APPRAISAL ON MORPHOMETRY, GAS EXCHANGE CHARACTERISTICS, AND IONS UPTAKE UNDER CADMIUM STRESS IN EARLY- AND LATE-SOWN OF COTTON

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Abstract. Cadmium (Cd) is a water-soluble metal pollutant that is not required for plants, but its mobility in the soil-plant continuum has lately attracted substantial interest due to its harmful effects on plants. It may cause serious morphological and physiological abnormalities in addition to inhibiting cotton growth. Thereby, the present study was conducted to explore the effect of different concentrations of Cd on the growth, morphological, biochemical, and physiological processes of cotton (*Gossypium hirsutum* L.) varieties. Understanding the morphological, biochemical, and physiological responses to Cd stress is necessary for a holistic approach to plant resistance mechanisms to Cd stress. A net house experiment was conducted to investigate the growth and adaptation mechanism of *G. hirsutum* varieties (V_1: KL-MNH-142 and V_2: KL-FH-886) with different sowing times (i.e., early and late) under Cd stress (250 µM, 500 µM, 750 µM, and 1000 µM). The results showed that Cd predominately accumulated in the root at a higher dose, which consequently led to a reduction in the root biomass. During the late sowing time, transpiration rate (1.01 ± 0.04) and stomatal gas exchange rate (0.01 ± 0.003) were recorded to be significantly decreased by the application of Cd at 1000 µM to variety V_2 (KL-MNH886) as compared to the early sowing interval and respective control (1.63 ± 0.06). Calcium contents (1.4 ± 0.5) in the root were decreased in the early sowing period in V_1 as compared to the late sowing period. Besides, the application of Cd (1000 µM) has significantly decreased root soluble sugar (0.13 ± 0.001) in V_1 under the late sowing period compared with the early sowing period and respective control. Cd not only led to the decrease in root anthocyanin but also changed the chlorophyll content. Our study proved that *G. hirsutum* has good tolerance to Cd stress during early time intervals as compared to the late time interval and is the best species for soil and ecological environment restoration.
Keywords: *heavy metals, cadmium, pollution, cotton, physiological and biochemical features, tolerance*

Introduction

Heavy metal contamination in soil is a serious environmental issue that affects agricultural productivity and even threatens human health through the food chain as a result of the effects of industrialization and geological activities (Sharma and Pandey, 2014; Adrees et al., 2015; Nazir et al., 2023). Cadmium (Cd) is a natural element in the earth's crust and is usually combined with other elements (such as oxygen, chlorine, or sulfur) to form minerals. A significant amount of Cd has been emitted and deposited in the soil environment during the past two centuries (Ben et al., 2007). In this context, according to the US geological survey (USGS), the assessed Cd reserves are 500,000 tons (USGS, 2013). Further, $\sim 1.3 \times 10^5$ km^2 of agricultural soils are sternly affected by Cd in China (Ren et al., 2008).

Being a stressor to plants, metals are not degradable and can persist in the soil for many years, potentially having detrimental effects on the soil ecology (Khan et al., 2010; Mehmood et al., 2023). If the level of Cd is more than the permissible limit, it is reported to exert harmful effects on the growth, physiological, and biological functions of the plants (Deng et al., 2014; Sergeant et al., 2014). Meanwhile, Cd could hamper photosynthesis, hence inhibiting plant growth and adaptability (Ahmad et al., 2012; Ahmad et al., 2016). The common symptoms of Cd toxicity in plants are inhibition of chlorophyll pigments, photosynthesis, root elongation, plant biomass, ultrastructural changes, reduction of seedling growth and development, cell division, and lipid peroxidation (Benavides et al., 2005; Gill et al., 2013). At the cellular level, Cd accelerates reactive oxygen species (ROS) production, interrupts the cellular redox state, and deteriorates the synthesis of macromolecules (Ahmad et al., 2016; Rahman et al., 2021; Majeed et al., 2022). Furthermore, an excessive amount of Cd severely affects the soil microorganisms which may lead to dysfunction, protein denaturation, and destruction of cell membrane integrity (Tang et al., 2018). In plants, the phytotoxicity-mediated by potentially toxic elements (PTEs) causes various disorders resulting in chlorosis, decreased nutrient uptake, and plant growth, development, and yield. PTEs

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can accumulate plants and their product that consequently causes a reduction in agricultural productivity, as well as lead to biomagnification at different trophic levels of an ecosystem that can impose uncertain ill-health effects on humans (Carbonell et al., 1998; Liu et al., 2008).

The conventional approach of minimizing toxicity by replacing soil contaminated with PTEs is a costly alternative since the replaced contaminated soil may need to be remedied soon to prevent secondary contamination. Thus, the optimization of the tolerance mechanism in plants can enhance their ability to endure harmful soil conditions as well as act as an economical and ecologically safe approach. At present, phytoremediation has become a more promising method of environmental restoration in an eco-friendly way (Jan et al., 2021; Yasir et al., 2022). However, due to the low biomass of hyperaccumulators, the use of phytoremediation technology is currently quite limited for real-time applications (Liu et al., 2008; Thind et al., 2021). Various mechanisms are involved at the cellular level to minimize the high concentrations of metals and metalloids (Liu et al., 2020; Emanuil et al., 2022). One of these mechanisms is the compartmentalization of Cd in specific tissues or cellular organelles (Isaure et al., 2006). In this context, cotton is considered a suitable crop for phytoremediation in industrially polluted regions. Cotton (*Gossypium hirsutum* L.), a member of the Malvaceae family is primarily cultivated in subtropical and tropical areas all over the world. Due to its high tolerance against PTEs, it is well known as a soil remediation crop and can absorb, translocate and accumulate such contaminants (Chen et al., 2014).

