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Research Article

Silver Nanoparticles Reduce the Toxic Effects of Cadmium on *Datura stramonium* Callus Culture

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Cadmium (Cd) is one the most toxic metals harmful for both animals and plants. In this study, we test whether silver nanoparticles (AgNPs) can protect plants from cadmium toxicity. AgNPs treatments (0 and 100 μg/L) were applied to *Datura stramonium* calli grown in different cadmium metal environments (0, 150, 300, 450, and 600 μM). Cd application led to a decrease in fresh weight (FW), dry weight (DW), relative water content (RWC), total chlorophyll content (Chl.$_T$), tolerance index (Ti), and bioaccumulation factor (BCF). The Cd treatment increased the hydrogen peroxide (H_2O_2), catalase (CAT), and guaiacol peroxidase (GPX) contents and Cd accumulation (Cd con). These results show the positive role of AgNPs in protecting the callus cultures from oxidative stress. The AgNPs pretreatment improved the growth of tissue cultures compared to nontreatment, increasing FW, DW, RWC, and Chl.$_T$; the highest CAT and GPX activities were detected in the AgNPs pretreatment condition; and AgNPs pretreatment improved Ti and BCF, despite increased Cd. Also, this treatment caused a decrease in H_2O_2. Based on these results, we propose AgNPs as an effective agent to reduce the toxic effects of Cd metal on *D. stramonium*.

1. Introduction

Cadmium (Cd) is a nonessential metal, and its excess amount hinders normal growth and development, even causing death at high concentrations [1, 2]. Its danger stems from its difficulty to biodegrade and long-term persistence, creating a chronic environmental threat that negatively impacts human and animal health [3–5].

Its negative effect on plants is represented by a decrease in photosynthesis [6], a weak ability to absorb the necessary nutrients, and an increase in the production of reactive oxygen species (ROS), which leads to damage to cellular components as a result of increased damage due to oxidative stress [7, 8].

Adapting plants to resist heavy toxins is one of the most sustainable methods for removing these pollutants while preserving environmental diversity. The integrated plant system provides a unique opportunity to preserve the environment from heavy metal contamination through its ability to withstand this toxicity and transform it into a safe biological state, which cleans the environment of toxins. The enzymatic and nonenzymatic antioxidant systems meet this challenge [9].

Nanoparticles (NPs) are nanomaterials with a diameter ranging from 1 to 100 nm [10]. They have unique physical and chemical properties that make them highly effective in various fields, such as agriculture, food, and industry [11, 12]. In addition, nano-elements contribute to the mitigation or detoxification of chemical pollutants produced by the action of trace metals [13]. Therefore, they are one of the most promising methods to reduce the risk caused by toxic metals, including Cd [14].

Silver nanoparticles (AgNPs) have significant physiological potential. They have been widely used in resisting

various abiotic stresses because they can control a plant's cell enzymatic and nonenzymatic oxidative systems. AgNPs have been reported to reduce ROS and increase the stability of cellular membranes, contributing to the increase in growth and the accumulation of biomass [15].

The genus Datura is one of the most famous plants of the Solanaceae family and includes several important species, including stramonium. It is a rich source of biologically active chemical compounds, including various chemical components, such as alkaloids, steroids, phenols, amides, and acyl sugars [16]. These compounds have therapeutic properties, including antioxidant, anti-inflammatory, antiulcer, and analgesic [17], as well as antibacterial, antifungal, anticancer, and antiviral properties [18, 19].

When searching the literature, we found no study addressing the role of AgNPs in reducing the effect of Cd metal on the tissue culture of plants. The current study aimed to explore the contribution of laboratory-manufactured AgNPs to enhance metal tolerance and accumulation based on physiological and biochemical parameters and the bioaccumulation capacity of the *D. stramonium* cultures grown under cadmium stress conditions.

2. Materials and Methods

2.1. Plant Material and Callus Induction. *D. stramonium* seeds were obtained from the Center of Desert Studies, University of Anbar. They were washed with water and surface sterilized with 70% ethanol for one minute. The sterilization process was then completed with a 2.0% NaOCl solution for 15 min in a laminar flow cabinet. Then, it was rinsed with sterile distilled water three times. They were cultured on MS medium-containing sucrose 30 g and agar 7.0 g free of plant growth regulators. The seeds were incubated to obtain sterilized seedlings at 25°C and photoperiod 16/8 h dark. For callus induction, the cotyledon of the obtained *D. stramonium* seedlings was grown in the MS medium with the same components and conditions indicated for seed germination with the addition of growth regulators 1.0 mg·L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg·L^{-1} α-naphthalene acetic acid (NAA), and 1.0 mg·L^{-1} 6-benzylaminopurine (BA).

2.2. Establishment of Callus Culture. A homogeneous callus culture was obtained from several subcultures on the MS media supplemented with the same components of the medium indicated for seed germination, taking into account the addition of growth regulators, and incubated at 25°C in the dark. Experimental treatments were prepared by dividing fresh three-month-old calli into two groups.

