Interactive Effect of Light and CdO Nanoparticles on *Dodonaea viscosa* Morphological, Antioxidant, and Phytochemical Properties

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**ABSTRACT:** Cadmium nanoparticles (NPs) used in semiconducting devices are photosensitive and optically active. The objective of this study was to investigate the interactive effect of different spectral lights and CdO NPs on morphological, antioxidant, and phytochemical characteristics of *Dodonaea viscosa*. The plants were grown on media in the presence of green and chemically synthesized CdO NPs and under red, yellow, green, blue, and white light intensities. Results illustrated that plant morphological parameters changed in the presence of different spectral lights and NPs behaved differentially under different spectral lights. Fresh and dry weights of plants decreased in the presence of NPs in the media; however, the concentration and route of synthesis of NPs have a significant effect on these parameters. The same was observed in the case of shoot and root lengths; however, green synthesized NPs were found to be less toxic under different spectral lights. The total antioxidant response increased under yellow, blue, and white lights, while the total reducing potential of plant extracts significantly varied depending upon the NP concentration and light spectrum. Different spectral lights significantly influenced the syntheses of phenolics and flavonoids under CdO NP stress and light regimes. It is concluded that toxicity of NPs also depends upon the wavelength of striking light that varies the morphological, biochemical, and antioxidative response of the plants. Furthermore, the white light might have synergistic effects of different wavelengths.

1. **INTRODUCTION**

Light is an important microenvironmental factor for plant growth, morphology, and physiology. Fluorescent lamps with the spectral range 350−750 nm are being used as a light source in plant tissue culture techniques. Light emitting diodes (LEDs) are a superlative light source, having narrow bandwidth, solid-state construction, long life, minimum heating, small mass/volume ratio, and wavelength specificity, to study physiological, morphological, and biochemical responses of plants.2,5 Plants have photoreceptors to respond to spectral ranges of blue (400−495 nm), green (495−570 nm), yellow (570−590 nm), red (590−710 nm), and UV-light (below 400 nm) wavelengths. Gene expression and chloroplast movement are according to the signals that photoreceptors (including phytochromes) perceive from the environment.3 Therefore, different light intensities can increase the secondary metabolite production and accumulation in plants, which paves the way for their commercial use.4 In the presence of any contaminant, the system either does not confer toxicity and becomes well adopted or tries to adjust its physiology by becoming tolerant or there might be synergetic, adoptive, or antagonistic effect of the change of environmental conditions and contaminant.5 Regarding the light factor, photosynthetically active radiation and artificial light have different effects due to the presence of UV radiation, difference in wavelength, and variation in intensities during day time that cause difference in both spectral lights, making it effective for plant adaptation.6,7 The difference in the response to the light spectrum and metal contaminant represents the species as tolerant or sensitive.8,9 However, no studies are undertaken yet on the effect of light and nanoparticles on oxidative damage and reactive oxygen species (ROS) production. ROS generation and oxidative stress are the best accepted paradigm to assess and compare the toxicity of different contaminants.10,11 The plant changes its metabolism, especially secondary metabolism, response on environmental changes including light and metal contaminant.12,13 Phenolics are
among the most ubiquitous groups of secondary metabolites that have plasticity and enable the plants to adapt to biotic and abiotic environmental changes. Phenolics exhibit radical scavenging activity that protects them from different biotic and abiotic stresses.

CdO is an n-type II–IV semiconductor with a band gap of 2.5 eV. Its large linear refractive index (n₀ = 2.49), high electrical conductivity, high carrier concentration, and high transparency in the visible range of the electromagnetic spectrum make it a suitable candidate for use in optoelectronic devices. CdO is widely used in solar cells, phototransistors, electrical conductivity, high carrier concentration, and high scavenging activity that protects them from oxidative stress. CdO NPs have antimicrobial properties, low electrical resistivity, photoluminescence, optical transmittance, and photocatalytic properties.

