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Research Article

Mitigating Cadmium-Induced Stress in Chickpea (*Cicer Arietinum L.*) Through 5-Aminolevulinic Acid: Effects on Growth, Water Status, And Photosynthetic Performance

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Abstract

Cadmium (Cd) is a highly toxic, non-essential heavy metal that poses a serious threat to crop productivity, particularly in soil-based cultivation systems. This study evaluated the potential of 5-Aminolevulinic Acid (ALA), a known plant growth regulator, to reduce the adverse effects of cadmium stress in chickpea (*Cicer arietinum L.*). Chickpea plants were exposed to cadmium stress with and without ALA application, and key morphological and physiological traits were assessed at the vegetative (50 days after sowing) and reproductive (115 days after sowing) stages. Cadmium treatment led to pronounced reductions in shoot and root growth, lowered relative leaf water content, and increased electrolyte leakage, reflecting impaired water relations and membrane damage. In contrast, application of ALA improved plant growth, enhanced leaf water status, and reduced membrane injury under cadmium stress. In addition, ALA application restored chlorophyll and carotenoid contents that were otherwise diminished by cadmium exposure. The results demonstrate that ALA plays an effective role in improving cadmium stress tolerance in chickpea by supporting water balance, protecting membrane integrity, and maintaining photosynthetic pigment levels.

Keywords: 5-aminolevulinic acid, Cadmium stress, Legumes, Water deficit, Photosynthesis

Introduction

Modern pollutants such as heavy metals, pesticides, petroleum residues, and other hazardous chemicals have become widespread in water, soil, and air because of rapid urbanization and industrial growth [1]. Heavy metals are commonly described as elements with a density greater than 5 g cm^{-3} and are grouped into essential and non-essential types [2]. Among them, Cadmium (Cd) has emerged as a major concern in agricultural systems because it does not degrade and severely affects plant growth and productivity [3]. Cadmium interferes with plant physiological, biochemical, and molecular processes, impairing growth from germination to reproduction [4,5]. In addition to its harmful effects on plants, Cd accumulation in edible crops poses serious risks to human health, as it can build up in organs such as the liver, kidneys, lungs, bones, and gastrointestinal tract, contributing to several chronic diseases [6,7].

Chickpea (*Cicer arietinum L.*), also known as gram or Bengal gram, is a self-pollinating, diploid legume belonging to the Fabaceae family and is one of the most important pulse crops worldwide [8,9]. Originating in the Fertile Crescent, chickpea is now cultivated extensively, with global production exceeding 15 million tonnes, making it the third most important pulse crop. India is the largest producer, followed by countries including Pakistan, Canada, Australia, and the United States [10]. Despite its economic and nutritional importance, chickpea is particularly sensitive to heavy metal stress, especially cadmium, which can adversely affect plant growth, yield, and grain quality when grown in contaminated soils [11,12].

5-Aminolevulinic Acid (ALA) is a naturally occurring compound in plants and a key precursor in the tetrapyrrole biosynthesis pathway, playing an important role in chlorophyll formation and stress regulation [13,14]. At low concentrations, externally applied ALA enhances photosynthesis, biomass production, and overall plant performance, whereas excessive doses may induce oxidative damage [15]. Under cadmium stress, ALA helps protect plants by maintaining membrane stability, preserving chloroplast structure, and enhancing antioxidant activity, thereby reducing oxidative injury [16]. Based on these roles, ALA was considered a potential regulator for alleviating cadmium toxicity in chickpea. Its effects were examined through various growth and physiological parameters, including photosynthetic pigment content, shoot and root length, biomass accumulation, electrolyte leakage, and relative leaf water content.

Material and Methods

Chickpea seeds (*Cicer arietinum* L. var. PBG-8) were obtained from Punjab Agricultural University, Ludhiana, India. Uniform and healthy seeds were surface sterilized with 0.01% mercuric chloride, thoroughly washed with distilled water, and soaked overnight in a concentrated *Rhizobium* inoculum mixed with activated charcoal. The experiment was carried out in earthen pots containing approximately 5 kg of washed river sand. To ensure adequate drainage, the pots were lined with perforated polythene sheets.