2.3. Preparation of AgNPs. AgNPs colloidal solution was prepared using the laser ablation in liquid phase (LAL) technique in DDDW. A rotating (2 cycles/min) Ag plate (1.5 × 1.5 cm) was shot by a 1064 nm laser wavelength with 6 Hz, 100 pulses, and 100 mJ in 15 mL of double deionized distilled water (DDDW). The height of water above the Ag billet surface was 8.0 mm. However, the color appearance of the colloidal material changed from transparent to a yellowish liquid. The flask was covered by foil to protect it from light. The estimated concentration of Ag in the colloidal was about 20 ppm.

2.4. Characterization of AgNPs. The primary test of AgNPs to confirm the formation of nanoparticles was the measurement of the UV-Vis absorbance to determine the surface plasmon resonance peak using a PG80 UV-Vis spectrophotometer with wavelengths from 300 to 800 nm. The concentration of Ag was estimated using atomic absorption spectroscopy (Perkin-Elmer, 300), and the topographical properties were determined by transmission electron microscopy (TEM) analysis. The surface plasmon resonance peak was located at a wavelength of 410 nm (Figure 1), which is in the reported range [20, 21].

The formed AgNPs appeared as spherical particles, as shown in the TEM image in Figure 2 (2.5 to 28 nm of the size range with an average of 9 nm).

2.5. AgNPs Treatment. A group was grown on the media with different levels of Cd, including 0, 150, 300, 450, and 600 μM, which were performed by adding $CdCl_2$ to the media solution. The second group was treated with the same Cd concentrations, and 100 μg·L^{-1} of AgNPs was added to the MS medium. The callus was harvested after 28 days and analyzed.

2.6. Measurements of Parameters. The studied parameters were calculated for all treatments after 28 days of cultures in the previously mentioned conditions; these traits included the following:

2.6.1. Fresh and Dry Weight. The fresh weight (FW) of callus samples was determined after separating them from the culture medium and removing the remnants of the media attached to the callus. The dry weight (DW) was calculated after drying the samples in an oven at 50°C for 72 h.

2.6.2. Relative Water Content. The relative water content (RWC) of the callus was calculated for all treatments based on the following formula:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100, \quad (1)$$

where (TW) is the weight of the fresh callus immersed in distilled water at room temperature for 4 h in the growth chamber under constant light.

2.6.3. Total Chlorophyll Content. Total chlorophyll (Chl.$_T$) was determined using 250 mg callus samples according to the method proven by Lichtenthaler [22]. Briefly, the samples were centrifuged to obtain the extract using an 80% (v/v) acetone solution at 2500 rpm for 10 min. Then, the

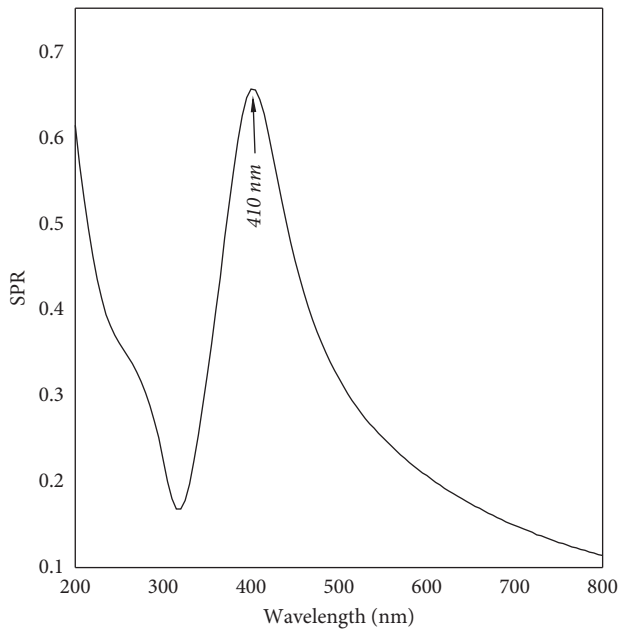


Figure 1: The surface plasmon resonance of the prepared AgNPs colloidal solution.

