was also increased by the application of Ag NPs (16%). Furthermore, the addition of Ag NPs decreased the plant Cd content. According to the current study, adding Ag NPs to plants under Cd stress improved their growth and photosynthesis by reducing Cd absorption and improving plant stress tolerance.

INTRODUCTION

Heavy metal (HM) pollution is a significant environmental concern, threatening ecosystems and human health.[1] Heavy metals are released into the environment from a variety of sources. Heavy metals can be released into the atmosphere by natural occurrences, such as volcanic eruptions. Excessive accumulation of HMs can degrade soil quality and damage plants' surface.[2] Cadmium (Cd), a prevalent HM, is of particular concern due to its extensive distribution and high toxicity. It obstructs various important plant processes such as photosynthesis, seed germination, plant growth, and chlorophyll degradation.[3−5] According to Kanu et al.,[6] chlorosis, reduced development, and plant death are among the most significant and frequent symptoms seen in rice under Cd stress. The excess Cd in grains has a significant negative impact on human health, leading to various diseases and problems with the body.[7,8] Thus, the toxicity of Cd has become a significant obstacle to preventing its negative impacts on plants and animals.[9] In order to avoid detrimental impacts on chickpea plants and human health, it is essential to stop the absorption of Cd in chickpeas and its subsequent transport.

Nanotechnology has recently been used in a variety of fields, such as manufacturing, chemical synthesis, drug delivery, medicine, and agriculture.[10] Nanoparticles (NPs) are widely used in agriculture primarily because they are readily absorbed by different plant components and interact with a wide range of live cells and tissues.[11] NPs are tiny particles that are 1 to 100 nm in size and have large surface areas.[12] Different materials have been used to create both metallic and nonmetallic NPs.[13] It has been observed that these modified NPs have great potential for both safeguarding and raising agricultural yields.[14] Among the different NPs, silver nanoparticles (Ag NP) are the most well-known metallic NPs and are used in a variety of agricultural functions and stress tolerance. Ag NPs have shown promising effects as fertilizers, insecticides, and abiotic stress tolerance.[15] Ag NPs have been used to improve seed germination and plant development, as seen by increased leaf area, root length, and shoot length in

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Zeya mays, *Phaseolus vulgaris*, *Brassica juncea*, and water hyacinth.[16−18] According to Yosefzaei et al.,[19] proline and glucose concentrations rise in response to Ag NP exposure, and antioxidant enzyme activity, such as catalase and guaiacol peroxidase, was also raised. A concentration of 75 mg of Ag NPs improved growth characteristics in rice plants.[20] On the other hand, sorghum plants treated with 1000 μM Ag NPs showed a notable rise in root length as well as increased activity of catalase and ascorbate peroxidase.[21] Plants have evolved complex antioxidant defense mechanisms to combat oxidative damage and control the quantities of reactive oxygen species within their cells. Enzymatic and nonenzymatic antioxidants are the two groups into which these systems fall. Nonenzymatic antioxidants are defined as small molecules such as glutathione, β-carotene, α-tocopherol, ascorbate, and proline.[22] However, specific enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) are examples of enzymatic antioxidants. $O_2^{\bullet-}$ or H_2O_2 is neutralized by the combined action of these enzymes.[22]

The foliar application of Ag NPs may have the potential to provide considerable tolerance against Cd stress in chickpea plants. Keeping this in mind, the present work was designed with the aim of investigating the role of Ag NPs on photosynthetic efficiency, antioxidant enzyme activity, and carbohydrate metabolism in chickpeas under Cd stress.

MATERIALS AND METHODS

Experimental Layout. Chickpea (*Cicer arietinum*) seeds were purchased from the local market of Hyderabad-500032, India. Seeds were surface-sterilized with 0.01% sodium hypochlorite (NaOCl) for 10 min and rinsed 3−4 times with double-distilled water (DDW). Sterilized seeds were sown in earthen pots filled with soil. In chickpea seedlings, cadmium (Cd) stress was given through the soil by adding 10 mL of $CdCl_2$ (100 μM) 10 days after sowing (DAS). Silver nanoparticles (Ag NPs) purchased from the Nano Research Lab, H21 Gopalpur East Singhbhum, Jamshedpur, Jharkhand, India−832102, were prepared at concentrations of 50, 100, and 200 μM by diluting the stock solution. The RBD statistical design was applied with 5 replicates of each treatment, and experiment encompasses eight treatments in total in this study: Set 1: Control treated only double-distilled water; Set 2: Cd (100 μM) stress through the soil at 10 DAS; Set 3: Ag NPs (50 μM) foliar application at 26−30 DAS (5 successive days); Set 4: Ag NPs (100 μM) foliar application at 26−30 DAS (5 successive days); Set 5: Ag NPs (200 μM) foliar application at 26−30 DAS (5 successive days); Set 6: Ag NPs (50 μM) + Cd (100 μM); Set 7: Ag NPs (100 μM) + Cd (100 μM); and Set 8: Ag NPs (200 μM) + Cd (100 μM).

Using a sprayer that was adjusted to dispense 1 mL every push, the foliar spraying was done carefully. Plants were gently removed from the pots at 35 DAS to determine the length, fresh and dry weight, antioxidant enzyme activity, biochemical characteristics, protein content, and rubisco activity. The water level in the pots was maintained at that level during the entire plant growth, and sample analysis was completed after 40 days of DAS.

Growth Analysis. The plants' length was precisely measured with a meter scale, and their fresh and dry weights were carefully calculated with a precision weighing device. After their fresh weight was measured, the plants were dried for 48 h at 70 °C in an oven. This allowed for the measurement of the dry weight.

SPAD Value of Chlorophyll Content. Using an SPAD chlorophyll meter (SPAD-502Plus; Konica, Minolta Sensing, Inc., Japan), the amount of chlorophyll in leaves was measured.

Total Carotenoid Content and Rubisco Activity. Total carotenoid content was extracted from 1 g of leaf samples that were collected at 1 p.m. The materials were homogenized in aqueous acetone [7 mL, 80% (v/v)], filtered, and then added to a total volume of 20 mL with 80% acetone. In order to determine the concentration of carotenoids and chlorophylls at 470, 647, and 663 nm in a spectrophotometer.[23] For Rubisco activity, a total of 0.1 g of fresh leaf samples were weighed and ground in an ice bath and determined activity by using the Rubisco Kit (Ge Ruisi, Suzhou, China).[24]

Photosynthesis and Related Attributes. Using a portable infrared gas analyzer (Li-COR 6200, Portable Photosynthesis System, Lincoln, NA), important physiological parameters such as the net photosynthesis rate (P_N), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and transpiration rate (E) were determined on the 40 DAS.

Antioxidant Enzymatic Activity Assay. Fresh leaf samples (0.5 g) containing 1% poly(vinylpyrrolidone) were pulverized in 50 mM phosphate buffer in order to measure the antioxidant enzymatic activity. After centrifuging the homogenate for 10 min at 4 °C at 15,000 g, the supernatant was collected. This was used as the source of enzymes to measure the activities of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD). For these findings, the procedure outlined by Faizan and Hayat[25] was adhered to. An enzyme extract (0.1 mL), phosphate buffer, and hydrogen peroxide (H_2O_2, 0.1 M) were combined to create a reaction mixture for CAT analysis. After 1 min, the mixture was mixed with sulfur dioxide (H_2SO_4), and the potassium permanganate solution was used for titration.[26] In order to determine POX activity, 0.1 mL of the enzyme extracts were combined with a 1% solution of phosphate buffer, pyrogallol, and H_2O_2, and the absorbance at 420 nm was measured using a spectrophotometer.[26] A combination containing phosphate buffer (50 mM), riboflavin (20 μM), NBT (75 mM), methionine (13 mM), and ethylene diamine tetra acetic acid (0.1 mM) was produced for the purpose of determining SOD activity. A spectrophotometer was used to measure the mixture's absorbance at 560 nm after it was exposed to fluorescent light.[27]

Proline Content. The procedure described by Bates et al.[28] was followed in order to determine the proline content of leaves. First, 50 mg of fresh leaves were extracted in sulfosalicylic acid. Next, the same amounts of ninhydrin and glacial acetic acid were added. The sample was then heated to 100 °C, and 5 mL of toluene were added. The aspirated layer's absorbance was determined at 525 nm using a spectrophotometer. The proline content was given in $\mu g\ g^{-1}$ (FW) units.

