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Protective role of gallic acid against cadmium-induced toxicity in snapdragon (*Antirrhinum majus* L.)

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Abstract
This study aims to evaluate the anatomical adaptive strategies of *Antirrhinum majus* under cadmium (Cd) toxicity in the presence of exogenous gallic acid (GA). The experiment was designed as a CRD (completely randomized design) to assess the morphological and anatomical traits under different concentrations of Cd toxicity (0 mM, 20 mM, 40 mM, 60 mM, and 80 mM) along with two levels of GA (10 mM and 20 mM). It was hypothesized that *A. majus* developed structural adaptations to overcome Cd stress with exogenous GA. Morpho-anatomical attributes of *A. majus* were negatively affected due to Cd toxicity by disrupting nutrient uptake, inducing oxidative damage, inhibiting photosynthesis, and altering tissue structure. The results of this study showed that at 80 mM Cd, root length was reduced by 44%, and a 22% decline was assessed in shoot length. A decrease was observed in plant height by 57%, leaf area by 42%, and dry weight by 77% in the 80 mM Cd compared to the control group. Gallic acid at 10 mM improved growth attributes under Cd stress, and 20 mM GA offered the maximum resistance. Plant height, leaf area, and biomass were improved, and they followed similar trends. Anatomical parameters were also adversely affected under Cd stress. At 80 mM Cd, maximum reductions were noted in the thicknesses of epidermis (36%), cortex (22%), xylem (12%), phloem (79%), and pith (14%) compared to the control group. Conclusively, Cd stress alone severely impaired snapdragon's morphology and anatomical structures, whereas 20 mM gallic acid application mitigated these adverse effects.

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Introduction

Plants in their natural environment are constantly subjected to biotic and abiotic stressors. Human activities such as the discharge of industrial waste, fertilizer activities, and the dumping of amalgam and sewage contain long-term deposition of heavy metals in soil, such as Cu, Ni, Co, Cd, Hg, and As (Nawaz et al., 2023). The specific heavy metals that are dangerous for plants are mainly cadmium (Hameeda et al., 2024). Cadmium (Cd) is a heavy metal that is toxic and has negative effects on the growth and development of plants. Cadmium toxicity in different plants depends on different factors, including the concentration and the exposure period, the stage of plant development, and the plant genotype (Anwar et al., 2024). Cadmium stress decreases seed sprouting and decreases root and shoot growth. It disrupts photosynthesis, respiration, and nutrient uptake, leading to oxidative stress, proteins, and cell walls, ultimately causing cell death (Ismael et al., 2019). Plant exposure to heavy metals causes chlorosis, necrosis, leaf deformation, decreased pollen

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viability and germination, and abnormal seed development (Vasilaci et al., 2023). Cadmium is a toxic metal that harms plants by reducing growth, altering physiological attributes, and causing cell damage. The heavy metal toxicity reduces growth by interfering with cell division and elongation, inhibiting root growth, and limiting nutrient intake and water absorption (Li et al., 2023). Cadmium harms the physiological and metabolic processes of plants by modifying gene expression, affecting growth, and impacting stress response (Deng et al., 2025). It is critical to reduce Cd pollution in soil and water as a developing remediation solution (Shiyu et al., 2020).

A variety of approaches are being exploited these days to mitigate the impact of heavy metals on plants; one such approach is the exogenous application of different growth regulators that can mitigate the hazardous effects of heavy metal toxicity (Yang et al., 2024). Of such bioregulators, gallic acid (GA) is being exploited as a potential plant growth regulator (Xu et al., 2024). Gallic acid has been reported to effectively mitigate the effects of heavy metals on plants (Saidi et al., 2021). Gallic acid is used in the food, dye, and pigment industries as an inhibitor and antioxidant; it possesses anti-inflammatory, anti-bacterial, anti-cancer, and antioxidant properties (Harwansh et al., 2024; Xiang et al., 2024).

Heavy metal stress (especially Cd) induces structural changes in roots and shoots that reduce plant function and growth (Hu et al., 2025; Song et al., 2025). For example, roots often show thickened and lignified endodermal and exodermal cell walls, reduced xylem vessel diameter, fewer and smaller vascular bundles, and increased deposition of barrier materials (like suberin or lignin) in cell walls (Enstone et al., 2002). These changes help limit metal translocation but also reduce water and nutrient flow, leading to stunting, leaf loss, and reduced biomass (Pandey et al., 2022; Mohamed et al., 2025).

Antirrhinum majus (Snapdragon) was used as a model for biochemical and developmental genetics (Farooq et al., 2025). It has long been used in medicine as a diuretic, to treat liver problems, tumors, scurvy, and as an astringent and detergent (Al-Sanfi, 2015). Snapdragon contains 2.79–5.69% free amino acids, 2.15–4.69% soluble carbohydrates, and carotenoids 0.22 to 0.27% (Azam et al., 2025). Snapdragon also contains anthocyanidins, flavanols, flavones, aurones, flavanones, and cinnamic acids, as well as a good source of natural antioxidants (Kumar, 2022). Snapdragon seeds contain a range of neutral lipids, glycolipids, and phospholipids, making them a good source of fixed oil that can be used as a substitute for olive oil in meals and cooking (Kiymaz and Acemi, 2024).

Limited studies explored the adaptability of ornamental plants, such as *Antirrhinum majus*, to cadmium (Cd) contamination and how this stress could be mitigated through the foliar application of gallic acid (GA). Thus, this study aimed to explore the effects of cadmium stress on morpho-anatomical features of *A. majus* and to assess the role of GA in mitigating Cd-induced stress.

Materials and Methods

Plant material and experimental design

The present research was conducted in the research area of the Islamia University of Bahawalpur, during the winter season. The research involved *Antirrhinum majus* L., a popular winter annual plant. The seedlings of snapdragon were purchased from a local nursery in Bahawalpur and planted in polythene pots filled with 5 kg of canal soil. The soil was kept moist for five days, under a completely randomized design (CRD). Pots were treated with $CdCl_2$ at 0, 20, 40, 60, and 80 mM. Gallic acid concentrations of 10 and 20 mM were applied exogenously under control and Cd stress conditions. There were five replications. Each pot received 100 mL of Cd solution and 10 mL of GA solution via foliar spray (Anwar et al., 2024)

Morphological analysis

Different morphological attributes of *Antirrhinum majus* were studied. Root length (cm), shoot length (cm), plant height (cm), and leaf area (cm^2) were studied by the manual scale method. Fresh weight (g) and dry weight (g) were determined using a digital weighing balance. The number of leaves and the number of branches were counted manually.

Anatomical analysis

For anatomical research, samples were obtained from the mid-portion of the lamina, the internodal base of the main tiller, the thickest root present at the junction of the main stem and root, and stem samples from the main tiller's third internode. Tissue samples were preserved immediately following receipt for 24 hours in a FAA solution of formaldehyde (5%), glacial acetic acid (5%),

distilled water (35%), and ethanol (70%) by volume). Tissue samples were placed in a solution with 75% ethyl alcohol by volume and 25% acetic acid for long-term preservation. Permanent slides were made by applying various grades of ethyl alcohol for dehydration, and fixed samples were manually sectioned and doubly stained with safranine andfast green. A phone camera was used to capture digital photographs of preserved slides. The following anatomical traits of the roots (epidermis thickness, cortex thickness, endodermis thickness, xylem thickness, phloem thickness, epidermis cell area, cortex cell area, metaxylem cell area, and pericycle cell area), stems (epidermis thickness, cortex thickness, hypodermal thickness, hypodermal cell area, xylem thickness, phloem thickness, epidermal cell area, collenchyma cell area, endodermis thickness, metaxylem cell area, and protoxylem cell area.), and leaves (midrib thickness, epidermal thickness, cortex thickness, spongy mesophyll thickness, palisade mesophyll thickness, xylem thickness, phloem thickness, lamina thickness, collenchyma cell area, epidermal cell area, metaxylem cell area, and phloem cell area) were noted.