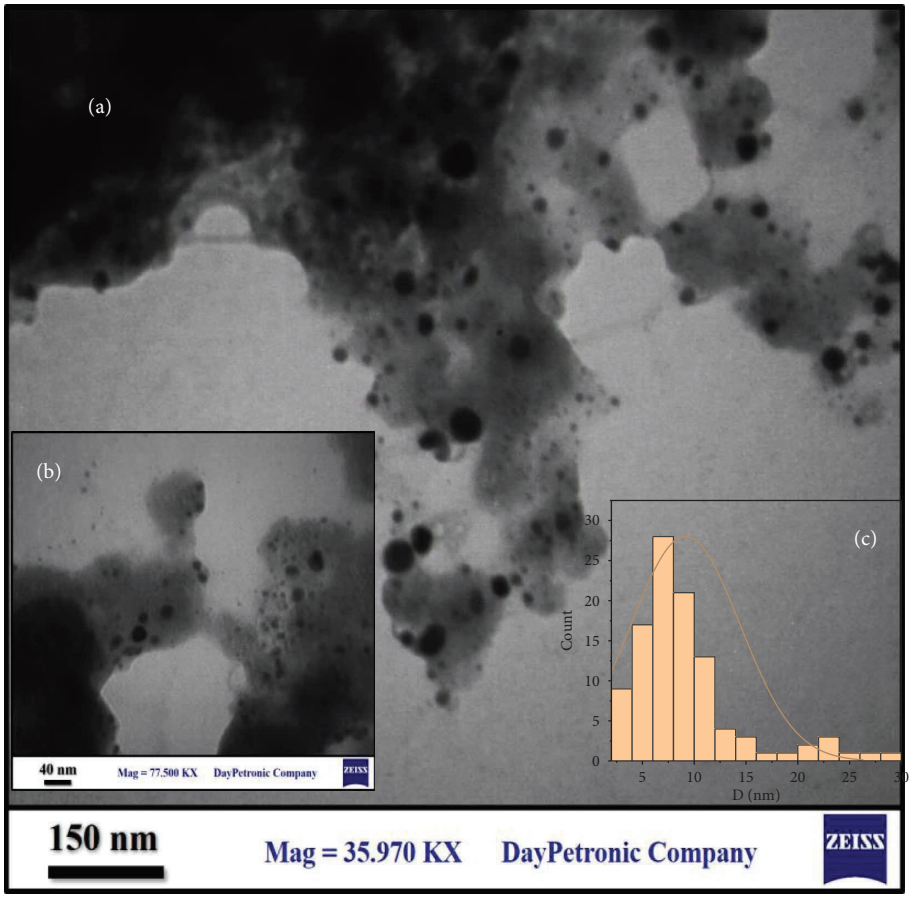


Figure 2: AgNPs treatment: (a and b) TEM image of the prepared AgNPs; (c) the size distribution of particles.

Chl._T content was recorded for each sample based on the wavelengths shown in the following equations:

$$\begin{aligned}\text{Chlorophyll } a\left(\text{mg.ml}^{-1}\right) &= (12.24\,A663.2 - 2.79\,A646.8),\\ \text{Chlorophyll } b\left(\text{mg.ml}^{-1}\right) &= (21.21\,A646.8 - 5.1\,A663.2),\\ \text{Chl.}_T\left(\text{mg.ml}^{-1}\right) &= (\text{Chlorophyll } a + \text{Chlorophyll } b).\end{aligned} \tag{2}$$

2.6.4. Determination of H_2O_2. The report of Sergiev et al. [23] was followed to estimate the hydrogen peroxide (H_2O_2) content of the callus samples. Briefly, it was centrifuged at 12,000 rpm for 15 min to produce 500 mg of homogeneous callus tissue supplemented with 0.1% TCA. Then, 500 μl of the supernatant was added to 500 μl of potassium iodide (1 M) and 500 μl of phosphate buffer (10 mM). The absorbance of the solution was recorded at 390 nm.

2.6.5. Determination of Enzyme Activities. For the purpose of extracting antioxidant enzymes, a report was based on Gapinska et al. [24]. In short, 500 mg of fresh callus were homogenized in 50 mM K-phosphate buffer (pH 7) containing 1 mM EDTA and 1% PVP. After centrifugation (10,000 × g, 15 min, 4°C), the supernatant fraction was used to determine enzyme activities.

2.6.6. Catalase (CAT). The CAT activity was determined based on the Aebi [25]. In short, 15 mM of H_2O_2 was mixed with 50 mM K-phosphate buffer at pH 7.0 and 25°C with 50 μl of enzyme extract. The absorption at 240 nm for 30 s was achieved using 39.4 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ as an extinction coefficient.

2.6.7. Guaiacol Peroxidase (GPX). The GPX activity was quantified by adding 10 mM of guaiacol, 50 mM of phosphate buffer, and 40 mM of H_2O_2. The assay, using 2.5 ml of the reaction mixture and 75 μl of the enzyme extract at 470 nm for 3 min, was determined, and the extinction coefficient (25.5 $\text{mM}^{-1}\cdot\text{cm}^{-1}$) was used to calculate enzyme activity [26].

2.6.8. Accumulation of Cd. The Cd concentration (Cd con.) in 100 mg dried callus samples was estimated for acid digestion according to the method modified by Hernández Arteaga et al. [27]. Briefly, 10 ml of a mixture of hydrochloric acid (HCl) and nitric acid (HNO_3) in a ratio of 1 : 3 was added to the homogenized sample in a hot plate stirrer at 100°C. The product was diluted with volumetric 10 ml deionized water. The sample was analyzed by atomic absorption spectrometry.