Malondialdehyde and Hydrogen Peroxide. Malondialdehyde equivalents were measured to determine lipid peroxidation rates in accordance with the methodology developed by Hodges et al.[29] Hydrogen peroxide (H_2O_2) accumulation was computed using the method developed by Jana and Choudhuri.[30]

Protein Content. The protein content of chickpea fresh leaves was measured by employing the Bradford[31] method. Using a mortar and pestle, 1 g of fresh leaves were first ground in the buffer before being centrifuged at 20,000*g* for 10 min. In

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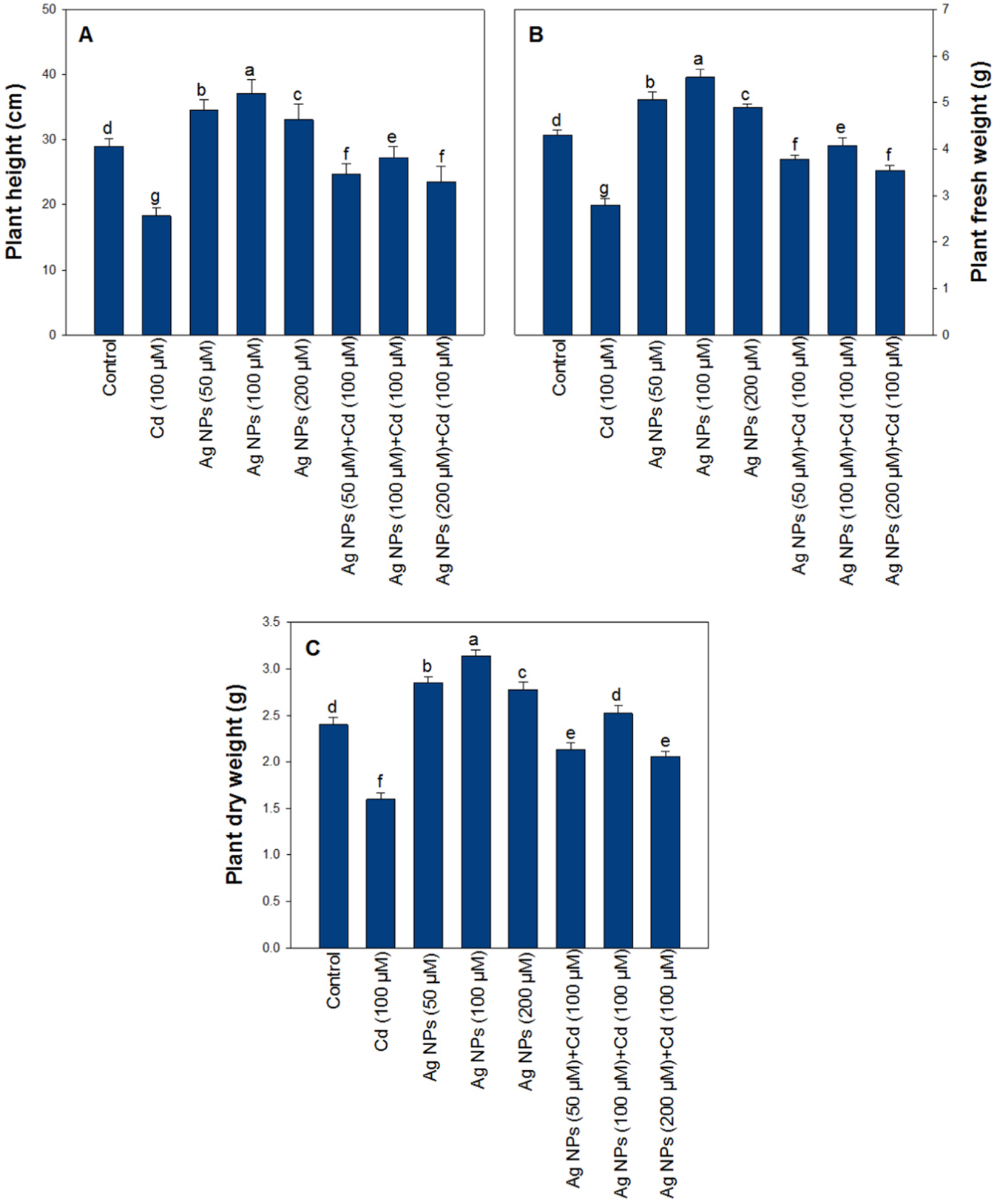

Figure 1. Impact of foliar spray of Ag NPs (50, 100, and 200 μM) on (A) plant height, (B) plant fresh weight, and (C) plant dry weight in chickpea plants in the presence/absence of Cd (100 μM) stress at 35 DAS. Values are mean ± SE (n = 5) and are statistically significant (p < 0.05).

order to create color development, the resultant supernatant was combined with the Bradford reagent, and the absorbance was determined using a spectrophotometer. The protein content was given in mg g^{-1} (FW) units.

Cd Content. To assess the Cd content, the plant sample was cleaned by running tap water and then dried in the incubator for 48 h at 80 °C. The dried material was weighed, ground into a fine powder using a motor and pestle, and mixed

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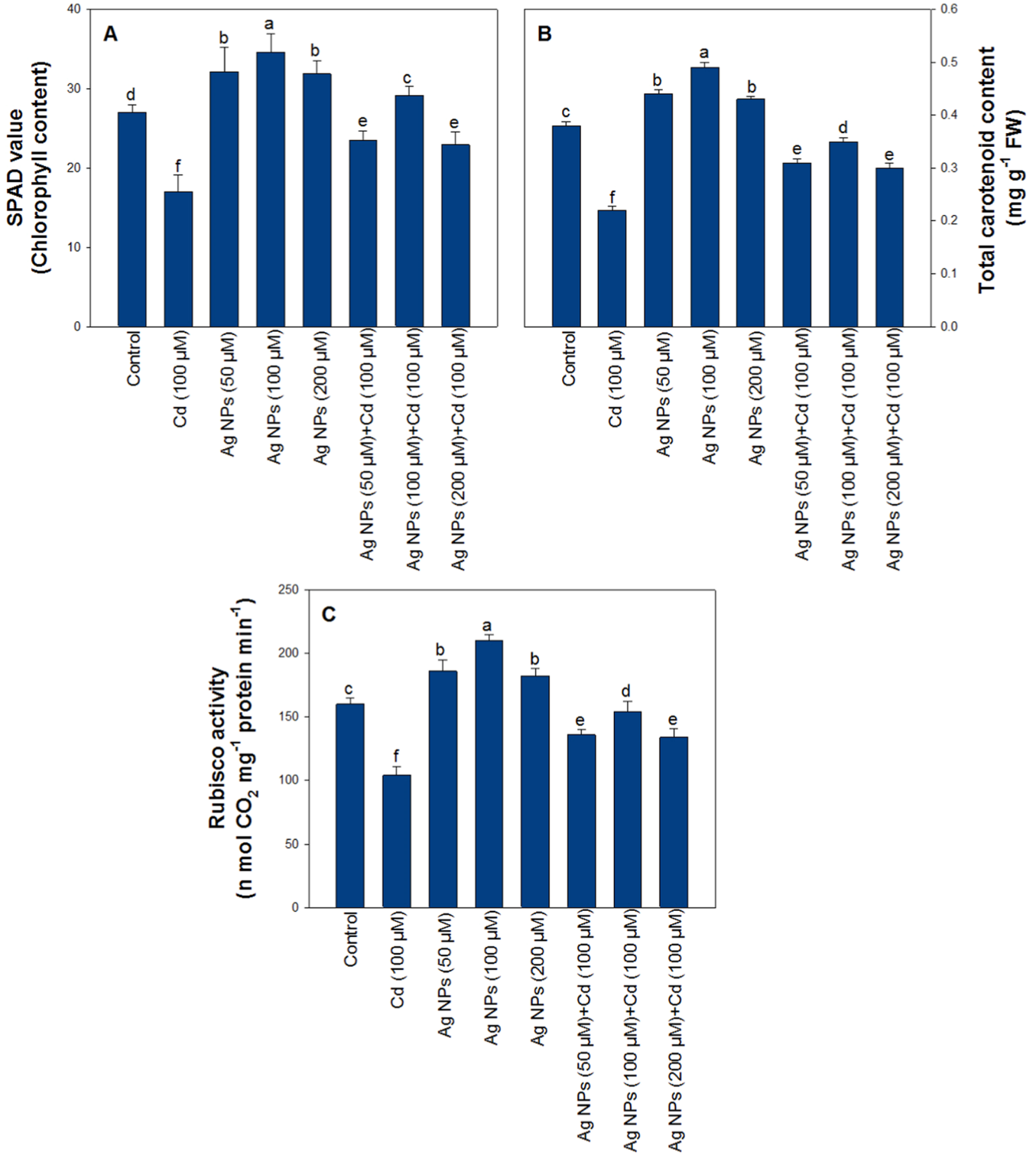