Dodonaea viscosa L. (Sanatha) is a shrub that belongs to the Sapindaceae family and is wildly grown in Australia but also found in tropics, sub-tropics, and temperate regions. D. viscosa has been used by native people since centuries in ethnobotany practices to treat sore throats, cold, malaria, rheumatism, itching, wounds, aches, digestive disorders, trachoma, and respiratory distress syndrome. D. viscosa has been used by native people since centuries in ethnobotany practices to treat sore throats, cold, malaria, rheumatism, itching, wounds, aches, digestive disorders, trachoma, and respiratory distress syndrome. D. viscosa has been used by native people since centuries in ethnobotany practices to treat sore throats, cold, malaria, rheumatism, itching, wounds, aches, digestive disorders, trachoma, and respiratory distress syndrome.

Due to photoluminescence properties of CdO NPs, these NPs were selected to study the toxic effect on plant growth in the presence of different spectral lights. The plants grown on MS media having NP concentration 2.5–20 mg/L of green and chemically synthesized CdO NPs showed differences in morphological and biochemical responses. The antioxidants (phenolics and flavonoids) and antioxidative response varied due to the spectral light and presence of NPs. The spectral light, in some cases, also reduced the toxic effect of NPs, which might be due to variation in the band gap of NPs at different light wavelengths. The study will raise our understanding about plant responses under NP stress and different light regimes. Further, it will spotlight the stress mechanism and also enhanced production of metabolites (phenolics and flavonoids in this case) under these stresses. The metabolites produced under such stresses can be further isolated and used by humans as nutraceuticals or drugs.

2. MATERIALS AND METHODS

2.1. Synthesis and Characterization of CdO NPs. CdO NPs synthesized through the coprecipitation method and green chemistry were used in this study as previously reported. In short, for chemical synthesis, 50 mL of 0.5 M Cd(NO₃)₂·4H₂O was reacted with 50 mL of NaOH (0.5 M). However, for green synthesis, 50 mL of the extract of Artemisia scoparia (30 g DW/150 mL) was reacted with 0.5 M Cd(NO₃)₂·4H₂O. The NPs were collected by centrifugation and calcinated at 400 °C for 2 h.

X-ray diffraction of CdO NPs was carried out using a D8 Advance diffractometer having a Cu Kα radiation source of 1.54 Å with 1200 W X-ray energy. Scanning was done in the range of 2θ from 10 to 70°. NicoletTM380 was used for Fourier transform infrared (FTIR) spectroscopy of CdO NPs in the range of 1000–3500 cm⁻¹ in the transmittance mode. Morphology of CdO NPs was investigated by a scanning electron microscope (SEM, MIRA3 TESCAN) operating at an acceleration voltage of 20 kV.

2.2. Preparation of CdO NP-Supplemented Media. Four concentrations (2.5, 5, 10, and 20 mg/L) of each chemically synthesized CdO NPs (Ch-CdO NPs) and green synthesized CdO NPs (As-CdO NPs) were used to analyze plant response under in vitro conditions. The MS medium (4.4 g/L, PhytoTech) was dissolved in 30 mg/L sucrose. NPs were added, and pH was adjusted at 5.6. The MS medium without addition of NPs worked as a control. The medium was sonicated for 20 min in a water bath to minimize agglomeration of NPs. After addition of gelrite (0.44 g/L), the medium was heated and then poured at 30 mL/100 mL in conical flasks with constant shaking. The flasks were autoclaved at 121 °C and 15 psi for 20 min and allowed to solidify at room temperature.