Statistical analysis

All data were analyzed using One-Way-ANOVA with Statistix 8.1 software (Muhammad et al., 2025). The least significant difference (LSD) test was used for pairwise comparisons of means to determine treatment significance at $p \leq 0.05$. The cost-effectiveness of each treatment was also computed to assess additional benefits. Microsoft Excel was used for data alignment and data representation by bar graphs.

Results

Morphological attributes

Cadmium stress significantly ($p \leq 0.05$) reduced morphological attributes of *Antirrhinum majus* (**Figures 1-4**). Root and shoot lengths were decreased under Cd stress. The maximum reductions were observed at 80 mM Cd by 44% and 22%, respectively. Gallic acid mitigated this toxicity at 80 mM Cd; plants with 10 mM GA showed slighter reductions in root length by 6% and 7% in shoot length, while 20 mM GA provided even better protection with 6% and 14% decreases. Plant height and leaf area followed the same trend. The greater reduction was assessed in plant height and leaf area by 57% and 42%, respectively, at Cd 80 mM. Supplementation with 10 mM GA reduced these reductions (43% and 55%), while 20 mM GA showed stronger mitigation (52% and 53%). Fresh weight and dry weight also declined with Cd toxicity, with maximum losses at 80 mM Cd by 77% dry weight reduction and 57% fresh weight, respectively. In contrast, plants with 80 mM Cd + 10 mM GA showed comparatively lower reductions (54% dry weight and 50% fresh weight), while 20 mM GA-treated plants still performed better than Cd alone, though decreases remained high (61% dry weight and 54% fresh weight). Similarly, the number of leaves (NOL) and branches (NOB) dropped sharply at 80 mM Cd, 45% reduction in the number of leaves and complete absence of branches. With GA, reductions were mitigated: 80 mM Cd + 10 mM GA reduced NOL and NOB by 88% and 82%, while 80 mM Cd + 20 mM GA plants showed slightly smaller reductions (94% and 73%). Overall, 80 mM Cd severely impaired growth, but GA, particularly at 20 mM, improved morphological parameters, partially mitigating Cd-induced toxicity even at the highest stress level.

Anatomical attributes

Stem

There were significant changes observed in the stem traits of snapdragon under Cd concentrations alone and with GA (**Table 1**; **Figures 1-4**). The maximum reduction in stem anatomical parameters was observed at 80 mM Cd by 36–97%, while minimal reductions occurred at 20 mM Cd. Exogenous GA mitigated these effects by enhancing epidermal and cortical thickness. The results also showed that the hypodermal and endodermal thickness decreased by 97% and 91%, respectively, at 80 mM Cd. With the greatest protection at 80 mM Cd + 20 mM GA, GA reversed this drop, raising hypodermal thickness (HT) and endodermis thickness (EnT) by up to 20% and 40%, respectively. When compared to Cd alone, combined GA levels at 80 mM Cd improved HT and EnT, although the reductions were still significant. Similarly, xylem (XT) and phloem thickness (PT) decreased with Cd stress, most severely at 80 mM Cd (12% in XT, 79% in PT). GA enhanced all three parameters, with 20 mM GA showing greater improvements than 10 mM, even at 80 mM Cd. Cell areas of epidermis, collenchyma, hypodermis, endodermis, metaxylem, and protoxylem also declined, with maximum losses at 80 mM Cd (up to an average 98%). GA application reduced these effects, with 20 mM GA

markedly improving cell areas across tissues. At 80 mM Cd, GA-treated plants maintained higher values than Cd alone, although reductions were still considerable. Overall, increasing Cd severely impaired stem anatomy, while GA, particularly at 20 mM, partially restored tissue thickness and cell areas, even under 80 mM Cd stress.

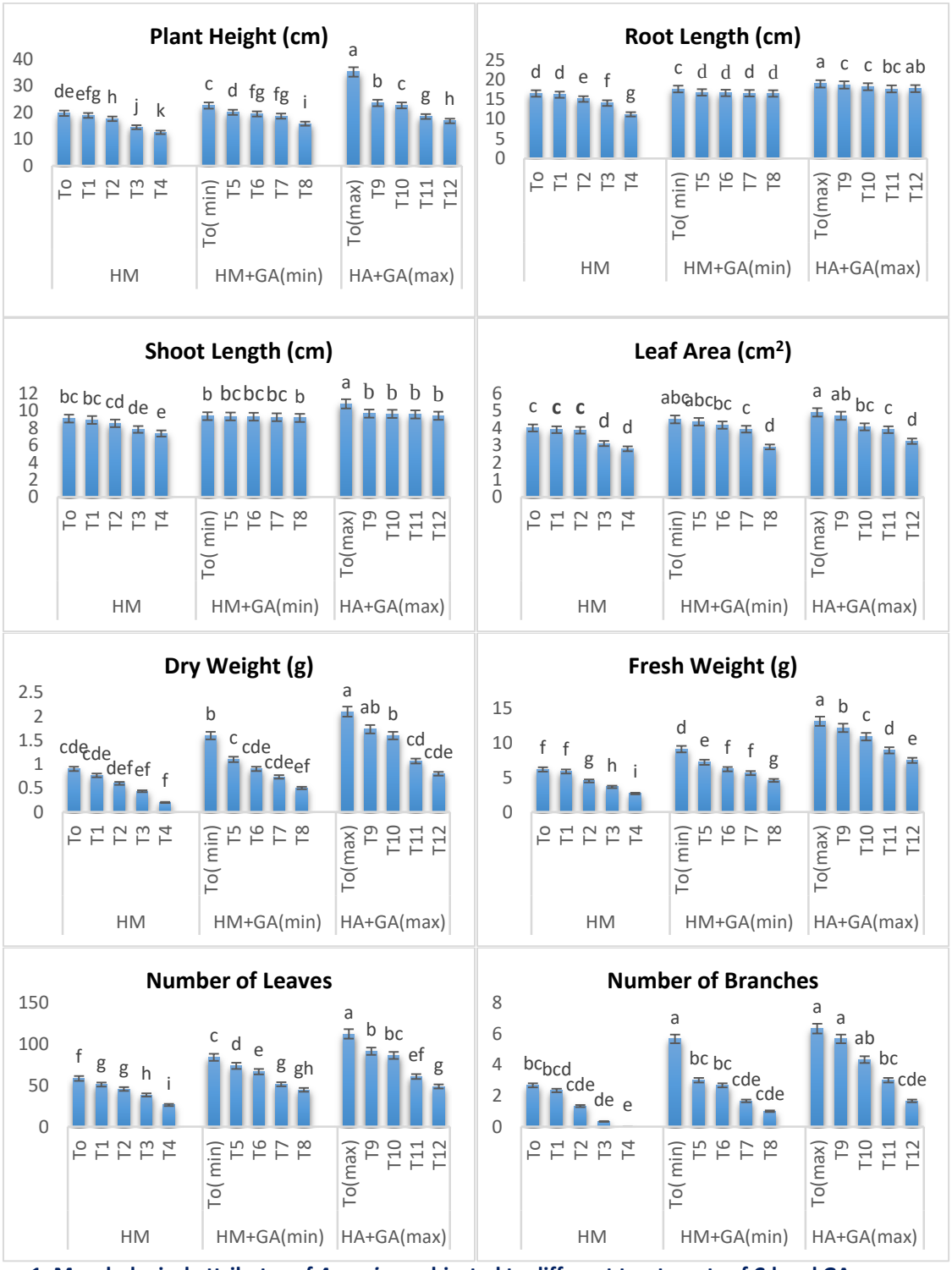


Figure 1: Morphological attributes of *A. majus*, subjected to different treatments of Cd and GA.
The graph represents means (±SE) and treatments represented as T0 = Control, T1 = Cd 20 mM, T2 = Cd 40 mM, T3 = Cd 60 mM, T4 = Cd 80 mM, T0 $_{(min)}$ = GA 10 mM, T5 = Cd 20 mM + GA 10 mM, T6 = Cd 40 mM + 10 mM, T7 = Cd 60 mM + GA 10 mM, T8 = Cd 80 mM + GA 10 mM, T0 $_{(max)}$ = GA 20 mM, T9 = Cd 20 mM + GA 20 mM, T10 = Cd 40 mM + GA 20 mM, T11 = Cd 60 mM + GA 20 mM, T12 = Cd 80 mM + GA 20 mM