Figure 2. Impact of foliar spray of Ag NPs (50, 100, and 200 μM) on (A) SPAD value of chlorophyll content, (B) total carotenoid content, and (C) rubisco activity in chickpea plants in the presence/absence of Cd (100 μM) stress at 35 DAS. Values are mean ± SE (n = 5) and are statistically significant ($p < 0.05$).

with an $HNO_3/HClO_4$ 3:1, v-(v) concentration. Using an atomic absorption spectrophotometer (GBC, 932 plus; GBC Scientific Instruments, Braeside, Australia), the amount of Cd was calculated and given as $\mu g\ g^{-1}$ of DM.

Statistical Analysis. The statistical analysis of the data was conducted using analysis of variance (ANOVA) in SPSS ver. 17.0 (SPSS, Chicago, IL). To accurately discern any statistically significant differences among the means, the least significant difference (LSD) test was employed as a posthoc analysis.

RESULTS

Effect of Exogenous Ag NP Application on Chickpea Morphology under Cd Stress. The results demonstrated

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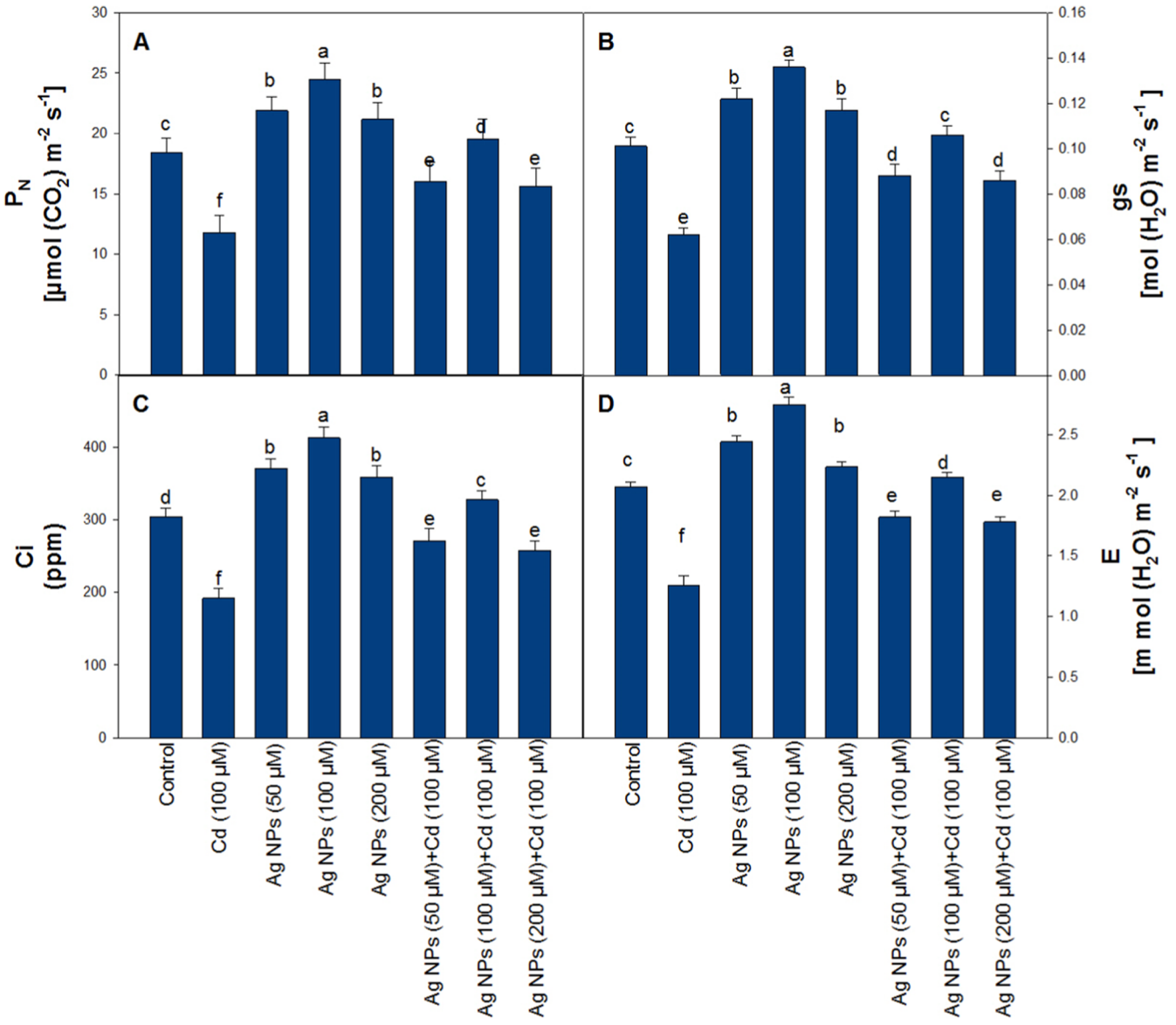

Figure 3. Impact of foliar spray of Ag NPs (50, 100, and 200 μM) on (A) net photosynthetic rate; P_N, (B) stomatal conductance; gs, (C) intercellular carbon dioxide concentration; Ci, and (D) transpiration rate; E in chickpea plants in the presence/absence of Cd (100 μM) stress at 35 DAS. Values are mean ± SE (n = 5) and are statistically significant (p < 0.05).

that cadmium (Cd) toxicity decreased the morphological parameters in chickpea plants (Figure 1A−C). The decline was more pronounced in plants exposed to 100 μM Cd by 37% in plant height, 35% in plant fresh weight, and 33% in plant dry weight compared to control plants. It is interesting to note that foliar application of silver nanoparticles (Ag NPs) increased all of the morphological attributes in a concentration-dependent manner in plants produced in the presence/absence of Cd toxicity (Figure 1A−C). The maximum increase in height, fresh weight, and dry weight was noted in the plants treated with 100 μM Ag NPs, and the increase was 28% (height), 29% (fresh weight), and 31% (dry weight) over water-treated plants. Under Cd stress, Ag NPs also exerted beneficial roles, reduced toxicity, and increased all growth attributes in a concentration-dependent manner.

