



# Attenuating Effect of an Extract of Cd-Hyperaccumulator *Solanum nigrum* on the Growth and Physio-chemical Changes of *Datura innoxia* Under Cd Stress

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## Abstract

**Purpose** The use of plant extracts obtained from plants that are highly tolerant to heavy metal toxicity has been beneficial in improving the growth of plants grown under metal toxicity conditions. A lab experiment was performed to elucidate the alleviating role of foliar applied cadmium (Cd)-hyperaccumulator *Solanum nigrum* (*S. nigrum*) extract on *Datura innoxia* (*D. innoxia*) plants grown under Cd stress (0, 50, and 100 mg Cd kg<sup>-1</sup> soil).

**Methods** Growth parameters, photosynthetic pigment content, osmo-metabolic compounds, reduced glutathione and phytochelatin content, oxidative damage, and lignin content and its related enzyme (cell wall-bound peroxidase, POX) were determined.

**Results** Apart from the foliar application response of *S. nigrum* leaf extract (SNE) in either Cd exposed or non-Cd exposed plants, growth parameters of *D. innoxia* plant grown under both Cd concentrations (50 and 100 mg Cd kg<sup>-1</sup> soil) in terms of root and shoot fresh, dry weight, length, and leaf area were noticeably diminished by 29 and 51%, 29 and 54%, 35 and 70%, 40 and 53%, 30 and 69%, 40 and 60%, and 11 and 23%, respectively, compared with untreated control plants. Foliar delivered SNE secured the photosynthetic pigment, free amino acids, soluble proteins, and soluble sugar content. Additionally, it lessened the adverse effects of Cd stress on *D. innoxia* plants by curtailing the content of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) by 28 and 27%, and 21 and 23%, respectively, compared with the plants subjected to 50 and 100 mg Cd kg<sup>-1</sup> only. The findings herein indicated that the plant water extract and their interactions in the investigated Cd rates significantly augmented phenolics, alkaloids, reduced glutathione and phytochelatin content. Cell wall stiffening in *D. innoxia* indicated that lignin content and POX were significantly higher in plants exposed to 100 mg Cd kg<sup>-1</sup> soil displaying increase values of 275 and 300%, respectively, against non-Cd treated control. The magnitude of increment imposed by Cd stress was lessened by using SNE that reflects on adequate cell growth advocated by limited lignification, in terms of lignin content, and downregulated POX activity. Owing to SNE application, root and leaves Cd contents were efficiently reduced reflecting apparent plant liveliness compared with the SNE non-treated Cd-stressed plants.

**Conclusions** The outcomes of this study designate that foliar application of the Cd-hyperaccumulator *S. nigrum* leaf extract can be counted as an unconventional and innovative approach in the alleviation of Cd stress and can be employed as integrated practice when Cd-contaminated regions were exploited for sustainable agriculture of the multipurpose plants.

**Keywords** *Datura innoxia* · Lignin · Phytochelatin · *Solanum nigrum*

## 1 Introduction

Toxic metals have been added to agricultural soils due to an increased reliance on sewage wastewater irrigation and chemical fertilizers, as well as quick industrialization, producing harmful impacts on plant and human health. Cd is one of the metal contaminants; it ranks seventh on a list of the most harmful toxins to crops in Egypt and around the world, and its long residence time in soil makes it a common

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environmental distress for the agricultural system (Ibrahim et al. 2022). Nowadays, plants may encounter more Cd due to the use of phosphorites and phosphatic fertilizers which contain threshold levels of Cd ( $4.77 \text{ mg kg}^{-1}$ ). In plants, Cd exposure affects a wide range of physiological and biochemical processes, resulting in stunted growth, chlorosis, and oxidative stress initiated by the generated of reactive oxygen species (ROS) (Gutsch et al. 2018). The photosynthetic apparatus and water balance are also affected by stomatal closure in plants (Perfus-Barbeoch et al. 2002). Cd stress can be mitigated in living cell by minimizing the toxic effects, such as detoxification through compartmentation, chelation, and sequestration in extra cytoplasmic compartments such as the cell wall (Jha and Bohra 2016).

Toxic metal tolerance comprises several adaptive scenarios at the organelle, cellular, and plant levels to cope up with metal toxicity including overproduction of soluble sugars, free amino acids, soluble proteins and medicinally active alkaloids (Saad-Allah and Elhaak 2017), osmoprotectants and antioxidant molecules (proline, betaine, glycine, phenolic compounds, flavonoids) (Khalid et al. 2019) in all plant tissues. Accordingly, it is very imperative to explore the mechanisms of Cd tolerance to ascertain which plants have ability to elicit physiological and biochemical mechanisms in response to Cd exposure. Furthermore, the practice of sustainable agricultural approaches to recuperate plant growth and productivity even under environmental stress can maintain the gaining of plants' yield under predicted climatic fluctuations (Li et al. 2021).