2.2.1. Seed Germination. Seeds of D. viscosa were collected from the Quaid-i-Azam University, Islamabad. Viability of seeds of D. viscosa was checked by the water float method that showed germination efficiency >98%. Under aseptic conditions, seeds were sterilized by immersing in freshly prepared mercuric chloride for 20 s. After that, seeds were treated with 70% ethanol for 1–2 min and washed thrice with distilled autoclaved water. Four seeds were inoculated in each flask, were kept in the dark for 5 days to break dormancy, and then shifted to chambers (36 cm length, 36 cm width, 50 cm height) having different spectral lights. Inoculated flasks were placed under illumination lights (1000 lux each) including red LEDs (610–715 nm, photosynthetic photon flux density (PPFD) 24.11 μmol/m²/s, and photosynthetically active radiation (PAR) 4.62 m⁻² s⁻¹ at 623 nm), yellow LEDs (530–780 nm, PPFD 6.72 μmol/m²/s, and PAR 1.40 m⁻² s⁻¹ at 571 nm), green LEDs (480–670 nm, PPFD 5.24 μmol/m²/s, and PAR 1.14 m⁻² s⁻¹ at 547 nm), blue LEDs (380–560 nm, PPFD 19.3 μmol/m²/s, and PAR 19.3 m⁻² s⁻¹ at 480 nm), and white LEDs (380–780 nm, PPFD 15 μmol/m²/s) under 16/8 h photoperiod at 25 °C. After 28 days, plantlets were harvested for root analysis by GIA root software, shoot length, fresh weight, and leaf shape examination. Plants were then dried in an incubator at 45 °C for 48 h for dry biomass calculation.

2.3. Biological Properties. The dried plant material was ground using a mortar and pestle, and 100 mg of plant material was suspended in 1 mL of dimethyl sulfoxide (DMSO). After 24 h incubation at room temperature, tubes were centrifuged at 8000 rpm at room temperature and the supernatant was used for determination of antioxidative activities (2,2-diphenyl-1-picryl hydrazyl (DPPH)-based free radical scavenging activity, total antioxidant potential, total reducing power) and nonenzymatic antioxidants (phenolics and flavonoids).

2.3.1. Determination of Free Radical Scavenging Potential. To determine free radical scavenging activity, 190 μL of DPPH solution (2,2-diphenyl-1-picryl hydrazyl) prepared in methanol was mixed with 10 μL of extract in 96-well microtiter plates. The reaction mixture was incubated for 1 h in the dark, and the optical density was measured at 515 nm using a microplate reader. DMSO was used as a negative control, while ascorbic acid was taken as a positive control. Percent inhibition was calculated as follows

\[
\%\text{ inhibition} = \frac{A_b - A_t}{A_b} \times 100
\]
where Abs is the absorbance of DPPH solution with sample and Abc is the absorbance of the negative control.

2.3.2. Determination of Total Antioxidant Activity (TAC).

To estimate total antioxidant activity of the samples, 100 μL of the test sample was mixed with 900 μL of reagent solutions (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated for 90 min at 95 °C. After cooling to room temperature, absorbance was measured at 695 nm using a microplate reader. DMSO was used as a negative control, while ascorbic acid was used as a positive control. TAC is expressed as μg ascorbic acid equivalent per mg dry weight (μg AAE/mg DW).

2.3.3. Determination of Total Reducing Power (TRP).

The reduction potential of samples was determined according to the protocol reported by Zafar et al. B briefly, 100 μL of the test sample was mixed with 200 μL of phosphate buffer and 250 μL of potassium ferricyanide and incubated at 50 °C for 20 min. The mixture was acidified with addition of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. The supernatant layer (150 μL) was mixed with 50 μL of ferric chloride, and the optical density was measured at 630 nm. Results are expressed as μg ascorbic acid equivalent per mg dry weight (μg AAE/mg DW). DMSO was used as a negative control, and ascorbic acid was used as a positive control.

2.3.4. Determination of Total Phenolic Content.

Total phenolic contents were determined using the Folin–Ciocalteu (FC) reagent. In short, 20 μL of the extract and 90 μL of the FC reagent were poured in the wells of a microtiter plate. After 5 min, 90 μL of Na₂CO₃ was added to each well and incubated for 1 h. Absorbance was measured at 650 nm using a microplate reader. Gallic acid was used as a standard, so the resultant TPC was determined as μg gallic acid equivalent per mg dry weight (μg GAE/mg DW).