Effect of Exogenous Ag NP Application on Chickpea SPAD Value of Chlorophyll Content, Total Carotenoid Content, and Rubisco Activity under Cd Stress. The findings displayed in Figure 2A−C demonstrate that Cd had a toxic effect on the SPAD value of chlorophyll content, total carotenoid, and rubisco activity of chickpea plants. In comparison to control plants, the Cd (100 μM) stress reduces the SPAD value by 37%, the total carotenoid content by 40%, and the rubisco activity by 35% (Figure 2A−C). When plants under Cd stress were sprayed with Ag NPs, the SPAD value of chlorophyll content, total carotenoid, and rubisco activity increased dramatically. Nonetheless, at different concentrations of Ag NPs (50, 100, and 200 μM), all of the above parameters were increased in a concentration-dependent manner. In comparison to plants treated with sole Cd, the application of Ag NPs (100 μM) increased the SPAD value by 71%, the total carotenoid content by 59%, and the rubisco activity by 48% in Cd- and Ag NPs-treated chickpea plants (Figure 2A−C).

Effect of Exogenous Ag NP Application on Chickpea Photosynthesis and Related Attributes under Cd Stress. Cd (100 μM) toxicity reduced photosynthesis and related attributes in chickpea plants compared to control plants (Figure 3). Compared to control plants, there was a startling

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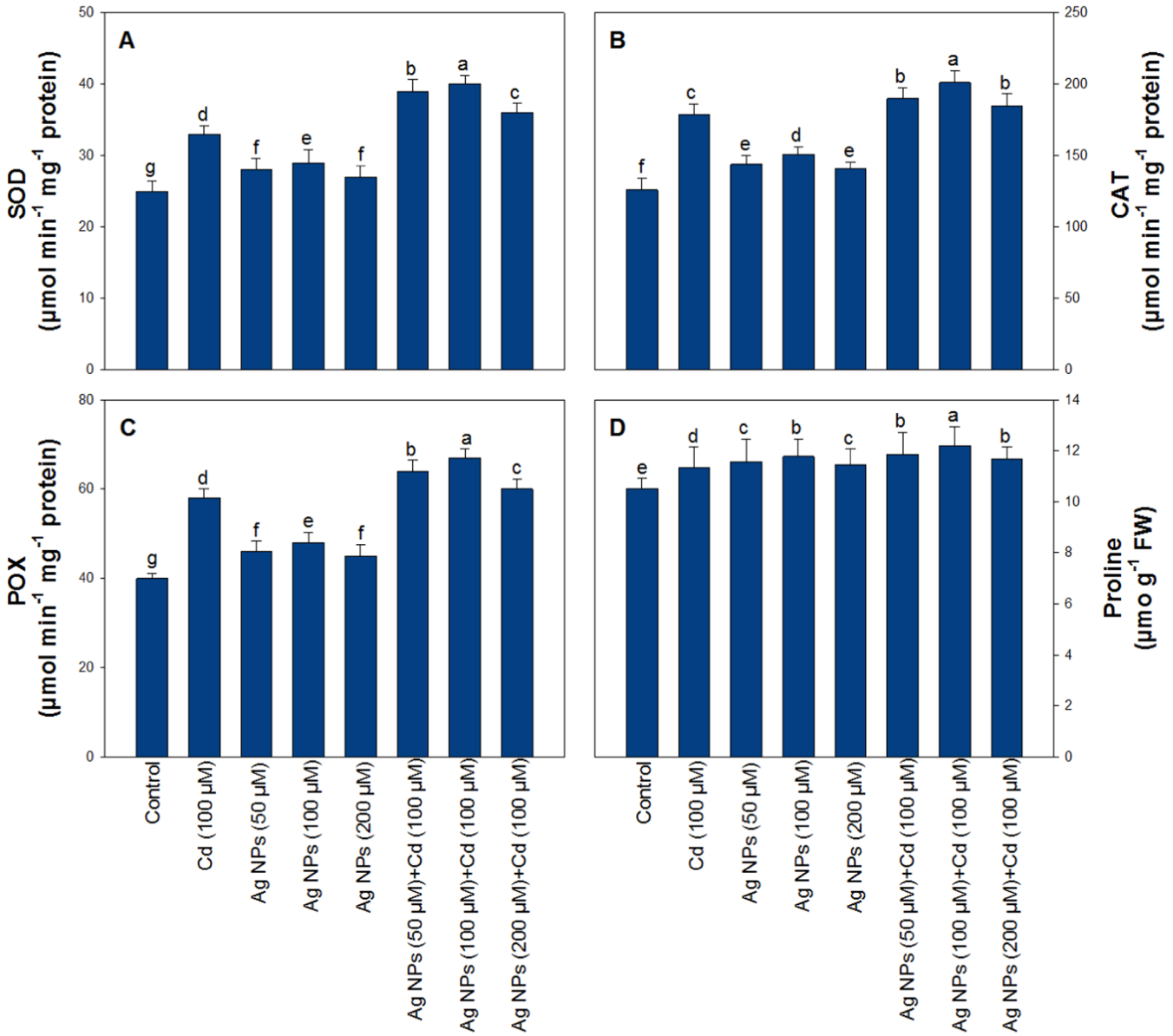

Figure 4. Impact of foliar spray of Ag NPs (50, 100, and 200 μM) on (A) superoxide dismutase; SOD, (B) catalase; CAT, (C) peroxidase; POX, and (D) proline in chickpea plants in the presence/absence of Cd (100 μM) stress at 35 DAS. Values are mean ± SE (n = 5) and are statistically significant ($p < 0.05$).

36% reduction in P_N, a significant 38% decline in gs, a notable 37% decline in Ci, and a significant 39% drop in E in Cd-treated chickpea plants (Figure 3). However, the consequences of Cd toxicity were successfully alleviated by the exogenous application of Ag NPs in a concentration-dependent manner, leading to enhanced P_N, gs, Ci, and E. Plants treated with Ag NPs (100 μM) under Cd stress increased P_N by 65%, gs by 71%, Ci by 70, and 72% by E compared to only Cd-treated plants. Among the different tested (50, 100, and 200 μM) concentrations of Ag NPs, 100 μM proved to be the best and increased all of the photosynthetic attributes significantly (Figure 3A−D).

Effect of Exogenous Ag NP Application on Chickpea Antioxidant Enzyme Activity under Cd Stress. Plants typically develop many antioxidant enzymes in response to Cd stress in order to counteract the excess reactive oxygen species that are produced as a result of oxidative stress. We saw significant improvement in the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Figure 4). At Cd stress treatment (100 μM), the increase in enzymatic activity is notable; it is 31% (SOD), 42% (CAT), and 45% (CAT) higher than that in control (Figure 4A−C). When the Ag NPs applied to the leaves of plants had been Cd-stressed, the enzymatic activities displayed a distinct reaction in a concentration-dependent manner. When Ag NPs (100 μM) are added, it is discovered that SOD activity increases in both stressed and nonstressed plants by 61 and 18%, respectively, over control plants (Figure 4A). The same results are also displayed with CAT and POX and increased their activity by 60% (with Cd-stressed), 20% (without Cd-stressed), 68% (with Cd-stressed), and 21% (without Cd-stressed), respectively, over their control plants (Figure 4B,C).

