

Research paper

**Impact of cadmium stress on growth and physiological responses of fenugreek (*Trigonella foenum-graecum* L.)**

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**Abstract**

Pollution of the environment by toxic metals creates stress conditions that generally negatively affect plant growth and development. Cadmium is a highly toxic metal that enters the environment mainly through industrial activities and affects crops and other agricultural plants. In the current investigation, the influence of different concentrations of CdCl<sub>2</sub> on Cd uptake, growth parameters, and antioxidant responses of *Trigonella foenum-graecum* L. were investigated. Results showed that H<sub>2</sub>O<sub>2</sub> and malondialdehyde contents were increased by Cd treatments compared to the control plants. Cd stress differently altered the activity of antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, and polyphenol oxidase. The content of antioxidant compounds such as phenols, flavonoids, anthocyanins, soluble proteins, and proline was also increased by the Cd treatment. In addition, our results demonstrated the decrease in seed germination percentage, growth parameters, and chlorophyll and carotenoid contents that may be considered circumstantial evidence for the toxicity of cadmium.

**Keywords:** antioxidant enzymes; cadmium; fenugreek; nonenzymatic antioxidant; phenolic compounds; proline

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**Introduction**

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family Fabaceae and is widely distributed in the world (Branch 2013). It is well known as a source of many potent drugs for a diversity of its constituents, such as steroids, N-compounds, polyphenolic substances, volatile constituents, amino acids, etc (Mehrafarin *et al.* 2011). The seeds and leaves of fenugreek plants are useful in the treatment of multiple diseases including diabetes and cancer. Fenugreek is widely cultivated in Iran as a vegetable and spice crop and its seeds are used as a tonic and blood sugar lowering (Branch 2013; Zandi *et al.* 2017).

Environmental pollution due to heavy metals in soil is an increasingly serious worldwide threat to the environment, which leads to toxicity, risk to human health, and disrupts the ecosystem (Begum *et al.* 2018; Riskuwa-Shehu *et al.* 2020). Some of the heavy metals, such as cadmium (Cd), mercury (Hg), and lead (Pb) are highly toxic even in small amounts. Cd is the most toxic and is relatively mobile in soils. Cd, as a non-nutritive heavy metal, causes plant growth inhibition, chlorosis uptake limitation, alterations in activated oxygen metabolism, cell disturbances, and homeostasis by high stability. Cd contamination in the environment also has negative effects on the

biodiversity and activity of plant-associated microbial communities (Begum *et al.* 2018; Jan *et al.* 2019; Wang *et al.* 2020).

Stress conditions including Cd stress produce an ionic imbalance that stimulates the generation of reactive oxygen species (ROS) leading to disruption of cell and organelle membranes, which changes the metabolic activity of the cell via alteration of molecular signaling, osmoregulation, and secondary metabolites production. The plant defense system through antioxidant response, which consists of antioxidant components (enzymatic and non-enzymatic), is generated to cope with ROS and neutralize the damaging effects of the ROS (Ullah *et al.* 2019). The present study was conducted to evaluate the impact of different Cd concentrations on Cd uptake, plant growth parameters, and antioxidant responses of *Trigonella foenum-graecum* plant.

## Material and Methods

### *Plant materials and growth conditions*

*Trigonella foenum-graecum* seeds were collected from the Hamedan province, Iran. The seeds were surface sterilized in 75% ethanol for 10 min and 0.5% calcium hypochlorite for 15 min, and then washed 3 times with sterile distilled water. Seeds were cultured in 9×10 cm plastic pots containing windy sand, perlite, and cocopeat 1:1:1 (v/v/v), and watered with 1/2 MS solution twice a week until the seedling emergence. The growth chamber was set at 250 mmol m<sup>-2</sup>s<sup>-1</sup> light intensity, 16/8 h light/dark photoperiod, 25±1 °C temperature, and 80-90% relative humidity. At the four-leaf stage, plants were subjected to four levels of CdCl<sub>2</sub> (0, 100, 200, and 400 µM) twice a week with irrigation

for two weeks. The experiment was performed in triplicate and each replicate contained 15 seedlings. At the end of the experiment, one-month plants were collected frozen in liquid nitrogen and then transferred to -80 °C until analyses.

### *Cadmium content*

For total Cd determination, plant samples (0.05 g) were dried at 80 °C for 48 h. The homogenate powder was mixed with 3 mL of 65% HNO<sub>3</sub> and kept under the hood for 12 h. Then, the samples were boiled in a water bath (90 °C) for at least 2 h. After cooling at room temperature, 1 mL of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added and incubated at 90 °C for at least 1 h in a water bath. After increasing the total volume to 10 mL with the distilled water, the Cd content was analyzed using an atomic absorption spectrophotometer (Shimadzu AA-6200).