2.3.5. Determination of Total Flavonoid Content (TFC).

TFC was determined using the protocol described earlier. The sample (20 μL) was mixed with reaction solutions (10 μL of aluminum chloride and potassium acetate). Distilled water was added to raise the final volume up to 200 μL, and the plate was incubated for 30 min. Absorbance was measured at 415 nm. Quercetin was used as a standard, and the resultant TFC is expressed as μg quercetin equivalent per mg dry weight (μg QE/mg DW).

2.4. Statistical Analysis.

To study the interactive effect of CdO NPs and spectral lights on D. viscosa, five flasks of each concentration were inoculated, each containing four seeds for every light intensity. Results are expressed as mean with standard error. Origin (4.5) was used for graphic representation of parameter interaction. All tests were performed in triplicate, and means were analyzed using the analysis of variance (ANOVA) and least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of NPs.

CdO NPs were synthesized using green and chemical chemistry under the same conditions and using the same procedure. Both methodologies resulted in formation of brown NPs. SEM disclosed clear and distinct images of green and chemically synthesized CdO NPs (Figure 1A,D) with the size range 10–
30 nm. The intense and sharp peaks by XRD indicate that chemically synthesized particles are crystalline in nature (Figure 1B), while As-CdO NPs (CdO NPs synthesized by A. scoparia) are crystalline cum amorphous in nature (Figure 1E). FTIR confirmed attachment of functional groups responsible for capping and stabilization of the NPs. FTIR spectra of Ch-CdO NPs (Figure 1C) show prominent peaks at 3561, 3245, 1739, 983, and 691. Prominent peaks at 2938, 2355, 1482, 1062, and 614 were shown by FTIR spectra of As-CdO NPs (Figure 1F). The characterization patterns of chemically and biologically synthesized NPs match the reference patterns for CdO nanoparticles and published data.

3.2. Effect on D. viscosa Growth and Root and Shoot Morphology. Length and biomass are different parameters, and light affects both independently. The light spectrum affects plant growth and development, and different spectral lights from LEDs change plant height, biomass, anatomy of leaf and stem, photosynthesis and respiration characteristics, etc.25−28 Plantlet health indicators like leaf shape, biomass accumulation, and root and shoot morphology were recorded after 28 days of seed germination (Figures 2 and 3). The fresh weight of plants was significantly greater in the presence of red light followed by green light (Figure 2A,B). The same trend was also observed for the dry weight parameter. ANOVA analysis also showed a significant difference between morphological parameters as compared to the control (Tables 1 and 2) in the presence of both types of nanoparticles. The fresh weight of plants decreased in the presence of Cs-CdO NPs and different spectral lights except 2.5 mg/L, where a nonsignificant increase was observed in white light. However, there was significant variation in the fresh weight in the presence of Cs-CdO NPs irrespective of the light regimes. In the case of Ch-CdO NPs, a nonsignificant difference was observed in the plant fresh weight in some cases. It was also observed that the plant fresh weight was greater in the presence of Ch-CdO NPs as compared with Cs-CdO NPs. The dry weight of plants decreased in all of the cases except yellow and blue lights, where at some Cs-CdO NP concentrations, the difference was not significant. However, in the case of red, green, and white lights, a highly significant difference was observed as compared with control. A significant difference in the dry weight was observed in the presence of Ch-CdO NPs and light spectra, but in the case of yellow light, the dry weight of plants grown in the presence of 2.5 and 5 mg/L significantly increased than in control (Figure 2). It was also observed that the plant dry weight was mostly better in the case of chemically synthesized CdO NPs as compared with green synthesized NPs. Lin et al. reported the alteration of biomass accumulation in lettuce in the presence of different LEDs.29 Biomass production significantly increased under different LEDs in basil cultivars compared with fluorescent lights.30 Rapeseed length was maximum under red light; however, it gained maximum dry mass under the blue and red light combination.31 Red light also caused increase in biomass of roses, chrysanthemums, lettuce.26,32 Among various factors, C and N metabolisms are fundamentally coordinated for the optimal growth of plants.33 It can be concluded that biomass accumulation is linked to the availability of nutrients in media. CdO NPs in nutrient media along with light intensity are associated with the biomass accumulation. The light penetrates into the leaf, and different wavelengths have different effects on metabolic pathways in the upper epidermis layer and palisade mesophyll layer. It has been documented that green light drives the metabolic pathways deep within spinach leaves as compared to red and blue lights.34 Red light is the primary light source affecting biomass production and elongation through the phytochrome photoreceptor,35 while blue light affects independently and/or in a synergistic manner with the phytochromes.36,37 Metallo et al. also reported a decrease in plant height under blue light treatment in Kale.38