Effect of Exogenous Ag NP Application on Chickpea Proline Content under Cd Stress. Plants exposed to Cd (100 μM) showed a notable increase (8%) in proline accumulation compared to that in the corresponding control

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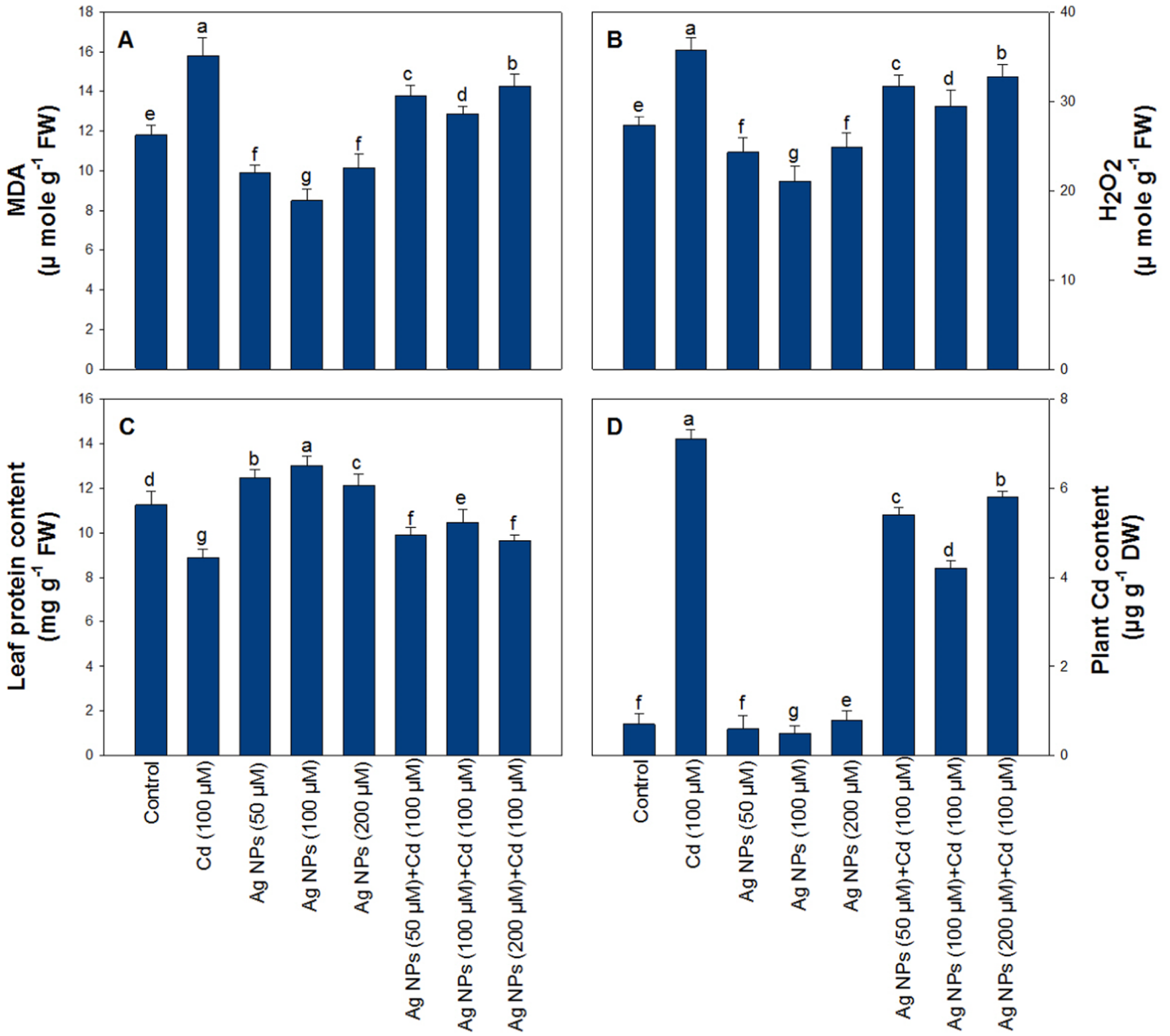

Figure 5. Impact of foliar spray of Ag NPs (50, 100, and 200 μM) on (A) malondialdehyde; MDA, (B) hydrogen peroxide; H_2O_2, (C) leaf protein content, and (D) plant Cd content in chickpea plants in the presence/absence of Cd (100 μM) stress at 35 DAS. Values are mean ± SE (n = 5) and are statistically significant ($p < 0.05$).

group (Figure 4D). Different concentrations (50, 100, and 200 μM) of Ag NPs further increased the proline content in chickpea leaves. A maximum increase in the proline content was noted in the plants treated with 100 μM Ag NPs in addition to 100 μM Cd (Figure 4D).

Effect of Exogenous Ag NP Application on Chickpea MDA and H_2O_2 Content under Cd Stress. The MDA level increased when Cd (100 μM) was applied to plants by 34% compared to their control (Figure 5A). Ag NPs application in a concentration-dependent manner resulted in a considerable reduction in MDA levels and almost completely abolished the negative effects of Cd. When Ag NPs (100 μM) were supplemented to chickpea plants, it reduced 86% of the MDA content compared to Cd-stressed plants (Figure 5A). The exposure to Cd on its own caused a substantial increase in H_2O_2 buildup in the chickpea plant by 31% compared to the control (Figure 5B). A significant decrease in the H_2O_2 content was produced by Ag NPs in a concentration-dependent manner, which also nearly eliminated the harmful effects of Cd stress. In comparison to Cd-stressed plants, 100 μM Ag NPs reduced the H_2O_2 content by 70% (Figure 5B).

Effect of Exogenous Ag NP Application on Chickpea Protein Content under Cd Stress. The amount of protein in the leaves of the seedlings exposed to Cd dramatically decreased (Figure 5A). On the other hand, the exogenous administration of Ag NPs greatly enhanced the protein content in a concentration-dependent manner and lessened the adverse effects of Cd stress. Compared to untreated plants, the plants exposed to Ag NPs had a somewhat higher protein content (16%) (Figure 6).

Effect of Exogenous Ag NP Application on Chickpea Cd Content. Plants supplemented with Cd (100 μM) showed a significant increase in metal content by 238% compared to control ones (Figure 5D). In addition, the Cd content was lowered in stressed and nonstressed plants treated with Ag NPs in a concentration-dependent manner. A noteworthy decrease

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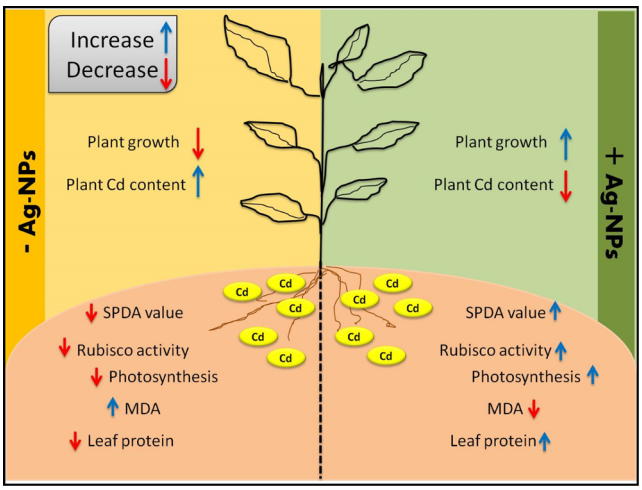

Figure 6. Schematic representation of Ag NP-mediated stress tolerance in chickpeas under Cd stress. MDA: Malondialdehyde; Cd: Cadmium; Ag NPs: Silver nanoparticles.

was observed in the plants treated with 100 μM Ag NPs when compared to the control (Figure 5D).