### *Growth parameters*

Seeds of each treatment were germinated on filter paper in a petri dish at 25±1 °C in the dark. The rate and percentage of seed germination were recorded every 24 h for 7 days. Germination was defined by the appearance of the emerging radicle. The germination rate was calculated according to the following equation (Bradbeer 1988):

$$GR = \sum (N_i / T_i)$$

where N<sub>i</sub> is the number of new emerging seeds on day T<sub>i</sub> and T<sub>i</sub> is the time from the start of the experiment to the i<sup>th</sup> interval.

Growth characteristics including shoot and root length (cm), shoot and root fresh and dry weight (DW)(gr), and shoot and root torsion weight (gr) were calculated at the time of harvest.

To calculate torsion weight, the plant samples were cut into pieces and placed in distilled water, and kept in the dark for 72 h. Then the total weight of the parts was measured and their average was calculated. To measure the DW, the plant parts at the previous stage were kept in an oven at 70 ° C for 48 h, and then their weights were calculated.

#### ***Photosynthetic pigment content***

Photosynthetic pigments were extracted from the leaves by using 80% acetone and the optical density of extracts was read by UV-visible spectrophotometer (Perkin Elmer, USA) at 470, 647, and 663 nm. The pigment content was calculated as mg g<sup>-1</sup> fresh weight (FW) (Sumanta *et al.* 2014).

#### ***H<sub>2</sub>O<sub>2</sub> content and lipid peroxidation***

Hydrogen peroxide level was estimated by a UV-visible spectrophotometer (Perkin Elmer, USA) at 412 nm and expressed as μmol g<sup>-1</sup> FW (Velikova *et al.* 2000). The content of H<sub>2</sub>O<sub>2</sub> was calculated using the molar extinction coefficient of 0.28 mol<sup>-1</sup> cm<sup>-1</sup> (ε<sub>530</sub> = 0.28 mol<sup>-1</sup> cm<sup>-1</sup>). Lipid peroxidation was calculated in terms of malondialdehyde (MDA) content using thiobarbituric acid (TBA) based on Heath and Packer (1968). The absorbance of the colored reaction product was determined at 532 nm, and the extinction coefficient of 155 mmol<sup>-1</sup>cm<sup>-1</sup> was used for measurement of the MDA level (Heath and Packer 1968).

#### ***Antioxidant enzymes activities***

The activity of the peroxidase (POD) enzyme was measured by a spectrophotometer with guaiacol as the substrate. The reaction combination contained

100 mM phosphate buffer (pH 7), guaiacol (1%), H<sub>2</sub>O<sub>2</sub> (0.3%), and enzyme extract in a final volume of 1 mL. Changes in the absorbance, due to the oxidation of guaiacol, were measured at 436 nm for 1 min (MacAdam *et al.* 1992). Catalase (CAT) activity was determined by checking the disappearance of H<sub>2</sub>O<sub>2</sub> at 240 nm for 1 min (Azarmehr *et al.* 2013). A reaction mixture and 50 μL of the enzyme extract were used. Polyphenol oxidase (PPO) activity was measured by a spectrophotometer at 430 nm according to the method described by Raymond *et al.* (1993), and was expressed as ΔAbs μg<sup>-1</sup> protein min<sup>-1</sup> (Raymond *et al.* 1993).

#### ***Total phenol, flavonoid, and anthocyanin contents***

Total phenol content was extracted with acidic methanol (1 ml HCl and 99 mL pure methanol) solution as described by Plessi *et al.* (2007) for determining the total phenol and flavonoid and anthocyanin contents. Total phenol content was determined by a UV-visible spectrophotometer (Perkin Elmer, USA) and Folin-Ciocalteu reagent method at 765 nm (Plessi *et al.* 2007) using the gallic acid calibration curve. The total flavonoid content was measured at 415 nm using the quercetin calibration curve (Chang *et al.* 2002). The content of total anthocyanins was estimated according to Wagner *et al.* (1979). For this purpose, acidic methanol extract was centrifuged and the upper solution was transferred to new pipes, then kept in the dark overnight and the absorbance was measured at 550 nm. For calculation, an extinction coefficient of 33000 mmol<sup>-1</sup>cm<sup>-1</sup> was used for anthocyanin content and

expressed as  $\mu\text{g g}^{-1}$  FW (Wagner 1979).

