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The Impact of Priming with Al_2O_3 Nanoparticles on Growth, Pigments, Osmolytes, and Antioxidant Enzymes of Egyptian Roselle (*Hibiscus sabdariffa* L.) Cultivar

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Abstract: Lower concentrations of nanoparticles (NPs) could have positive effects on plants. In the present experiment, we tested the efficacy of seed priming Egyptian roselle cultivar with aluminum oxide nanoparticles (Al_2O_3 NPs). Plants grown with different concentrations (0.01, 0.05, 0.1, and 0.5%) of Al_2O_3 NPs-primed seeds showed varied responses. An increasing impact with 0.01% Al_2O_3 NPs was noticed on growth traits, such as fresh weight, dry weight, shoot length, root length, and leaf area, and physio-biochemical activities like chlorophyll a, chlorophyll b, carotenoid contents, soluble sugars, protein, amino acid, proline, and the activities of defense enzymes viz-superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Nevertheless, a decrease was noted in malondialdehyde (MDA) when plants were primed with 0.01% Al_2O_3 NPs. Seed priming with 0.05, 0.1, and 0.5% Al_2O_3 NPs caused the negative effects in the aforementioned parameters. The principal component analysis revealed significant correlations among the various studied parameters. Therefore, seed priming with Al_2O_3 NPs at 0.01% was expected to serve as an effective measure for inducing positive effect in Egyptian roselle cultivar.

Keywords: aluminum oxide nanoparticles; seed priming; growth and physio-biochemical traits; roselle cultivars

1. Introduction

The field of nanotechnology in the current times is gaining attention due to continuous usage of a wide range of different nanoparticles (NPs), viz- titanium oxide ($nTiO_2$), cerium dioxide ($nCeO_2$), zinc oxide (nZnO), lead oxide (nPbO), iron oxide (nFe_2O_3), copper oxide (nCuO), aluminum oxide (Al_2O_3), and silicon dioxide ($nSiO_2$) or the products derived from them [1–11]. Nowadays, various NPs are contaminating various sectors, including agriculture and other allied sectors, because they are regarded as excellent alternatives, as their biosynthesis can be undertaken at low cost and easy scaling. However, their continuous use may also pose a great risk to the environment and public

health. Plants' exposure to various NPs poses both beneficial and negative effects on their growth and development. The effects of NPs are dependent on the source, type, size, plant species, exposure duration, and concentration. Therefore, there is a need to do a careful examination of their application, economic feasibility, and eco-toxicological hazards. The presence of NPs in the soil has been increasing dramatically due to their release, which can take place intentionally, naturally, and accidentally [12]. NPs are known to accumulate in different organs of plants [13]. Various doses of NPs cause negative effects in plants due to their nano size. Once taken inside plant tissues, NPs may induce damage to DNA, disrupt the bio-membranes, and enhance the uncontrolled biosynthesis of reactive oxygen species (ROS), such as superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), etc., which is regarded as one of the main reasons of nano-phytotoxicity [6,14–18]. Plants neutralize the effect of ROS by activating the antioxidant defense system, which consists of various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Various reports have demonstrated the negative impacts of nano-toxicity in plants. For example, varying concentrations of nCuO (25–2000 mg/L) and Al_2O_3 (2000 mg/L) were found to impose phytotoxic effects in maize and rice plants [19]. Wang et al. observed that various doses of nZnO (200, 400, and 800 mg dm^{-3}) significantly reduced tomato growth, contents of chlorophyll a and b, photosynthetic efficiency, and the chlorophyll fluorescence parameters in a concentration-dependent manner [20]. Rastogi et al. applied AgNPs (1 and 5 mM) on wheat seedlings and found a negative significant impact on growth parameters, biochemical parameters, and chlorophyll concentrations [17]. The phytotoxicity was more prominent on photosynthetic activity, and marked destruction of photosystems was observed at 5 mM concentration. In a study conducted on *Allium cepa, Nicotiana tabacum*, and *Vicia faba*, Ghosh et al. exposed the roots of these plants to nZnO (0.2, 0.4, and 0.8 g/L) [21]. The results suggested the chromosome aberrations, loss of membrane integrity, DNA strand breaks, and cell-cycle arrest of *Allium cepa*. In *Vicia faba* and *Nicotiana tabacum*, excess ROS production, de-regulation of ROS-antioxidant battery, and lipid peroxidation were observed. Iftikhar et al. evaluated the phytotoxic effect on growth, photosynthesis, and antioxidant defense system of wheat plants grown in pots spiked with nZnO (0, 300, 600, 900, and 1200 mg/kg) [22]. In a yet recent study, the results of Azhar et al. advocated that nCuO adversely affected the Arabidopsis biomass, chlorophyll contents, guard cells, stomatal pore, and various cell organelles, with a consequent increase in the ROS accumulation in all four Arabidopsis genotypes studied. These reports suggested that various NPs had negative impacts on different crop plants [23].

On the other hand, abundant literature suggests that various NPs have positive effects on plants as well [3,4,24]. Priming seeds of wheat plants with different concentrations of either nZnO (0, 25, 50, 75, and 100 mg L^{-1}) or nFe_2O_3 (0, 5, 10,15, and 20 mg L^{-1}) for 24 h increased the growth, chlorophyll contents, gas exchange attributes, Fe and Zn concentrations while reduced the oxidative stress under cadmium (Cd) contamination [7]. The ameliorative role of SiNPs (100, 200, 300, and 400 mg Kg^{-1}) under water deficit and salinity stresses in cucumber plants was reported [25]. The results showed that SiNPs improved the growth and productivity of cucumber plants; however, the greatest increase corresponded at a rate of 200 mg Kg^{-1} in terms of plant height, chlorophyll, nitrogen, potassium, and silicon contents in root, stem, leaf, and fruit. Pullagurala et al. showed that nZnO increased the photosynthetic pigments and decreased lipid peroxidation in *Coriandrum sativum* plants. However, despite these observations, the systematic and finer details of priming-mediated Al_2O_3 impact of Egyptian roselle cultivars have not been reported yet [26].

Hibiscus sabdariffa (roselle plant) is commonly known as "Karkadeh" in Egypt [27]. This plant belongs to the *Malvaceae* family and is one of the potential summer growing medicinal and industrial plants. Most of the research on this fascinating plant has so far been conducted related to its antioxidant activity, nutritional, and health benefits. In our earlier studies, we evaluated the underlying mechanisms of priming plants (lupine and broad bean) with nZnO and $nTiO_2$ for mitigating the negative impacts of salinity stress [28,29]. In the present communication, the efficacy of various doses of Al_2O_3 was tested on Egyptian roselle cultivar to unravel their potential of tolerance. With this aim, the study was planned and executed.