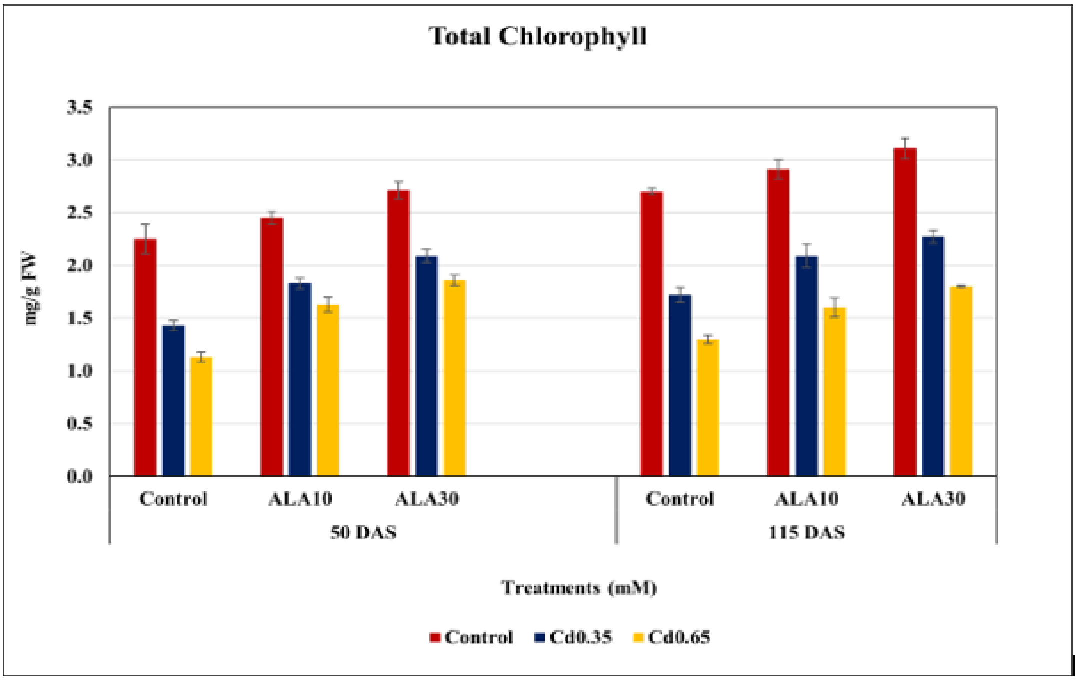
After germination, seedlings were thinned to retain five healthy plants per pot and grown under natural conditions in a dome-shaped outdoor structure. Cadmium was applied as $CdSO_4$ at concentrations of 0.35 and 0.65 mM, while 5-Aminolevulinic Acid (ALA) was supplied at 10 and 30 mg L^{-1}, both individually and in combination at 20-day intervals. All treated plants received a standard nutrient solution, whereas control plants were provided only with water and nutrients. Leaf samples were collected at the vegetative (50 DAS) and reproductive (115 DAS) stages for analysis of chlorophyll, carotenoids [17]; electrolyte leakage [18] and relative leaf water content [19] using standard methods. Root and shoot length as well as fresh biomass were also measured at the time of sampling.

Statistical Analysis

The observations made from single sample were obtained in triplicate and expressed as mean ± SE (Standard Error). Data was analyzed statistically using one-way ANOVA in SPSS-27 with a 5% probability level. The post-hoc Least Significant Difference (LSD) test has been used.

Observations and Results

Chlorophyll Content: At 50 Days After Sowing (DAS), exposure to cadmium markedly reduced the total chlorophyll content in chickpea plants, and the decline intensified with increasing Cd concentration. Plants treated with 0.35 mM Cd showed a reduction of about 37%, while those exposed to 0.65 mM Cd recorded nearly a 50% decrease compared with the control. In contrast, application of 5-Aminolevulinic Acid (ALA) alone resulted in a noticeable improvement in chlorophyll content, with greater enhancement observed at the higher dose. When ALA was supplied along with cadmium, the extent of chlorophyll loss was considerably reduced, indicating that ALA helped sustain pigment levels under metal stress. The mitigating effect was more pronounced at 30mg L^{-1} ALA, which substantially limited the decline in chlorophyll even under higher Cd exposure.



Figures 1: Effect of Cd stress alone, ALA alone and their combination on chlorophyll content in chickpea plants at 50 DAS. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =0.22). At 115 DAS each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =0.22).

A similar trend was observed at 115 DAS, where cadmium stress again caused a significant reduction in chlorophyll content relative to untreated plants, with stronger effects at the higher Cd level. Foliar application of ALA alone continued to promote chlorophyll accumulation at this later growth stage. Importantly, the combined application of ALA with cadmium effectively restrained chlorophyll degradation, particularly at the lower Cd concentration. Overall, plants receiving ALA alongside Cd maintained higher chlorophyll levels than those exposed to Cd alone, demonstrating the sustained protective influence of ALA on the photosynthetic pigment system during prolonged cadmium stress (Figure 1).