Different light regimes influenced the length of plants (Figure 3A,B). Root length was maximum (8.7 cm) in the presence of white light, while shoots attained the maximum height (10.9 cm) in the presence of red light. The chemically and green synthesized CdO NPs differentially affected plant morphological parameters. In all of the cases, As-CdO NPs had a toxic effect on root length except under green and blue lights, where in the presence of 2.5 mg/L NPs, root length significantly increased as compared with controls. Yet, there was variation in root length response under different NP concentrations and light intensities, i.e., root length was 0.1 cm at 20 mg/L NPs under green light, and this concentration was less toxic in the presence of other light spectra. A nonspecific trend was also observed in the case of shoot length under different light regimes and As-CdO NP concentrations (Figure 3). The toxicity of 20 mg/L As-CdO NPs showed the following order: white > red > yellow > blue > green. The root length, in the case of Ch-CdO NPs, increased under red, green, and blue lights at 2.5, 5, 5, and 10 mg/L concentrations, respectively, as compared to respective controls. Ch-CdO NPs displayed maximum toxicity in the white light regime. The shoot length also significantly varied in the presence of different Ch-CdO NP concentrations and light regimes, and in most of the cases, a stimulatory effect of Ch-CdO NPs was

![Figure 2](https://example.com/figure2.png)
observed on shoot lengths. In different plant species, LED lights of varying wavelengths have different effects, i.e., lettuce attained more stem length under red light as compared to blue or fluorescent light; however, rapeseed shoot length did not get affected by red light. The plants grown under low radiation have compact roots and elongate quickly due to less absorption of water and nutrients from the rooting system. However, this response is not necessary and variations in results have been documented.

Cadmium stress to plants inhibits root and shoot elongation by inhibiting mitotic activities of meristematic cells. Many ion and protein transporters are present in plants for Cd transport via apoplastic and symplastic pathways. Lower activity of H⁺-ATPase decreases root length, which participates in the uptake of elements by roots. Toxicity of nanoparticles depends upon their composition, size, and shape and ion release from NPs. More ion release and existence of NPs around vascular bundles decrease plant growth. Meanwhile,

Table 1. ANOVA Analysis of Morphological Parameters of *D. viscosa* Plants Grown in the Presence of Ch-CdO NPs and Different Light Spectra

| source of variation | SS     | df  | MS    | F       | P-value  | F crit      |
|---------------------|--------|-----|-------|---------|----------|-------------|
| morphology          | 319.797| 3   | 106.599| 290.617 | 3.85 × 10⁻⁴⁰ | 2.731807    |
| light × conc.       | 13768.12| 24  | 573.6716| 1.563979| 0.075467  | 1.669456    |
| error               | 26409.79| 72  | 366.8027|         |          |             |
| total               | 359975.3| 99  |       |         |          |             |

Table 2. ANOVA Analysis of Morphological Parameters of *D. viscosa* Plants Grown in the Presence of Cs-CdO NPs and Different Light Spectra

| source of variation | SS     | df  | MS    | F       | P-value  | F crit      |
|---------------------|--------|-----|-------|---------|----------|-------------|
| morphology          | 1490.902| 2   | 745.4512| 87.14521| 2.26 × 10⁻¹⁶| 3.199582    |
| light × conc.       | 302.6455| 23  | 13.1585| 1.538263| 0.105979  | 1.766805    |
| error               | 393.4898| 46  | 8.554127|         |          |             |
| total               | 2187.038| 71  |       |         |          |             |
under high light intensity, sucrose transport increases from leaves to roots and influences the root system growth in tobacco. It was assumed that different light intensities along with CdO NPs in nutrient media alter the nutrient uptake and transport by interacting with plant machinery. Under metal and light stresses, the synergistic effect provokes differences in cellular functions. These may include growth variation, decrease in chlorophyll content, oxidative stress, and lipid peroxidation.34