Several studies have shown the effects of Cd stress induced by $CdCl_2$ on seed germination, growth, and physiological parameters of various crops (Ambede et al., 2012; Aliu et al., 2014). Unfortunately, no study has been conducted on the impact and tolerance level of different cotton varieties to explore their potential for phytoremediation under Cd stress. Therefore, it is imperative to explore the impact of Cd stress on different cotton varieties regarding their growth, physiological, and biochemical attributes. Based on this hypothesis, the present study was conducted to investigate the tolerance of the cotton varieties under early and late sowing conditions under different levels of Cd ($CdCl_2.H_2O$). The objective of the current study was to evaluate and compare the effect of various levels of Cd on cotton growth, physiology, and nutrient contents under different cotton (*G. hirsutum*) varieties and sowing times.

Materials and methods

Plant growth conditions and experimental design

The experiment was carried out in the net house of the botanical garden of the University of Agriculture, Faisalabad. The net house experiment was laid out in a completely randomized design (CRD) with three replications. This experiment was done to screen the cotton varieties (KL-FH142 and Kl-MNh886) against the Cd effect in the net house, and irrigation was done regularly to avoid dryness in all the pots. The cotton varieties (KL-FH142 and Kl-MNh886) were selected due to their lack of response reported under different levels of Cd stress. The soil was treated with different concentrations of the Cd viz., control 250, 500, 750, and 1000 µM based on preliminary experiments.

The seeds of both, cotton varieties were sown at different time intervals with a gap of fifteen days in normal soil which were taken from the uncontaminated site of the botanical garden of the University of Agriculture Faisalabad. The plastic pots (total 60)

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of 20 × 13 cm (length × diameter) were filled with sun-dried soil. Early crop seeds were sown on 9 July 2015 in 30 plastic pots and late crop seeds were sown on 24 July 2015 in another 30 pots after 15 days of the early crop. Ten holes were made in each pot, about 2 cm deep at the same distance. Each hole had a seed and put a small amount of soil on it. After three days of sowing, seeds germinated and fifteen days old plant seedlings were treated with different doses of Cd (250, 500, 750, and 1000 μM). For the early sowing experiment, 24 pots were treated other 6 were left untreated that severed as a control for V_1 (variety 1) and V_2 (variety 2). After 15 days, a similar procedure was repeated, and this experimental set was referred to as a late-sowing crop. Thus, the experiment was designed in a completely randomized design (CRD) with a two-factor factorial arrangement. The maximum rainfall, mean daily rainfall intensity, optimum temperature, and average daily temperature of the experimental site were, 35 mm (May to October), 14.04 mm, 40°C, 34-40°C, and 31.1-35.2°C, respectively.

Measurement of morphological parameters

Different parameters were analyzed at a single harvest with an applied treatment period of 30 days. Five plants were picked randomly and isolated their roots. Plant length is measured by a meter rod from the base of the plant to the terminal of each plant's youngest leaf. The fresh weight of roots and shoots was valued using weight balance in grams, and their mean value was measured. For the observation of the dry weight of roots and shoots (g), they were desiccant for 72 h in an oven at 65°C, and weight was measured using an analytical balance.

Gas exchange parameters

The exchanging gas parameters such as (net CO_2 assimilation rate (A) (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs) (mmol m^{-2} s^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), and sub-stomatal CO_2 concentration (Ci) (μmol mol^{-1}) were calculated using an open system LDA-24ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). These observations were made on the second leaf from the terminal of each plant shoot. With these specific adjustments viz., the superficial area of the leaf was 6.25 cm2, ambient CO_2 concentration (Cref) 290.1 mmol-1, the temperature of the leaf cavity (Tch) ranged from 41 to 43.80°C, and the movement rate of the leaf cavity gas (V) 394 mL min-1, stomatal conductance (gs) (mmol m^{-2} s^{-1}, the frequency of gas flow in leaf cavity (U) 256.66 m molS-1, ambient pressure (P) 98.9 kPa, water vapor pressure (e ref) into chamber extended from 4.4 to 6.6 m bar, the molar flow of air per unit leaf area (US) 410.6 mol m^{-2} s^{-1}, the analyses were performed.

Pigments analysis

The fresh leaves (0.25 g) were chopped into pieces and extracted with acetone (80%). Using a spectrophotometer (Hitachi Model-U 2001 Japan) the absorbance was recorded at 645, 663, and 480 nm for the determination of chlorophyll a, chlorophyll b, and total chlorophyll content (Arnon, 1949). The amount of chl a, chl b, and carotenoids were determined using the following equations:

$$\text{Chl. a (mg)} = [12.7(OD663) - 2.69(OD645)] \times V/1000 \times W \quad \text{(Eq.1)}$$

$$\text{Chl. b (mg)} = [22.9(OD645) - 4.68(OD663)] \times V/1000 \times W \quad \text{(Eq.2)}$$

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$$\text{Carotenoids (mg)} = [\text{OD480} + (0.114 \times \text{OD663}) - (0.638 \times \text{OD645})] \quad \text{(Eq.3)}$$

where OD = optical density V = volume of sample W = weight of sample.