Carotenoid Content: At 50 Days After Sowing (DAS), cadmium exposure caused a clear decline in carotenoid content in chickpea leaves, and the effect increased with higher metal concentration. Plants treated with 0.35 mM Cd showed a moderate reduction, while those receiving 0.65 mM Cd exhibited a much sharper decrease compared with the control. In contrast, application of 5-Aminolevulinic Acid (ALA) alone resulted in improved carotenoid levels, particularly at the higher dose. When ALA was applied along with cadmium, the loss of carotenoids was noticeably reduced, indicating that ALA helped protect these pigments under metal stress. The higher ALA concentration was more effective in limiting carotenoid degradation, even under elevated Cd levels.

A similar response pattern was recorded at 115 DAS. Cadmium stress continued to suppress carotenoid content, with stronger reductions observed at the higher Cd concentration. Foliar treatment with ALA alone enhanced carotenoid accumulation at this later growth stage as well. More importantly, combined application of ALA and Cd substantially minimized pigment loss compared to Cd treatment alone. The protective effect of ALA was evident at both Cd levels, with greater stabilization of carotenoids observed at the higher ALA dose. Overall, these results indicate that ALA plays an important role in preserving carotenoid content in chickpea plants subjected to cadmium-induced stress (Figure 2).

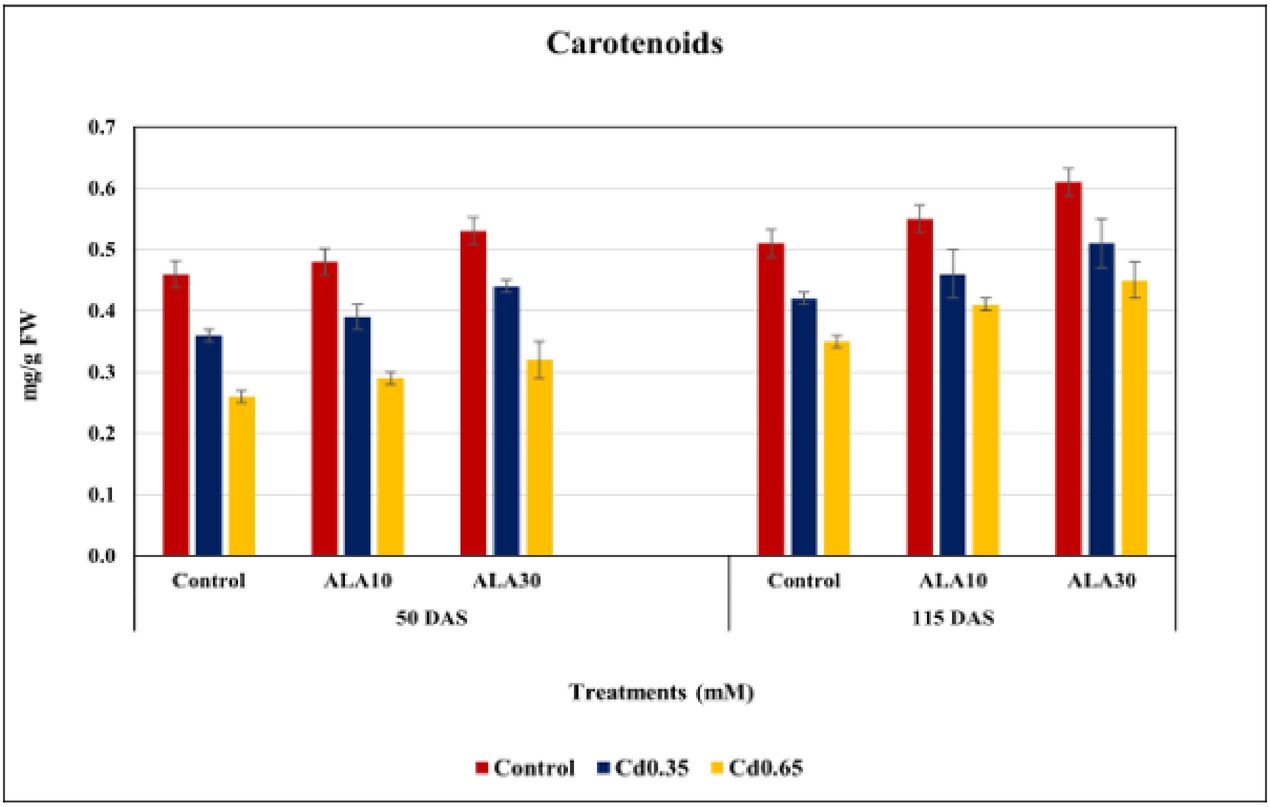


Figure 2: Effect of Cd stress alone, ALA alone and their combination on carotenoids in chickpea plants at 50 DAS. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =0.055). At 115 DAS each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =0.09).