Cd-hyperaccumulators are the only species that can survive and reproduce in soil solutions containing Cd concentrations above  $35 \mu\text{M}$  (Dobrikova et al. 2021). The Cd tolerance in hyperaccumulators plants mostly relays on the complexation of metalloids in vacuoles and osmotic adjustment (Xu et al. 2009). Moreover, the mechanism of tolerance of hyperaccumulators plants is accomplished through the manufacturing of low molecular weight components viz. vitamins E and C, glutathione, and phytochelatins, as well as phenolic compounds that quash ROS (Xu et al. 2009). Cd-hyperaccumulators are focused owing to their photosynthesis, physiological properties, and Cd resistance along with their responding to oxidative stress (ur Rehman et al. 2019). It can also be used in sustainable agriculture under conditions of heavy metal stress and integral ultrastructure and anatomy (Tao et al. 2020). Some Cd-accumulator plants have various potentials benefits viz. potential source of a Cd-resistant gene (Kumar et al. 2021) as well as being an perfect source of generating several medicinal components (Pandey et al. 2019). Recently, utilizing plant extracts derived from plants that are highly tolerant to heavy metal toxicity has been beneficial in improving the growth of plants grown under metal toxicity conditions (Yang et al. 2021). Several studies has declared that hyperaccumulator

intercropping (Lin et al. 2018) or applying hyperaccumulator straw or extracts to soil significantly enhance growth and Cd tolerance capacity of the target plants (Han et al. 2020). Also, foliar application of hyperaccumulator extracts can be more feasible and efficient as absorption of extract's nutrients by leaves is vastly diverse from absorption of the same nutrients by roots. Roots are bathed in a soil solution that is usually relatively dilute. The concentration of the nutrients is controlled to some extent by the surrounding soil. In foliar applications, the aqueous state evaporates rapidly after application and absorption must happen from concentrated solution (Gray and Akin 1984).

*Solanum nigrum* L. named as black nightshade was for the first time recognized as a Cd hyperaccumulator by Wei et al. (2005). Further investigations verified *S. nigrum* to be a Cd hyperaccumulator with Cd concentrations of above  $100 \text{ mg kg}^{-1}$  leaf DW ( $> 0.01\%$ ) and bio-concentration factor (BF) for its roots higher than 1 (Wang et al. 2015). The ability to uptake and accumulate bulky amounts of Cd in its leaves ( $124.6 \text{ mg kg}^{-1}$  DW) assigns this plant an effective phytoremediator (Wei et al. 2005) as well. The tolerance mechanisms of Cd-mediated stress in *S. nigrum* are ascribed to the following: (1) release of organic acids that involved in coordinated and complexed with metal in vacuole away from active cellular components; heavy metal complexation by organic acids may play an important role in metal detoxification, transportation, and storage (Sun et al. 2006). (2) Activation of natural resistance-associated macrophage proteins (NRAMPs) (Song et al. 2014). (3) Antioxidants; phytochemical analysis of SNE revealed the presence of various bioactive compounds such as tannins, flavonoids, phenol, glycosides, steroids, coumarins, and quinones which shoulder an important role in Cd-binding (Kumar et al. 2020).

*Datura innoxia* belongs to solanaceae family has received high awareness all over the world (Jamshidi-Kia et al. 2018). It is cultivated for the production of secondary metabolites with commercial importance due to their extensive use in medicine, which comprise torpane alkaloids such as atropine, scopolamine, and hyoscyamine. They are exploited as parasymphatholitics for their ability to suppress parasymphathetic nerve activity (Maheshwari et al. 2013). On the other hand, after alkaloid extraction, the remaining biomass can be also used for industrial applications such as bioethanol production. The less digestible stems counted more than 50% of its biomass, with high-yielded cell wall material (Wiar 2006).

To cope up with global growing demands of the multi-purpose plants including *D. innoxia*, farmers and breeders in arid and semi-arid regions are prompted to often engage with the cultivation in low-grade soils such of those are heavy metal-contaminated ones. Consequently, plant will instantly combat the heavy metal stress. Since the plant chemical composition and the structure and composition of

cell wall are likely affected by modified environmental conditions, this may influence plant potential value and quality as well. Thus studying the changes in lignin content and its mediated enzyme (cell wall-bound peroxidase, POX) under HMs stress may stipulate information on the effect of on cell wall structure as a mechanical barrier against Cd experience and a valuable criterion that imparts the plant quality.