3.3. Leaf Phenotype of *D. viscosa* Under CdO NPs and Light Stress. In response to abiotic stress, leaves exhibit phenotypic plasticity, which changes the chemical, functional, and structural characteristics of plants. D. *viscosa* plantlets under the stress of CdO NPs and different intensities of light showed altered leaf shape under different concentrations (Table 3). In the presence of As-CdO NPs, more change in leaf morphology was observed depending upon the concentration of NPs and light intensity. However, no specific trend in change in leaf morphology was observed in the presence of green and chemically synthesized NPs. It has been reported that red and blue LEDs boost the leaf area, which captures more light energy and increases biomass.36 Nanoparticles affect the morphology and physiology of leaves by altering the leaf length and width and chlorophyll, enzymatic, and protein contents.37

Plant leaves respond differentially to light wavelengths. It may change leaf shape, size, and even number of leaves per plant. Lettuce revealed greater leaf area when cultivated under fluorescent light, whereas white fluorescent lamps induced the formation of smaller leaves compared to other lights; thus, size of leaves changed under different spectral regimes.48 However, sweet basil did not show any difference under fluorescent and LED lights.28 In Phalaenopsis, rose, and cucumber, greater leaf formation was observed on increasing red light.39,40 Light affects the photosynthesis ability of the plants and changes carbohydrate quantity and other metabolite concentrations in the cell or tissue. Studies have shown that combinations of red and blue lights are favorable for carbohydrate production in plants.49,51 It has also been reported that morphological and phytochemical characteristics of the same species but different cultivars change under the same light regime.43 Light spectrum also affects the transpiration rate from leaf along with the structure and texture of the leaves, i.e., blue light is approximately 10 times effective for stomatal opening as compared with red and green lights that change overall leaf morphological characteristics.52

| light | conc. (mg/L) | leaf shape As-CdO NPs | leaf shape CdO NPs | phenotypic plasticity, which changes the chemical, functional, and structural characteristics of plants.45 D. *viscosa* plantlets under the stress of CdO NPs and different intensities of light showed altered leaf shape under different concentrations (Table 3). In the presence of As-CdO NPs, more change in leaf morphology was observed depending upon the concentration of NPs and light intensity. However, no specific trend in change in leaf morphology was observed in the presence of green and chemically synthesized NPs. It has been reported that red and blue LEDs boost the leaf area, which captures more light energy and increases biomass.36 Nanoparticles affect the morphology and physiology of leaves by altering the leaf length and width and chlorophyll, enzymatic, and protein contents.37

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Table 4. ANOVA Analysis of Phytochemical and Antioxidant Assays of the *D. viscosa* Plant Grown in the Presence of Ch-CdO NPs and Different Spectral Lights

| source of variation | SS   | df  | MS   | F    | P-value | F crit |
|---------------------|------|-----|------|------|---------|--------|
| light × conc.       | 5411.516 | 24  | 225.4798 | 3.474608 | 7.56 x 10^0 | 1.63128 |
| antioxidative       | 2391.93  | 4   | 5972.981 | 92.04268 | 5.5 x 10^-32 | 2.466476 |
| error               | 6229.786 | 96  | 64.89361 |       |         |        |
| total               | 35 533.23 | 124 |      |       |         |        |
Ch-CdO NPs exerted more stress on *D. vicosa* in the presence of blue and white lights where a significant difference was observed except at 20 mg/L NP concentration. Under other light regimes, a less significant difference was observed. It was also observed that light intensity had a minor effect as 41.1–50.1% free radical scavenging activity was observed among the control plants (Figure 4). Much more variation in free radical scavenging activity was observed in *D. vicosa* plants when grown in the presence of As-CdO NPs under different light regimes. The radical scavenging potential decreased by increasing the concentration of NPs up to 10 mg/L, but the 20 mg/L concentration exerted more stress that resulted in an increase in DPPH activity. Nam et al. reported a significant effect of light sources on antioxidant activity in buckwheat sprouts.53 Findings of Naznin et al. indicated that LEDs have a vital role in antioxidant and scavenging activities of plants.54

However, metal and metallic oxide NPs increase the production of antioxidants to combat the stressful environment.