Electrolyte Leakage: At 50 Days After Sowing (DAS), chickpea plants exposed to cadmium showed a pronounced increase in electrolyte leakage, reflecting damage to cell membrane integrity. The rise in leakage was moderate at 0.35 mM Cd and became much more pronounced at 0.65 mM Cd when compared with untreated plants. In contrast, plants receiving 5-Aminolevulinic Acid (ALA) alone exhibited lower electrolyte leakage, indicating better membrane stability, particularly at the higher ALA dose. When ALA was applied together with cadmium, the extent of membrane injury was noticeably reduced. This protective effect was stronger at 30 mg L^{-1} ALA, which effectively limited electrolyte loss even under higher cadmium stress.

At 115 DAS, cadmium stress continued to enhance electrolyte leakage, confirming sustained membrane damage at both Cd concentrations. However, foliar application of ALA alone consistently lowered electrolyte leakage, suggesting improved cellular protection at this later stage of growth. More importantly, the combined application of ALA and cadmium significantly restrained electrolyte loss compared with cadmium treatment alone. The reduction in leakage was more evident at the higher ALA concentration, highlighting its dose-dependent role in maintaining membrane integrity during prolonged exposure to cadmium stress (Figure 3).

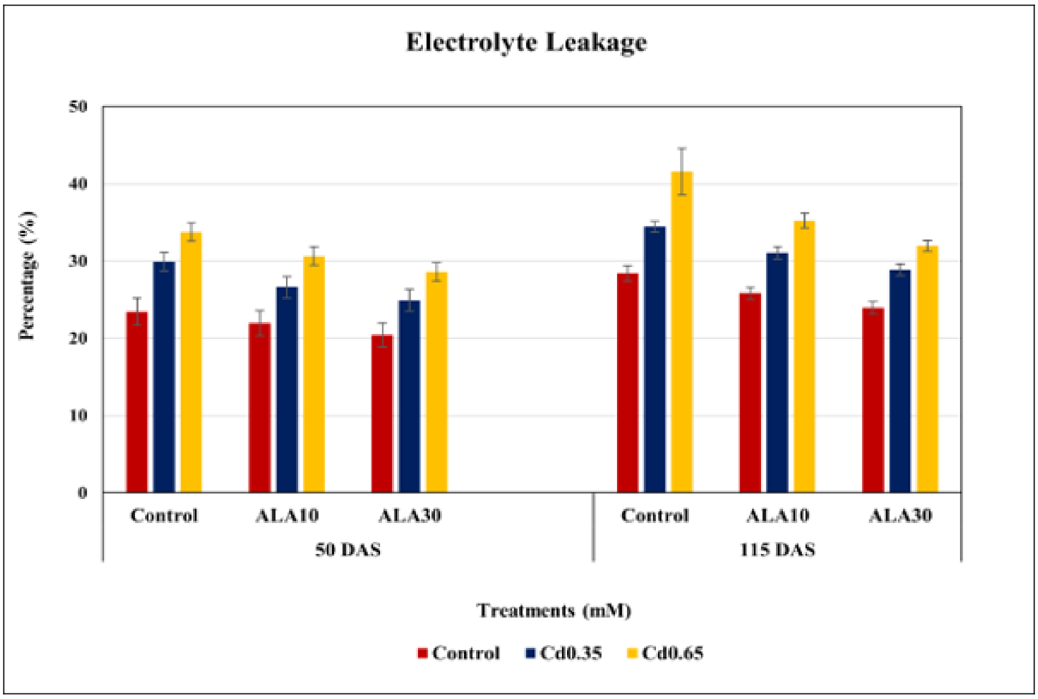


Figure 3: Effect of Cd stress alone, ALA alone and their combination on electrolyte leakage in chickpea plants at 50 DAS. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =4.16). At 115 DAS each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =4.42).

Relative Leaf Water Content: At 50 Days After Sowing (DAS), chickpea plants exposed to cadmium showed a clear decline in relative leaf water content, and the reduction became more pronounced as the cadmium level increased. This response suggests that cadmium adversely affected the plant's capacity to regulate water balance. In contrast, plants treated with 5-Aminolevulinic Acid (ALA) alone maintained slightly higher leaf water content, particularly at the higher application rate. When ALA was supplied together with cadmium, the loss of leaf water content was noticeably reduced, indicating that ALA helped plants cope with cadmium-induced water stress.

By 115 DAS, cadmium stress continued to negatively influence leaf water status, with a marked decrease observed under higher metal exposure. Foliar application of ALA alone remained effective in improving water retention at this growth stage. Moreover, plants receiving both ALA and cadmium maintained higher relative leaf water content than those treated with cadmium alone. The higher ALA dose was consistently more effective in preserving leaf hydration, highlighting its role in supporting cellular water balance and enhancing stress tolerance during prolonged cadmium exposure (Figure 4).