Determination of ions

The dried plant material was digested using H_2SO_4 and H_2O_2 (Wolf, 1982). The resultant was incubated overnight at room temperature. Then 0.5 ml of HNO_3 was poured down into digestion tubes and distributed via the slides of tubes, waited for the reaction, place the tubes in a digestion unit and heated up to 350C, and then heated further for 30 min until fumes were formed. Digestion tubes were removed from the slab and steadily chilled for 10 min. 0.5 ml of H_2O_2 is added gradually and placed in the tubes exclusively in the digestion unit for the next 20 min. Then, the digestion tubes detached from the slab and cooled. Put the tubes back into the digestion block with the addition of 0.5 ml of HNO_3 and place them back into the digestion unit. These steps were repetitive until the cold component was colorless. Distilled water is used to make volume up to 50 ml, and then filtered and used to determine the K^+, Ca^{2+}, and Cd. Determination of K^+ and Ca^{2+} by using the protocol of Yoshida et al. (1976). A flame photometer (Sherwood model, 410 UK) was used to estimate Ca^{2+} and K^+. Standard curves were formed by running the Ca^{2+} and K^+ series of different grades (10, 20, 30, 40, and 50 ppm). Cd was measured by an Atomic Absorption Spectrophotometer.

Soluble sugars, anthocyanins, and phenolic contents

Soluble sugars were calculated using Yoshida et al. (1976) procedure. Fresh plant material (0.1 g) was taken and boiled in 5 ml deionized water, filtered, and diluted up to 50 ml, 1 ml dilute filtrate, 5 ml Anthrone reagent was applied followed by heating at 90°C for 20 min and absorbance was detected at 620 nm using a spectrophotometer. Glucose with different concentrations (0, 20, 40, 60, 80, and 100 μM) was used as a standard. The content of anthocyanin was analyzed using the standard method of Mancinelli (1949). The estimation of phenolic content was performed using the method of Singleton and Rossi (1965).

Statistical analysis

Statistically, two-way variance analysis and CO-STAT computer program Duncan's Multiple Range tests (DMRT) were used to analyze data for morphological parameters, ion quality, and gas exchange parameters.

Results

Plants growth parameters

The biomass attributes (fresh and dry weight of roots and shoots) were found to be significantly ($p \leq 0.05$) affected under different levels of Cd and sowing times (*Fig. 1*). In late sowing conditions, the declining trend with an increase in the concentration of Cd was more prominent in V_2 (8.5 ± 1.6), whereas the maximal significant decline in the shoot fresh weight was observed under 1000 μM Cd stress as compared to respective control (10.3 ± 2.6). Likewise, during the early sowing period,

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root fresh weight of V_2 (0.41 ± 0.09) showed a significant ($p \leq 0.05$) reduction at a higher concentration (1000 µM) of Cd over the control (1.16 ± 0.06). Meanwhile, shoot dry weight (1.74 ± 0.21) significantly ($p \leq 0.05$) decreased in the case of V_1 during the early sowing time as compared to the late sowing period and respective controls (4.2 ± 0.14). Likewise, the root length (15.1 ± 0.33) was reduced with the application of Cd (1000 µM) in the case of V_1. In the late sowing interval, Cd at 1000 µM significantly ($p \leq 0.05$) reduced the shoot length (27.6 ± 1.3) of V_2. Less number of leaves (4 ± 0.51) was observed in the late sowing plants in V_2 as compared to V_1 and early sowing plants. Under Cd stress, toxic implications were more apparent at higher doses (500, 750, and 1000 µM) than at lower doses except for a few parameters.

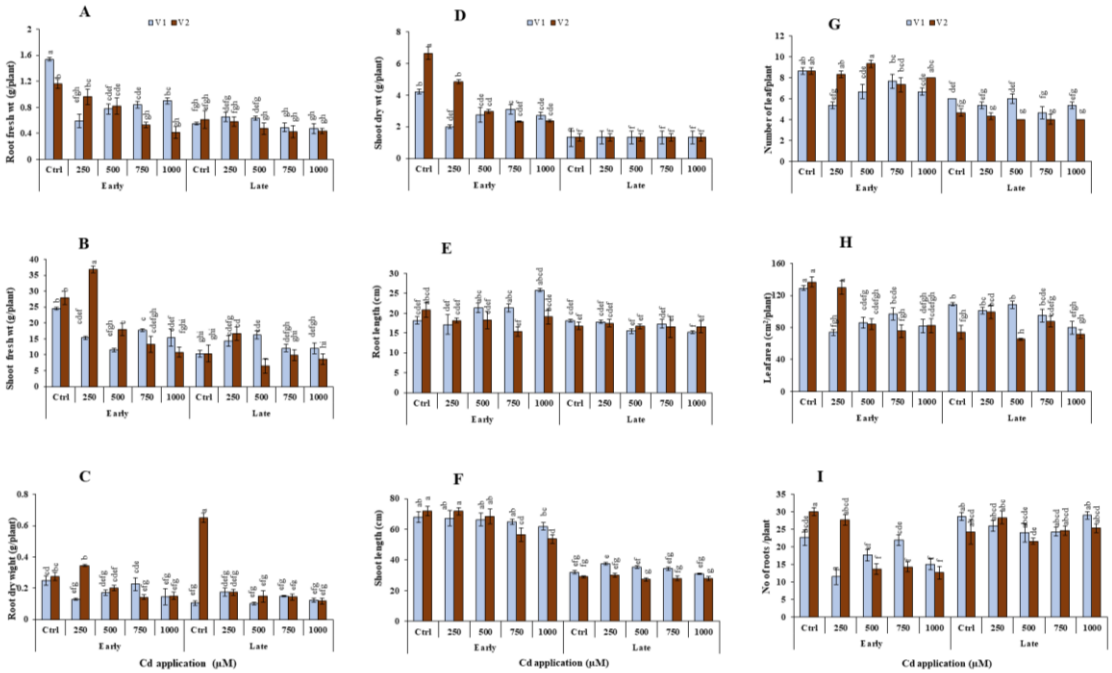

Figure 1. *Root fresh weight (A), shoot fresh weight (B), root dry weight (C), shoot dry weight (D), root length (E), shoot length (F), number of leaf plant^{-1} (G), leaf area (H), and number of roots plant^{-1} (I) of the cotton plant (two cotton cultivars, namely, V_1 = KL-MNH-886 and V_2 = KL-FH-142) treated with $CdCl_2$ treatment was applied to 15 days old plants and early and late crop sowing with the difference of 15 days. Mean values sharing different letter (s) on the bars are significantly different from each other at $p \leq 0.05$*