2.6.9. Tolerance Index. The tolerance index (TI) of biomass after exposure to Cd metal was calculated as follows [28]:

$$\text{TI} = \frac{\text{FW of callus treated in Cd}}{\text{FW of callus in control medium}} \times 100. \tag{3}$$

2.6.10. Bioaccumulation Factor. The bioaccumulation factor (BCF) of callus culture for Cd metal was calculated using the formula [29]:

$$\text{BCF} = \frac{\text{Cd con. in the callus}}{\text{Cd con. in the medium}}. \tag{4}$$

2.7. Experimental Design and Statistical Analysis. The experiment was conducted in a completely randomized design (CRD). The data were analyzed for three replicates using a two-way ANOVA to analyze variance (Table 1). The experimental error value (±SE) was calculated based on the mean values of the three replicates. Duncan's multiple range test (DMRT) was conducted to determine the differences between the mean values of the statistically significant coefficients ($p \leq 0.05$).

3. Results

The growth of the *D. stramonium* calli culture was negatively affected by increasing Cd treatment, which caused all growth characteristics to decrease significantly. The higher concentration of Cd treatment of 600 μmol reduced these characteristics compared to the control treatment by about 29.64%, 24.32%, 22.48%, and 54.06% for FW, DW, RWC, and Chl._T, respectively. However, a significant improvement of these parameters was observed once treated with a concentration of 100 $\mu\text{g}\cdot\text{l}^{-1}$ of AgNPs, to reduce that effect at the same concentration to 7.88%, 11.53%, 9.72%, and 16.63%, respectively (Figures 3 and 4).

To verify the effect of Cd concentrations on membrane damage, H_2O_2 was monitored. The Cd-treated cultures showed a continuous increase in the mean values with the increase in the treatment concentration. The increase reached 1.7-fold for the 600 μM concentration compared to the control treatment. Relative stabilization occurred in the level of H_2O_2 with treatment with AgNPs (Figure 5(a)).

This stability may be due to the increase in the activity of CAT and GPX enzymes, as treatment with AgNPs contributed significantly to this increase. Treatment with a high level of Cd with AgNPs achieved the highest enzyme activity with an increase of 2.4- and 1.6-fold compared to the control not treated with silver particles (Figures 5(b) and 5(c)).

Cd treatment showed an increase in the metal concentration in callus cultures with increasing treatment, reaching its highest level for the tissue cultures treated with a concentration of 600 μM. The pretreatment of AgNPs allowed increasing Cd in callus cultures under low concentrations of 150 and 300 μM. At the same time, the nanomaterial contributed to reducing the tissue Cd content under high concentrations of 450 and 600 μM (743.3 and 846.7 mg/kg DW) compared with untreated counterparts with nanoparticles (Table 2).

Table 1: Analysis of variance for the effects of Cd concentrations, AgNPs, and their interaction on the characteristics.

Characteristics	Cd	AgNPs	Cd × AgNPs
FW	***	***	*
DW	***	***	*
RWC	***	***	NS
$Chl._T$	***	***	***
H_2O_2	***	***	***
CAT	***	***	***
GPX	*	***	NS
Ti	**	***	NS
Cd	***	***	NS
BCF	***	***	*