Shoot and Root Length: Cadmium exposure resulted in a clear suppression of shoot growth in chickpea plants, and the extent of reduction increased with higher Cd concentration. Plants treated with 0.35 mM Cd showed a moderate decline in shoot length, while a much stronger inhibition was observed at 0.65 mM Cd compared with the control. In contrast, foliar application of 5-Aminolevulinic Acid (ALA) alone markedly promoted shoot elongation, with the higher dose producing greater improvement. When ALA was applied along with cadmium, the negative effect of Cd on shoot growth was substantially reduced. At the higher ALA level, shoot length was effectively restored and even exceeded that of the control under lower Cd stress, demonstrating the strong growth-promoting and protective influence of ALA (Figure 5).

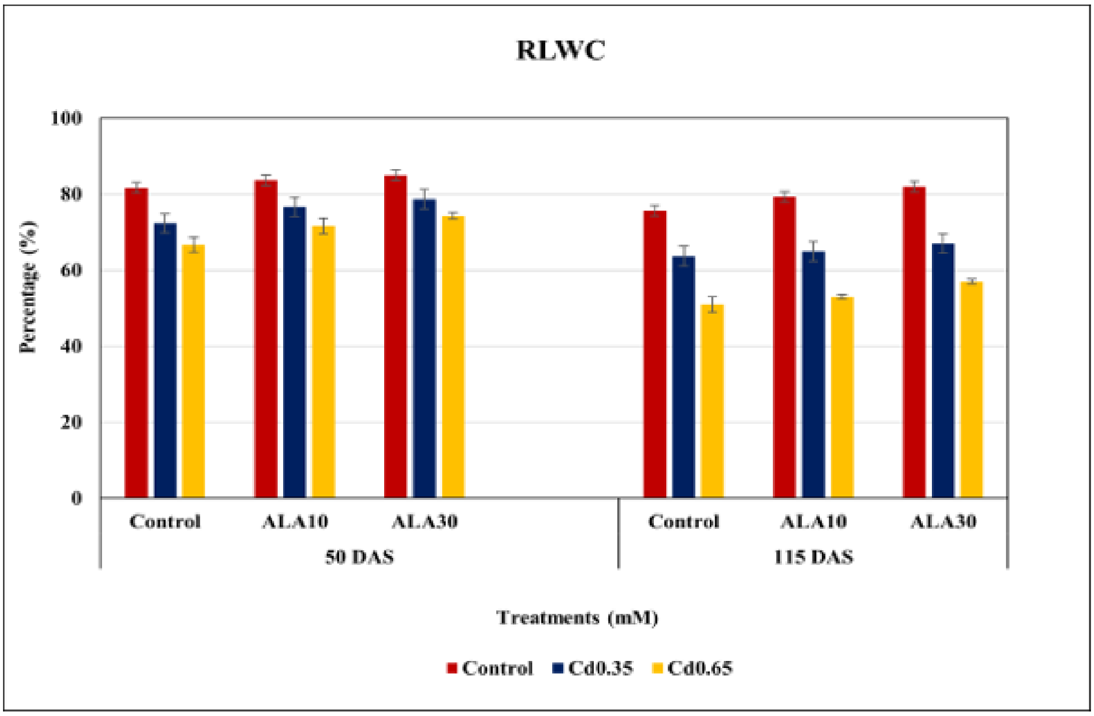


Figure 4: Effect of Cd stress alone, ALA alone, and their combination on RLWC in chickpea plants at 50 DAS. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =4.63). At 115 DAS each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =3.81).