Table 1: Stem anatomical parameters of *A. majus*, subjected to different levels of Cd and GA.

Treatments	ET (µm)	CT (µm)	HT (µm)	HCA (cm^2)	XT (µm)	PT (µm)
T0	0.2bcd	1.69cde	0.29de	1.33cde	2.34abc	0.68de
T1	0.19cd	1.68de	0.19h	1.28def	2.26bcd	0.61ef
T2	0.17d	1.47f	0.11i	1.2efgh	2.23d	0.52fg
T3	0.16d	1.46f	0.11i	1.11gh	2.14ef	0.45g
T4	0.12d	1.38g	0.1i	0.85h	2.09f	0.29h
$T0_{(min)}$	0.22abc	1.78b	0.35c	1.45abc	2.36a	0.79bc
T5	0.21bcd	1.72cde	0.32cd	1.37bcd	2.35ab	0.74cd
T6	0.19cd	1.69cde	0.29cde	1.29def	2.34bcd	0.66de
T7	0.19d	1.68de	0.26efg	1.17fgh	2.21de	0.54fg
T8	0.19d	1.69e	0.21gh	1.05h	2.13ef	0.49g
$T0_{(max)}$	0.25a	1.91a	0.44a	1.59a	2.39a	0.92a
T9	0.23ab	1.79b	0.39ab	1.49ab	2.38a	0.92a
T10	0.23bcd	1.82b	0.38bc	1.38bcd	2.37a	0.86ab
T11	0.22d	1.74bc	0.27def	1.25defg	2.26ed	0.68de
T12	0.21d	1.72bcd	0.25fgh	1.2efg	2.24d	0.59ef
Treatments	**ECA (cm^2)**	**CCA (cm^2)**	**ENCA (cm^2)**	**MCA (cm^2)**	**PCA (cm^2)**	
T0	1.54c	2.35a	1.39cd	0.96d	0.55b	
T1	1.5bc	1.91b	1.32de	0.89d	0.46bc	
T2	1.26e	1.73c	1.26e	0.742e	0.35de	
T3	1.14f	1.41de	1.15f	0.61fg	0.27efg	
T4	0.88g	1.3e	0.99h	0.58g	0.2g	
$T0_{(min)}$	1.69b	2.2a	1.5b	1.15ab	0.65a	
T5	1.67b	2.1a	1.41bc	0.95cd	0.55b	
T6	1.55c	2.2a	1.39cd	0.87d	0.54b	
T7	1.24e	2.6d	1.24f	0.7ef	0.39cd	
T8	1.13f	2.5de	1.03gh	0.66efg	0.24fg	
$T0_{(max)}$	1.89a	2.4a	1.59a	1.23a	0.72a	
T9	1.71b	2.4a	1.48b	1.21a	0.65a	
T10	1.64b	2.3a	1.39cd	1.14ab	0.51b	
T11	1.36d	2.4b	1.26e	1.06bc	0.41cd	
T12	1.15f	2.4d	1.09fg	0.73e	0.32def	

The table represented as T0 = Control, T1 = Cd 20 mM, T2 = Cd 40 mM, T3 = Cd 60 mM, T4 = Cd 80 mM, $T0_{(min)}$ = GA 10 mM, T5 = Cd 20 mM + GA 10 mM, T6 = Cd 40mM + 10 mM, T7 = Cd 60 mM + GA 10 mM, T8 = Cd 80 mM + GA 10 mM, $T0_{(max)}$ = GA 20 mM, T9 = Cd 20 mM + GA 20 mM, T10 = Cd 40 mM + GA 20 mM, T11 = Cd 60 mM + GA 20 mM, T12 = Cd 80 mM + GA 20 mM.

Parameters of the stem are represented as ET = Epidermis thickness, CT = Cortex thickness, HT = Hypodermal thickness, HCA = Hypodermal cell area, XT = Xylem thickness, PT = Phloem thickness, ECA = Epidermal cell area, CCA = Collenchyma cell area, EnT = Endodermis thickness, MCA = Metaxylem cell area, ProCA = Protoxylem cell area. Means following different alphabets differ significantly at $p < 0.05$.

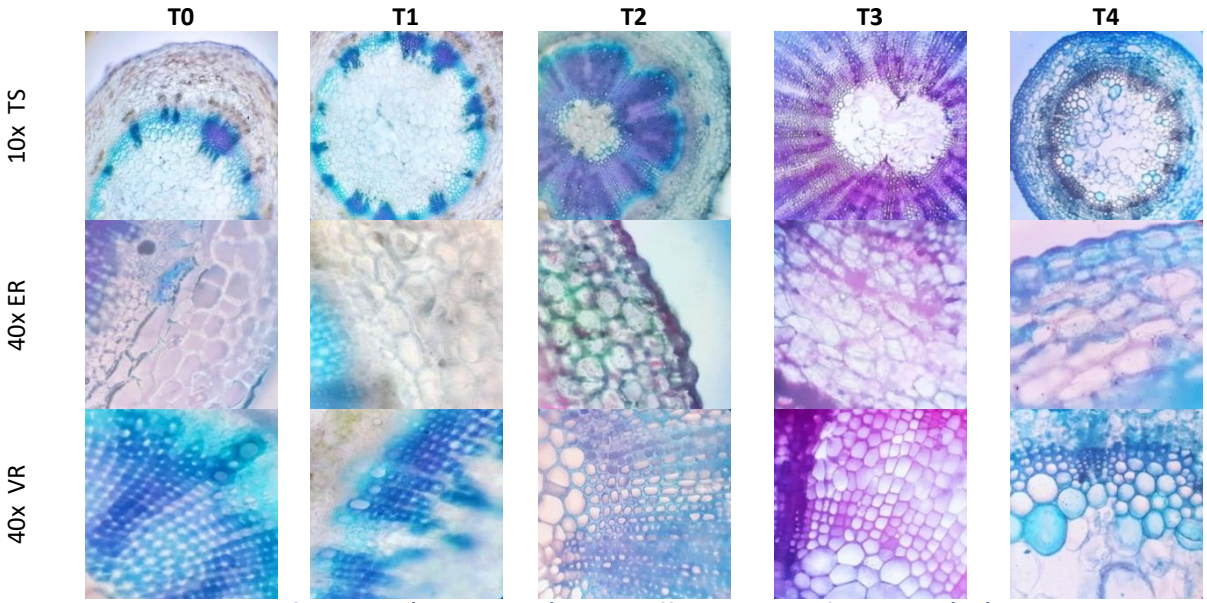


Figure 2: Stem Anatomy of *A. majus* (Snapdragon) under different levels of cadmium (Cd) stress. Treatments represented as T0 (Control), T1 (Cd 20 mM), T2 (Cd 40 mM), T3 (Cd 60 mM), and T4 (Cd 80 mM).