NS, not significant; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

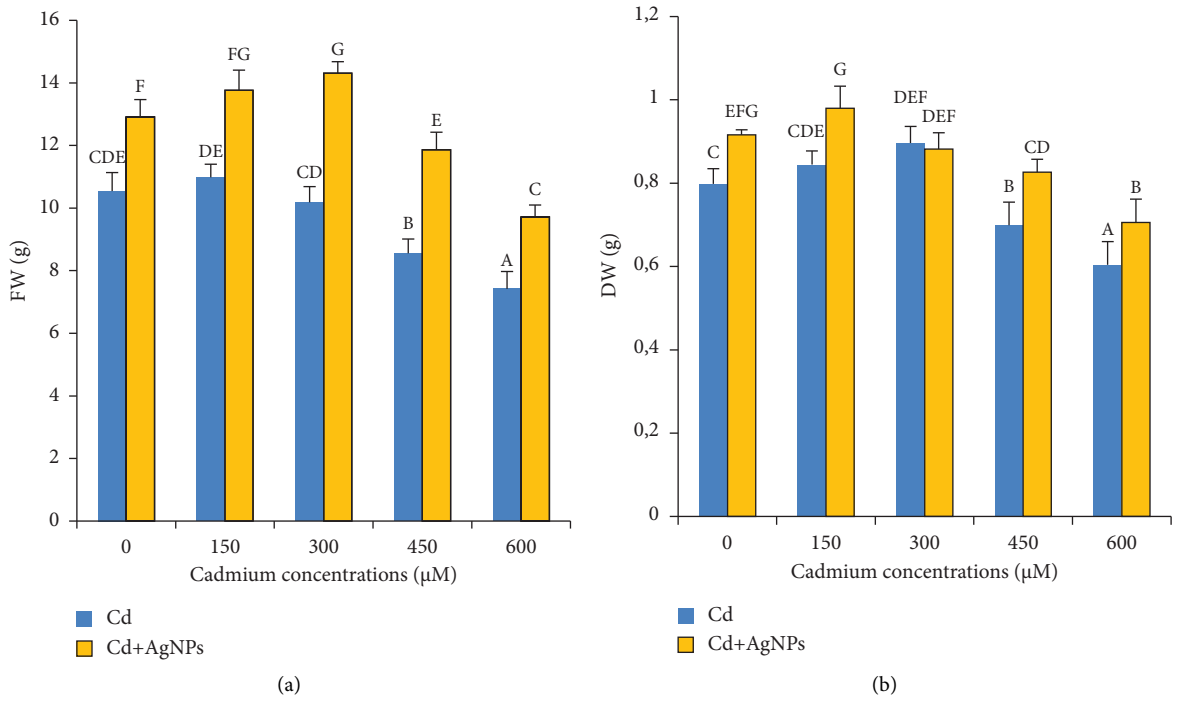


Figure 3: Continued.

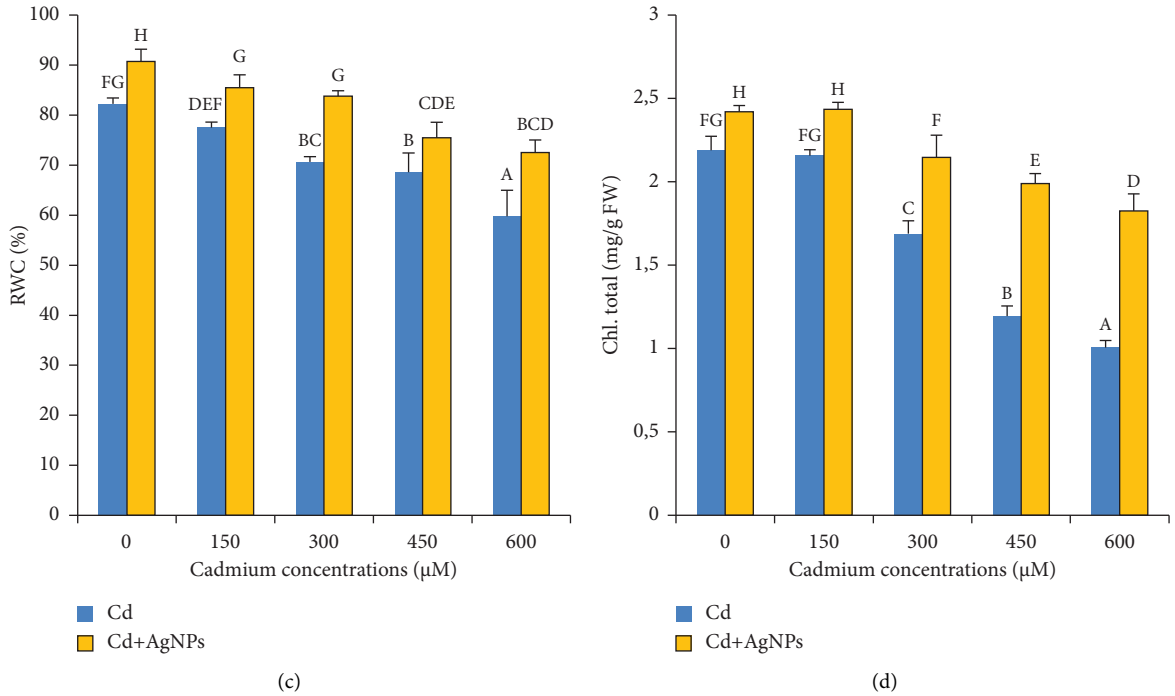
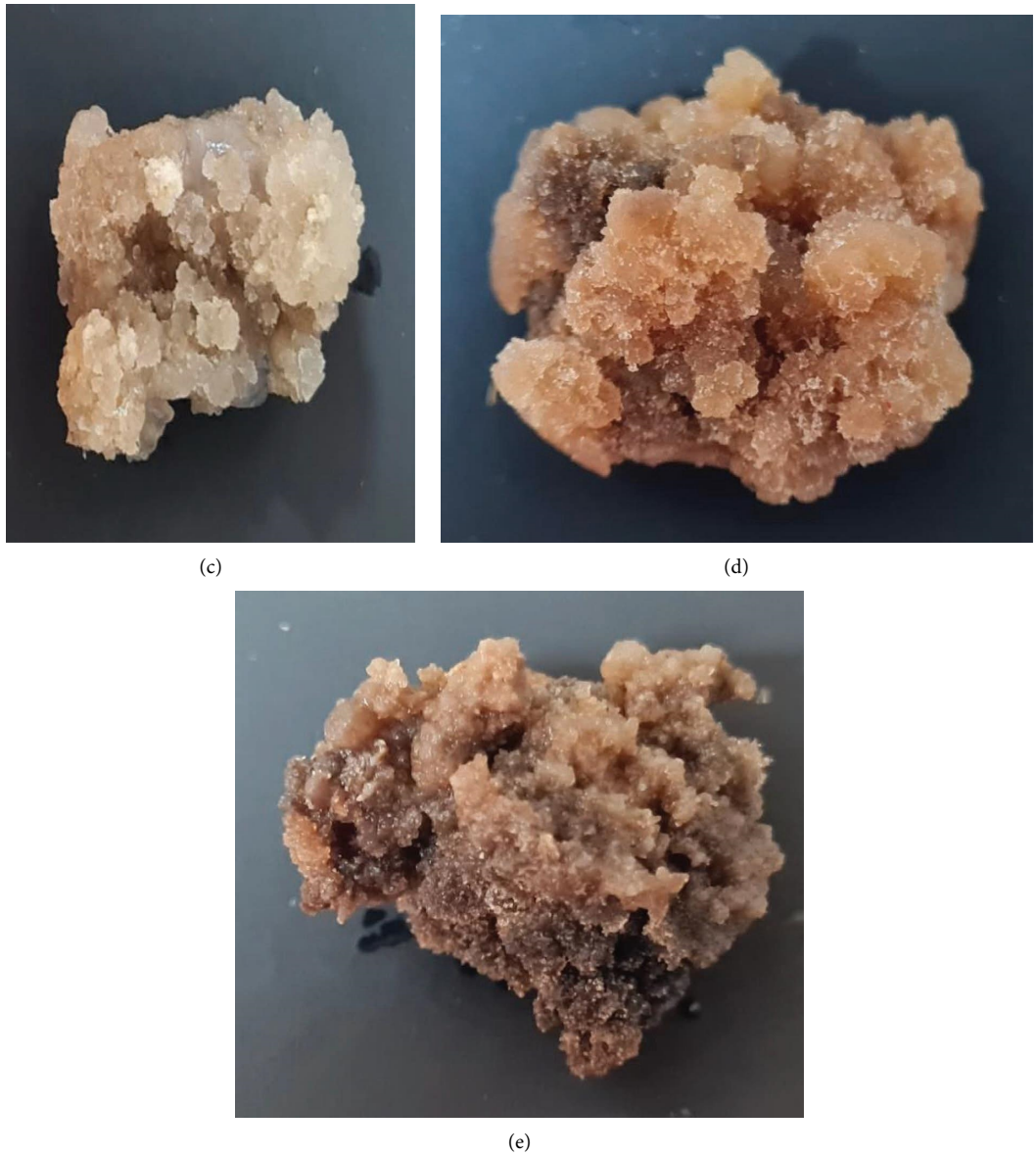