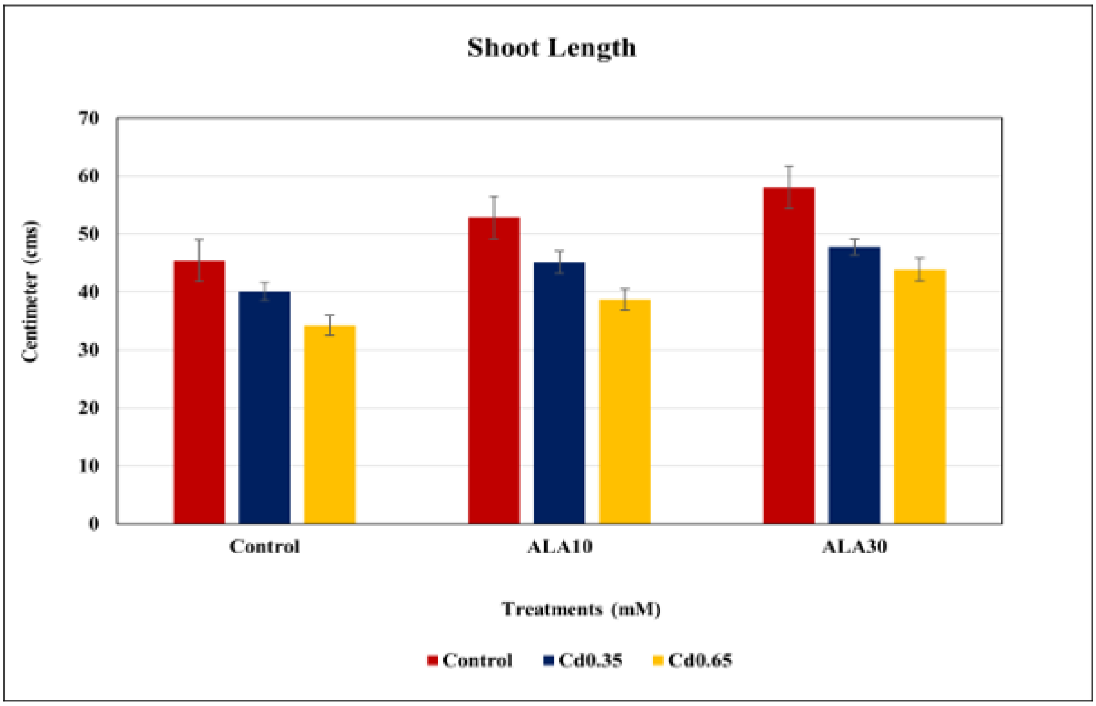


Figure 5: Effect of Cd stress alone, ALA alone, and their combination on shoot length in chickpea plants. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =5.84).

Root growth was also adversely affected by cadmium, with pronounced reductions observed under both Cd concentrations, particularly at the higher level. However, plants treated with ALA alone exhibited enhanced root development, and this response was more evident at 30 mg L^{-1}. Combined application of ALA with cadmium significantly alleviated Cd-induced inhibition of root elongation. The reduction in root length was progressively minimized with increasing ALA concentration, indicating a dose-dependent protective effect. Overall, these observations suggest that ALA plays an important role in supporting both shoot and root growth of chickpea plants under cadmium stress (Figure 6).

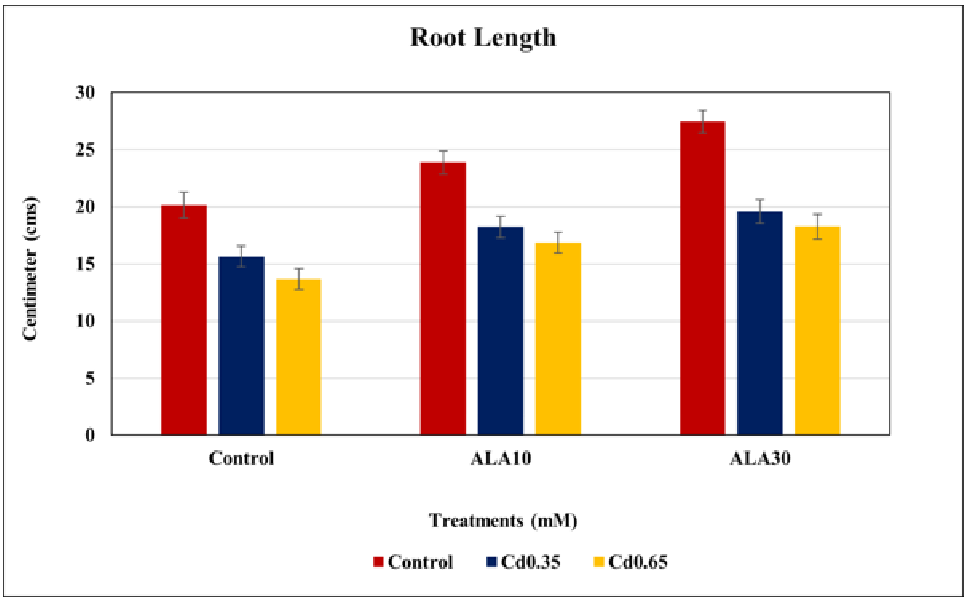


Figure 6: Effect of Cd stress alone, ALA alone, and their combination on root length in chickpea plants. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =5.13).