TS = Transverse section; ER = Epidermal region; VR = Vascular region

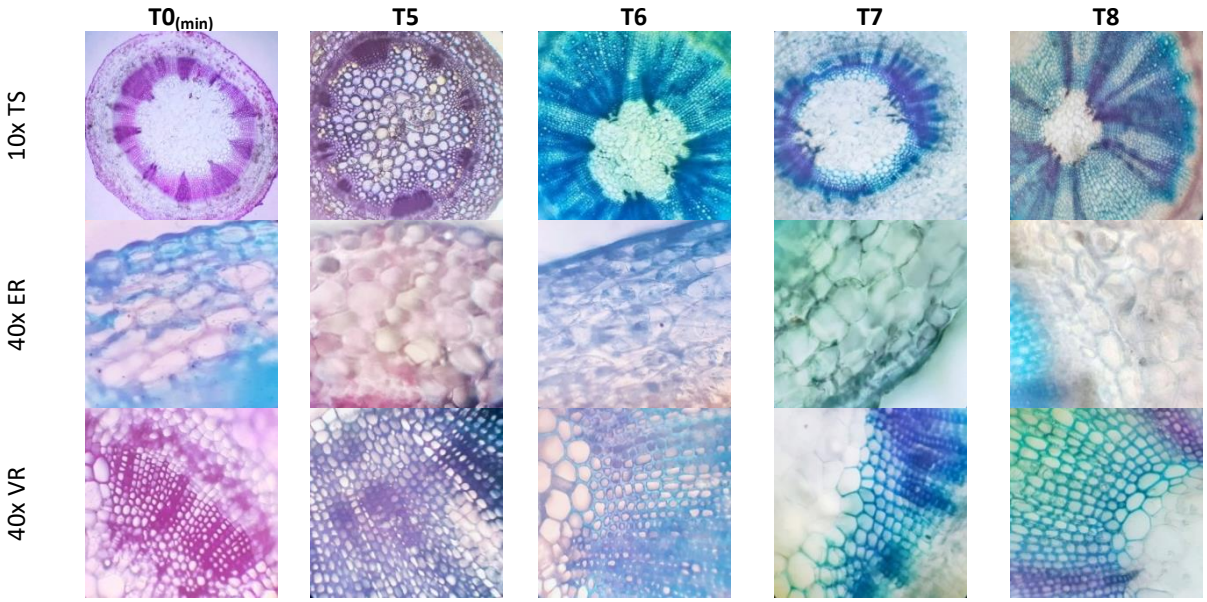


Figure 3: Stem anatomy of *A. majus* under different Cd and GA levels.
Treatments represented as $T0_{(min)}$ (GA 10 mM), T5 (Cd 20 mM + GA 10 mM), T6 (Cd 40 mM + GA 10 mM), T7 (Cd 60 mM + GA 10 mM), T8 (Cd 80 mM + GA 10 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

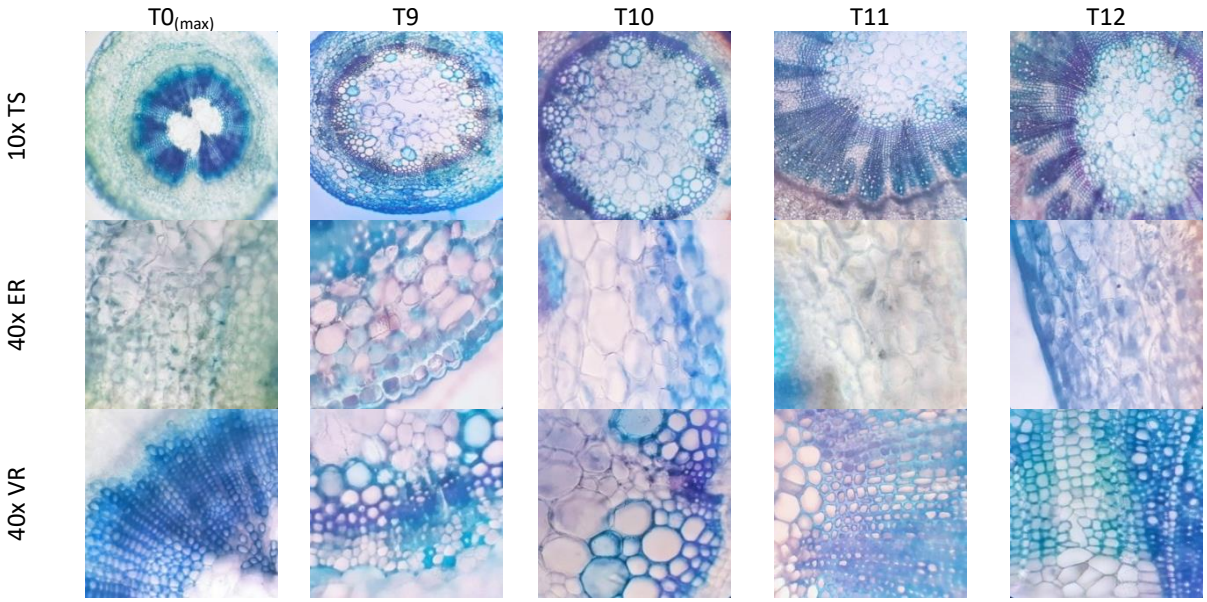


Figure 4: Stem anatomy of *A. majus* (Snapdragon) under different cadmium (Cd) and gallic acid (GA) levels.
Treatments represented as $T0_{(max)}$ (GA 20 mM), T5 (Cd 20 mM + GA 20 mM), T6 (Cd 40 mM + GA 20 mM), T7 (Cd 60 mM + GA 20 mM), T8 (Cd 80 mM + GA 20 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

Leaf

Cadmium stress significantly reduced leaf anatomical traits (**Figures 5-8**). At 80 mM Cd, maximum decreases were observed in midrib thickness (3%), epidermal thickness (38%), cortical thickness (2%), spongy mesophyll (17%), and palisade mesophyll (24%) compared to the control. While minimal reductions occurred at 20 mM Cd. GA mitigated these effects, enhancing tissue thickness. In contrast, plants treated with 20 mM Cd and 20 mM gallic acid (GA) (T9) showed the minimum reduction. Similarly, Cd stress significantly reduced cell areas, with the greatest reductions observed at 80 mM Cd for epidermal cell area (58%), collenchyma cell area (8%), metaxylem cell area (82%), and phloem cell area (76%). The smallest decreases occurred at Cd 20 mM. Gallic acid treatments improved these parameters, where 10 mM and 20 mM GA enhanced epidermis cell area by 19% and 9%, collenchyma cell area by 2%, metaxylem cell area by 19% and 39%, and phloem cell area by 32%, respectively. Among combined treatments, 20 mM Cd + 10 mM GA minimized reductions, while 80 mM Cd + 20 mM GA showed comparatively higher reductions in these parameters of the leaf. Overall, the application of gallic acid, particularly at 20 mM, effectively mitigated Cd-induced damage, maintaining greater stem thickness and cell area under high Cd stress.