2. Materials and Methods

2.1. Synthesis of Al_2O_3 Nanoparticles

For the preparation of Al_2O_3 nanoparticles, aluminum isopropoxide [$C_6H_{21}AlO_3$] (0.5 moles, 5.1062 g; sigma-aldrich, St. Louis, MI, USA) was dissolved in deionized water (50 mL). The solution was kept under constant stirring at room temperature using a magnetic stirrer at 11,000 rpm for 4 h to be precipitated. The white precipitation was filtered and washed with distilled water and ethyl alcohol several times (after 24 h). Then, the precipitations were dried in an oven at 50 °C to obtain white powder. The x-ray diffraction (XRD) profiles observed for Al_2O_3 nanoparticles are shown in Figure 1. The patterns displayed several peaks, which indicated that the sample was single having a tetragonal structure. Crystallite size (D_{size}) of the obtained Al_2O_3 nanoparticles was calculated from the following Debye–Scherrer's formula:

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

where β is the full width at half-maximum, D is the average crystallite size, and λ and θ are the wavelength of X-ray and the diffraction angle, respectively [30]. The crystallite size of the obtained Al_2O_3 nanoparticles was found to be 30.38 nm. The Fourier transform infrared (FTIR) spectra confirmed the purity of the prepared Al_2O_3 nanoparticles, where the obtained bands revealed to the (O–Al–O) functional groups. A transmission electronic microscope (TEM) was done to know the size of the Al_2O_3 nanoparticles. Figure 1 presents a typical TEM image of the Al_2O_3 sample, which confirmed that the prepared sample was in the nanoscale, and the particle size obtained from the TEM image was around 10 nm. The particle size was smaller than the crystallite size.

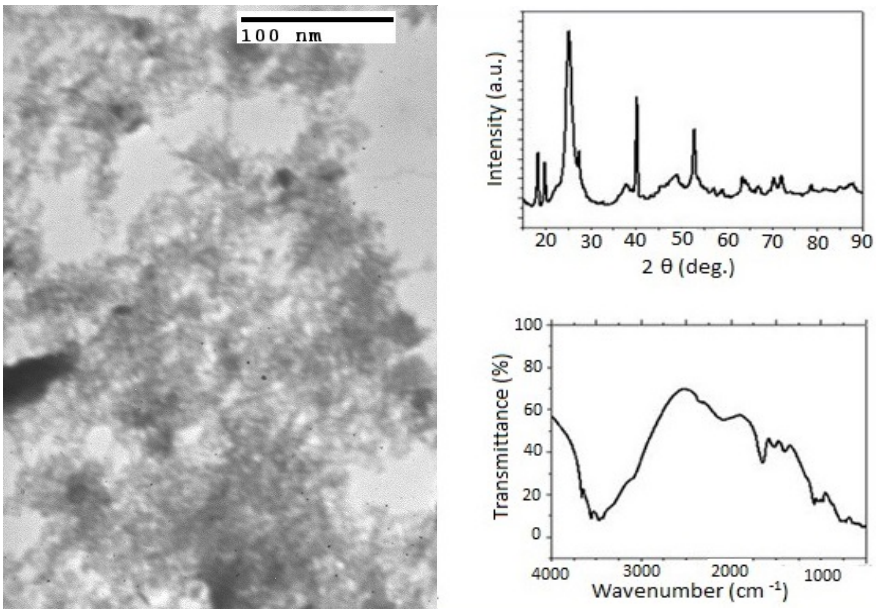

Figure 1. XRD, FTIR, and TEM for the prepared Al_2O_3 nanoparticles.

2.2. Plant Growth Conditions

The seeds of roselle (*Hibiscus sabdariffa* L.) cultivar (cv. light red sepals) were obtained from the breeding program of Agriculture Research, Dokky, Cairo, Egypt. Firstly, surface sterilization of seeds was carried out with 70% ethyl alcohol for 2 min, then washed thrice with sterilized water. After sterilization, the seeds were transferred on nylon mesh placed on the surface of medium containing 0.8% agar in Petri dishes. The seeds were categorized into five groups according to the priming solution as follows:

1. The 1st (control) set was priming with distilled water for 12 h.
2. The 2nd set was primed with 0.01% Al_2O_3 NPs for 12 h.
3. The 3rd set was primed with 0.05% Al_2O_3 NPs for 12 h.
4. The 4th set was primed with 0.1% Al_2O_3 NPs for 12 h.
5. The 5th set was primed with 0.5% Al_2O_3 NPs for 12 h.

The thoroughly washed seeds after doing priming were sown (5 five seeds/pot) in plastic pots filled with soil. The physical and chemical characteristics of the soil were sand 20.91%, silt 29.97%, clay 49.12%, pH 7.13, electrical conductivity (EC) 1.20 dSm^{-1}, Cl^- 1.51, Na^+ 1.62, K^+ 0.94, Ca^{2+} 1.87, and Mg^{2+} 1.12 mg 100 g^{-1} soil, respectively. The experiment was conducted in the wire house of the experimental farm of South Valley University, Qena, Egypt in a completely randomized block design in a factorial arrangement. In all treatments, three replicates were used. The pots were normally irrigated at field capacity with water through the whole duration of the experiment (30 days). After thirty days of seed sowing, the roselle plants were harvested for determining different traits.

2.3. Growth Traits

By using a measuring scale, the lengths of roots and shoots were determined. After recording the fresh weights of plants, the samples were dried at 80 °C in an oven, and then dry weights were measured.

2.4. Photosynthetic Pigments

The contents of photosynthetic pigments (chlorophyll a and b, and carotenoids) were determined, according to Lichtenthaler and Wellburn [31] method, spectrophotometrically (Spectronic Genesys ZPC, Rochester, NY, USA) at 663, 644, and 452.5 nm, respectively. The pigment extract was determined by using pure 80% acetone as a blank.

2.5. Organic Solutes (Soluble Sugar, Soluble Protein, Total Free Amino Acids, and Proline)

The method based on anthrone sulfuric acid, as described by Irigoyen et al. [32], was employed to determine the content of soluble sugar by measuring absorbance spectrophotometrically at 620 nm against a blank (distilled water + anthrone reagent). The method of Bradford [33] was used to determine soluble protein content by using bovine serum albumin as a standard. The method of Lee and Takanashi [34] was used to assess total free amino acid content by taking the absorbance at 570 nm against a blank (distilled water and the same reagent). The procedure of Bates et al. [35] was followed to estimate the proline content, and the absorbance was noted at 520 nm using toluene as a blank.