Plant biomass has antagonistic effects with Cd stress at lower doses, however, at higher doses i.e., 750 and 100 µM, there was significant ($p \leq 0.05$) suppression recorded in the plant biomass. Thus, the impacts were found to be more toxic in the second variety (V_2) than in the first variety (V_1). Also, the early sowing crop exhibited better resistance to Cd toxicity than the late sowing crop, therefore it can be inferred that time interval plays a major influence in plant development. The observations of shoot length revealed significant findings among interaction means. It was recorded that Cd at 250 µM significantly ($p \leq 0.05$) increased the shoot length in V_2 as compared to V_1. Whereas the application of Cd at 500, 750, and 1000 µM decreased

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the shoot length of both early and late varieties (*Fig. 1*). In the case of root length, similar observations to that of shoots were recorded i.e., Cd (250 µM) significantly ($p \leq 0.05$) improved the root length in V_1 as compared to V_2 and then declined the shoot length in both varieties when compared to control. Moreover, the alike trends of variation were recorded for the number of leaves, leaf area, number of roots, and number of branches (*Fig. 1; Table 1*).

Table 1. *Effect of Cd on the number of leaves, leaf area, number of roots, and number of branches at early and late sown cotton varieties*

Sowing time	Variety	Treatment	Number of leaves	Leaf area	Number of roots	Number of branches
Early	V_1	Control	8.6 ± 0.3^{ab}	92.7 ± 2.0^{a}	$22.6 \pm 2.1^{b-e}$	$7.0 \pm 1.0^{a-f}$
		250 µM	$5.3 \pm 0.3^{e-g}$	$73.8 \pm 3.6^{f-h}$	11.6 ± 2.4^{f}	4.3 ± 0.6^{g}
		500 µM	$6.6 \pm 0.8^{c-e}$	$86.1 \pm 6.9^{c-g}$	17.6 ± 1.6^{ef}	$5.6 \pm 0.8^{b-g}$
		750 µM	7.6 ± 0.8^{bc}	$96.8 \pm 7.8^{b-e}$	$22.0 \pm 1.5^{c-e}$	$7.3 \pm 1.2^{a-g}$
		1000 µM	$6.6 \pm 0.3^{c-e}$	$81.5 \pm 8.0^{d-h}$	15.0 ± 1.5^{f}	$5.6 \pm 0.8^{c-g}$
Early	V_2	Control	8.6 ± 0.3^{ab}	136.5 ± 6.3^{a}	30.0 ± 1.1^{a}	8.3 ± 0.6^{ab}
		250 µM	8.3 ± 0.3^{ab}	129.7 ± 8.1^{a}	$27.6 \pm 3.7^{a-d}$	$8.3 \pm 0.3^{e-g}$
		500 µM	9.3 ± 0.3^{a}	$83.7 \pm 6.7^{c-h}$	13.6 ± 2.3^{f}	$9.3 \pm 0.3^{e-g}$
		750 µM	$7.3 \pm 0.6^{b-d}$	$75.4 \pm 8.1^{f-h}$	14.3 ± 1.4^{f}	$7.3 \pm 0.3^{d-g}$
		1000 µM	$8.0 \pm 0.3^{a-c}$	$82.3 \pm 8.8^{c-h}$	12.6 ± 1.7^{f}	7.0 ± 1.0^{fg}
Late	V_1	Control	$6.0 \pm 0.2^{d-f}$	108.4 ± 2.2^{b}	28.6 ± 1.2^{ab}	4.6 ± 0.3^{ab}
		250 µM	$5.3 \pm 0.3^{e-g}$	101.0 ± 4.1^{bc}	$26.0 \pm 1.5^{a-d}$	$5.3 \pm 0.3^{a-c}$
		500 µM	$6.0 \pm 0.5^{d-f}$	108.4 ± 4.4^{b}	$24.0 \pm 2.6^{a-e}$	$4.3 \pm 0.3^{a-e}$
		750 µM	4.6 ± 0.8^{fg}	$94.8 \pm 8.1^{b-e}$	$24.3 \pm 1.2^{a-d}$	$5.0 \pm 0.5^{a-e}$
		1000 µM	$5.3 \pm 0.3^{e-g}$	$79.6 \pm 8.1^{e-h}$	29.0 ± 1.0^{ab}	4.0 ± 0.5^{ab}
Late	V_2	Control	4.6 ± 0.3^{fg}	$74.1 \pm 8.6^{f-h}$	$24.3 \pm 1.1^{a-d}$	$4.3 \pm 0.3^{a-e}$
		250 µM	4.3 ± 0.3^{g}	$99.3 \pm 8.6^{b-d}$	$28.3 \pm 1.5^{a-c}$	3.6 ± 0.3^{ab}
		500 µM	4.0 ± 0.1^{g}	65.1 ± 1.6^{h}	21.6 ± 1.5^{de}	$3.3 \pm 0.3^{a-g}$
		750 µM	4.0 ± 0.7^{g}	$87.6 \pm 6.7^{c-g}$	$24.6 \pm 1.4^{a-d}$	$3.0 \pm 0.2^{a-e}$
		1000 µM	4.0 ± 0.1^{g}	71.8 ± 5.5^{gh}	$25.3 \pm 1.7^{a-d}$	$3.0 \pm 0.5^{a-d}$