Figure 5a: Different leaf anatomical parameters of *A. majus*, subjected to different levels of Cd and GA.
The graph represents means (±SE) and treatments represented as T0 = Control, T1 = Cd 20 mM, T2 = Cd 40 mM, T3 = Cd 60 mM, T4 = Cd 80 mM, T0 $_{(min)}$ = GA 10 mM, T5 = Cd 20 mM + GA 10 mM, T6 = Cd 40 mM + 10 mM, T7 = Cd 60 mM + GA 10 mM, T8 = Cd 80 mM + GA 10 mM, T0 $_{(max)}$ = GA 20 mM, T9 = Cd 20 mM + GA 20 mM, T10 = Cd 40 mM + GA 20 mM, T11 = Cd 60 mM + GA 20 mM, T12 = Cd 80 mM + GA 20 mM.

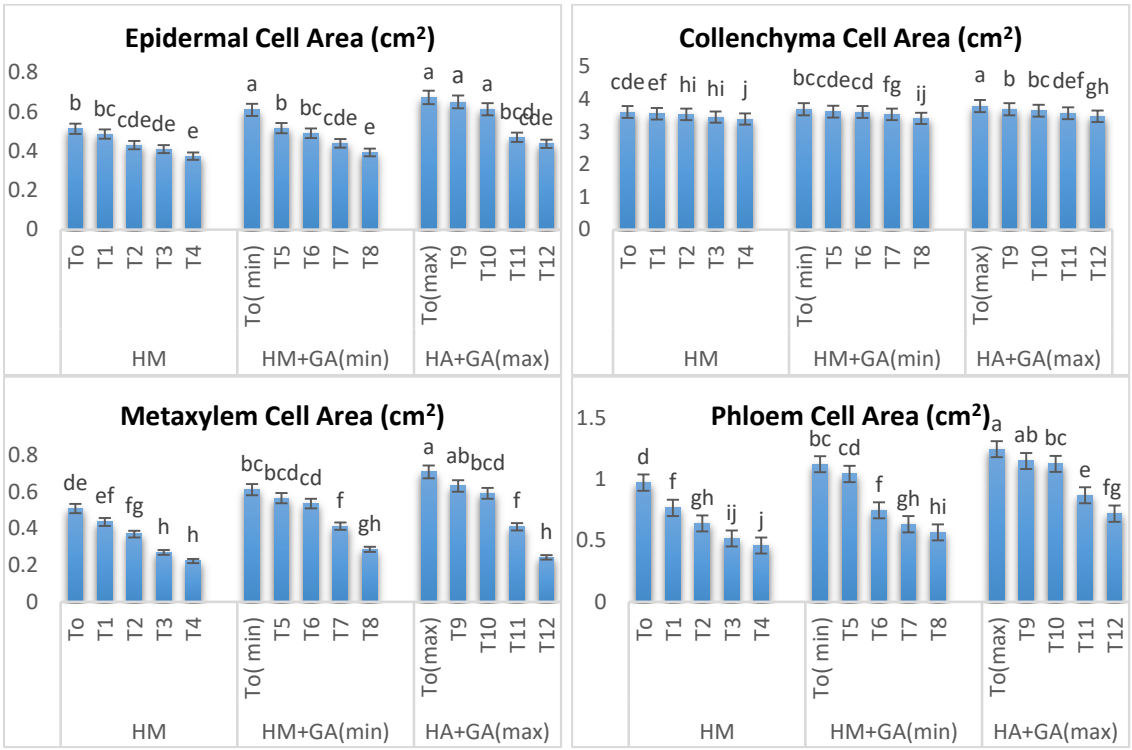


Figure 5b: Different leaf anatomical parameters of *A. majus*, subjected to different levels of Cd and GA.
The graph represents means (±SE) and treatments represented as T0 = Control, T1 = Cd 20 mM, T2 = Cd 40 mM, T3 = Cd 60 mM, T4 = Cd 80 mM, $T0_{(min)}$ = GA 10 mM, T5 = Cd 20 mM + GA 10 mM, T6 = Cd 40 mM + 10 mM, T7 = Cd 60 mM + GA 10 mM, T8 = Cd 80 mM + GA 10 mM, $T0_{(max)}$ = GA 20 mM, T9 = Cd 20 mM + GA 20 mM, T10 = Cd 40 mM + GA 20 mM, T11 = Cd 60 mM + GA 20 mM, T12 = Cd 80 mM + GA 20 mM.

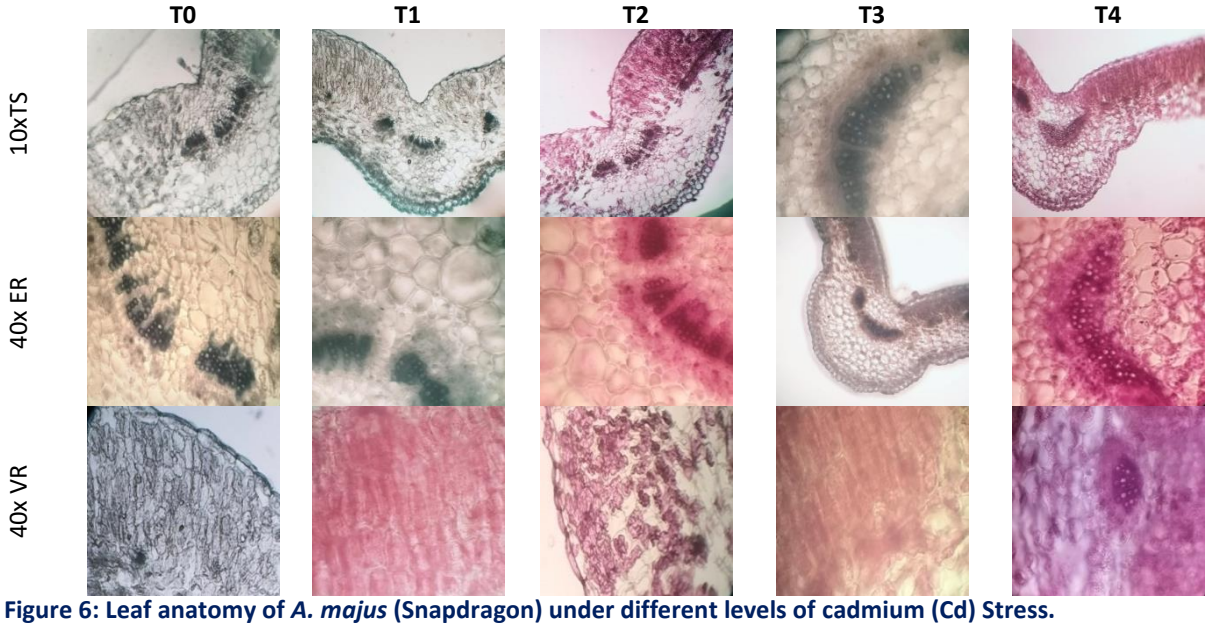


Figure 6: Leaf anatomy of *A. majus* (Snapdragon) under different levels of cadmium (Cd) Stress.
Treatments represented as T0 (Control), T1 (Cd 20 mM), T2 (Cd 40 mM), T3 (Cd 60 mM), T4 (Cd 80 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