Figure 3: The effect of Cd treatment and AgNPs on: (a) FW; (b) DW; (c) RWC; (d) $Chl._T$ of *D. stramonium* callus culture after 28 days. Values represent the mean ± SE different letters indicate a significant difference between the treatments ($p < 0.05$, Duncan's test).

(a) (b)

Figure 4: Continued.



(c) (d)

(e)

Figure 4: Callus culture induced from the cotyledon of *D. stramonium* on MS media by 1.0 mg·L^{-1} 2,4-D, 0.5 mg·L^{-1}, and 1.0 mg·L^{-1} (a). Callus cultured on MS media with Cd (150 μM) þand þAgNPs 100 μg·L^{-1} (b). Callus cultured on MS media with Cd (300 μM) þand þAgNPs 100 μg·L^{-1} (c). Callus cultured on MS media with Cd (450 μM) þand þAgNPs 100 μg·L^{-1} (d). Callus cultured on MS media with Cd (600 μM) þand þAgNPs 100 μg·L^{-1} (e).

Despite the continuous decrease in the tolerance to the effects of Cd treatment by callus culture, the pretreatment of callus culture of *D. stramonium* with AgNPs showed a significant tolerance towards Cd. The prominent role of pretreatment with AgNPs was increasing the tolerance of plant tissues to this stress by 2.20%, 14.65%, 13.18%, and 6.96% more than their counterparts untreated with nanoparticles, respectively.

In this study, a prominent role was observed for the treatment with AgNPs in the phytoremediation of Cd metal. At low levels of 150 and 300 μM, phytoremediation of 2.24 and 1.939 were found, respectively. However, Cd levels of 450 and 600 μM did not differ significantly from the low-concentration products of the untreated Cd treatments (Table 2).