2.6. Malondialdehyde (MDA)

The thiobarbituric acid reactive substance (TBABS) was determined, and the procedure is cited in Abdel Latef and Tran [36]. The absorbances were taken at 532, 600, and 450 nm.

2.7. Assays of Antioxidant Enzyme Activities

Fresh leaf samples were used for assaying the activity of antioxidant enzymes. The superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the nitro blue tetrazolium (NBT) method of Giannopolitis and Ries [37]. Aebi [38] method was used for measuring the activity of catalase (CAT; EC 1.11.1.6). Peroxidase (POD; EC 1.11.1.7) activity was estimated according to the method described by Maehly and Chance [39]. The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was determined according to the method of Chen and Asada [40].

2.8. Statistical Analysis

Data were statistically analyzed by the analysis of variance (ANOVA) with SAS software (Version 9.1; SAS Institute, Cary, NC, USA), and Duncan's multiple range test was calculated at the 0.05 level of significance ($p < 0.05$). All data shown here are the mean ± standard deviation (SD)

of three independent replicates of each treatment. Principal component analysis (PCA) was done by Minitab software.

3. Results

3.1. Effect of Priming Plants with Different Al_2O_3 NPs Doses on Growth Traits

The weights (fresh and dry) and lengths of roots and shoots and leaf area of roselle plants were observed to study the impact of priming with different concentrations of Al_2O_3 NPs (Figure 2A–D). The low dose (0.01%) of Al_2O_3 provoked a significant increase in fresh weight (14.45%), dry weight (14.28%), root length (14.37%), shoot length (17.17%), and leaf area by 13.25% as compared to control plants. Seed-priming with 0.05, 0.1, and 0.5% Al_2O_3 caused a significant decrease in the formerly mentioned growth characteristics. The dose of 0.05% caused a decrease in fresh weight (25.73%), dry weight (25.00%), root length (20.54%), shoot length (19.86%), and leaf area (19.74%). Fresh weight decreased by 46.95%, dry weight by 58.33%, root length by 47.30%, shoot length by 42.44%, and leaf area by 47.25% on subjecting plants to 0.1% Al_2O_3 NPs. The higher dose of Al_2O_3 NPs further imposed a decreasing trend, resulting in the decrease of fresh weight by 64.24%, dry weight by 83.33%, root length by 66.32%, shoot length by 60.61%, and leaf area by 67.46% in comparison to control plants (Figure 2A–D). This showed that 0.01% of Al_2O_3 NPs were the only best effective NP among other NPs treatments to boost the growth traits.

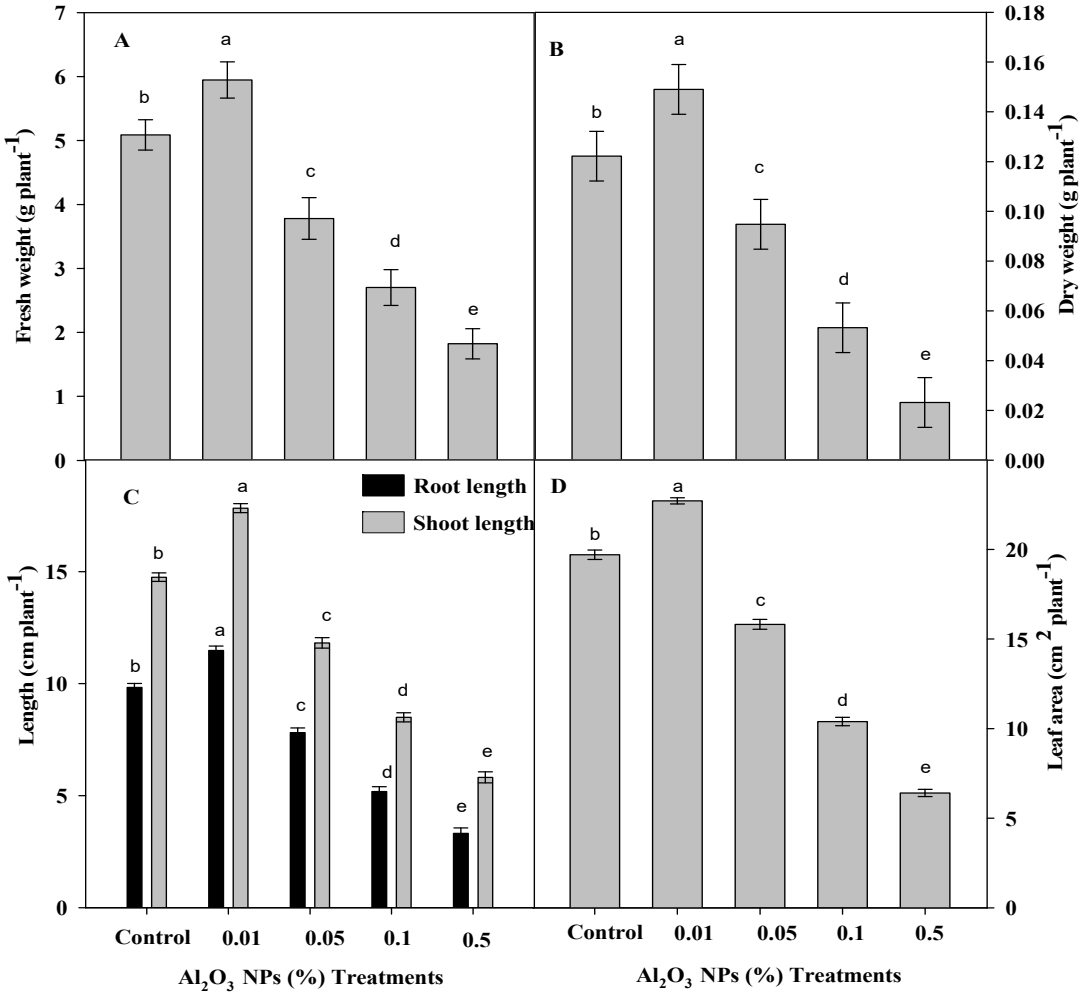

Figure 2. Effects of seed-priming with aluminum nanoparticles (Al_2O_3 NPs) on (**A**) fresh weight, (**B**) dry weight, (**C**) root length and shoot length, and (**D**) leaf area in roselle plants. Bars represent standard deviation (±SD) of the means (n = 3). Different letters indicate significant differences among the treatments at $p < 0.05$, according to Duncan's multiple range test.