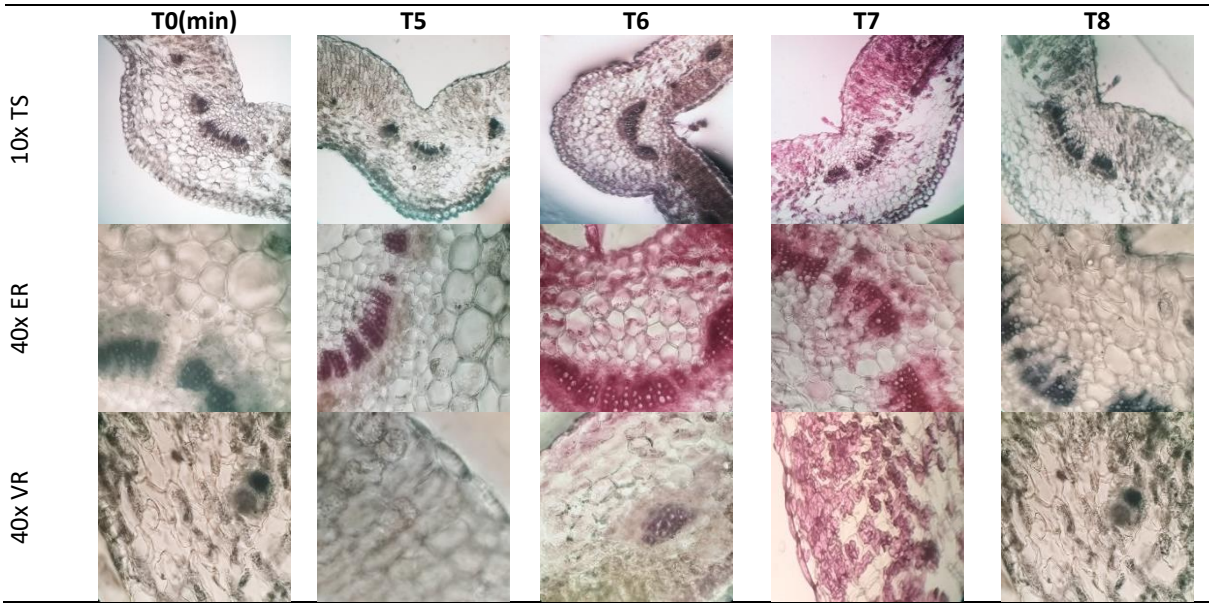


Figure 7: Leaf anatomy of *A. majus* (Snapdragon) under Different Levels of Cadmium (Cd) and Gallic Acid (GA) treatment.

Treatments represented as $T0_{(min)}$ (GA 10 mM), T5 (Cd 20 mM + GA 10 mM), T6 (Cd 40 mM + GA 10 mM), T7 (Cd 60 mM + GA 10 mM), T8 (Cd 80 mM + GA 10 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

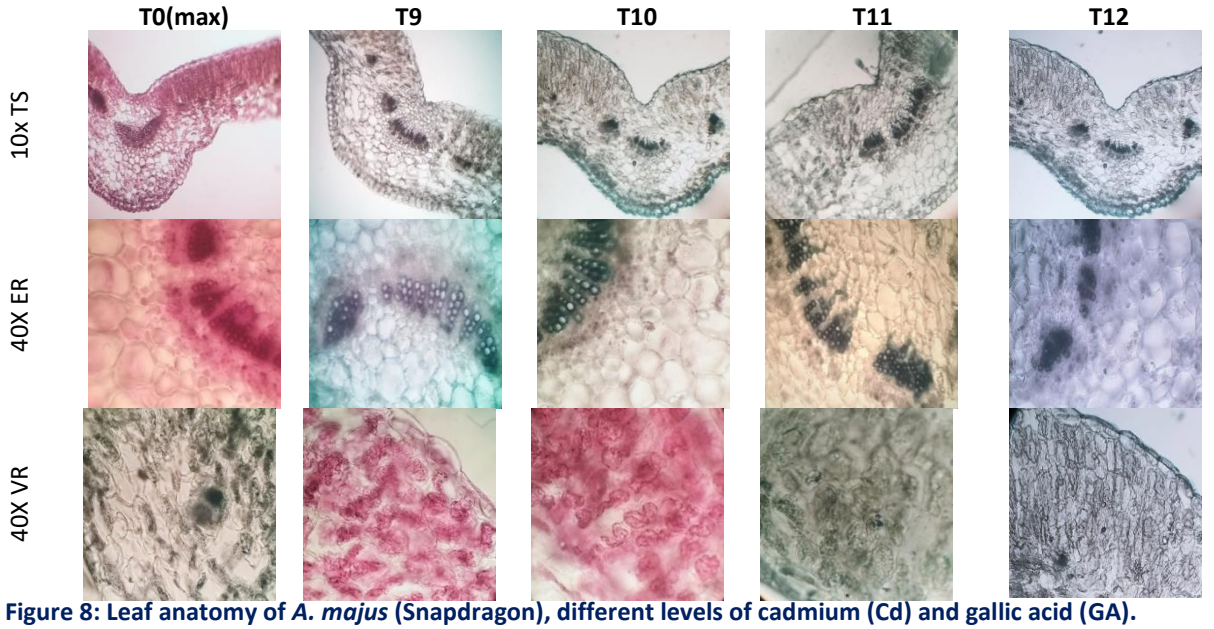


Figure 8: Leaf anatomy of *A. majus* (Snapdragon), different levels of cadmium (Cd) and gallic acid (GA).

Treatments represented as $T0_{(max)}$ (GA 20 mM), T9(Cd 20 mM + GA 20 mM), T10 (Cd 40 mM + GA 20 mM), T11 (Cd 60 mM + GA 20 mM), T12 (Cd 80 mM + GA 20 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

Root

Cadmium (Cd) stress significantly reduced root anatomical thickness in *Antirrhinum majus* (**Table 2**; **Figures 9-11**). The greatest reductions were observed at 80 mM Cd with 66% epidermal thickness (ET), 15% cortical, 96% endodermal, 43% xylem, and 85% phloem as compared to the control group. Minimal reductions were noted at Cd 20 mM. Gallic acid (GA) improved root anatomy, with 10 mM and 20 mM GA increasing epidermis thickness (66% and 93%) and phloem thickness (36% and 56%) compared to the control. Among combined treatments, 20 mM Cd + 20 mM GA showed the least reduction (38% ET, 1.3% CT, 2.6% EnT, 2% XT, 23% PT), while Cd 80 mM + GA 20 mM showed the highest, confirming GA's protective effect even under severe Cd stress. Cd stress also reduced root cell areas, with the highest decline at 80 mM Cd. The smallest decline was observed at 20 mM Cd. These parameters were improved by GA; 10 mM and 20 mM GA increased MXCA by 16% and 12%, and EPCA by 22% and 12% over the control group. There was also a reduction observed in epidermal cell area by 12%, collenchyma cell area by 1.4%, metaxylem cell area by 12%, and pericycle cell area by 10% when 20 mM Cd and 20 mM GA were combined. Overall, GA improved structural parameters under different concentrations of Cd stress.

Table 2: Different root anatomical parameters of *A. majus*, subjected to varying levels of Cd and GA.