Mean values sharing different letter (s) in a column are significantly different from each other at $p \leq 0.05$

Gas exchange parameters and pigments' responses

At 250 and 500 µM of Cd, there was a significant ($p \leq 0.05$) decrease recorded in the net photosynthetic rate, stomatal conductance, and transpiration rate, however, the inhibitory effect was more prominent at 750 to 1000 µM of Cd. In late sowing time, V_2 showed a significant ($p \leq 0.05$) reduction in net photosynthetic rate and stomatal conductance at 1000 µM of Cd when compared to the early sowing interval and respective control. Furthermore, V_2 showed more Cd-induced effects as compared to V_1 as well as the late crop is notably more affected by Cd stress than the early crop. On the other hand, during the early sowing time interval, the

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transpiration rate (E) had significantly ($p \leq 0.05$) decreased by the application of Cd at 1000 µM in V_2 as compared to the late sowing time interval and respective control. In the late sowing period, V_1 showed a significant ($p \leq 0.05$) reduction in net transpiration rate (A) as compared to the early sowing period and respective control (*Fig. 2A-C*).

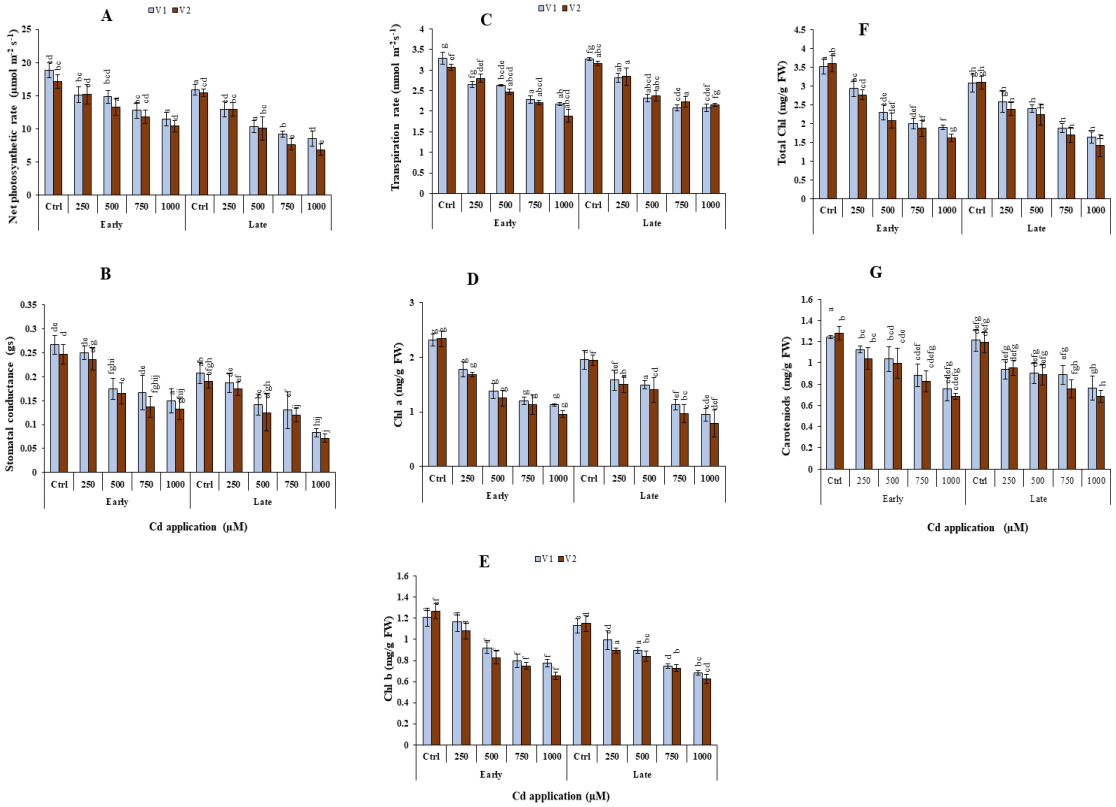

Figure 2. *Net photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), Chl a (D), Chl b (E), total Chl (F), and carotenoids (G) of the cotton plant (two cotton cultivars, namely, V_1 = KL-MNH-886 and V_2 = KL-FH-142) treated with CdCl2 treatment was applied to 15 days old plants and early and late crop grow with the difference of 15 days. Mean values sharing different letter (s) on the bars are significantly different from each other at $p \leq 0.05$*

Photosynthetic pigments play important roles in maintaining plant growth and are essentially composed of Chl a, Chl b, total Chl, and carotenoids. The effects of Cd concentrations are shown in negative linear relation with photosynthetic pigments (Chl a, Chl b, total Chl, and carotenoids). Chl contents (0.4 ± 0.06) were lowered in V_2 during the early sowing time as compared to the late sowing period. Chl b contents (0.8 ± 0.01) were also decreased in the early sowing in V_2 than in late sowing varieties. Total Chl con-tents were reduced during early sowing time (V_1 and V_2) varieties than late sowing interval. Carotenoids were also recorded to decrease to a greater extent in early-sowing varieties than in late-sowing varieties. The photosynthetic pigments content of the cotton varieties gradually decreased and showed a comparable relationship between the pigments with the increase of Cd concentration (*Fig. 2D-G*). Furthermore, late crops are more affected as compared to early crops as well as V_2 is more affected as compared with V_1 so environmental conditions (temperature, light

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intensity, length of day) also play a major role in photosynthetic pigment contents. Photosynthesis is directly reported to be dependent on photosynthetic pigments so the reduction in pigments can be reported to be associated with photosynthetic rate as well as low production.