3.2. Effect of Priming Plants by Al_2O_3 NPs on Contents of Chlorophyll a, b and Carotenoids

The contents of chlorophyll a, chlorophyll b, and carotenoids were found to be decreased by 10.49, 11.58, and 16.43%, respectively, in plants primed with 0.05% Al_2O_3 NPs relative to control plants (Figure 3A–C). A higher decrease was noticed in plants primed with 0.1% Al_2O_3 NPs, which showed 35.91, 39.63, and 45.20% respective decrease in chlorophyll a, chlorophyll b, and carotenoids contents as compared to control plants. A significant higher decrease in the above-mentioned traits was found in plants treated with 0.5% Al_2O_3 NPs. However, seed-priming with 0.01% Al_2O_3 NPs enhanced these photosynthetic pigments. An increase by 12.90, 16.32, and 31.13% in chlorophyll a, chlorophyll b, and carotenoids, respectively, was observed in treatment compared to the control (Figure 3A–C).

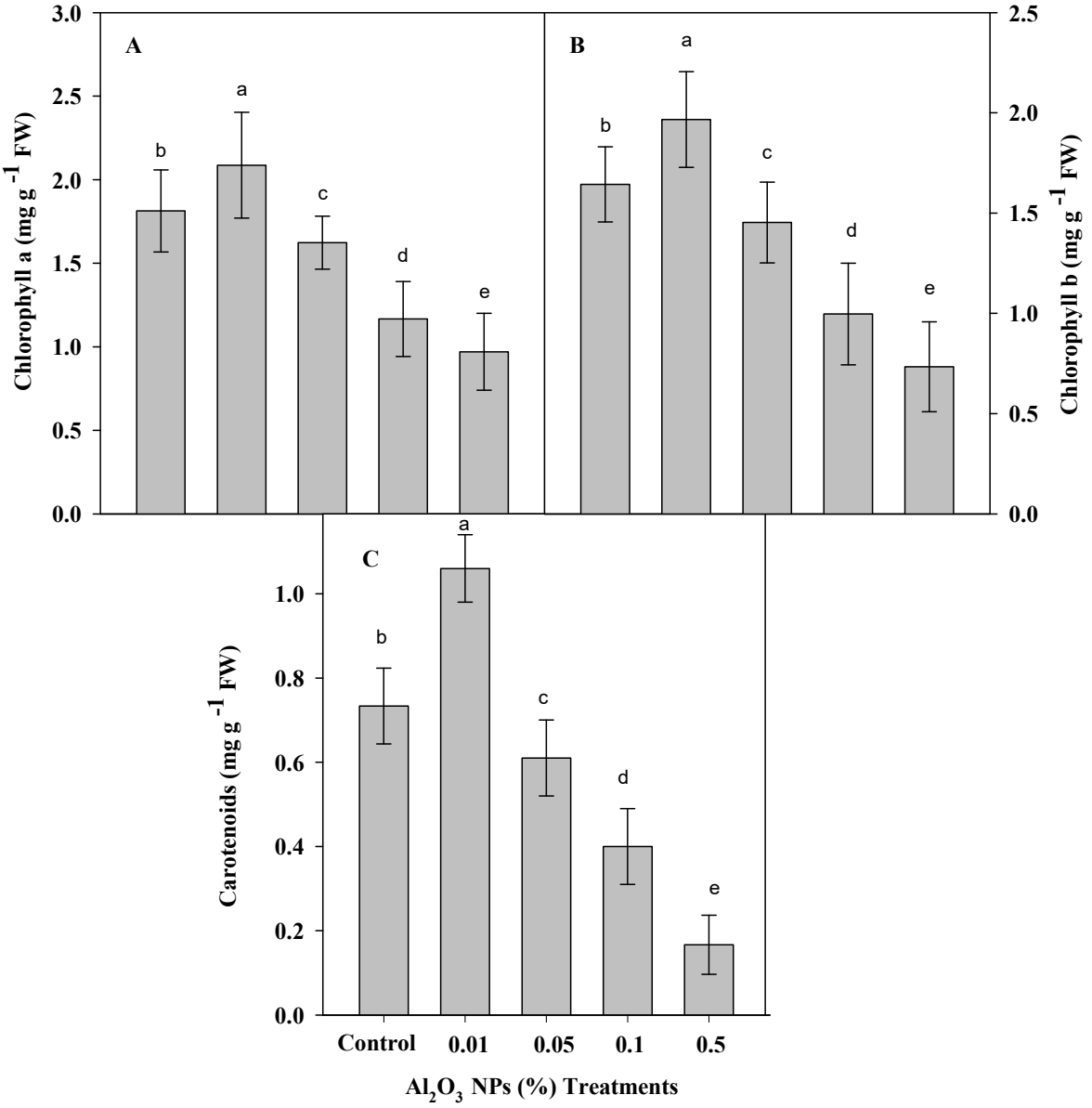

Figure 3. Effects of seed-priming with aluminum nanoparticles (Al_2O_3 NPs) on (**A**) chlorophyll a, (**B**) chlorophyll b, and (**C**) carotenoids content in roselle plants. Bars represent standard deviation (±SD) of the means (n = 3). Different letters indicate significant differences among the treatments at $p < 0.05$, according to Duncan's multiple range test.

3.3. Effect of Priming with Graded Levels of Al_2O_3 NPs on Organic Solutes

The concentration 0.01% Al_2O_3 NPs resulted in a significant increase in soluble sugar (18.09%), soluble protein (18.83%), total free amino acids (25.06%), and proline (36.67%) over control plants (Figure 4A–D). Seed-priming with 0.05, 0.1, and 0.5% Al_2O_3 NPs caused a marked diminution in organic solutes compared to control plants (Figure 4A–D). A decrease by 18.34, 48.87, and 66.93% in a soluble sugar was noted when plants were primed in 0.05, 0.1, and 0.5% Al_2O_3 NPs in comparison to control plants (Figure 4A). The increasing concentration of Al_2O_3 NPs also resulted in a significant decrease in soluble protein in a dose-dependent manner. A significant decrease by 12.16, 31.75, and 59.78%, respectively, was noticed with 0.05, 0.1, and 0.5% Al_2O_3 NPs application (Figure 4B). A similar significant decreasing trend in total free amino acids was also noticed with 0.05, 0.1, and 0.5% Al_2O_3 NPs, compared to the control plants (Figure 4C). The proline content increased by 36.67%, with 0.01% Al_2O_3 NPs. A significant decrease in proline content was recorded when plants were primed with other Al_2O_3 NPs doses; priming with 0.05, 0.1, and 0.5% Al_2O_3 NPs caused a dramatic decrease in proline content by 12.72, 55.90, and 69.54%, respectively, compared to control (Figure 4D).