### ***Proline and soluble protein contents***

The proline content was measured using ninhydrin (Bates *et al.* 1973). A mixture of proline, ninhydrin acid, and glacial acetic acid (1:1:1) solutions was incubated at 95 °C for 1 h. After cooling the reaction in an ice bath, the reaction combination was mixed with 2 mL toluene and then the absorbance of the chromophore phase was determined by the spectrophotometer. The amount of free proline was calculated from the standard curve at 520 nm and expressed as  $\mu\text{mol g}^{-1}$  FW. The protein content of the extracts was measured by a BioRad Protein Assay Reagent (Bradford 1976) using bovine serum albumin as the standard. Briefly, for determination of total protein, 2.5 mL Bradford reagent was added to 100  $\mu\text{L}$  of the extract into a tube, and quickly vortexed. Soluble proteins were placed at lab temperature in dark for 25 min, then absorbed by the spectrophotometer at 595 nm.

### ***Statistical analysis***

All assayed characteristics were measured in triplicate. Data were subjected to analysis of variance using SPSS software (SPSS Inc, version 20, SPSS Inc., Chicago, USA). A comparison of the means was carried out by Duncan's multiple-range test with the same software. Significance was determined at  $p \leq 0.05$  level. The results were expressed as mean values and standard error of the means.

## **Results**

### ***Cd content***

Results from ICP analysis of *T. foenum-graecum* plants treated with  $\text{CdCl}_2$  for two weeks showed Cd absorption and accumulation by roots and shoots (Figure 1). Cd content of the shoots and roots increased with Cd (200  $\mu\text{M}$ ) 15 and 18 folds compared to the control plants, respectively.

### ***Growth parameters***

Plant growth characteristics were used as one of the important criteria to assess the plants' responses to the Cd stress. The data associated with growth (germination, root and shoot length, plant weight, and shoot and root torsion weight) were assembled in Table 1. The results showed that increasing the level of Cd decreased significantly the germination percentage (up to 69%) and rate (up to 63%) in comparison with the control groups. It was observed that the increase of Cd concentration in the nutrient solution, induced a significant decrease in the root and shoot length up to 67% and 70%, respectively. The increase in Cd concentration also caused a significant reduction in other considered growth characteristics such as plant weight (fresh and dry), and the torsion weight of the shoots and roots (Table 1). This reduction was 83 and 78% for FW, and 83 and 76% for DW of the shoots and roots, respectively. In addition, a 54 and 55% reduction were observed in torsion weights of shoots and roots, respectively, at 400  $\mu\text{M}$  Cd compared to the control plants.

### ***Photosynthetic pigment content***

Chlorophylls a and b and carotenoid contents significantly declined in a dose-dependent manner. The highest dose of Cd (400  $\mu\text{M}$ ) reduced the chlorophylls a and b, and carotenoid contents to 69,

Table 1. Growth parameters of *Trigonella foenum-graecum* plants at different Cd concentrations

Cd concentration (mM)	Germination rate	Germination percentage (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (gr)	Root fresh weight (gr)	Shoot dry weight (gr)	Root dry weight (gr)	Shoot turgid weight (gr)	Root turgid weight (gr)
0	9.44 ± 0.46 <sup>a</sup>	75.33 ± 5.03 <sup>a</sup>	14.66 ± 0.57 <sup>a</sup>	13.33 ± 0.57 <sup>a</sup>	1.20 ± 0.20 <sup>a</sup>	0.12 ± 0.02 <sup>a</sup>	0.46 ± 0.07 <sup>a</sup>	0.008 ± 0.01 <sup>a</sup>	0.67 ± 0.06 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>
100	7.21 ± 0.71 <sup>b</sup>	60.00 ± 0.00 <sup>b</sup>	11.66 ± 0.57 <sup>b</sup>	8.66 ± 0.57 <sup>b</sup>	0.86 ± 0.10 <sup>b</sup>	0.07 ± 0.00 <sup>b</sup>	0.33 ± 0.04 <sup>b</sup>	0.004 ± 0.00 <sup>b</sup>	0.55 ± 0.04 <sup>b</sup>	0.07 ± 0.00 <sup>b</sup>
200	5.30 ± 0.46 <sup>c</sup>	46.66 ± 5.77 <sup>c</sup>	8.66 ± 0.57 <sup>c</sup>	6.66 ± 1.52 <sup>c</sup>	0.50 ± 0.02 <sup>c</sup>	0.04 ± 0.00 <sup>c</sup>	0.19 ± 0.00 <sup>c</sup>	0.003 ± 0.00 <sup>c</sup>	0.43 ± 0.02 <sup>c</sup>	0.06 ± 0.01 <sup>c</sup>
400	3.44 ± 0.41 <sup>d</sup>	23.33 ± 5.77 <sup>d</sup>	4.33 ± 1.15 <sup>d</sup>	4.33 ± 0.57 <sup>d</sup>	0.20 ± 0.25 <sup>d</sup>	0.02 ± 0.00 <sup>c</sup>	0.07 ± 0.00 <sup>d</sup>	0.002 ± 0.00 <sup>d</sup>	0.30 ± 0.02 <sup>d</sup>	0.03 ± 0.00 <sup>d</sup>

Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

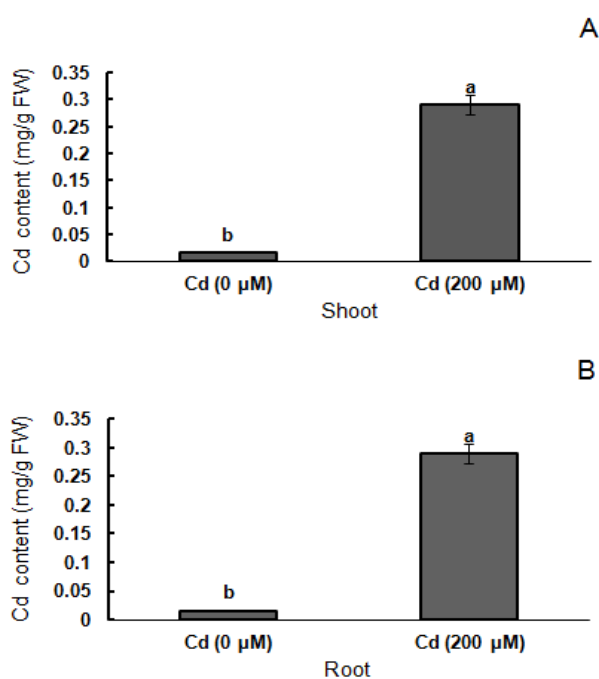


Figure 1. Cd content of shoot (A) and root (B) of *Trigonella foenum-graecum* plants at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

77, and 67%, respectively (Figure 2).

#### *H<sub>2</sub>O<sub>2</sub> content and lipid peroxidation*

In our study, the content of H<sub>2</sub>O<sub>2</sub> and MDA rose

depending on the Cd concentration (Figure 3). The highest concentration of Cd (400 μM) had the most pronounced effect (2.75 and 2.83 folds), respectively, on the MDA and H<sub>2</sub>O<sub>2</sub> contents

compared to the control groups.

#### ***Antioxidant enzymes activities***

Cd at 100  $\mu\text{M}$  had no significant impact on the POD activity, while higher concentrations of Cd (200 and 400  $\mu\text{M}$ ) declined the POD activity by about 11 and 29%, respectively compared to the control plants (Figure 4). In comparison with the control plants, 100 and 200  $\mu\text{M}$  Cd-induced SOD activity, whereas no significant change was observed in the activity of this enzyme at 400  $\mu\text{M}$  Cd. CAT activity declined at a higher Cd dose, and there were no significant alterations at 100 and 200  $\mu\text{M}$  Cd. In this study, PPO activity decreased with the increase in Cd dose (Figure 4). The activity of PPO declined to 31% compared to the control plants.

#### ***Total phenol, flavonoid, and anthocyanin contents***

The total content of phenols, flavonoids, and anthocyanins of *T. foenum-graecum* leaves was significantly increased by increasing the Cd concentration (Figure 5). The total phenol content at the highest Cd dose (400  $\mu\text{M}$ ) increased up to 3.57 folds compared to the control plants. The total flavonoid and anthocyanin contents significantly increased up to 6.38 and 4.74 folds, respectively, with an increase in the Cd concentration compared to the control plants (Figure 5).

#### ***Proline and soluble proteins***

The results showed that proline and soluble proteins were significantly increased with increasing the Cd concentration compared to the control plants, with 2.23 and 3.31 folds higher in

the highest concentration of Cd, respectively (Figure 6).

#### **Discussion**

Cadmium is one of the most toxic environmental and industrial pollutants that causes severe physiological disorders of growth and development in all plants (Salarizadeh *et al.* 2016; Jan *et al.* 2018). Excessive Cd ions are taken up readily by the plant roots, then translocate to the shoots, and induce intricate changes in plants at different levels (Salarizadeh *et al.* 2016). Our results confirmed the Cd absorption and accumulation by roots and shoots of *T. foenum-graecum* plants under the Cd stress (Figure 1). In our experiment, Cd application harmed *T. foenum-graecum* seed germination (Table 1). The percentage and germination rate of seeds significantly decreased by increasing levels of Cd (Table 1). A reduction of seed germination by cadmium has been shown in fenugreek (Zayneb *et al.* 2015) and cumin (Salarizadeh *et al.* 2016) plants. The high inhibitory effect of Cd on the have some defense mechanisms against metal pollution. Also, decreasing in seed germination may be related to the negative effects of cadmium on the uptake and movement of water or breakdown and/or change in permeability characteristics of the cell membrane (Zayneb *et al.* 2015; Salarizadeh *et al.* 2016).