To the best of the authors' knowledge, this is the first study figuring the impact of foliar spraying with SNE on Cd-stressed *D. innoxia*. Therefore, the purpose herein is to examine the attenuating effect of foliar applied extract of Cd-hyperaccumulator *S. nigrum* on Cd-stressed *D. innoxia* performances that might be evaluated through some physio-biochemical indices viz. growth traits, photosynthetic pigment content, osmo-metabolic compounds, phenolics, alkaloids, phytochelatin and reduced glutathione content, oxidative damage, and lignin content and its mediated enzyme, POX, in Cd-stressed *D. innoxia* plant.

## 2 Materials and Methods

### 2.1 *S. nigrum* Collection and Extract Preparation

The current species were gathered from its natural habitat at Wadi Al-Assiuty (31°18' and 31°48' E and 27°10' and 27°45' N), Assiut Governate, Egypt. The Flora of Egypt (Hepper and Boulos 2001) was used for authentication of the plant. Samples were air-dried and ground to a grainy powder. The powder was then mixed with bidistilled water in ratio of a weight: volume (W/V) of 1:10 and put in a water bath at 80 °C for 25 min. The fresh extract was filtered through a Watman filter paper and left to cool at room temperature (Abeed et al. 2021). The resultant filtrate was handled as 100% *S. nigrum* water leaves extracts and diluted to 50% for the usage as foliar spraying. The fresh prepared water extract was immediately used for foliar spraying. This concentration was chosen based on a previous preliminary experiment including five concentrations, i.e., 10, 20, 30, 40, 50%. The plants received 50% *S. nigrum* water leaves extract recorded a significant positive response based on biomass weight and chlorophyll concentration. *S. nigrum* leaf extract was analyzed and its biochemical constituents were as illustrated in Table 1.

### 2.2 Plant Materials, Growth Conditions, and Treatments

Two-week-old uniform *D. innoxia* plantlets were collected on summer 2021 from botanical garden of faculty of agriculture, Assiut University, and were transplanted in plastic pots (35 cm diameter) filled with 5 kg of sandy-clay soil in rate of 4 plantlets/pot. The pots were arranged in a completely

**Table 1** Some biochemical constituents of *Solanum nigrum* leaf extract (SNE)

Constituents	Values
Citric acid ( $\mu\text{mol g}^{-1}$ )	40.0
Ascorbic acid ( $\text{mg } 100 \text{ g}^{-1}$ )	16.3
Proline ( $\mu\text{g g}^{-1}\text{DW}$ )	24.0
Ca ( $\text{mg } 100 \text{ g}^{-1}$ )	60.0
Mg ( $\text{mg } 100 \text{ g}^{-1}$ )	260.0
Fe ( $\text{mg } 100 \text{ g}^{-1}$ )	21.8
Zn ( $\text{mg } 100 \text{ g}^{-1}$ )	8.7

random arrangement with four replicates. Seven days after transferring, three Cd concentrations (0, 50, and 100 mg Cd  $\text{kg}^{-1}$  soil) were delivered to the soil in a form of water solution. Cd application was introduced as cadmium dichloride ( $\text{CdCl}_2$ ). The developed plants of both control (0 mg Cd  $\text{kg}^{-1}$  soil) and Cd (50 and 100 mg Cd  $\text{kg}^{-1}$  soil) treated pots were sprayed with SNE after one week of treatment with Cd, while the untreated control plants were sprayed with distilled water only. Two foliar spray treatments (water, SNE) were applied using hand pump trigger sprayers. Each replicate was sprayed by 250 ml of sprays per pot and the soil surface was covered by polyethylene bag to avoid foliar treatment reached soil. Plants were received sprays for two consecutive days. The addition of cadmium was based on our previous studies (Eissa and Abeed 2019); also, a preliminary experiment has revealed a high threshold of *D. innoxia* against Cd stress. Plant samples were harvested after 14 days of Cd exposure for analyzing the following parameters.

### 2.3 Analysis of Plant Growth Parameters

Plants were randomly selected from each treatment to estimate shoot and root length, fresh and dry weight of shoot and root, and leaf area at the end of the experiment. The length of shoot and root of *D. innoxia* plants were manually determined using a measuring tape. Fresh weight of the harvested plants was measured immediately then oven dried at 60 °C for 48 h to a constant weight to assess dry weight. Leaf area ( $\text{cm}^2$ ) was estimated via the product of the maximum length and maximum width of the leaf. The length and the width in cm were measured using a measuring tape.