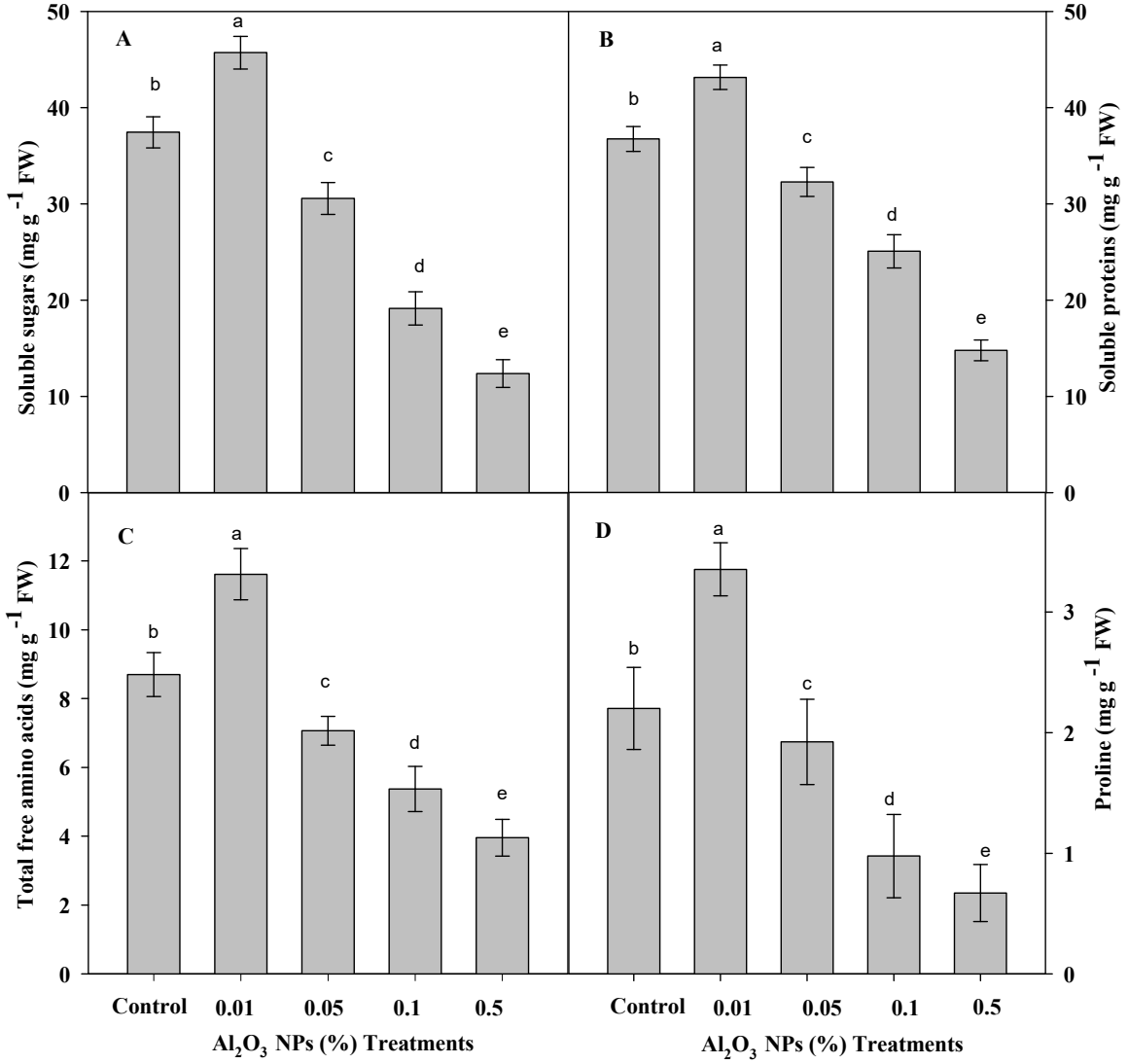

Figure 4. Effects of seed-priming with aluminum nanoparticles (Al_2O_3 NPs) on (**A**) soluble sugar, (**B**) soluble protein, (**C**) total free amino acid, and (**D**) proline in roselle plants. Bars represent standard deviation (±SD) of the means (n = 3). Different letters indicate significant differences among the treatments at $p < 0.05$, according to Duncan's multiple range test.

3.4. Effect of Priming with Al_2O_3 NPs Doses of Roselle Plants on MDA

Exposure of roselle plants to 0.01% Al_2O_3 NPs showed a significant decrease in MDA content by 18.74% over control plants (Figure 5). Priming with 0.05, 0.1, and 0.5% Al_2O_3 NPs caused a dramatic increase in MDA content by 30.11, 35.76, and 46.62%, respectively, compared to control (Figure 5).

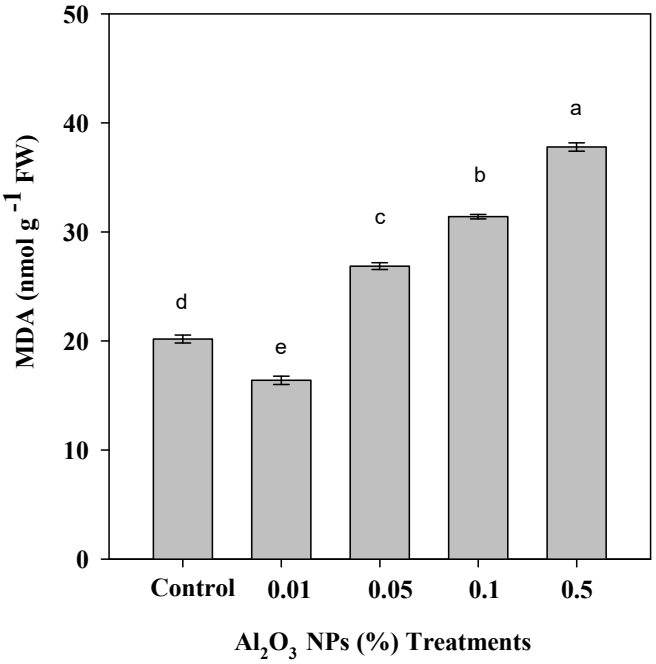

Figure 5. Effects of seed-priming with aluminum nanoparticles (Al_2O_3 NPs) on malondialdehyde content (MDA) in roselle plants. Bars represent standard deviation (±SD) of the means (n = 3). Different letters indicate significant differences among the treatments at $p < 0.05$, according to Duncan's multiple range test.