Soluble sugar, anthocyanins, and phenolic contents

Soluble sugar under Cd stress showed an increasing trend at 250, 500, and 750 µM and was found maximal at 1000 µM in both varieties as well as early and late crops respectively as compared with the control. Shoot soluble sugar inhibited slightly at 750 µM in both V_1 and V_2 of the early crop as compared with the control. The research showed that root soluble sugar in cotton plants showed a slight variation different from the shoot, in root soluble sugar varied parallelly to the Cd application. Time intervals have no significant effect on soluble sugar in both early and late crops (*Fig. 3*).

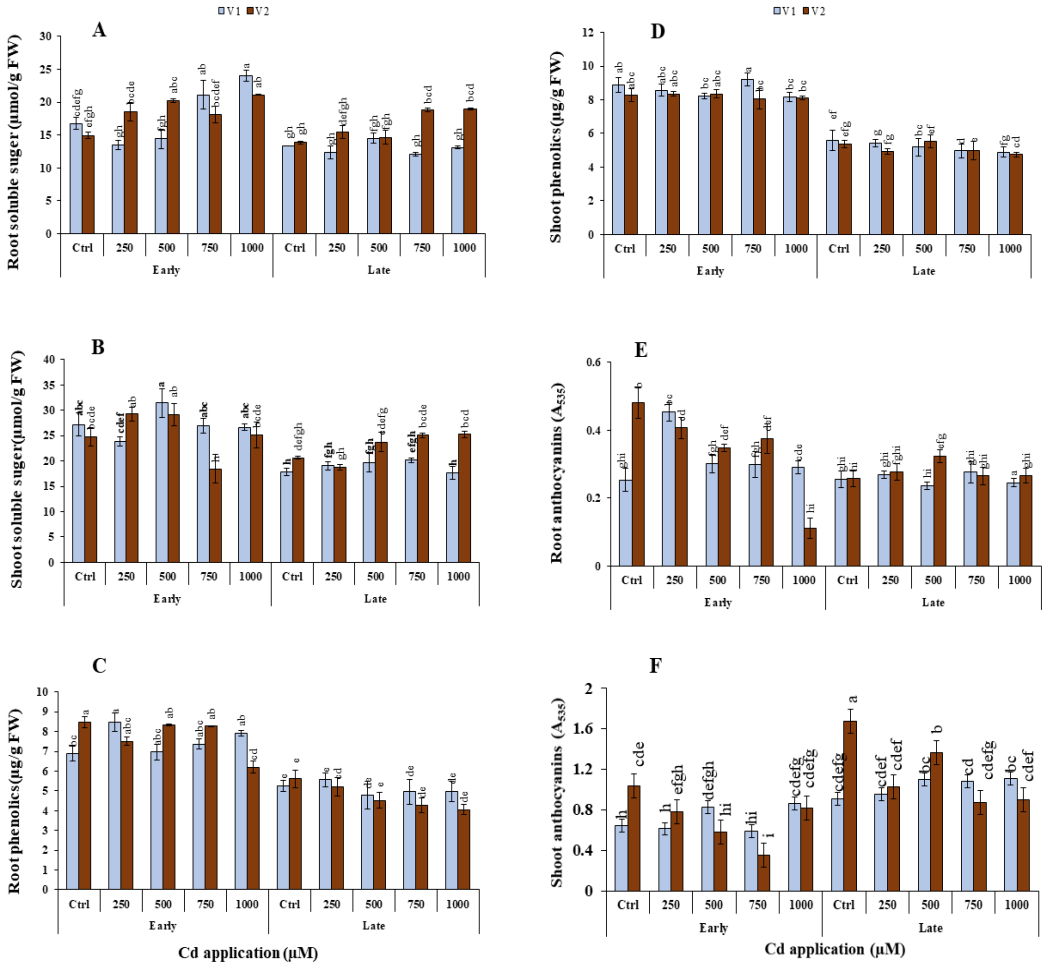

Figure 3. *Root soluble sugar (A) shoot soluble sugar (B), root phenolics (C), shoot phenolics (D), root anthocyanins (E), and shoot anthocyanins (F) of the cotton plant (two cotton cultivars, namely, V_1 = KL-MNH-886 and V_2 = KL-FH-142) treated with $CdCl_2$ treatment was applied to 15 days old plants and early and late crop grow with the difference. Mean values sharing different letter (s) on the bars are significantly different from each other at $p \leq 0.05$*

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The phenolic parameter in the response of Cd. Moreover, shoot phenolic had significantly ($p \leq 0.05$) decreased during the late sowing period in both varieties V_1 (0.24 ± 0.01) and V_2 (0.26 ± 0.02) in the presence of higher Cd levels (750 μM and 1000 μM) as compared to early sowing. In addition, root phenolics also decreased in both varieties (0.21 ± 0.01) under the presence of high Cd application during late time intervals as compared to early sowing.

For root and shoot phenolic, although varieties and treatments showed significant differences. Shoot and root phenolic significantly ($p \leq 0.05$) reduced with high Cd treatment (*Fig. 3A-B*). The shoot and root phenolic have a reverse relation with Cd stress and findings showed that the Cd effect on phenolic content was more prominent at 750 and 1000 μM Cd. Shoot phenolics content significantly ($p \leq 0.05$) decreased in V_1 during the late sowing interval as compared to the early interval and respective control. Similarly, the application of 1000 μM Cd has significantly ($p \leq 0.05$) decreased root soluble sugar (0.13 ± 0.001) in V_1 under the late sowing period than in the early sowing period and control.