### 2.4 Analysis of Photosynthetic Pigment Content

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in dry leaves were determined by suspending 0.25 g leaf sample in 10 ml ethyl alcohol (95%) in water bath at 60–70 °C. The absorbance of the extract was measured using a Unico UV-2100 spectrophotometer

at wavelengths 663 and 644. Lichtenthaler (1987) equations were used for chlorophyll determinations as  $\text{mg g}^{-1}$  DW.

## 2.5 Analysis of Osmo-metabolic Compounds

Proline content was quantified in the fresh leaves according to Bates et al. (1973). Total free amino acids content was measured in the fresh leaves by the method of Moore and Stein (1948). The method of Lowry et al. (1951) was used to estimate soluble proteins content of fresh leaves. Soluble sugar content of the fresh leaves was determined by adopted the method of Fales (1951).

## 2.6 Quantification of Phenolics, Alkaloids, Reduced Glutathione, and Phytochelatins Content

Phenolic content was estimated according to Kofalvi and Nassuth (1995) using the Folin-Ciocalteu's phenol reagent. A routine quantification method for analysis of the total alkaloidal content spectrophotometrically based on Dragendorff's reagent (DR) as described by Sreevidya and Mehrotra (2003) was carried out. Non-enzymatic antioxidant as reduced glutathione (GSH): the supernatant of grinding fresh leaves in trichloroacetic acid was utilized for the quantification of reduced glutathione (GSH) by protocols of Ellman (1959). Phytochelatins (PCs) evaluated by subtracting the amount of GSH from non-protein thiols as mentioned by Nahar et al. (2016) which attained by mixing supernatant of leaves grounded in sulfosalicylic acid with Ellman's reaction mixture (Ellman 1959).

## 2.7 Analysis of Oxidative Stress Markers; Hydrogen Peroxide, and Malondialdehyde Content

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content of fresh leaves was spectrophotometrically estimated as described by Mukherjee and Choudhuri (1983). Malondialdehyde (MDA) content in the fresh leaves of *D. innoxia* plant was measured following to Zhang and Qu (2004).

## 2.8 Analysis of Lignin Content and Its Mediated Enzyme (Cell Wall-Bound Peroxidase, POX)

Lignin content was quantified via the thioglycolic acid reaction adopted by Kováčik and Klejdus (2008). The activity of peroxidases (POX,  $\mu\text{mol mg}^{-1}$  protein  $\text{g}^{-1}$  FW  $\text{min}^{-1}$ ) was measured after the extraction of the enzymes from leaves according to cited by Ghanati et al. (2002). The activity peroxidases (PO) was evaluated based on the increase in the absorbance at 470 nm using 168 mM guaiacol in 100 mM phosphate buffer and 30 mM  $\text{H}_2\text{O}_2$ . The change in absorbance was modified to units (U) utilizing an extinction coefficient of  $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

## 2.9 Analysis of Cadmium Content of Root and Leaf of *D. innoxia* Plants

Cadmium (Cd) content was determined by following the method adopted by Eissa and Abeed (2019) in which dried leaf and root samples (0.5g) were ground and exposed to the acid-digestion using  $\text{HNO}_3:\text{HClO}_4$  mixture (2,1). Cd concentrations were detected by atomic absorption spectrophotometer (PerkinElmer A Analyst 200) which has a detection limit of  $0.001 \text{ mg L}^{-1}$  for cadmium. The translocation factor (TF) of Cd was estimated by applying the following equation:  $\text{TF} = \text{Cd leaves content}/\text{Cd root content}$  (Li et al. 2021)