DISCUSSION

The accumulation of cadmium (Cd) in plant tissues inhibits growth and causes a number of toxicity symptoms. The diminish in growth and yield is the very first symptom of Cd accumulation.[32] Cd translocation within plant tissues interferes with the photosynthetic process, alters nutrient profiles of nutrients and enzyme activity, and negatively affects crop quality by reducing yield generation.[33] In plants, growth was suppressed under Cd toxicity due to changes in the rates of cell division, growth, and structure, as reported by Ashraf et al.[7] There were also changes in the rates of water and critical mineral nutrient uptake by the root, and the disruption of the hydrogen−sulfur bond caused the denaturation of proteins. Our findings clearly show that Cd had a detrimental impact on every development parameter, including plant height, fresh weight, and dried weight of chickpeas (Figure 1A−C). The present investigation demonstrated how Ag NPs improve growth metrics like length, FW, and DW. This could be because relative water content (RWC) has improved, which is the primary factor in its primary function in polysaccharide synthesis.[34] Additionally, Ag NP function in preserving the hormonal balance adversely impacted by stressors improves the metabolic and physiological conditions of calli cultures. Researchers have discovered that the positive effects of Ag NPs on plant physiochemical characteristics and growth may be linked to changes in the expression levels of genes involved in cellular growth, water uptake, nutrients, and seed germination.[35] Additionally, the growth and length were significantly influenced by the Ag NP treatment. Prior research on rice[36] and Arabidopsis[37] revealed a similar form of Ag NP-induced root growth, which was ascribed to ROS accumulation and Ag NPs interaction with several cellular signaling pathways, such as cell proliferation, ROS scavenging, and hormone signaling pathways, which include auxin, abscisic acid, and ethylene.[36−38]

Since chlorophyll is a vital pigment involved in light absorption and the subsequent conversion of light energy into chemical energy during photosynthesis, the relationship between leaf chlorophyll content and photosynthesis is well-established.[39] Our observations demonstrate that under Cd stress, a decrease in chlorophyll content is frequently seen, resulting in decreased photosynthetic rates and related attributes in chickpea plants (Figure 3A−D). This reduction in chlorophyll content is because of reduced mobility of pigment−protein complexes, structural disruption in chlorophyll molecules, and impairment of enzymes involved in chlorophyll synthesis. The results of this study revealed that Ag NPs significantly increased the chlorophyll content and photosynthetic rate in a dose-dependent manner. Ag NPs increased photosynthesis and related attributes by accelerating water-splitting processes and promoting electron exchange through redox reactions.[40] According to earlier research, using Ag NPs can successfully counteract the decline in chlorophyll content and promote photosynthesis during stress.[41] This is consistent with findings of Farghaly and Nafady,[42] who found that Ag NPs strongly enhance photosynthesis and are closely linked to changes in nitrogen metabolism. Additionally, Racuciu and Creange[43] observed that while chlorophyll content was decreased at higher doses of NPs, it increased at low concentrations (10−50 μL/l) of Ag NP treatment on maize plants. Additionally, Nghia et al.[44] verified Ag NPs' beneficial effects. Govorov and Carmeli[45] claimed that metal NPs can increase photosynthetic systems' capacity to produce chemical energy efficiently.

Plants use the antioxidant system as a major defense mechanism to deal with elevated quantities of harmful metals in the growth medium, including Cd contamination. According to earlier research, Ag NPs have strong antioxidant qualities because they scavenge reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical ($OH^•$), which lowers oxidative stress and shields biomolecules from harm.[46] They stimulate the production of genes involved in detoxification and antioxidant response, including the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Ag NPs increase Nrf2 signaling, which causes genes that encode important antioxidants including SOD, CAT, GP*x*, and HO^{-1} to be transcriptionally activated.[46] Additionally, they alter intracellular signaling networks that control antioxidant gene expression and cell survival, including phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase (MAPK).[47] Together, these processes strengthen the antioxidant defenses of cells and provide cytoprotective benefits against damage caused by oxidative stress. Ag NPs therefore have the potential for use in the amelioration of oxidative stress brought on by exposure to Cd. The antioxidative trend shows the equilibrium between ROS generation and scavenging, as well as the distinct roles played by these enzymes in Ag NP-induced stimulation.[48,49] At the gene transcript level, the role of antioxidant defense in fostering chickpea growth may also be evident.

Proline is an osmoprotectant biomolecule. It aids in the maintenance of plant cells' osmotic pressure and controls their metabolism. The stability of the cell membranes is enhanced by it. When plants are exposed to metal poisoning, their capacity to absorb and move water is negatively impacted. Consequently, increased proline synthesis helps to preserve the osmotic equilibrium inside cells. According to Rady and Hemida,[50] plants often accumulate larger quantities of proline when they are under Cd stress. According to Szabados and Savouré,[51] proline preserves protein integrity by functioning as a molecular chaperone. Proline also possesses a chelating

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property, which allows it to form bonds with heavy metals to immobilize them and reduce metal toxicity in plants.

The predominant consequence of metal toxicity in plants is the generation of surplus ROS, leading to oxidative harm to cellular components such as proteins, lipids, and DNA[52,53] ROS interacts with fatty acids and phospholipids to promote lipid peroxidation, which raises MDA levels. Because of this, MDA content is typically used to quantify lipid peroxidation.[54,55] MDA is a marker for lipid peroxidation that takes place inside cellular organelles. It was observed that there was a significant increase in H_2O_2 and MDA levels in plant tissues after Cd treatment (Figure 5A,B). Cd stress severely damaged the cells of chickpea seedlings, as evidenced by reduced root cell viability that followed growth retardation and elevated MDA contents. The current results are consistent with earlier research on soybeans,[56] tomatoes,[57] rice,[58] and mungbean.[59] On the other hand, the extra Ag NP supply in conjunction with the Cd treatment decreased the levels of H_2O_2 and MDA accumulation in plant tissues. This was accompanied by increased root cell viability, suggesting that the presence of Ag NP supplies shields chickpea seedlings from oxidative stress when subjected to Cd stress. Additionally, Ag NPs reduced ROS-induced damage in chickpea seedlings, mostly because they decreased the uptake of Cd into plant tissues (Figure 5D). Ag NPs have dramatically lowered MDA levels in metal-treated plants, protecting cellular membrane integrity and positively impacting growth and production in the process. Our findings are consistent with a prior work that found that Ag NPs improved the antioxidant defense mechanisms of wheat plants and lowered MDA levels during stress.[60] These findings support recent research on the impact of heavy metal stress on several crop plants, such as rice.[56]

The current study found that when chickpeas were exposed to Cd through the soil, their protein content dropped (Figure 5C). Increased rates of protein denaturation brought on by protease activity breaking down proteins and a reduction in the synthesis of protein macromolecules during stressful situations could be the cause of this. Moreover, foliar spraying Ag NPs increased the protein content in both stressed and nonstressed plants (Figure 5C). The increase in protein content under NP administration is explained by their role in regulating transpiration and translation processes involved in the synthesis of the protein and other membrane enzymes.[61,62] Furthermore, these compounds influence the structure or activity of proteins by directly interacting with sterols, which form protein structures and control the activity of the enzymes involved in protein synthesis.

CONCLUSIONS

Cadmium (Cd) stress reduced growth, photosynthesis, and physiochemical functions of chickpea plants. Nevertheless, the application of Ag NPs alleviated Cd toxicity and helped the applied plants sustain their physio-biochemical attributes, resulting in improved growth and weight production. The Ag NPs exhibited increased chlorophyll content and photosynthetic parameters and improved protein content in plants under Cd stress. Furthermore, Ag NP treatment reduced MDA and H_2O_2 levels while enhancing antioxidant enzyme activity to help the chickpea plant cope with Cd toxicity. This study highlights the need for further research in this area and offers insightful information on the potential of Ag NPs as a protective agent against Cd toxicity. Additionally, understanding the molecular mechanisms behind different stresses and crops is crucial to fully appreciating these protective advantages.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Alsafran, M.; Usman, K.; Ahmed, B.; Rizwan, M.; Saleem, M. H.; Al Jabri, H. Understanding the Phytoremediation Mechanisms of Potentially Toxic Elements: A Proteomic Overview of Recent Advances. *Front. Plant Sci.* **2022**, *13*, No. 881242.

(2) Aslam, M. M.; Okal, E. J.; Waseem, M. Cadmium toxicity impacts plant growth and plant remediation strategies. *Plant Growth Regul.* **2023**, *99*, 397−412.

(3) Alam, P.; Faizan, M. Glucose modulates photosynthesis, antioxidant system and morpho-physiology in tomato (*Solanum lycopersicum*) plants under cadmium stress. *Egypt. J. Soil Sci.* **2024**, *64* (3), 845−854.