Data statistically analyzed for root anthocyanins showed highly significant results among interaction means. Results showed that Cd (250 μM) significantly ($p \leq 0.05$) increased the root anthocyanins in V_1 as compared to V_2. While the application of Cd (500, 750, and 1000 μM) decreased the root anthocyanins in both early and late varieties as compared to the control. Moreover, root anthocyanins were significantly ($p \leq 0.05$) lowered in V_2 during the late sowing interval as compared to the early sowing period and their respective control (*Fig. 3*). Comparison of early and late crops indicated that the early sown crops had high resistance to Cd stress than the late crops. Thus, these findings illustrated that a favorable environment could be beneficial in providing resistance to Cd stress.

Ionic relations and Cd accumulation

The observations showed that Cd (250 μM) increased the root calcium in V_2 as compared to V_1 and the respective control, however, this impact was statistically non-significant. While the application of Cd (500, 750, and 1000 μM) also increased the root calcium in both early and late varieties as compared to the control. Calcium ions (in root were decreased in the early sowing period in V_1 as compared to the late sowing period. While calcium contents were higher in the late sowing time interval V_1 variety. Results showed that Cd at 250 μM significantly ($p \leq 0.05$) increased the calcium of shoot in early varieties (KL-FH142) as compared to (KL-MNH886). Whilst the application of Cd at higher doses (500, 750, and 1000 μM) decreased the shoot calcium in both early and late varieties as compared to the control. Furthermore, shoot calcium and potassium ion contents were not decreased as compared to root Ca ion contents. It was noted that the shoot and root potassium content declined with an increase in Cd stress level, however, Cd effects were more significant ($p \leq 0.05$) at higher levels in all plants as compared with the control. Hence, these results affirmed the better resistance of V_1 against Cd toxicity as compared to V_2. On the other hand, Cd had a direct relation with calcium contents in cotton plants. Calcium contents in the root were high with an increase in Cd treatment. Cd effect on calcium content in the shoot had no significant effects, calcium contents were slightly high when Cd concentrations increased from 250 to 1000 μM compared with the control in both varieties (*Fig. 4*).

The finding illustrated that root and shoot Cd contents was in direct relation to Cd treatment. Results showed that Cd (250 μM) significantly ($p \leq 0.05$) increased the root Cd

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in early varieties (KL-FH142) as compared to (KL-MNH886). While the application of Cd at 500, 750, and 1000 µM declined the root Cd in both early and late varieties as compared to the control. This relation increased with an increase in concentrations of Cd. Results showed that Cd (250 µM) significantly ($p \leq 0.05$) increased the shoot Cd in V_1 as compared to V_2. While the exposure of Cd at 500-1000 µM increased the shoot Cd in both early and late varieties as compared to the control. Thus, findings demonstrate that Cd levels in all plants (early and late, V_1 and V_2) were linearly enhanced.