## 2.10 Statistical Analysis

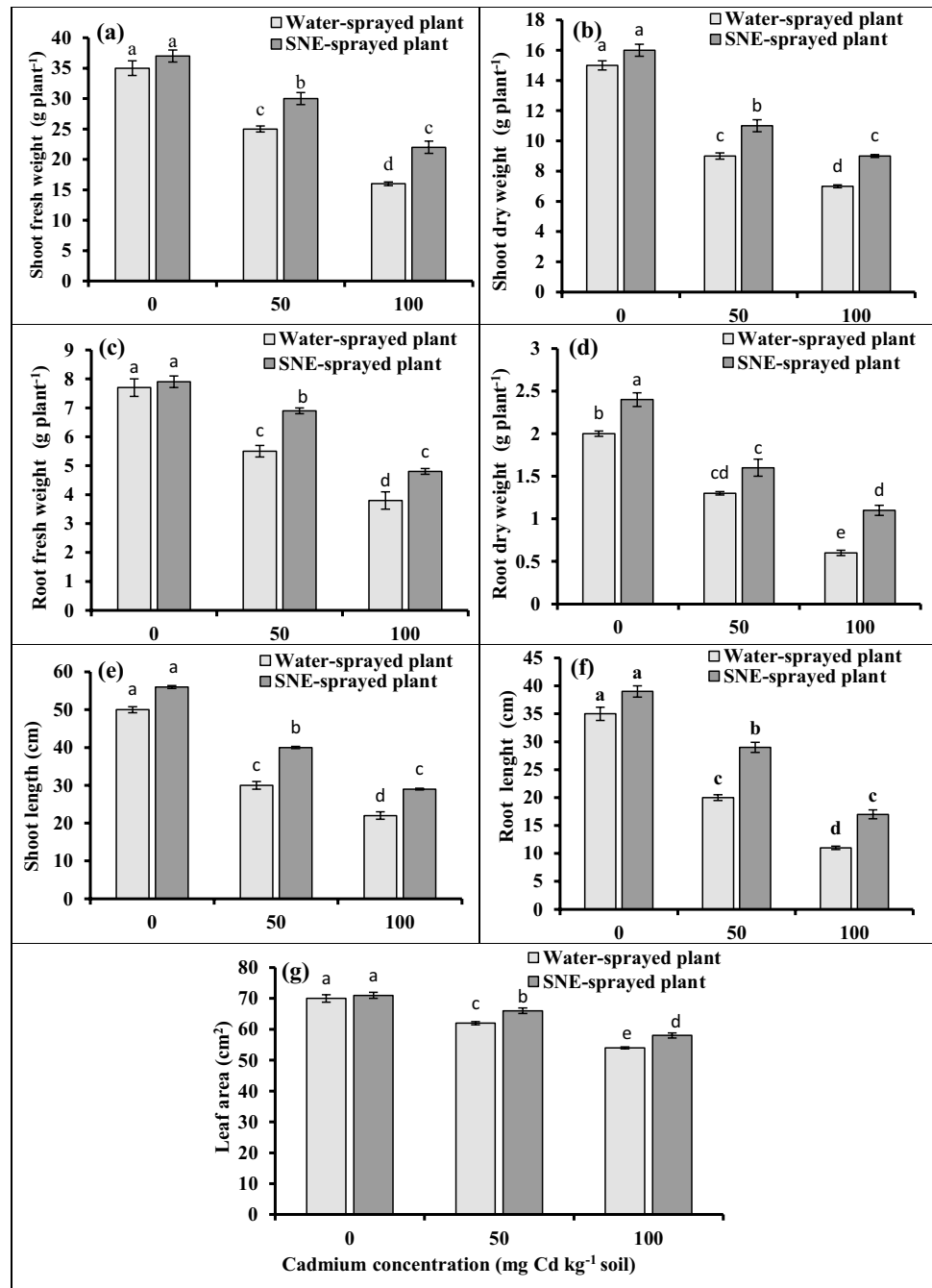
A randomized complete design (RCD) with four replicates was applied in the present experiment. Duncan's multiple range tests and one -ANOVA were proceed by SPSS 17.0 package (SPSS, Chicago, IL, USA) at probability level of 5%.

## 3 Results

### 3.1 *S. nigrum* Leaf Extract Improved *D. innoxia* Growth Under Cd Stress

To investigate how SNE application aided *D. innoxia* to combat Cd-induced adverse effect on the growth of *D. innoxia* plants, several growth-conjugated attributes were analyzed, including root and shoot fresh, dry weight, length, and leaf area, under both unstressed and stressed conditions with and without SNE foliar application (Fig. 1). In comparison with the control (0 mg Cd  $\text{kg}^{-1}$  soil), 50 and 100 mg Cd  $\text{kg}^{-1}$  soil decreased root FW (by 29 and 51%) and root DW (by 35 and 70%), shoot FW (by 29 and 54%), shoot DW (by 40 and 53%), root length (by 30 and 69%), shoot length (by 40 and 60%), area of leaves (by 11 and 23%), respectively (Fig. 1). Foliar spray of *D. innoxia* plants with SNE improved root FW (by 25 and 26%) and root DW (by 23 and 100%), shoot FW (by 20 and 38%), shoot DW (by 22 and 29%), root length (by 45 and 54%), shoot length (by 33 and 31%), area of leaves (by 6 and 7%), at 50 and 100 mg Cd  $\text{kg}^{-1}$  soil levels, respectively, versus plants subjected to Cd alone (Fig. 1). Furthermore, *D. innoxia* plants sprayed with SNE only exhibited increment in root FW, root DW, shoot FW, shoot DW, root length, shoot length, and leaf area by 3, 20, 6, 7, 11, 12, and 1%, respectively, over the control plants (Fig. 1).