In the present study, all Cd levels negatively influenced the plant growth of *T. foenum-graecum*, causing a significant reduction in the height, fresh and dry weight, and the torsion weight of shoots and roots (Table 1). Plant growth reduction is a common response of many plant species exposed to varying levels of heavy metals (Hatamian *et al.*

2020). Various biochemical mechanisms of the plants such as photosynthesis and translocation of photosynthetic products and nutrient elements may be involved in growth reduction under heavy metal toxicity. There might be a general reduction in leaf morphophysiological characteristics, such as chlorosis, through the prevention of cell division and enlargement, hydraulic conductance, and water potential of plant tissues (Guo *et al.* 2016; Shah *et al.* 2017, Hatamian *et al.* 2020; Ozyigit *et al.* 2021). Moreover, the inhibitory effect of Cd ions on plant

growth is mediated through alteration in the activity of many key enzymes involved in various metabolic pathways and auxin metabolism or auxin carriers (Prasad 1995). Guo *et al.* (2016) found that Cd ions negatively reduced plant growth and dry biomass of *M. sinensis* and *M. floridulus* plants. Hatamian *et al.* (2020) reported that plant growth characteristics were adversely influenced by 6 months-application of cadmium in irrigation water (Hatamian *et al.* 2020).

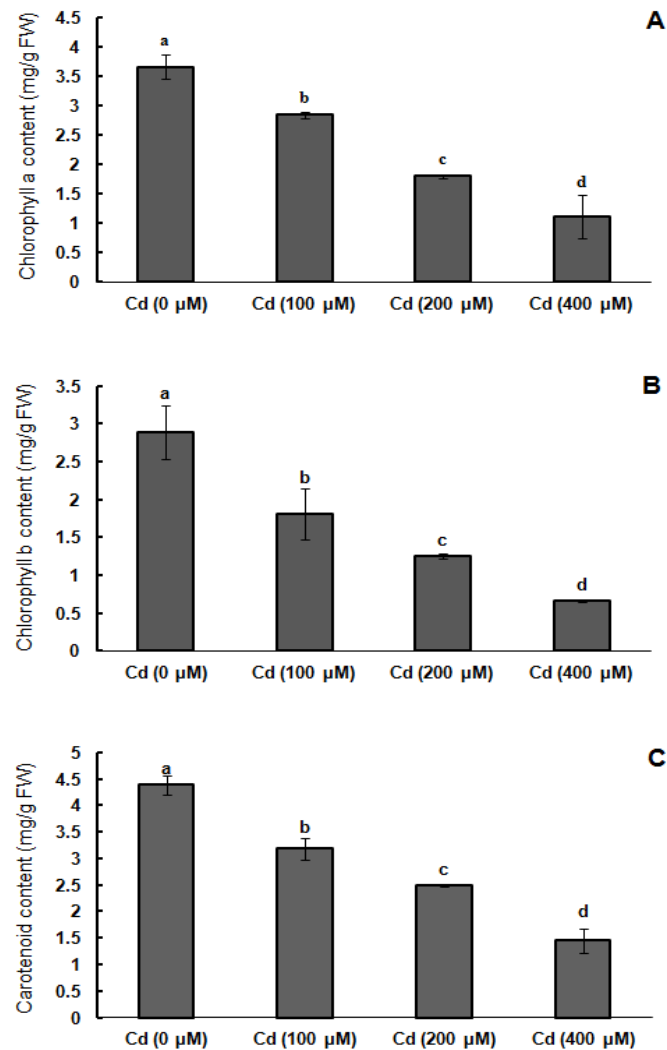


Figure 2. Chlorophyll *a* (A), chlorophyll *b* (B), and carotenoid (C) contents of *Trigonella foenum-graecum* leaves at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

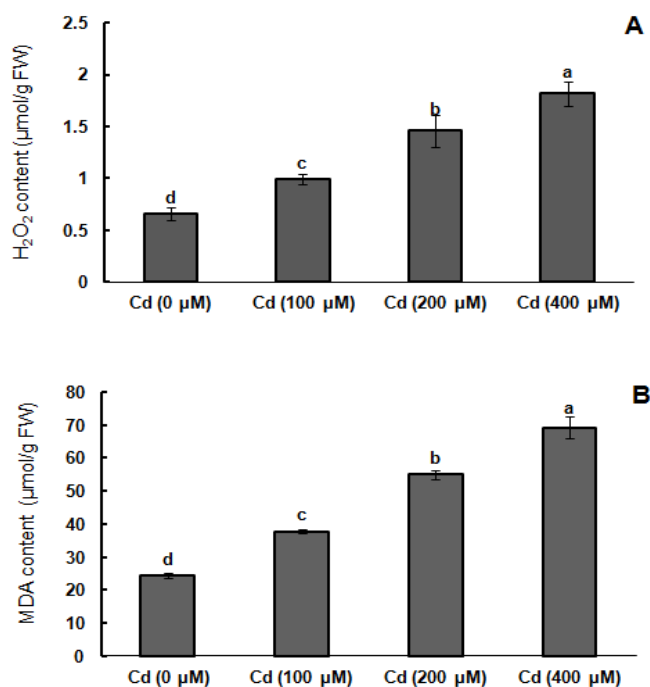


Figure 3. H<sub>2</sub>O<sub>2</sub> (A) and MDA (B) contents of *Trigonella foenum-graecum* leaves at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