(4) Faizan, M.; Rajput, V. D.; Al-Khuraif, A. A.; Arshad, M.; Minkina, T.; Sushkova, S.; Yu, F. Effect of foliar fertigation of chitosan

https://doi.org/10.1021/acsomega.4c07946
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nanoparticles on cadmium accumulation and toxicity in *Solanum lycopersicum*. *Biology* **2021**, *10*, No. 666.
(5) Faizan, M.; Faraz, A.; Mir, A. R.; Hayat, S. Role of zinc oxide nanoparticles in countering negative effects generated by cadmium in *Lycopersicon esculentum*. *J. Plant Growth Regul.* **2021**, *40*, 101−115.
(6) Kanu, U.; Ashraf, A.; Bangura, D. M.; Yang, A. S.; Ngaujah, X. Tang. Cadmium (Cd) stress in rice; phyto-availability, toxic effects, and mitigation measures-a critical review. *IOSR J. Environ. Sci., Toxicol. Food Technol.* **2017**, *11*, 7−23.
(7) Ashraf, U.; Kanu, A. S.; Mo, Z.; Hussain, S.; Anjum, S. A.; Khan, I.; Abbas, R. N.; Tang, X. Lead toxicity in rice: effects, mechanisms, and mitigation strategies − a mini review. *Environ. Sci. Pollut. Res.* **2015**, *22* (23), 18318−18332.
(8) Sebastian, A.; Prasad, M. N. V. Operative photo assimilation associated proteome modulations are critical for iron dependent cadmium tolerance in *Oryza sativa* L. *Protoplasma* **2015**, *252* (5), 1375−1386.
(9) Ali, K.; Ahmed, B.; Dwivedi, S.; Saquib, Q.; AlKhedhairy, A. A.; Musarrat, J. Microwave Accelerated Green Synthesis of Stable Silver Nanoparticles with Eucalyptus globulus Leaf Extract and Their Antibacterial and Antibiofilm Activity on Clinical Isolates. *PLoS One* **2015**, *10* (7), No. e0131178.
(10) Saratale, R. G.; Karuppusamy, I.; Saratale, G. D.; et al. A comprehensive review on green nanomaterials using biological systems: recent perception and their future applications. *Colloids Surf., B* **2018**, *170*, 20−35.
(11) Salem, S. S.; Fouda, A. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. *Biol. Trace Elem. Res.* **2021**, *199*, 344−370.
(12) Faraz, A.; Faizan, M.; Sami, F.; Siddiqui, H.; Pichtel, J.; Hayat, S. Nanoparticles: biosynthesis, translocation and role in plant metabolism. *IET Nanobiotechnol.* **2019**, *13* (4), 345−352.
(13) Iravani, S.; Korbekandi, H.; Mirmohammadi, S. V.; Zolfaghari, B. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res. Pharm. Sci.* **2014**, *9*, 385−406.
(14) Basavegowda, N.; Baek, K. H. Current and future perspectives on the use of nanofertilizers for sustainable agriculture: the case of phosphorus nanofertilizer. *3 Biotech* **2021**, *11*, No. 357.
(15) Alabdallah, N. M.; Hasan, M. M. Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. *Saudi J. Biol. Sci.* **2021**, *28*, 5631−5639.
(16) Rani, P. U.; Yasur, J.; Loke, K. S.; Dutta, D. Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: Eichhornia crassipes (Mart) Solms. *Acta Physiol. Plant.* **2016**, *38*, No. 58.
(17) Salama, H. M. H. 'Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.)'. *Int. Res. J. Biotechnol.* **2012**, *3*, 190−197.
(18) Sharma, P.; Jha, A. B.; Dubey, R. S.; et al. 'Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions'. *J. Bot.* **2012**, *12*, No. 217037.
(19) Yosefzaei, F.; Poorakbar, L.; Farhadi, K. The effect of silver nanoparticles on morphological and physiological indexes of Ocimum basilicum L. *Iran. J. Plant Physiol. Biochem.* **2016**, *1* (2), 63−73.
(20) Ejaz, M.; Raja, N. I.; Mashwani, Z. U. R.; Ahmad, M. S.; Hussain, M.; Iqbal, M. Effect of silver nanoparticles and silver nitrate on growth of rice under biotic stress. *IET Nanobiotechnol.* **2018**, *12* (7), 927−932.
(21) Ziotti, A. B. S.; Ottoni, C. A.; Correa, C. N.; de Almeida, O. J. G.; de Souza, A. O.; Neto, M. C. L. Differential physiological responses of a biogenic silver nanoparticle and its production matrix silver nitrate in Sorghum bicolor. *Environ. Sci. Pollut. Res.* **2021**, *28*, 32669−32682.
(22) Dumanović, J.; Nepovimova, E.; Natić, M.; Kuča, K.; Jaćević, V. The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Front. Plant Sci.* **2021**, *11*, No. 552969.
(23) Lichtenthaler, H. K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology*; Academic Press, 1987; Vol. *148*, pp 350−382.
(24) Wu, G. X.; Li, S. L.; Li, Y. M.; Bi, H. G.; Ai, X. Z. Effects of hydrogen sulphide, nitric oxide and their interaction on photosynthesis of cucumber seedlings under chilling stress. *Plant Physiol. J.* **2020**, *56*, 2221−2232.
(25) Faizan, M.; Hayat, S. Effect of foliar spray of ZnO-NPs on the physiological parameters efficiency and antioxidant systems of *Lycopersicon esculentum*. *Pol. J. Nat. Sci.* **2019**, *34* (1), 87−105.
(26) Chance, B.; Maehly, A. C. Assay of catalase and peroxidases. *Methods Enzymol.* **1955**, *2*, 764−775.
(27) Beauchamp, C. O.; Fridovich, I. Superoxide dismutase: improved assays and assays applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276−287.
(28) Bates, L. S.; Waldeen, R. P.; Teare, I. D. Rapid determination of free proline water stress studies. *Plant Soil* **1973**, *39*, 205−207.
(29) Hodges, D. M.; DeLong, J. M.; Forney, C. F.; Prange, R. K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **1999**, *207*, 604−611.
(30) Jana, S.; Choudhuri, M. A. Glycolate metabolism of three submersed aquatic angiosperms during ageing. *Aquat. Bot.* **1982**, *12*, 345−354.
(31) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(32) Faizan, M.; Bhat, J. A.; Hessini, K.; Yu, F.; Ahmad, P. Zinc oxide nanoparticles alleviates the adverse effects of cadmium stress on Oryza sativa via modulation of the photosynthesis and antioxidant defense system. *Ecotoxicol. Environ. Saf.* **2021**, *220*, No. 112401.
(33) Nazir, S.; Arif, Y.; Mir, A. R.; Siddiqui, H.; Faizan, M.; Alam, P.; Hayat, S. Comparative and interactive response of salicylic acid, 24−epibrassinolide or sodium nitroprusside against cadmium stress in Linum usitatissimum *Journal of Umm Al-Qura Univ. Appl. Sci.* 2024 DOI: 10.1007/s43994-024-00145-x.
(34) Arya, A.; Mishra, V.; Chundawat, T. S. Green synthesis of silver nanoparticles from green algae (*Botryococcus braunii*) and its catalytic behavior for the synthesis of benzimidazoles. *Chem. Data Collect.* **2019**, *20*, No. 100190.
(35) Gupta, S. D.; Agarwal, A.; Pradhan, S. Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: an insight from antioxidative enzyme activities and gene expression patterns. *Ecotoxicol. Environ. Saf.* **2018**, *161*, 624−633.
(36) Mirzajani, F.; Askari, H.; Hamzelou, S.; Farzaneh, M.; Ghassempour, A. Effect of silver nanoparticles on Oryza sativa L. and its rhizosphere bacteria. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 48−54.
(37) Syu, Y. Y.; Hung, J. H.; Chen, J. C.; Chuang, H. W. Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression. *Plant Physiol. Biochem.* **2014**, *83*, 57−64.
(38) Azeez, L.; Lateef, A.; Wahab, A. A.; Rufai, M. A.; Salau, A. K.; Ajayi, E. I. O.; Ajayi, M.; Adegbite, M. K.; Adebisi, B. Phytomodulatory effects of silver nanoparticles on Corchorus olitorius: its antiphytopathogenic and hepatoprotective potentials. *Plant Physiol. Biochem.* **2019**, *136*, 109−117.
(39) Seleiman, M. F.; Semida, W. M.; Rady, M. M.; Mohamed, G. F.; Hemida, K. A.; Alhammad, B. A.; Hassan, M. M.; Shami, A. Sequential application of antioxidants Rectifies ion imbalance and Strengthens antioxidant systems in salt-stressed cucumber. *Plants* **2020**, *9*, No. 1783.
(40) Pradhan, S.; Patra, P.; Das, S.; Chandra, S.; Mitra, S.; Dey, K. K.; Akbar, S.; Palit, P.; Goswami, A. Photochemical modulation of Biosafe Manganese nanoparticles on Vigna radiata: a Detailed molecular, biochemical, and Biophysical study. *Environ. Sci. Technol.* **2013**, *47*, 13122−13131.
(41) Khan, I.; Awan, S. A.; Rizwan, M.; Akram, M. A.; Zia-ur-Rehman, M.; Wang, X.; Zhang, X.; Huang, L. Physiological and transcriptome analyses demonstrate the silver nanoparticles mediated