**Fig. 1** Impact of Cd stress, foliar application with *Solanum nigrum* extract and their interactions on Fresh weight of shoots (g/plant) (a), dry weight of shoot (g/plant) (b), fresh weight of root (g/plant) (c), dry weight of root (g/plant) (d), shoot length (cm) (e), root length (cm) (f), and area of leaves (cm<sup>2</sup>) (g) in *Datura innoxia* plants. Water and *Solanum nigrum* leaf extract (SNE) indicate the plants sprayed with distilled water, *Solanum nigrum* extract, respectively. Bars donate averages and standard errors of four independent replicates ( $n = 4$ ). Different alphabetical letters designate significant differences among the treatments at  $P < 0.05$ , based on LSD test.

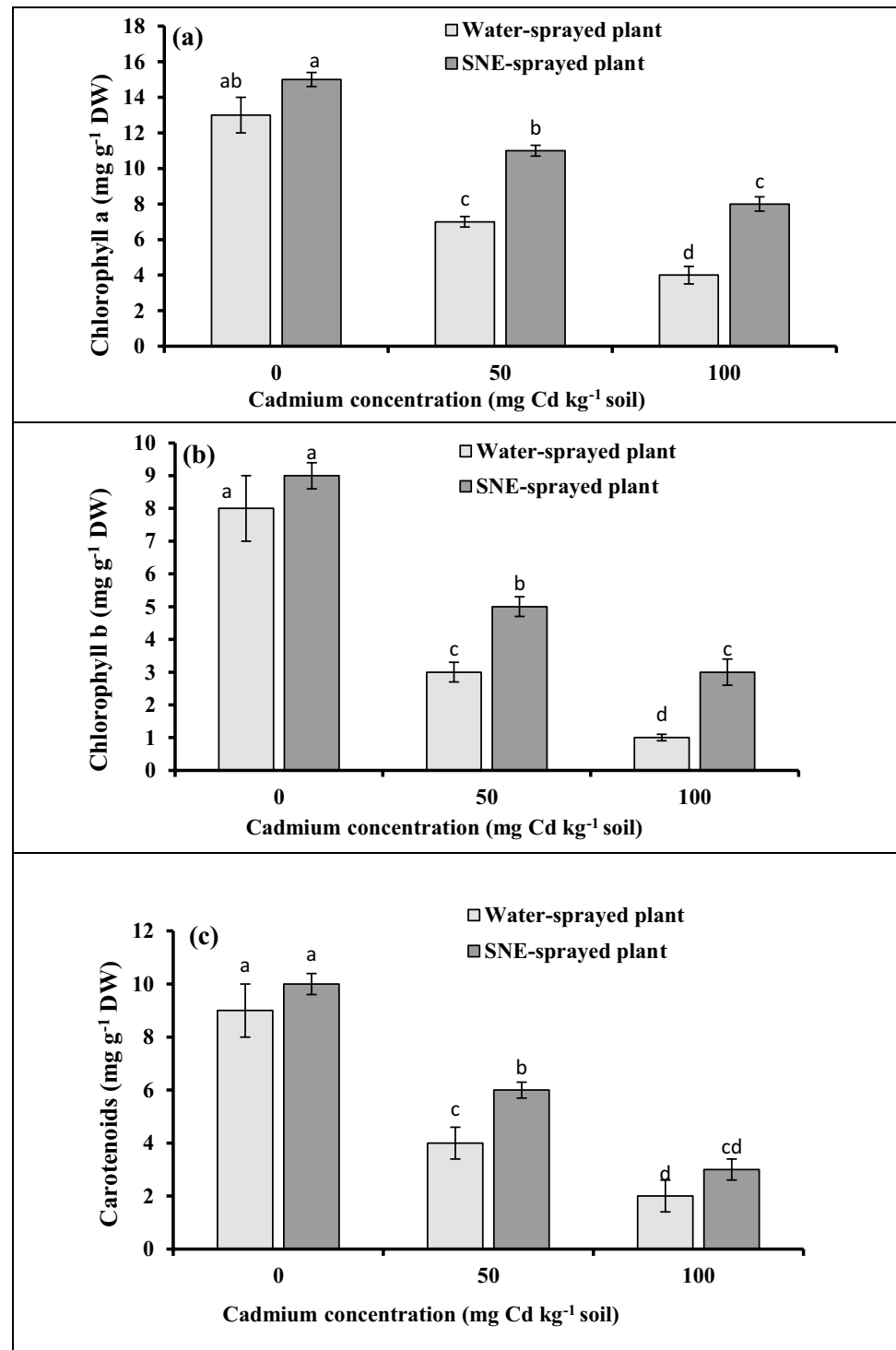


### 3.2 *S. nigrum* Leaf Extract Protected Photosynthetic Pigments in *D. innoxia* Leaves Under Cd Stress

To assess the protective impact of exogenous SNE on the photosynthetic pigments under Cd toxicity, the levels of photosynthetic pigments (chlorophyll a, b and carotenoids) in Cd-delivered *D. innoxia* leaves were evaluated (Fig. 2). In comparison to the untreated control plants, there was a drastic decrement in the content of chlorophyll a by 46 and 69%, chlorophyll b by 63 and 88%, and carotenoids by 56 and 78% in the plants subjected to 50 and 100 mg Cd kg<sup>-1</sup>

stress, respectively (Fig. 2). Contrary, spraying the extract of *S. nigrum* protected photosynthetic pigments from Cd-mediated injurious effects, as witnessed by the considerable increased contents of chlorophyll a (57 and 100%), chlorophyll b (67 and 200%), and carotenoids (200 and 50%) as response to 50 and 100 mg Cd kg<sup>-1</sup> rates, respectively, in comparison with only Cd-treated plants (Fig. 2). Non-stressed *D. innoxia* plants delivered SNE also exhibited enhanced contents of chlorophyll a (by 15%), chlorophyll b (by 13%), and carotenoids (by 11%), in comparison with water-sprayed non-Cd treated control (Fig. 2).