4. Discussion

In this study, although Cd improved the parameters of FW and DW under concentrations 150 and 300 μM, the negative effect of this mineral was evident at concentrations 450 and 600 μM. At the same time, Cd metal concentrations caused a significant reduction in physiological parameters, including RWC and Chl.$_T$. The inhibition of Cd for the mentioned indicators could be due to its effectiveness in preventing the elongation and division of cells that occur due to the inhibition of the proton pump [30, 31]. Moreover, the concentration of Cd caused a decrease in the water absorption of calli cultures due to the decrease in the osmotic potential in the media. Also, the effect of Cd on the damage

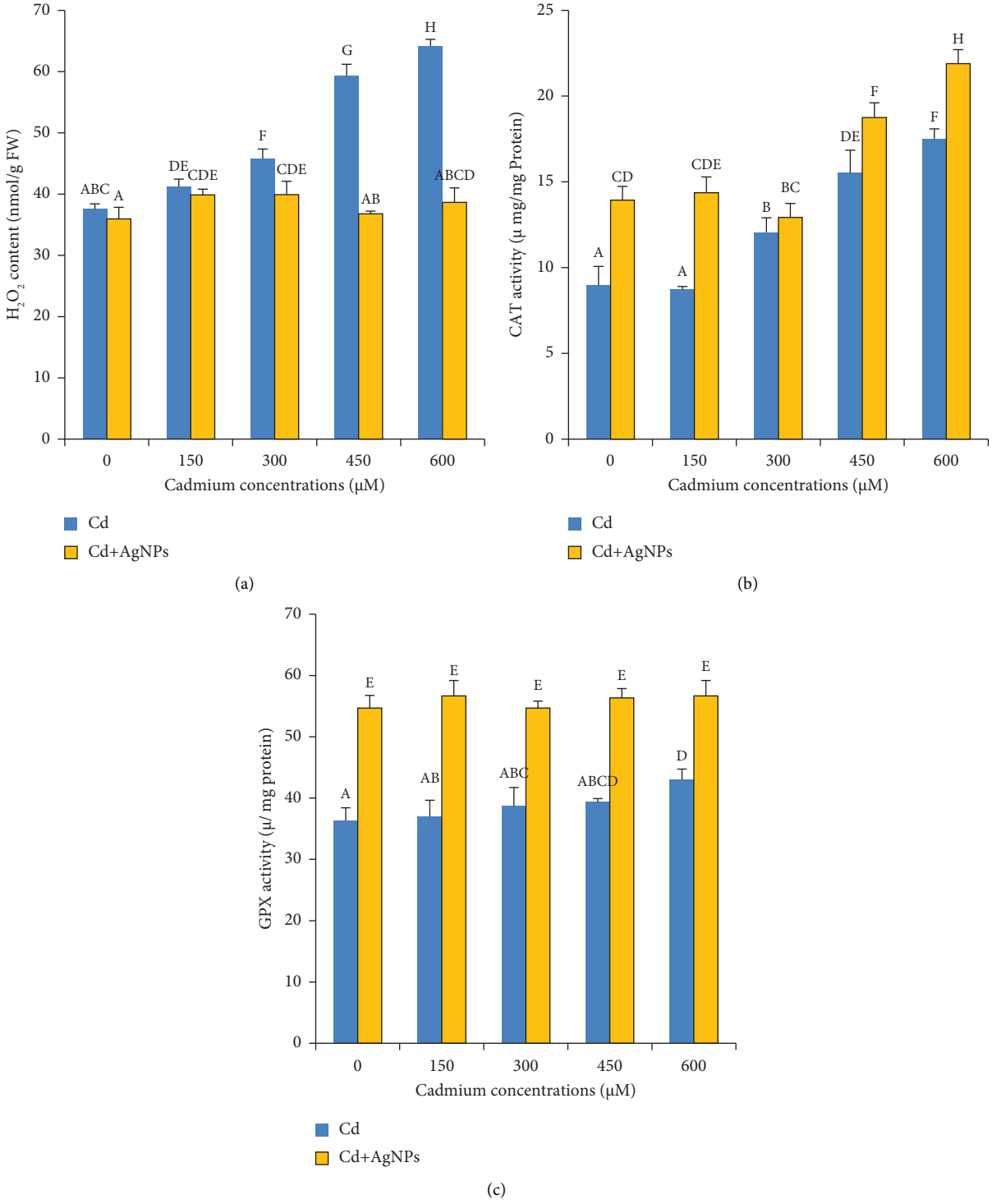


Figure 5: The effect of Cd treatment and AgNPs on: (a) H_2O_2 content; (b) CAT activity; and (c) GPX activity of *D. stramonium* callus culture after 28 days. Values represent the mean ± SE; different letters indicate a significant difference between the treatments ($p < 0.05$, Duncan's test).

of pigments, including chlorophyll pigment, was negatively reflected in the growth of calli cultures [32]. This result agreed with previous reports on different plant species such as *Brassica juncea* [28], *Brassica napus* L. [33], and *Plantago major* [34].