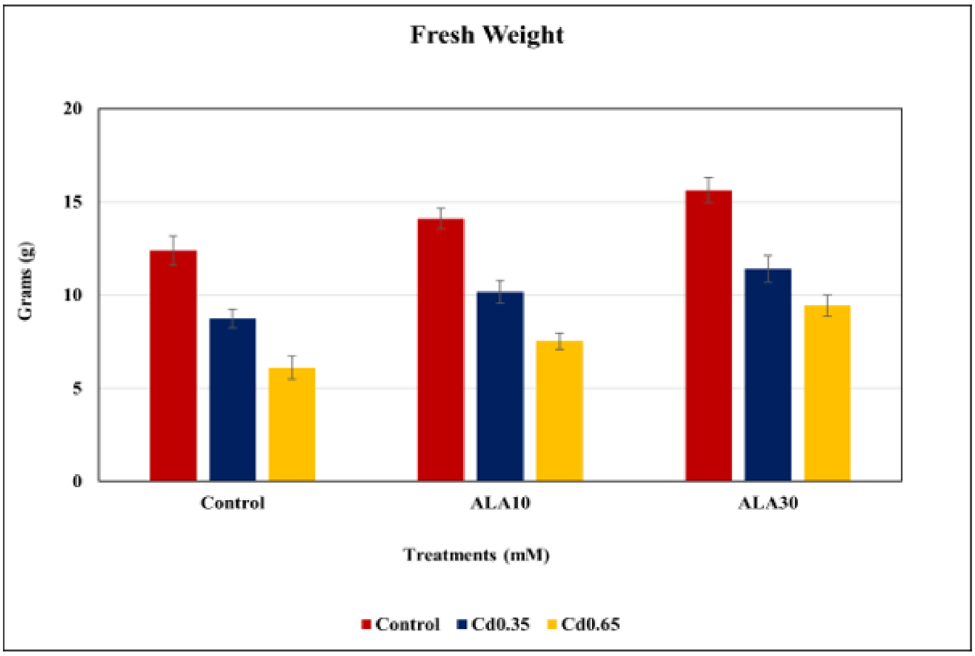


Figure 7: Effect of Cd stress alone, ALA alone, and their combination on fresh weight in chickpea plants. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =1.82).

Fresh and Dry Weight: Cadmium (Cd^{2+}) stress significantly reduced the fresh weight of chickpea plants, with values declining to 29.57% and 50.81% under 0.35 mM and 0.65 mM Cd treatments, respectively, compared to control plants. In contrast, exogenous application of 5-Aminolevulinic Acid (ALA) alone enhanced fresh biomass accumulation, showing increases of 13.71% at 10 mg/L and 26.08% at 30 mg/L relative to the control. When ALA was co-applied with Cd, the inhibitory effects on fresh weight were notably alleviated. Under 0.35 mM Cd, fresh weight reduction was mitigated to 18.01% with 10 mg/L ALA and further to 8.06% with 30 mg/L ALA. Similarly, at 0.65 mM Cd, the reduction was lessened to 50.81% and 23.92% with 10 mg/L and 30 mg/L ALA, respectively. These results underscore the protective role of ALA in counteracting Cd-induced biomass loss in a dose-dependent manner (Figure 7).

Cadmium (Cd^{2+}) exposure significantly reduced the dry weight of chickpea plants, with declines of 36.36% and 57.02% observed under 0.35 mM and 0.65 mM Cd treatments, respectively, compared to control plants. In contrast, exogenous application of 5-Aminolevulinic Acid (ALA) alone enhanced dry biomass accumulation, resulting in increases of 16.53% at 10 mg/L and 28.93% at 30 mg/L relative to the control. When ALA was co-applied with Cd, the negative impact on dry weight was notably alleviated. Under 0.35 mM Cd, dry weight improved to 21.49% and 41.32% with 10 mg/L and 30 mg/L ALA, respectively. Similarly, at 0.65 mM Cd, dry weight increased to 7.44% and 28.10% with corresponding ALA treatments. These results highlight the dose-dependent ameliorative effect of ALA in mitigating Cd-induced biomass reduction (Figure 8).