Trt	ET (µm)	CT (µm)	ENT (µm)	XT (µm)	PHT (µm)	EPCA (cm^2)	COCA (cm^2)	MXCA (cm^2)	PXCA (cm^2)
T0	0.15cd	1.33cd	0.87c	2.56cde	0.30de	0.28bc	1.25bc	0.62de	0.51cde
T1	0.14cd	1.31cd	0.63e	2.47ef	0.26f	0.18d	1.19c	0.53f	0.47ef
T2	0.12d	1.21ef	0.46f	2.35g	0.23g	0.16def	1.03d	0.39g	0.37gh
T3	0.11d	1.17ef	0.24g	2.28gh	0.17h	0.09fg	0.83f	0.28h	0.26i
T4	0.09d	1.15f	0.12h	1.78j	0.04i	0.06g	0.58g	0.19i	0.13j
$T0_{(min)}$	0.25ab	1.41abc	1.15a	2.64bc	0.41bc	0.33ab	1.35ab	0.72bc	0.58abc
T5	0.16cd	1.40abc	0.97b	2.59cd	0.32cd	0.32abc	1.33ab	0.64cd	0.55bcd
T6	0.15cd	1.36bcd	0.87c	2.54def	0.31d	0.29bc	1.25bc	0.62d	0.48def
T7	0.12d	1.19ef	0.64e	2.21h	0.19fg	0.17de	1.17c	0.47fg	0.38gh
T8	0.11d	1.16f	0.38f	1.86j	0.07h	0.09g	0.90cf	0.28h	0.22i
$T0_{(max)}$	0.29a	1.51a	1.18a	2.81a	0.65a	0.37a	1.40a	0.82a	0.64a
T9	0.21bc	1.49a	1.15a	2.74ab	0.43ab	0.34ab	1.38a	0.74ab	0.61ab
T10	0.16cd	1.46ab	1.15a	2.65bc	0.36ab	0.26c	1.36ab	0.77ab	0.59ab
T11	0.14cd	1.26de	0.74d	2.45f	0.26ef	0.17de	1.24bc	0.54ef	0.43fg
T12	0.13d	1.19ef	0.45f	2.04i	0.16g	0.10efg	0.99de	0.39g	0.35h

The table represented as T0 = Control, T1 = Cd 20 mM, T2 = Cd 40 mM, T3 = Cd 60 mM, T4 = Cd 80 mM, $T0_{(min)}$ = GA 10 mM, T5 = Cd 20 mM + GA 10 mM, T6 = Cd 40 mM + 10 mM, T7 = Cd 60 mM + GA 10 mM, T8 = Cd 80 mM + GA 10 mM, $T0_{(max)}$ = GA 20 mM, T9 = Cd 20 mM + GA 20 mM, T10 = Cd 40 mM + GA 20 mM, T11 = Cd 60 mM + GA 20 mM, T12 = Cd 80 mM + GA 20 mM.
ET = Epidermis thickness, CT = Cortex thickness, EnT = Endodermis thickness, XT = Xylem thickness, PT = Phloem thickness, EPCA = Epidermis cell area, COCA = Cortex cell area, MXCA = Metaxylem cell area, PXCA = Pericycle cell area. Means following different alphabets differ significantly at $p < 0.05$.

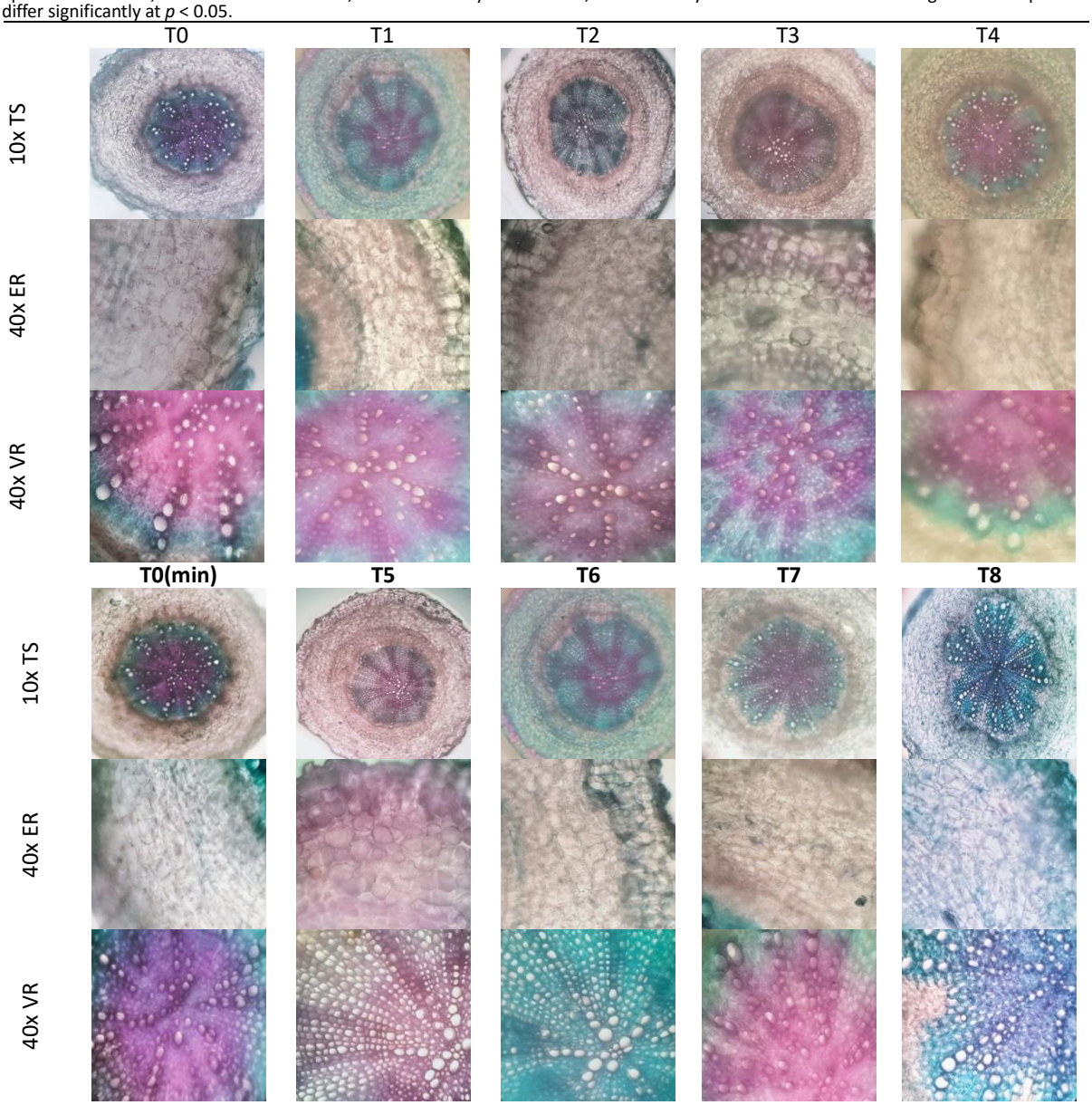


Figure 9 & 10: Root anatomy of *A. majus* (Snapdragon) under different levels of cadmium (Cd) and gallic acid (GA) treatment.

Treatments represented as $T0_{(min)}$ (GA 10 mM), T1 (Cd 20 mM), T2 (Cd 40 mM), T3 (Cd 60 mM), T4 (Cd 80 mM); T5 (Cd 20 mM + GA 10 mM), T6 (Cd 40 mM + GA 10 mM), T7 (Cd 60 mM + GA 10 mM), T8 (Cd 80 mM + GA 10 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