3.5. Effect of Al_2O_3 NPs Priming on Antioxidant Enzymes of Roselle Plants

The 0.01% Al_2O_3 NPs resulted in a significant increment in the activity of SOD, CAT, POD, and APX by 14.44, 27.40, 23.23, and 39.18%, respectively, over control. On the other hand, SOD activity decreased by 11.01, CAT by 8.87, POD by 12.39, and APX by 26.53%, respectively, in plants primed with 0.05% Al_2O_3 NPs with respect to control plants. Seed-priming with 0.1% Al_2O_3 NPs further decreased SOD activity by 16.28, CAT by 14.54, POD by 28.07, and APX by 37.77% as compared to control plants. A higher declining trend in the antioxidant enzymes' activity was noticed when plants were primed with 0.5% Al_2O_3 NPs. A significant decrease by 27.78, 33.94, 40.17, and 55.68%, respectively, was recorded in SOD, CAT, POD, and APX activities compared to control plants (Figure 6A–D).

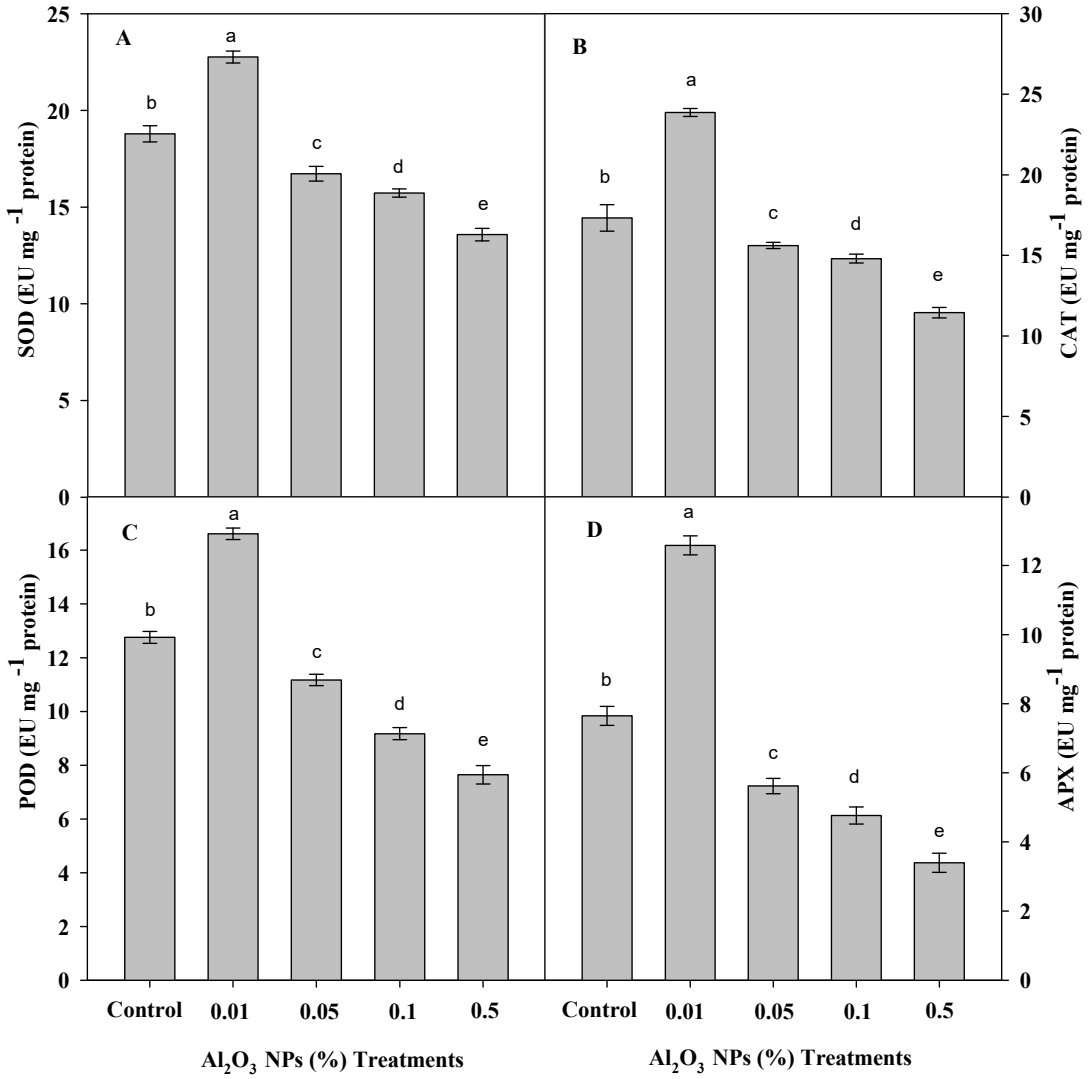

Figure 6. Effects of seed-priming with aluminum nanoparticles (Al_2O_3 NPs) on the activities of (**A**) superoxide dismutase (SOD), (**B**) catalase (CAT), (**C**) peroxidase (POD), and (**D**) ascorbate peroxidase (APX) in leaves of roselle plants. Bars represent standard deviation (±SD) of the means ($n = 3$). Different letters indicate significant differences among the treatments at $p < 0.05$, according to Duncan's multiple range test.

3.6. Understanding Interactions between Various Doses of Al_2O_3 NPs and Variables Through PCA-based Clustering Approach

A PCA was drawn to assess the maximum amount of data variability and to study the interaction between variables and doses of Al_2O_3 NPs treatments. The two principal components PC1 (66.80%) and PC2 (16.40%) explained a total of 83.2% overall data variability (Figure 7A). The loading plot of various variables indicated that growth, photosynthetic pigments, organic solutes, and antioxidants enzymes were positively correlated with each other and negatively with MDA content. The score plot of Figure 7B authenticated the grouping of 0.01, 0.05, 0.1, and 0.5% Al_2O_3 NPs treatments. The lower dose of 0.01% Al_2O_3 NPs was adjudged as the best treatment. Its effect was followed by that of control treatment. Both these treatments were clustered on left-hand rectangles of the score plot. The higher doses (0.05, 0.1, and 0.5%) of Al_2O_3 NPs posed negative impacts on roselle plants, and this was confirmed by the score plot. The highest dose 0.5% Al_2O_3 NPs induced maximum negative impacts on all studied variables, and this dose along with 0.1% were clustered in the negative component of the score plot (Figure 7B).