Our data revealed that chlorophylls (a, b) and carotenoids significantly declined in a dose-dependent manner of Cd application (Figure 2). In agreement with our result, the chlorophyll content in several other plant species was greatly reduced under Cd stress (Chaca *et al.* 2014; Mukherjee 2017; Dawuda *et al.* 2020). The decrease in pigments production by high Cd concentration could be attributed to a decrease in chlorophyll biosynthesis by interfering with the activity of the biosynthetic enzymes, particularly protochlorophyllide oxidoreductase and chlorophyll synthase (Dawuda *et al.* 2020). Recently, it was reported that chlorosis was caused by the loss of chlorophyll due to Mg losses caused by Cd in Cd-treated bean plants (Benabid and Fouzi Ghorab 2012).

In the Cd-stressed plants, the disturbance in numerous biochemical and physiological processes leads to enhanced biosynthesis of ROS such as H<sub>2</sub>O<sub>2</sub>, superoxide anion (O<sub>2</sub><sup>-</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>), and hydroxyl radical (OH<sup>•</sup>) in the cell causing oxidative damage (Dawuda *et al.* 2020; Shah *et al.* 2020). Although at normal levels, ROS act as important secondary messengers in several plant processes, including tolerance to various stresses, excessive amounts of ROS are harmful to cells mainly because of their reactions with lipids, proteins, and nucleic acids (Dawuda *et al.* 2020).

In agreement with our results, many studies have shown that the Cd-treated plants had higher MDA and H<sub>2</sub>O<sub>2</sub> contents than those of the control plants (Zayneb *et al.* 2015; Guo *et al.* 2016; Yildirim *et al.* 2019). H<sub>2</sub>O<sub>2</sub> as an oxidative agent



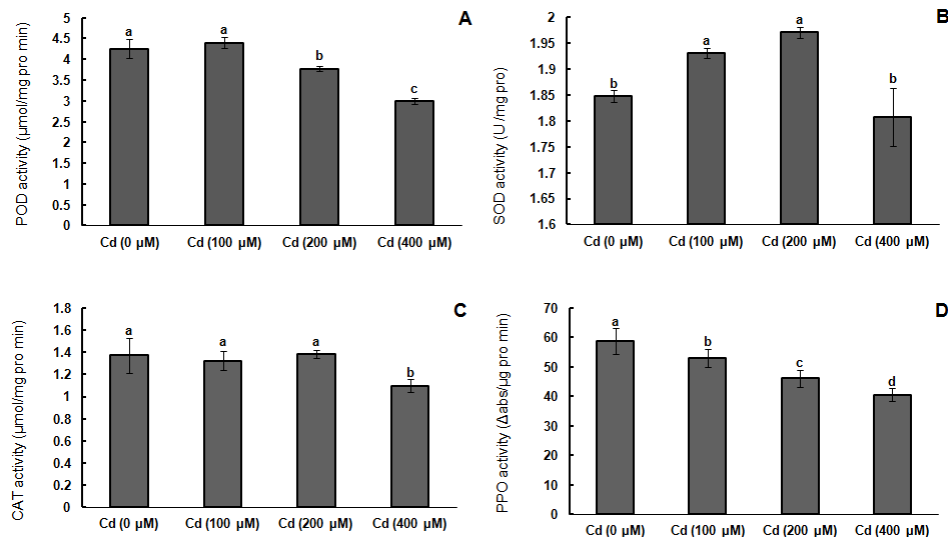


Figure 4. Peroxidase (POD) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C), and polyphenol oxidase (PPO) (D) activities of *Trigonella foenum-graecum* leaves at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

is relatively stable and able to penetrate the plasma membrane that activates various antioxidant enzymes, but over-accumulation of  $H_2O_2$  induces peroxidative reactions that damage plant cells (Zayneb *et al.* 2015; Guo *et al.* 2016). As a consequence of lipid peroxidation under oxidative stress, the enhanced production of MDA has already been reported in *T. foenum-graecum*, lettuce, and maize plants (Hussain *et al.* 2013; Mukherjee 2017; Dawuda *et al.* 2020). MDA acts as an indicator of free radical production, causes serious damage to the cell membrane, is negatively linked with plant growth and apoptosis, and premature senescence (Hussain *et al.* 2013; Zayneb *et al.* 2015; Guo *et al.* 2016).