alleviation of salt stress in pearl millet (Pennisetum glaucum L). *Environ. Pollut.* **2023**, *318*, No. 120863.
(42) Farghaly, F. A.; Nafady, N. A. Green synthesis of silver nanoparticles using leaf extract of Rosmarinus officinalis and its effect on Tomato and wheat plants. *J. Agric. Sci.* **2015**, *7* (11), No. 277, DOI: 10.5539/jas.v7n11p277.
(43) Racuciu, M.; Creanga, D. E. TMA-OH Coated magnetic nanoparticles internalized in vegetal tissue. *Rom. J. Phys.* **2007**, *52*, 395−402.
(44) Nghia, L. T.; Tung, H. T.; Huy, N. P.; Luan, V. Q.; Nhut, D. T. The effect of silver nanoparticles on growth of Chrysanthemum morifolium Ramat. cv. "JIMBA" in different cultural systems. *Vietnam J. Sci. Technol.* **2017**, *55* (4), 503−514.
(45) Govorov, A. O.; Carmeli, I. Hybrid structures composed of photosynthetic system and metal nanoparticles: plasmon enhancement effect. *Nano Lett.* **2007**, *7* (3), 620−625.
(46) Veeramani, S.; Shanmugam, K. Biosynthesis and characterization of silver nanoparticles coated with metabolites extracted from clerodendron phlomoides with its biochemical studies in liver tissue. *Proc. Anticancer Res.* **2023**, *7* (4), 1−19.
(47) Salama, B.; Alzahrani, K. J.; Alghamdi, K. S.; Al-Amer, O.; Hassan, K. E.; Elhefny, M. A.; et al. Silver nanoparticles enhance oxidative stress, inflammation, and apoptosis in liver and kidney tissues: potential protective role of thymoquinone. *Biol. Trace Elem. Res.* **2023**, *201* (6), 2942−2954.
(48) Faisal, M.; Faizan, M.; Alatar, A. A. Metallic allies in drought resilience: unveiling the influence of silver and zinc oxide nanoparticles on enhancing tomato (*Solanum lycopersicum*) resistance through oxidative stress regulation. *Plant Physiol. Biochem.* **2024**, *212*, No. 108722.
(49) Zhao, Z.; Xu, L.; Wang, Y.; Li, B.; Zhang, W.; Li, X. Toxicity mechanism of silver nanoparticles to Chlamydomonas reinhardtii: Photosynthesis, oxidative stress, membrane permeability, and ultrastructure analysis. *Environ. Sci. Pollut. Res.* **2021**, *28*, 15032−15042.
(50) Rady, M. M.; Hemida, K. A. Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. *Ecotoxicol. Environ. Saf.* **2015**, *119*, 178−185.
(51) Szabados, L.; Savouré, A. Proline: a multifunctional amino acid. *Trends Plant Sci.* **2010**, *15* (2), 89−97.
(52) Faizan, M.; Faraz, A.; Mir, A. R.; Hayat, S. Role of zinc oxide nanoparticles in countering negative effects generated by cadmium in Lycopersicon esculentum. *J. Plant Growth Regul.* **2021**, *40*, 101−115.
(53) Singh, D.; Sharma, N. L.; Singh, C. K.; Yerramilli, V.; Narayan, R.; Sarkar, S. K.; Singh, I. Chromium (VI)-induced alterations in physio-chemical parameters, yield, and yield characteristics in two cultivars of mungbean (*Vigna radiata* L.). *Front. Plant Sci.* **2021**, *12*, No. 735129.
(54) Ebrahimian, E.; Bybordi, A. Effect of salinity, salicylic acid, silicium and ascorbic acid on lipid peroxidation, antioxidant enzyme activity and fatty acid content of sunflower. *Afr. J. Agric. Res.* **2012**, *7*, 3685−3694.
(55) Hasanuzzaman, M.; Bhuyan, M. H.; Parvin, K.; Bhuiyan, T. F.; Anee, T. I.; Nahar, K.; Hossen, M.; Zulfiqar, F.; Alam, M.; Fujita, M. Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. *Int. J. Mol. Sci.* **2020**, *21*, No. 8695.
(56) Basit, F.; Bhat, J. A.; Hu, J.; Kaushik, P.; Guan, Y.; Ahmad, A.; Ahmad, P. Brassinosteroid supplementation alleviates chromium toxicity in soybean (Glycine max L.) via reducing its translocation. *Plants* **2022**, *11* (17), No. 2292.
(57) Javed, M. T.; Tanwir, K.; Abbas, S.; Saleem, M. H.; Iqbal, R.; Chaudhary, H. J. Chromium retention potential of two contrasting *Solanum lycopersicum* Mill. cultivars as deciphered by altered pH dynamics, growth, and organic acid exudation under Cr stress. *Environ. Sci. Pollut. Res.* **2021**, *28*, 27542−27554.
(58) Faizan, M.; Rajput, V. D.; Al-Khuraif, A. A.; Arshad, M.; Minkina, T.; Sushkova, S.; Yu, F. Effect of foliar fertigation of chitosan nanoparticles on cadmium accumulation and toxicity in *Solanum lycopersicum*. *Biology* **2021**, *10*, No. 666.
(59) Singh, D.; Singh, C. K.; Siddiqui, M. H.; Alamri, S.; Sarkar, S. K.; Rathore, A.; Prasad, S. K.; Singh, D.; Sharma, N. L.; Kalaji, H. M.; Brysiewicz, A. Hydrogen sulfide and silicon together alleviate chromium (VI) toxicity by modulating morphophysiological and key antioxidant defense systems in chickpea (*Cicer arietinum* L.) varieties. *Front. Plant Sci.* **2022**, *13*, No. 963394.
(60) Iqbal, M.; Raja, N. I.; Mashwani, Z. R.; Wattoo, F. H.; Hussain, M.; Ejaz, M.; Saira, H. Assessment of AgNPs exposure on physiological and biochemical changes and antioxidative defence system in wheat (Triticum aestivum L) under heat stress. *IET Nanobiotechnol.* **2019**, *13*, 230−236.
(61) Perrella, G.; Davidson, M. L.; O'Donnell, L.; Nastase, A. M.; Herzyk, P.; Breton, G.; Pruneda-Paz, J. L.; Kay, S. A.; Chory, J.; Kaiserli, E. ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115* (19), E4503−E4511.
(62) Ahmad, P.; Alam, P.; Balawi, T. H.; Altalayan, F. H.; Ahanger, M. A.; Ashraf, M. Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in Vicia faba through the modifications of biochemical attributes, antioxidants, ascorbate glutathione cycle and glyoxalase cycle. *Chemosphere* **2020**, *244*, No. 125480.

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