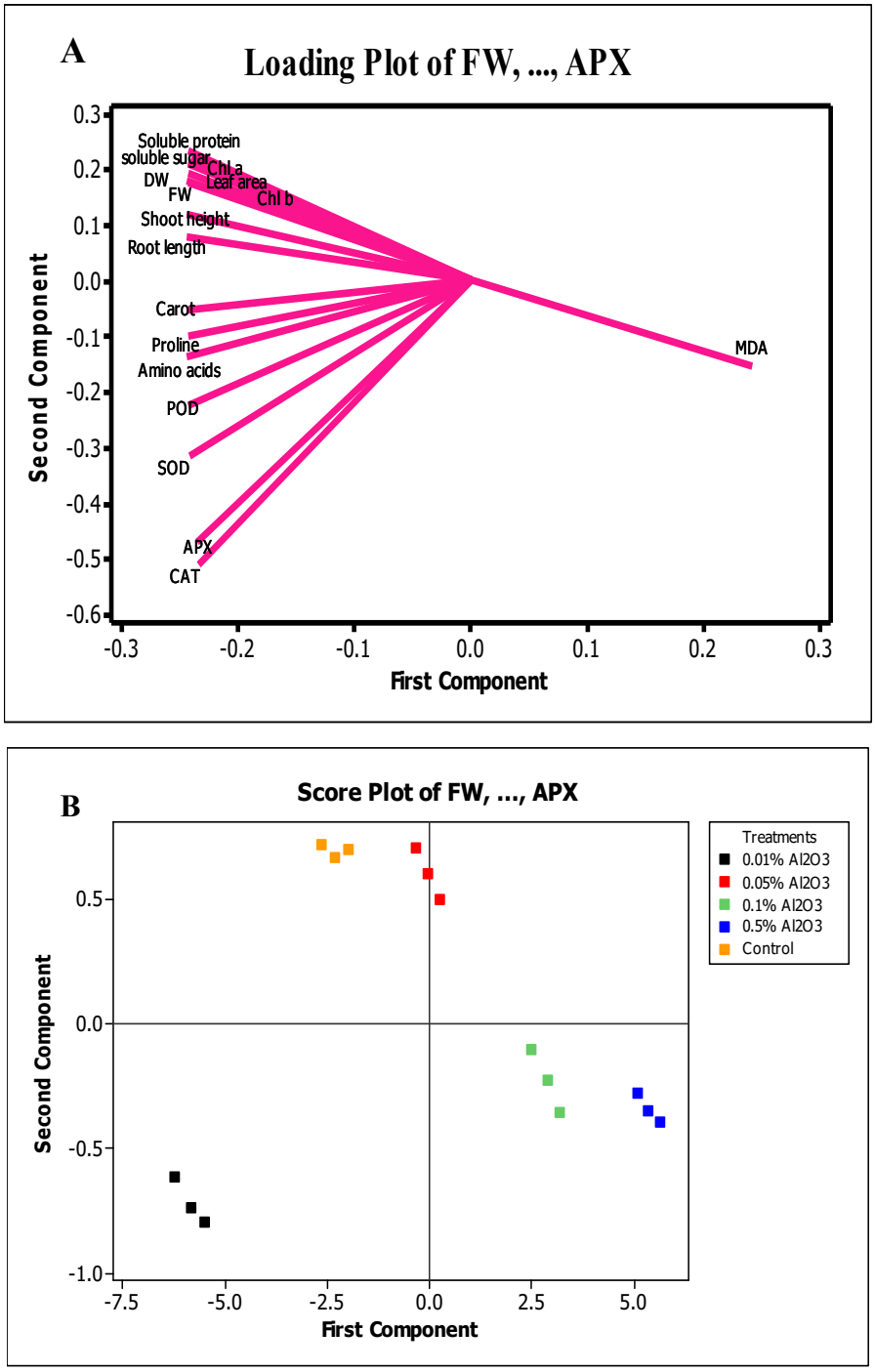

Figure 7. (**A**). Principal component analysis (PCA) to understand the variability relationships of parameters and treatments in roselle plants. The entire dataset was analyzed using a PCA clustering approach. The parameters included FW (fresh weight), DW (dry weight), root length, shoot height, leaf area, Chl a (chlorophyll a), Chl b (chlorophyll b), Carot (carotenoids), soluble sugars, protein, total free amino acids, proline, MDA (malondialdehyde), SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase), and POD (peroxidase). (**B**) The treatments' rectangles in different colors included (control) set was primed with distilled water for 12 h. The 2nd set was primed with 0.01% Al_2O_3 NPs for 12 h. The 3rd set was primed with 0.05% Al_2O_3 NPs for 12 h. The 4th set was primed with 0.1% Al_2O_3 NPs for 12 h. The 5th set was primed with 0.5% Al_2O_3 NPs for 12 h.

4. Discussion

Significant work has been carried out on the role of various NPs in different crop plants to unravel various physiological and molecular effects, including their synergistic and antagonistic effects individually. However, not many reports are currently available about the cumulative role of Al_2O_3 NPs as a 'growth-promoting' or 'growth-inducing' in Egyptian roselle plants. Therefore, the purpose of this experiment was to evaluate the potentiality of Al_2O_3 NPs-induced changes in roselle cultivar via the seed priming method. Seed-priming with 0.01% Al_2O_3 NPs positively affected all the growth traits by inducing growth-promoting effects, while other doses (0.05, 0.1, and 0.5%) retarded these traits in a concentration-dependent manner (Figure 2A–D). Al_2O_3 NPs provide Al^{2+} ions, an important plant micronutrient, thus fulfilling nutritional requirements, which enhanced plant growth traits like fresh and dry weight, length of root and shoot, and leaf area in the present study. The application of Al has increased plant growth traits in tea [41]. Exposure of plants to Al at lower concentrations has earlier been reported to accelerate the growth traits, while the higher concentration has limited the same [42]. Similar growth-promoting activity by Al_2O_3 NPs has also been reported in tobacco plants [43]. The concentration of 98 µM Al_2O_3 NPs has stimulated the root growth of *Arabidopsis thaliana* [44]. The growth retarding activity at higher doses in the present study could be attributed to the fact that Al_2O_3 NPs mediate the release of excess Al^{2+} ions. These ions induce a negative effect on macronutrients like Ca, Mg, and K, nitrogenous compounds, and activity of various enzymes [45]. Al_2O_3 NPs-mediated growth inhibition is also due to cellular damage via oxidative stress, DNA fragmentation, lignin accumulation, and callose deposition [46], which was in conformity with our results as higher doses of Al_2O_3 NPs decreased growth traits. A similar growth inhibitory effect on microalgae (*Scenedesmus* sp. and *Chlorella* sp.) by Al_2O_3 NPs has been reported [47]. Yang and Watts also reported root elongation inhibition effect of 13-nm-sized Al_2O_3 NPs (2 mg/mL) in five plant species—*Zea mays*, *Cucumis sativus*, *Glycine max*, *Brassica oleracea*, and *Daucus carota* [48].