**Fig. 2** Impact of Cd stress, foliar application with *Solanum nigrum* extract and their interactions on chlorophyll a ( $\text{mg g}^{-1}$  DW) (a), chlorophyll b ( $\text{mg g}^{-1}$  DW) (b), and carotenoids ( $\text{mg g}^{-1}$  DW) (c) in *Datura innoxia* plants. Water and *Solanum nigrum* leaf extract (SNE) designate the plants sprayed with distilled water, *Solanum nigrum* extract, respectively. Bars donate averages and standard errors of four independent replicates ( $n = 4$ ). Different alphabetical letters designate significant differences among the treatments at  $P < 0.05$ , based on LSD test. FW: fresh weight



### 3.3 *S. nigrum* Leaf Extract Modulated Osmo-metabolic Compound Accumulation in Cd-Exposed *D. innoxia* Plants

The imposition of 50 and 100  $\text{mg Cd kg}^{-1}$  Cd led to an augmentation in proline content by 147 and 293% and total free amino acids by 26 and 23%, whilst it resulted in a decrement

in soluble proteins content by 20 and 35% and soluble sugar content by 11 and 18%, respectively, compared with that of non-Cd treated control plants (Table 2). On the other hand, application of SNE to the plants exposed to 50 and 100  $\text{mg Cd kg}^{-1}$  resulted in an exacerbation of proline accumulation by 29 and 56%, and total free amino acids by 4 and 4%, and an increase in soluble proteins by 9 and 15%, and soluble

**Table 2** Impact of cadmium stress, foliar application with *Solanum nigrum* (*S. nigrum*) extract and their interactions on proline, total free amino acids, soluble proteins, and soluble sugar contents in *Datura innoxia* (*D. innoxia*) plants.

Treatments	Proline (mg g <sup>-1</sup> FW)	Total free amino acids (mg g <sup>-1</sup> FW)	Soluble proteins (mg g <sup>-1</sup> FW)	Soluble sugars (mg g <sup>-1</sup> FW)
Control	2.9±0.21 d	39.00±0.018 d	83.91±0.58 a	28.07±1.47 b
SNE	3.2±0.11 d	46.20±0.014 c	85.70±0.68 a	39.35±1.32 a
50 mg Cd kg <sup>-1</sup> soil	7.2±0.32 c	49.20±0.010 ab	66.78±0.66 b	25.43±0.23 c
50 mg Cd kg <sup>-1</sup> soil + SNE	9.3±0.31 c	51.60±0.015 a	72.82±0.55 ab	28.12±0.38 b
100 mg Cd kg <sup>-1</sup> soil	11.4±0.51 b	48.10±0.019 b	54.57±0.65 c	23.58±0.36 d
100 mg Cd kg <sup>-1</sup> soil + SNE	17.8±0.33 a	50.60±0.017 a	64.43±0.71 b	26.41±0.13 c