Plants have both enzymatic and non-enzymatic antioxidant systems to alleviate ROS

generated under biotic and abiotic stresses. Antioxidant enzymes such as SOD, POD, and CAT are involved in the oxidative defense system (Hussain *et al.* 2013). In the current study, the 400 µM Cd concentration decreased the activity of antioxidant enzymes (POD, CAT, PPO), while did not significantly alter the SOD activity (Figure 4). Cd affects plant metabolism through the inhibition of enzyme activity and protein denaturalization (Chaca *et al.* 2014). However, the activity of SOD was increased by 100 and 200 µM of Cd in the Cd-treated *T. foenum-graecum* plants. In previous studies, the activity of SOD and CAT in *T. foenum-graecum* plants declined under  $CdCl_2$  treatment. Dawuda *et al.* (2020) showed that 100 mM  $CdCl_2$  declined the SOD activity of the plants, while both CAT and POD activities in the roots and leaves of

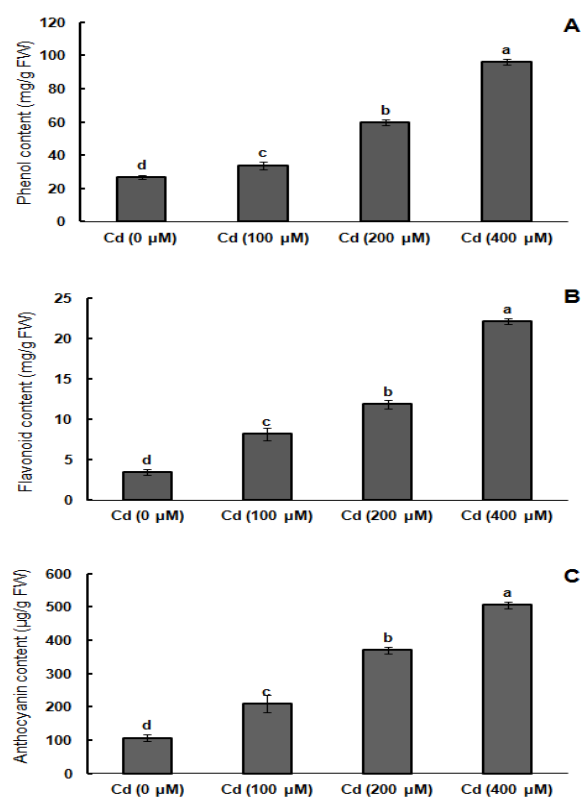


Figure 5. Phenol (A), flavonoid (B), and anthocyanin (C) contents of *Trigonella foenum-graecum* leaves at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

the plants enhanced (Dawuda *et al.* 2020).

Plants have resistance mechanisms to counter the damaging effects of ROS due to their high levels of antioxidant metabolites including phenols and flavonoids. They have been defined as antioxidants that rapidly quench free radicals due to radical chain reactions (Zayneb *et al.* 2015). Anthocyanins, as non-enzymatically ROS scavengers, have the potential role in response to stressed environments including heavy metals (Hussain *et al.* 2013). Our experiment revealed a Cd-induced increase in total phenol, flavonoids, and anthocyanins contents of *T. foenum-graecum* plants with an increase in Cd level compared to the

control plants (Figure 5). Our results on phenolic content are similar to some earlier reports on the increase in the endogenous levels of total phenols, flavonoids, and anthocyanins under cadmium stress (Hussain *et al.* 2013; Zayneb *et al.* 2015; Yildirim *et al.* 2019).

In the present study, compatible osmolytes such as proline and soluble proteins levels increased at the Cd-treated *T. foenum-graecum* leaves by increasing Cd doses (Figure 6). Accumulation of proline as a protein stabilizer is a common physiological response of plants under heavy metal stresses (Hussain *et al.* 2013; Yildirim *et al.* 2019; Hatamian *et al.* 2020). An increase in

proline content may be due to stimulation of the activity of proline synthesizing enzymes or de novo synthesis, and or decreased degradation or both under stress environments (Salarizadeh *et al.* 2016; Yildirim *et al.* 2019). Our results are in contrast with Shah *et al.* (2017) who reported a decrease in soluble proteins under cadmium stress in *Tagetes erecta*. This is possibly due to the protein degradation process as a result of increased protease activity (Salarizadeh *et al.* 2016; Shah *et al.* 2017). In a study, the protein content increased in *Trigonella foenum-graecum* at the initial

concentration of Cd ( $5 \text{ mg L}^{-1}$ ) after that, it decreased in the higher concentrations (10 and  $15 \text{ mg L}^{-1}$ ) (Mukherjee 2017).