CdO NPs varied TAC, TRP, and radical scavenging activity of *D. vicosa* plants under different light intensities. The same was observed in the case of total phenolic and flavonoid contents (Figure 5A,B). Total phenolics increased in all of the cases when Ch-CdO NPs were applied except under red light where a decrease was observed (Figure 5A). The phenolic contents increased to a great extent in the presence of yellow and blue lights irrespective of the concentration of NPs; however, in the presence of red light, a significant increase in phenolics was observed among controls. Due to application of As-CdO NPs, a significant increase in total phenolics was observed in all of the cases irrespective of the concentration of NPs and light regime (Figure 5B). Approximately 3-fold increase in phenolics was observed under yellow, green, blue, and white lights, while a minor but significant increase was observed under red light. Under red light, TPC decreased at 5, 10, and 20 mg/L concentrations under Ch-CdO NPs, while an increase was observed in the case of As-CdO NPs.53

### Table 5. ANOVA Analysis of Phytochemical and Antioxidant Assays of the *D. vicosa* Plant Grown in the Presence of As-CdO NPs and Different Spectral Lights

| source of variation | SS     | df  | MS   | F       | P-value | F crit |
|---------------------|--------|-----|------|---------|---------|--------|
| light × conc.       | 4797.155 | 23  | 208.572 | 2.699227 | 0.000797 | 1.686897 |
| assays              | 6238.368 | 3   | 2079.456 | 26.91121 | 1.23 × 10⁻¹¹ | 2.737492 |
| error               | 5331.699 | 69  | 77.27101 |         |         |        |
| total               | 16367.22 | 95  |        |         |         |        |

Figure 4. Mean TAC, TRP, and DPPH activity of *D. viscrosa* plants grown under different light intensities and Ch- and Cs-CdO NPs: (A) parameters of plants grown in the presence of Ch-CdO NPs and (B) parameters of plants grown in the presence of Cs-CdO NPs. The same alphabets marked on the top of each column show a significant difference in the total reducing power potential. The same alphabets marked at the bottom of each column show a significant difference in total antioxidant capacity.

Figure 5. Mean TPC and TFC of *D. viscrosa* plants grown under different light intensities and Ch- and Cs-CdO NPs: (A) parameters of plants grown in the presence of Ch-CdO NPs and (B) parameters of plants grown in the presence of Cs-CdO NPs. The same alphabets marked on the top of each column show a significant difference in total phenolic contents. The same alphabets marked at the bottom of each column show a significant difference in total flavonoid contents.
10, and 20 mg/L Ch-CdO NPs. Control plants had the highest TPC (33.4 ± 0.9 μg GAE/mg DW) under red light. Variation in total flavonoid contents was also observed under the NP and light treatment. In the presence of Ch-CdO NPs and red light, flavonoids increased to a great extent, while blue lights did not affect flavonoid contents as compared with control. A significant decrease in flavonoids was observed on increasing the concentration of Ch-CdO NPs under white light. A significant increase in TFC was observed under light regimes at low concentrations (2.5 and 5 mg/L) of As-CdO NPs (Figure 5B). However, under white light, TFC was significantly higher irrespective of NP concentration. In the presence of green light, TFC decreased by 15.3 and 20.1 μg QE/mg DW at 10 and 20 mg/L, respectively, in the presence of As-CdO NPs compared with that of control (29.5 μg QE/mg DW). Light plays an important role in plant metabolism and secretion of phytochemicals (Khan et al., 2019). Under stress conditions, more flavonoid and phenolic contents produce to boost the defense mechanism in plants. Phenolic production is directly related to phenylalanine ammonia-lyase enzyme activity and light intensity. Flavonoid production reduces under low light and nutrient conditions. High phenolic production causes low secretion of flavonoids in different circumstances. Toxic levels of NPs cause oxidative burst and alter secondary metabolism in plants. Tarrahi et al. reported the increase in secondary metabolite production in aquatic plant *Lemna minor* in the presence of CdSe NPs. The phenol-coupled APX reaction increases against oxidative stress to increase the phenolic production in plants under a stress environment. It can be assumed that different light intensities in the presence of CdO NPs alter phenolic and flavonoid production pathways to adjust their secretions during stress. Light intensity and metal contaminant have antagonistic actions in plants in gene regulation, stress mitigation, lipid peroxidation, and oxidative stress and damage. Depending upon the light intensity, the stress exerted on plants is mitigated by antioxidants. Different plants behave differentially toward wavelengths of light, e.g., secondary metabolites...
increased under the blue regime, while in carrots and grapes, enhanced production was observed under UV radiation.