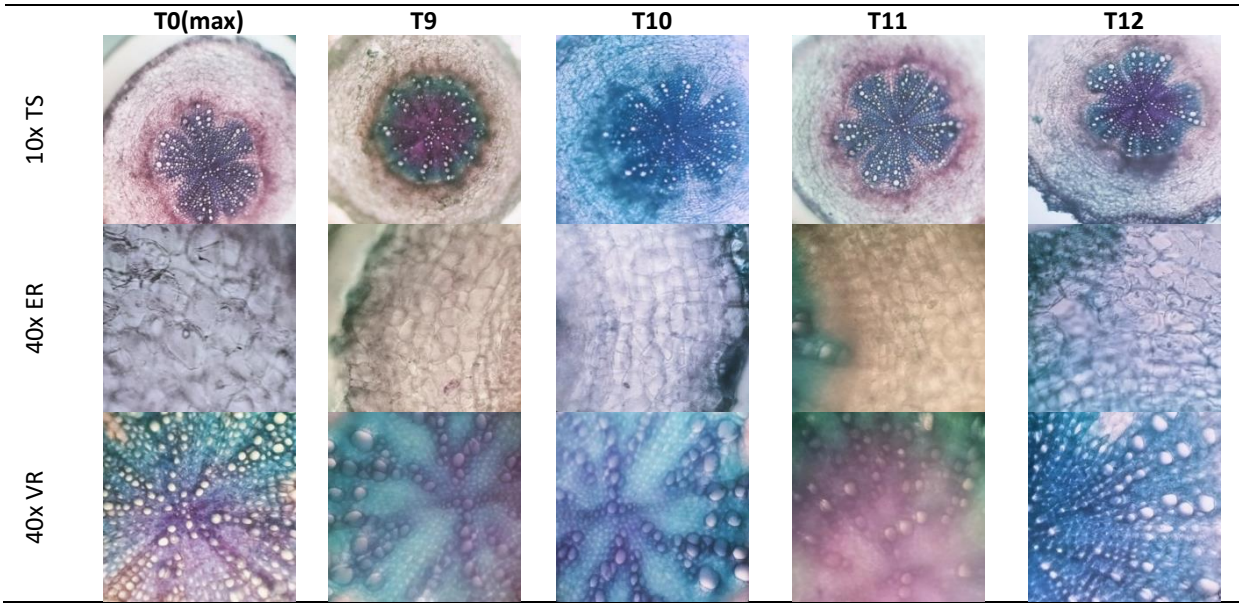


Figure 11: Root anatomy of *A. majus* (Snapdragon) under different levels of cadmium (Cd) and gallic acid (GA).

Treatments represented as $T0_{(max)}$ (GA 20 mM), T9(Cd 20 mM + GA 20 mM), T10 (Cd 40 mM + GA 20 mM), T11 (Cd 60 mM + GA 20 mM), T12 (Cd 80 mM + GA 20 mM). TS = Transverse section; ER = Epidermal region; VR = Vascular region

Discussion

Cadmium (Cd) is a non-essential and highly toxic heavy metal that disrupts plant mineral nutrition by competing with essential ions such as Zn, Fe, Mn, Ca, and K for absorption sites in roots (Vasilachi et al., 2023; Hu et al., 2025). This competition leads to nutrient imbalance, water deficiency, and oxidative stress, which collectively reduce metabolic activity and growth (Noor et al., 2022; Vasilachi et al., 2023). In fact, the inhibitory effects differed depending on the kind of heavy metal, concentration, length of exposure, and age of the plant (Ahmad et al., 2020). The present study suggested that the Cd stress caused significant reductions in plant height, shoot and root lengths, and biomass accumulation (**Figure 1**), similar to the findings in other crops (Jiao et al., 2024; Sheikh et al., 2025). Through disruptions in many morpho-physiological processes, including nutrient intake, cadmium stress impacts growth and yield (Gill et al., 2011). Both short-term and long-term exposure to Cd toxicity reduces photosynthetic activity in a variety of agronomic crops (Hu et al., 2025). Our results suggested the morphological performance under Cd stress was significantly enhanced by the exogenous gallic acid (GA). GA-treated plants showed an increase in biomass, longer shoots and roots, and stronger leaf growth (**Figures 1-11**). GA has the capacity to increase the antioxidant defense system, control hormone balance, and preserve cellular homeostasis (D-Agostino et al., 2025; Mendes et al., 2025). These findings support the potential of GA as an affordable growth enhancer to decrease the Cd toxicity in crops.

Heavy metal stress changes plant structure by altering epidermal and cortical layers and disrupting vascular systems (El-Okkiah et al., 2022; Gao et al., 2022). In the present study, the epidermal stem cells were thicker, and the cortical cells occupied less area in the Cd-treated stem. There were 10-12 cortical layers in the experimental plant compared to 14-16 in the control plant. The Cd concentrations significantly reduced stem thickness and xylem cell area in Snapdragon, enhancing mechanical strength and water transport capacity. Similar observations were reported by Zhang et al. (2024), who found that Cd stress inhibited vascular growth and reduced vessel diameter. However, the GA application counteracted these effects, enhancing vascular bundle integrity and xylem development. This may result from GA's antioxidant properties, which prevent lipid peroxidation and preserve cellular structures (Mendes et al., 2025). Cadmium toxicity reduced lamina thickness, mesophyll cell size, and vascular bundle area, due to decreased photosynthetic efficiency (**Figure 5 a & b**). GA treatment mitigated these effects by maintaining leaf structure, improving mesophyll density, and improving stomatal function. Similar results were reported in wheat and sunflower, where GA improved vascular and mesophyll organization under metal stress (Guo et al., 2023; Shivappa et al., 2025). The improved leaf anatomy indicates that GA helps sustain physiological functions such as gas exchange and transpiration even under Cd exposure (Mendes et al., 2025). Under combined abiotic stress, GA-treated plants showed a 123 µm increase in upper epidermis (UET) thickness in Neelam and a 75 µm increase in BSS 513. When compared to control

(untreated) plants, GA-treated plants showed much better leaf shape and survived these abiotic challenges. Similarly, following salinity and boron stress, cuticle thickness increased by 11.4 µm in Neelam and 9.0 µm in variety BSS 513 (Shumaila et al., 2023).

Cadmium (Cd) is quickly absorbed by roots and builds up in different plant tissues, impeding crop development and production all over the world (Gill and Tuteja, 2011). Different previous studies suggested that Cd, negatively affected plants, such as anatomical alterations which include smaller and degraded mesophyll tissue, parenchymatous tissue disintegration, loosening of cortical tissue in *Phaseolus vulgaris* roots (Talukdar, 2013), shriveling and cell breakdown, which caused *Phaseolus aureus* to lose the shape of its cortical cells (Singh and Agrawal, 2007), and decreased cortical thickness in maize roots (Gowayed and Almaghrabi, 2013). Similarly, root cortical and endodermal cell loss or disintegration was brought on by Cd accumulation in *Pteris vittata* (Armendariz et al., 2016). When compared to control plants, the root diameter of Cd-treated plants declined. Cortical cells were found in 14–16 layers in normal plant roots and 7–10 layers in Cd-treated plant roots (El-Okkiah et al., 2022). Roots are the first target of Cd accumulation, where toxicity disrupts epidermal, cortical, and vascular structures. In our study, Cd stress reduced the thickness of root tissues and distorted xylem and endodermal layers. The root structure showed better development and vascular systems when treated with GA, which supports elongation and cellular division under stress (Xu et al., 2024). Similar results were observed in the wheat plant as GA improved its growth under heavy melatonin stress (Mendes et al., 2025). The root diameters of plants declined with Cd concentration. The epidermis was single-layered with thin-walled parenchymatous cells in both the control and Cd-treated roots. Just below the epidermis lies a cortex made up of parenchymatous cells with thin walls (Liza et al., 2020). Our findings (**Table 2**) also showed that gallic acid (GA) improved root anatomical structures, with 10 mM and 20 mM GA increasing epidermis thickness by 66% and 93%, and phloem thickness by 36% and 56% compared to the control.

Conclusion

The current study found that the Cd stress had a significant negative impact on *Antirrhinum majus* growth indices, including fresh and dry weight and root-shoot length. The Cd stress also changed the anatomical characteristics of *A. majus*. Our study's findings suggest that foliar sprays of gallic acid at suitable quantities improved *A. majus* growth characteristics when exposed to Cd stress. Growth attributes and structural development are all greatly impacted by cadmium poisoning. Investigations on quick, economical, and effective ways to remove Cd from soil and other environmental areas are required.

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Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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