The decrement in photosynthetic pigments of roselle cultivar under the higher doses of Al_2O_3 NPs was in full conformity with the results of Sadiq et al., who reported a clear decrease of chlorophyll contents. This decrease could be attributed to the dissolution of Al_2O_3 NPs into Al^{2+} ions inside plant cells [47]. Griffitt et al. also reported that the dissolution of nano alumina was responsible for their toxic effects. Priming with Al_2O_3 NPs at higher concentrations resulted in a significant decrease in photosynthetic pigments in roselle plants (Figure 3A–C). This reduction has reached its maximum in plants primed with 0.5% Al_2O_3 NPs. Massive Al accumulation has been reported in a series of cellular toxic activities, including ROS uncontrolled production in plants, thus affecting cell division and cell expansion and production of photo-assimilatory substrates [49–53]. The increased ROS biosynthesis in plants disrupts normal vital activities in plants [54]. Excess ROS due to Al stress inside plant tissues damages cellular structures concerned with photosynthesis [55], and thus significant decrease was observed in photosynthetic pigments (Figure 3A–C). A similar decrease in chlorophyll a, b and total carotenoids contents by 50 mg mL^{-1} Al_2O_3 NPs has been reported in wheat [56]. In contrast, the dose of 0.01% Al_2O_3 NPs increased photosynthetic pigment constituents. This could be due to the beneficial effects of Al on roselle plants. There are also reports indicate that Al can increase chlorophyll, carotenoids, proline, cysteine, sugars, endogenous hormone levels, and metabolites of the Shikimic acid pathway in diverse crop plants [57–60].

In order to nullify the abiotic pressure-induced negative effects, plants activate antioxidant defense machinery, which, among others, is characterized by the enhanced accumulation of different osmolytic cytosolutes having orchestrated antioxidant capacity [54,61–64].The accumulated solutes bestow myriad benefits to plants, including cell turgor or osmotic balance maintenance, stabilization of bio-membranes to prevent their peroxidation, leakage of electrolytes, and scavenging overproduced ROS to regain oxidative signaling state lost due to oxidative burst [65–67]. Examination in this study showed that soluble sugars, proteins, total free amino acids, and proline were reduced in plants primed with 0.05, 0.1, and 0.5% Al_2O_3 NPs (Figure 4A–D), and this could be correlated from the less leaf area of roselle plants and photosynthetic pigment contents (Figures 2D and 3), observed under the same level

of Al_2O_3 NPs doses, which, in turn, could reduce net photosynthetic rate, as a consequence leading to reduced production of overall metabolites, thus causing negative effects in the tested plants. However, plants primed with 0.01% Al_2O_3 NPs, on the other hand, showed a marked increment in the levels of solutes, thereby aiding roselle plants to maintain the balance of water and minerals and, consequently, sustained growth. A dose-dependent increase in proline content in wheat plants has been noticed with the Al_2O_3 NPs supply [56], which was in agreement with our presented results. Nevertheless, Al-mediated significant increase in proline and soluble contents has been reported also in Fagopyrum species [53], which further authenticated our results.

The peroxidation of lipid membranes is a biomarker of the destruction of biological membranes under abiotic stress pressures [68,69]. MDA content is used as one of the oxidative stress markers and is an end product of oxidation of polyunsaturated fatty acids [62,70,71]. There was high MDA content when plants were primed with 0.05, 0.1, and 0.5% Al_2O_3 NPs, suggesting that Al_2O_3 NPs, and probably Al, induced the destruction of the cellular membranes, channel proteins, and lipids. In consistent with our results—an increase in lipid peroxidation under Al_2O_3 NPs—treatments to *Allium cepa* has been noticed, and the increment has been even greater than that observed with bulk Al_2O_3 alone [72]. A similar increment in MDA content has been reported in the plant cell suspension culture model with various doses of Al_2O_3 NPs [73]. Similarly, Al-mediated increased lipid peroxidation rate has been reported [53,74]. The application of 0.01% Al_2O_3 NPs significantly reduced the MDA content (Figure 5), thus showing the ameliorating capacity in reducing MDA content with respect to other doses. Results of Li et al. also showed that the presence of Al_2O_3 NPs reduced the Cu-induced toxicity towards *Scenedesmus obliquus* [75].

Plants possess versatile detoxification machinery to balance ROS biosynthesis, which consists of different enzymatic and non-enzymatic antioxidants. SOD, CAT, POD, and APX constitute the principal enzymatic antioxidant proteins in plants. The high significant activity of these enzymes under 0.01% Al_2O_3 NPs priming represented the ability of roselle plants to adapt to excess ROS. Therefore, the up-regulated activities of these antioxidants could be attributed to the modified defense system of the plants. The activated antioxidants' activity, in turn, also lowered down MDA levels (Figure 5). A similar increase in antioxidant activities has been reported by Al_2O_3 application in wheat plants [46,56]. On the other hand, the decrease in the activities of antioxidant enzymes by increasing levels of Al_2O_3 NPs priming in the present study might be due to the low ROS biosynthesis coupled with increased ROS scavenging rate by other antioxidants (Figure 6), and the values were even below control plants. This suggested the inefficiency of these in scavenging the ROS.

In order to authenticate our findings regarding the assessment of various Al_2O_3 NPs dose-priming in roselle plants, the entire data was subjected to a PCA-based clustering method (Figure 7A,B). Figure 7A suggests the effects of various doses of Al_2O_3 NPs on parameter studies. The growth traits, photosynthetic pigments, antioxidants, and osmolytes were strongly and positively correlated with one another and negatively with MDA content. Score plot grouped various Al_2O_3 NPs doses and showed the concentration-dependent effect on roselle plants. This figure clearly suggested that 0.01% Al_2O_3 NPs dose was one of the best doses in modulating all parameter studies as this was clustered in a left rectangle of the first component, whereas the doses of 0.1 and 0.5% Al_2O_3 NPs were clustered on the right-hand side of the first component.

5. Conclusions

The field of nano-biotechnology can offer various pros and cons in different sectors, of which agriculture is important. The use of NPs in different sectors is increasing nowadays. However, the dual effect of Al-based NPs has received little attention so far as their positive and negative aspects are concerned. Moreover, the priming method with NPs to improve growth and biochemical traits in crop plants has also gained momentum. Therefore, from the present study, it can be inferred that Al_2O_3 NPs have a dual effect on the growth and physiology of the test crop. A low dose of Al_2O_3 NPs imposes positive, whereas high doses show inhibitory effect. Therefore, priming plants with doses of

Al_2O_3 NPs can be suggested as a potential tool to induce positive changes in roselle and perhaps other crop plants.

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