Under different spectral lights, some enzymes stimulate, increasing the metabolites in some pathways, e.g., under blue light, phenylalanine ammonia-lyase enhances, which is a key enzyme in the phenyl-propanoid pathway.

The dendrograms depict that the synthesis route of NPs, chemical or green, has different effects on plant metabolic activities (Figure 6A,C). In the presence of chemically synthesized CdO NPs, TFC was equally with TAC & TPC and TRP & DPPH. However, in the presence of green synthesized CdO NPs, DPPH has varying response where TRP and TFC have major contribution as compared with TAC and TPC. Furthermore, light spectrum and NP concentration have different interactions based on the antioxidative activities and production of antioxidants (Figure 6B,D). Each spectrum of light has different photon energy, and the varying wavelength increases heat in media. Both these properties of light affect the valance bond and conduction bond of NPs (Figure 7). The excited electrons generate ROS by production of nascent oxygen or hydroxyl ions that generate oxidative stress on plants. The band gap of CdO is 2.5 eV, while the incident lights have different photon energies. Although the lights (i.e., white, blue, and to some extent green) with photon energies higher than or equal to 2.5 play influential role in generation of ROS, other lights with greater photon energies might also function as a driving force for ROS generation. At the same instance, under the experimental conditions, the photosynthetic photon flux density (PPFD) of the striking light also has an influential role. Although yellow and red lights have low band gap energies, the PPFD value of red light is maximum, while yellow light has a PPFD value greater than that of green light. Along with light wavelength and PPFD, photosynthetically active radiation (PAR) should also be considered because PPFD is the photon flux density of PAR. The striking light at a specific area of plant (both PPFD and PAR) determines ROS generated though the energy of that light is even low. Furthermore, the toxic effects of CdO NPs or dissolved Cd and biochemical changes due to these should also be considered for generation of ROS and antioxidative mechanisms. The oxidative stress is responsible for dysfunctioning of metabolic pathways, cellular damage, electrolyte misbalancing, and variation in molecular regulation. The production of antioxidants, phenolics and flavonoids, and antioxidative enzymes utilizes the ROS species, making the plant stress-tolerant. It is further concluded that photodynamic properties of nanoparticles should be addressed under different wavelengths of striking lights that exert different toxic effects on living systems. These specific stress conditions can also be used for enhanced production of secondary metabolites. This would be helpful for establishment of green bioprocess technologies that will not only produce the metabolites but also result in in vitro production of metabolites that will minimize the burden of plant collection for the sake of metabolite isolation. This will save the plants from extinction and help to establish the biotechnological aspects.

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Author Contributions

F.Z.G. and A.K. performed CdO NP toxicity studies under light spectra. S.H. and B.A. performed antioxidative assays. B.H.A. and M.Z. supervised the experiments and wrote the manuscript. C.H. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Notes

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