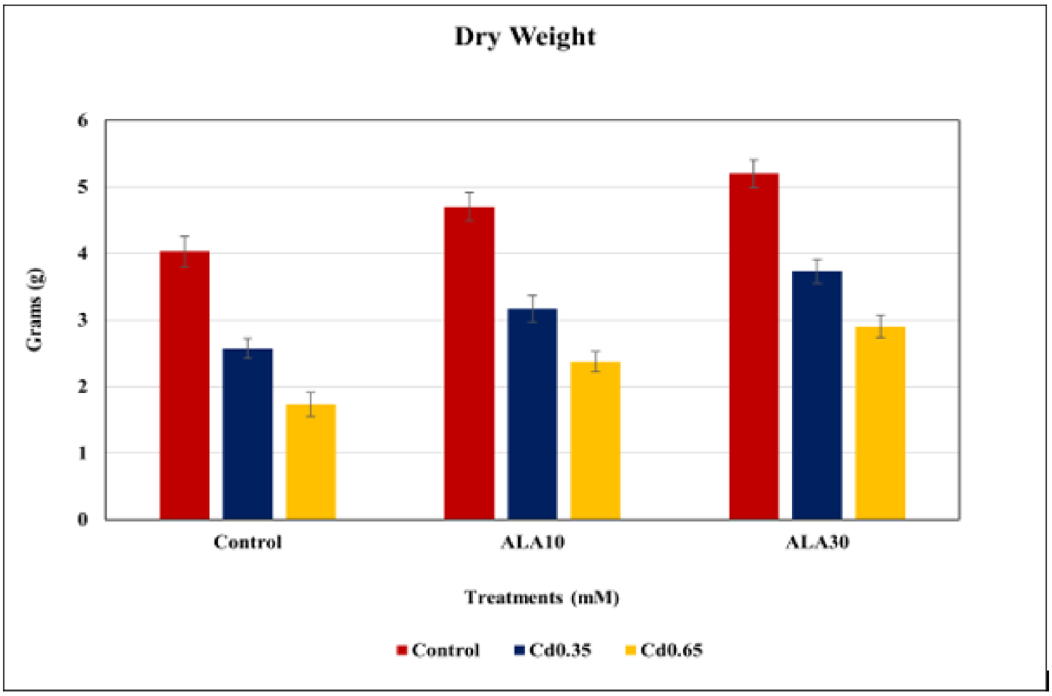


Figure 8: Effect of Cd stress alone, ALA alone, and their combination on dry weight in chickpea plants. Each value represents the mean ± SE of three replicates. The mean difference is significant at P≤0.05 (LSD 0.05 =0.56).

Discussion

Heavy metal stress, particularly due to cadmium, is widely recognized as a serious constraint on plant growth and productivity because it disrupts normal physiological, biochemical, and metabolic processes [20-23]. In the present study, exposure of chickpea plants to cadmium resulted in a marked increase in electrolyte leakage, indicating damage to cellular membranes, along with significant reductions in shoot and root length, relative leaf water content, and photosynthetic pigments such as chlorophyll and carotenoids. These responses suggest impairment of membrane integrity, water relations, and photosynthetic activity under Cd stress. Similar effects of cadmium toxicity have been reported earlier, where disturbances in cellular turgor, osmotic regulation, and enzyme functioning ultimately led to suppressed plant growth and physiological performance [24,25].

The application of 5-Aminolevulinic Acid (ALA) substantially alleviated the adverse effects of cadmium stress in chickpea. Plants treated with ALA in combination with Cd exhibited improved shoot and root growth compared to those subjected to Cd alone, indicating that ALA supports cell expansion and growth under stress conditions. As a key precursor in tetrapyrrole biosynthesis, ALA contributes to chlorophyll formation and indirectly supports photosynthetic efficiency and hormonal regulation, which explains its growth-promoting role under metal stress, as also observed in other plant species [26-29]. Cadmium-induced reduction in relative leaf water content was markedly improved by ALA application, reflecting enhanced water retention and stress tolerance, possibly through improved osmolyte accumulation and activation of antioxidant enzymes [30,31]. Moreover, the reduction in electrolyte leakage in ALA-treated plants suggests improved membrane stability, likely due to lowered lipid peroxidation and a strengthened antioxidant defence system [32,33]. Cadmium stress also caused a decline in chlorophyll and carotenoid content, indicating disruption of the photosynthetic apparatus [34-36] however, ALA application restored pigment levels at both vegetative and reproductive stages, consistent with its role in chlorophyll biosynthesis and protection of pigments from oxidative damage [37,38]. Although cadmium affected both growth stages, the reproductive phase appeared more sensitive, while the beneficial effects of ALA were slightly more pronounced during the vegetative stage, likely due to greater physiological flexibility during early growth. Overall, the results support the potential use of ALA as an effective plant growth regulator for reducing cadmium toxicity and enhancing stress tolerance in chickpea and other leguminous crops.

Conclusion

Cadmium stress markedly impaired chickpea growth by adversely affecting water status, membrane integrity, and photosynthetic pigment composition. The application of 5-aminolevulinic acid helped counter these effects by improving relative leaf water content, maintaining chlorophyll and carotenoid levels, and reducing electrolyte leakage. Overall, ALA proved effective in alleviating cadmium-induced damage and supporting better physiological performance in chickpea plants.

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Conflict of Interest

The authors declare no conflict of interest in this research article.

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