### Conclusions

Results from the present study showed that Cd stress affected morphophysiological and biochemical attributes in *T. foenum-graecum* plants. Increased  $\text{H}_2\text{O}_2$  generation and lipid peroxidation was related to the increased Cd concentration, which decreased seed germination percentage, growth characteristics, and chlorophyll

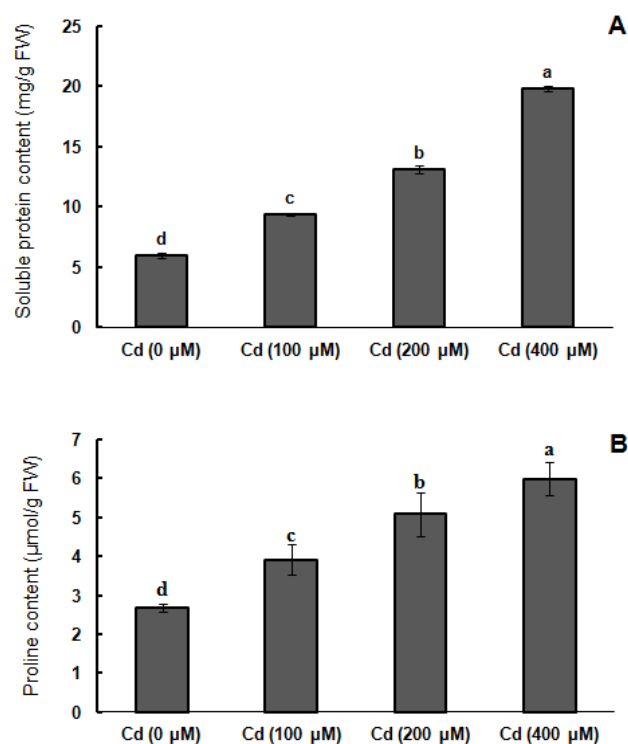


Figure 6. Soluble proteins (A) and proline (B) contents of *Trigonella foenum-graecum* leaves at different Cd concentrations. Means followed by different letters are significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

and carotenoid contents. This can be considered circumstantial evidence for the toxicity of cadmium. The increase in antioxidant compounds such as phenols, flavonoids, anthocyanins, soluble proteins, and proline and variation in the antioxidant enzymes activities demonstrates varied tolerance against the Cd stress. However, a much more detailed investigation at the cellular and molecular level will be required for a better understanding of the toxic effects of Cd on this

important plant.

### Acknowledgments

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### Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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## اثرات تنش کادمیوم بر پاسخ‌های رشدی و فیزیولوژیکی گیاه شنبلیله (*Trigonella foenum-graecum* L.)

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### چکیده

آلودگی محیط زیست با فلزات سمی شرایط تنش‌باری را به وجود می‌آورد که اغلب رشد و نمو گیاه را به شکل منفی تحت تاثیر قرار می‌دهد. کادمیوم فلزی بسیار سمی است که اساساً از طریق فعالیت‌های صنعتی وارد محیط زیست می‌شود و بر رشد گیاهان زراعی و سایر گیاهان تاثیر می‌گذارد. در پژوهش حاضر اثر غلظت‌های مختلف  $CdCl_2$  بر جذب کادمیوم، پارامترهای رشدی و پاسخ‌های آنتی‌اکسیدانی گیاه شنبلیله مورد مطالعه قرار گرفت. نتایج نشان داد که محتوای مالون‌دی‌آلدئید و پراکسید هیدروژن در گیاهان تیمار شده با کادمیوم در مقایسه با گیاهان شاهد افزایش یافت. تنش کادمیوم به طرز متفاوتی فعالیت آنزیم‌های آنتی‌اکسیدانی کاتالاز، سوپراکسید دیسموتاز، پراکسیداز و پلی‌فنل‌اکسیداز را تغییر داد. همچنین تیمار کادمیوم محتوای ترکیبات آنتی‌اکسیدانی غیر آنزیمی مانند فنل‌ها، فلاونوئیدها، آنتوسیانین‌ها، پروتئین‌های محلول و پرولین را افزایش داد. علاوه بر این، کاهش درصد جوانه‌زنی بذر و پارامترهای رشدی و نیز کاهش محتوای رنگیزه‌های فتوسنتزی کلروفیل و کاروتنوئید از اثرات سمی کادمیوم بود.

**واژه‌های کلیدی:** آنتی‌اکسیدان‌های غیر آنزیمی؛ آنزیم‌های آنتی‌اکسیدانی؛ پرولین؛ ترکیبات فنلی؛ شنبلیله