The current study showed the positive role of AgNPs in enhancing growth parameters, such as FW and DW. This may be due to the improvement of RWC, which is the main factor in its main role in the formation of polysaccharides [10]. As well as improving the metabolic and physiological

Table 2: The effect of Cd treatment and AgNPs on Cd accumulation, Ti, and BCF of *D. stramonium* callus culture after 28 days.

AgNPs	Con. Cd	Cd	Ti	BCF
0	0	ND	—	—
	150	259.0 ± 1.73a	1.044 ± 0.083def	1.727 ± 0.01b
	300	530.0 ± 26.46c	0.969 ± 0.101cde	1.767 ± 0.09b
	450	725.0 ± 18.52d	0.812 ± 0.042abc	1.611 ± 0.41b
	600	866.0 ± 8.54e	0.704 ± 0.045a	1.443 ± 0.14a
100	0	ND	—	—
	150	336.0 ± 35.68b	1.067 ± 0.043ef	2.240 ± 0.24d
	300	581.7 ± 51.31c	1.111 ± 0.074f	1.939 ± 0.17c
	450	781.0 ± 28.16d	0.919 ± 0.031bc	1.736 ± 0.06b
	600	916.7 ± 65.61e	0.753 ± 0.038ab	1.528 ± 0.11ab

Values represent the mean ± SE; different letters indicate a significant difference between the treatments ($p < 0.05$, Duncan's test).

state of calli cultures through the role of AgNPs in maintaining the hormonal balance negatively affected by stress factors. Previous literature indicated that AgNPs enhance the formation of essential pigments such as chlorophyll as the basis for photosynthesis and thus improve the rate of photosynthesis [35]. This result is similar to that reported by Ali et al. [15] on *Caralluma tuberculata* and de Andrade et al. [36] on *Helianthus annuus* L. and.

In this study, treatment with Cd significantly increased ROS generation, leading to increased H_2O_2 content in calli cultures. This contributes to the damage of various cellular components, including proteins, lipid membranes that form cell walls, and nucleic acids [37]. The activity of the antioxidant defense enzyme systems represented by CAT and GPX represents one of the defense mechanisms that can reduce the risk of oxidative stress and thus protect plant tissues. These results are similar to previous reports that showed the negative effect of H_2O_2 in decreasing cell viability, such as Daud et al. [38] in *Gossypium hirsutum* L. Also, previous literature showed a progressive activity in H_2O_2 degradation by enzymatic antioxidants [39–43].

The results of the current study showed that AgNPs had an effective role in protecting the plant cell from the negative effect of Cd stress, as they limited the increase of H_2O_2 by increasing the antioxidant enzymatic activities represented by CAT and GPX. This can be explained by the fact that AgNPs stimulate the vital pathways responsible for building antioxidant enzymes. Moreover, the role of AgNPs in the production of secondary metabolites has been studied [44]. The antioxidant activities contribute to reduce the effect of ROS by neutralizing the superoxide radicals (O_2) [45]. The results showed an increase in the activity of CAT treated with AgNPs with an increase in the level of stress induced by Cd. On the contrary, GPX activity was balanced with all levels of Cd, which may be explained by the convergence of mean values of GPX activity untreated with AgNPs. These results are similar to the results of previous studies that showed the role of the antioxidant enzyme system in counteracting various environmental influences [15, 46].

The accumulation of Cd, Ti, and BCF was estimated to verify heavy metal uptake by plant tissues and bioconcentration behavior [47]. Ti and BCF can be used to determine plant potential for phytoremediation purposes [48]. From the data, the mean values of BCF in Cd+ 100 treatment levels of AgNPs ranged from 1.528 to 2.24, which is indicative of significant bioaccumulation of Cd in plant tissues, which indicates a strong tolerance against heavy metals despite the strong accumulation compared to the control treatment of the nanomaterial [49]. In general, pretreatment with AgNPs led to a significant increase in the accumulation of Cd with an increase in the tolerance of Ti and BCF, thus contributing to the improvement of growth parameters for the efficiency of Cd phytoremediation.

5. Conclusions

There is an urgent need to understand the mechanisms by which plant cells can tolerate the effects of oxidative stress caused by trace metals, including Cd. Therefore, this study suggests a synergistic relationship between the parameters that would qualify the plant to play a role in phytoremediation. The current article showed that the inclusion of Cd in the cultivated media harmed most of the study parameters. However, it was found that the external application of AgNPs synthesized *in vitro* significantly improved all parameters through their stimulating effect of the antioxidant enzymatic system, which preserved the integrity of the cells. Moreover, the increase in the activity of the CAT and GPX enzymes contributed to regulating various physiological processes, which was positively reflected in the growth of callus culture. Thus, this contributes to prevent the risks of oxidative stress caused by Cd. This is the first study investigating the ability of AgNPs to mitigate Cd stress on the tissue cultures of plants, thus determining their role in plant bioremediation. Therefore, further studies can be suggested to understand the chemistry of compounds and food safety.

Data Availability

The findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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