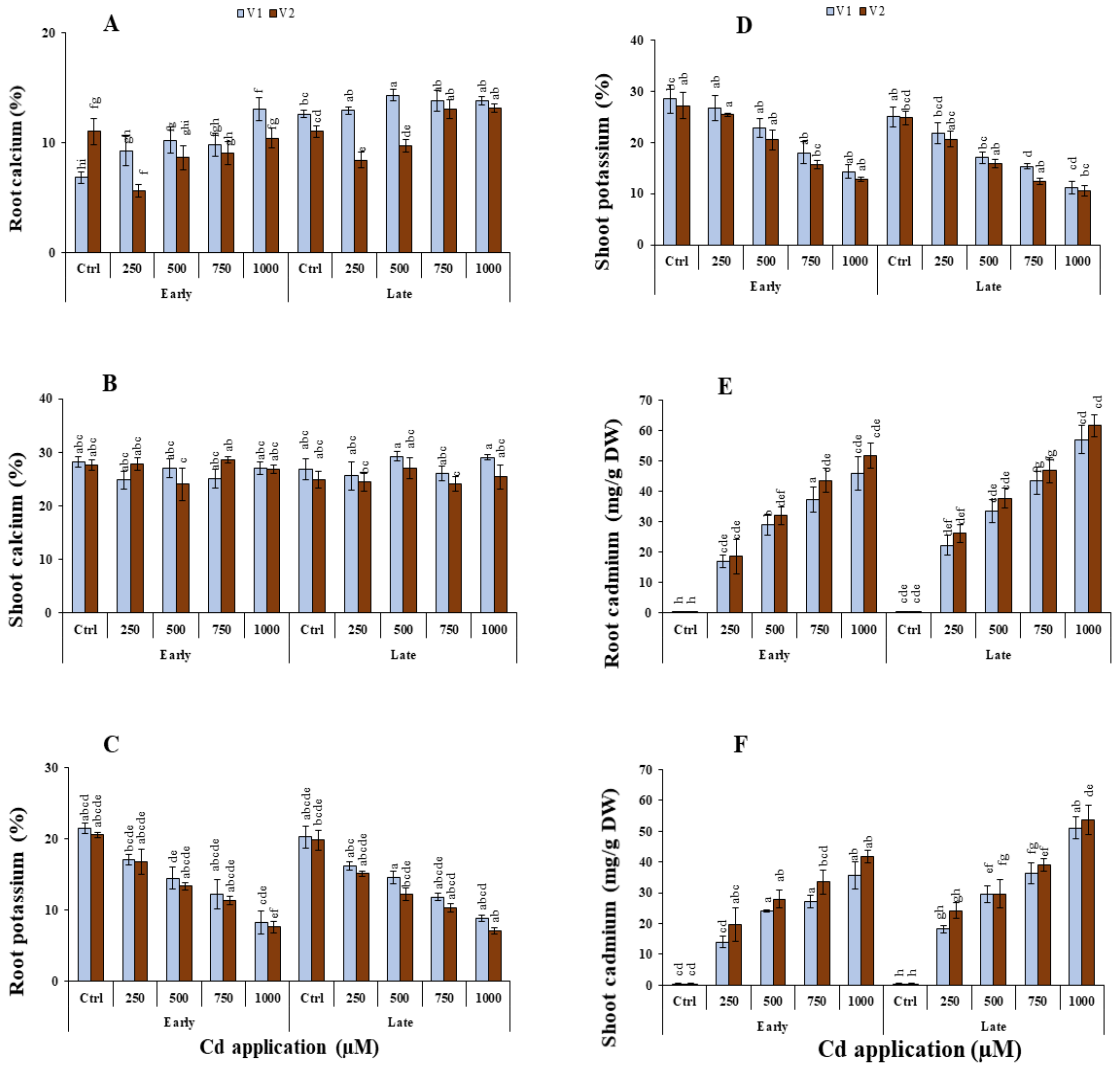

Figure 4. *Root potassium (A), shoot potassium (B), root calcium (C), shoot calcium (D), root Cd (E), and shoot Cd (F) of the cotton plant (two cotton cultivars, namely, V_1 = KL-MNH-886 and V_2 = KL-FH-142) treated with $CdCl_2$ treatment was practiced on 15 days old plants and early and late crop sow with the difference. Mean values sharing different letter (s) on the bars are significantly different from each other at $p \leq 0.05$*

Discussion

Cd is a toxic environmental pollutant that is reported to inhibit plant growth and development. In the present study, all Cd levels negatively affected the plant growth of

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G. hirsutum, causing significant reductions ($p \leq 0.05$) in plant growth and dry biomass. It has also been reported that plant varieties differ in their tolerance to Cd toxicity. Reduction in plant biomass and growth due to Cd toxicity as in different plants by a report (Hédiji et al., 2015). At higher Cd concentrations, the number of leaves and leaf surface area significantly ($p \leq 0.05$) reduced by the application of Cd in both cotton varieties (V_1, and V_2) during early and late time intervals might be attributed to a previous study by Guo et al. (2016), in which *M. sinensis* and *M. floridulus* showed a significant reduction in leaves under even at 200 μM Cd (Guo et al., 2016). However, higher Cd concentrations (50-100 μM) disturbed the tolerance mechanism and even plant death owing to reducing photosynthesis, respiration, water, and consequently nutrient uptake (Haider et al., 2021). This effect might be due to time intervals (early and late), during early July which is reported as a more favorable environment for the cotton plants due to better environmental conditions. Roots are more affected than shoots because of the higher accumulation of Cd than shoots which might be due to restricted transport to the upper parts of the plant (Sterckeman and Thomine, 2020).

Photosynthesis is also suppressed by Cd stress; this may be due to the destruction of chlorophyll synthesis (Vajpayee et al., 2000). Besides, it is also depicted that Cd has a harmful impact on carotenoid contents (Thapar et al., 2008). Photosynthetic pigments like (Chl a, Chl b, and carotenoids) are affected negatively in presence of Cd concentration compared with the control. With increasing Cd concentration, declining effects on pigments also became prominent in this work. Similar results have been discussed in other previous reports (Jiang et al., 2007). Also, our results showed that the photosynthetic rate, stomatal conductance, and transpiration rate declined with higher Cd levels. These observations of gas exchange attributes were found to be coherent with other studies (Vinit-Dunand et al., 2002).

Analysis of data showed the reduction of potassium content in the root and shoot of the cotton plant. It might be the result of high Cd stress. Root calcium was also severely affected by Cd stress. Calcium contents in roots were enhanced parallel to the Cd concentration, on the other hand, the impact of Cd on the shoot was less than on the root, roots are primarily an organ of plants where Cd is radially absorbed and transported to leaves by xylem sap, thereby roots are more affected by Cd than leaves in both varieties of the early and late crop. According to this investigation, root and shoot soluble sugar content increased with enhancing Cd toxicity. It can be seen from the change of soluble sugar content that with the increase in Cd concentration and the extension of stress time, cotton varieties (V_1 and V_2) maintained the osmotic pressure to adapt to environmental conditions by increasing the soluble sugar content (Kumari and Kaur, 2020). Soluble sugars in plants, such as sucrose, glucose, and fructose, can protect from freezing damage caused by low-temperature stress and can induce an increase in enzyme activity because of this reason, they are known as good membrane protectants (Kumari and Kaur, 2019). Increasing soluble sugar contents in the root was more obvious.

Shoot and root phenolics have an inverse relation with Cd stress. The negative effects of Cd stress were more prominent on late-crop plants. Similar trends of significant variations were also established in other plants under Cd stress (Mihaličová et al., 2014; Azimychetabi et al., 2021). All treatments of Cd significantly ($p \leq 0.05$) enhanced Cd levels in the roots and shoots of cotton plants. Cd concentration in roots was more than in shoots under Cd stress might be the approach of plants to overcome the heavy metal stress as reported by other researchers (Arshad et al., 2016; Khaliq et al., 2016; Hédiji et al., 2015).

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Conclusions

Our study showed that Cd had detrimental effects on plants' roots and shoots in both cotton varieties. The current findings also demonstrated that the growth of the cotton plant was retarded by rising Cd levels, which also constrained physiological and biochemical activities. Furthermore, more negative effects of varying doses of Cd were observed on the late crop as compared to the early plantation. Anyhow, early sowing of cotton was found better for more growth and nutritional value. Early-sowing crops had more resistance than late-sowing crops against Cd toxicity so time interval plays a significant role in plant growth. In conclusion, cotton had great potential in reducing the Cd concentration in the soil while maintaining the highest increase in physiological and biochemical attributes under early sowing time.

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