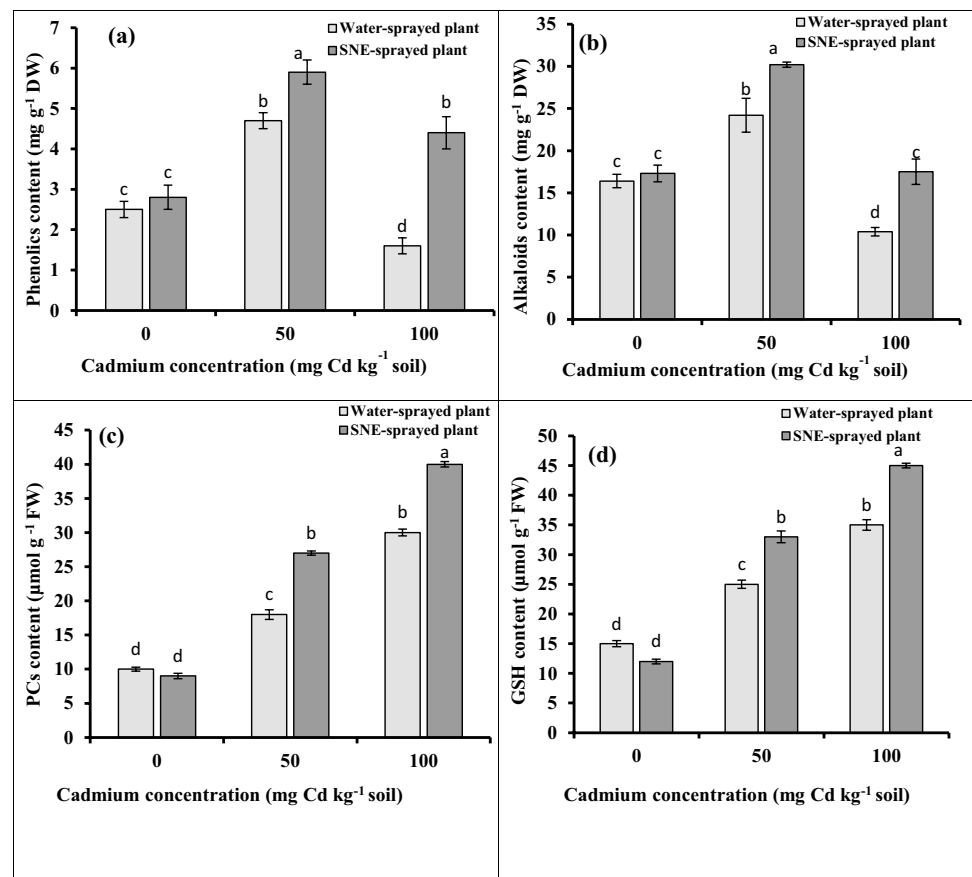
Each value donates average ± standard error of four independent replicates ( $n = 4$ ). a, b, c different letters within the same row designate significant divergent at  $P < 0.05$  among the treatments, based on LSD test. SNE, *Solanum nigrum* leaf extract; FW, fresh weight; mg, milligram; kg, kilogram; Cd, cadmium

sugars by 11 and 12%, respectively, when compared with the plants subjected to the same levels of Cd stress without SNE spraying (Table 2). SNE spraying treatment to non-Cd treated plants increased the contents of proline, total free amino acids, soluble proteins, and soluble sugars by 10, 18, 40, and 2%, respectively, versus control plants (Table 2).

### 3.4 *S. nigrum* Leaf Extract Enhanced Phenolics, Alkaloids, Reduced Glutathione, and Phytochelatins Content Under Cd Stress Conditions

*D. innoxia* plants that are grown in 50 mg Cd kg<sup>-1</sup> level exhibited a considerable increment in phenolics and alkaloid content (by 88 and 48%), respectively (Fig. 3a, b) versus the untreated control sample. Whereas plants grown in 100 mg Cd kg<sup>-1</sup> level displayed drastic depletion in phenolics and alkaloid content (by 36 and 37%), respectively (Fig. 3a)

**Fig. 3** Impact of Cd stress, foliar application with *Solanum nigrum* extract and their interactions on phenolics content (mg g<sup>-1</sup> DW) (a), alkaloid content (mg g<sup>-1</sup> DW) (b), reduced glutathione content (GSH; μmol g<sup>-1</sup>FW) (c), and phytochelatins (PCs; μmol g<sup>-1</sup>FW) (d) in *Datura innoxia* plants. Water and *Solanum nigrum* leaf extract (SNE) indicate the plants sprayed with distilled water, *Solanum nigrum* extract, respectively. Bars donate averages and standard errors of four independent replicates ( $n = 4$ ). Different alphabetical letters designate significant differences among the treatments at  $P < 0.05$ , based on LSD test. DW: dry weight

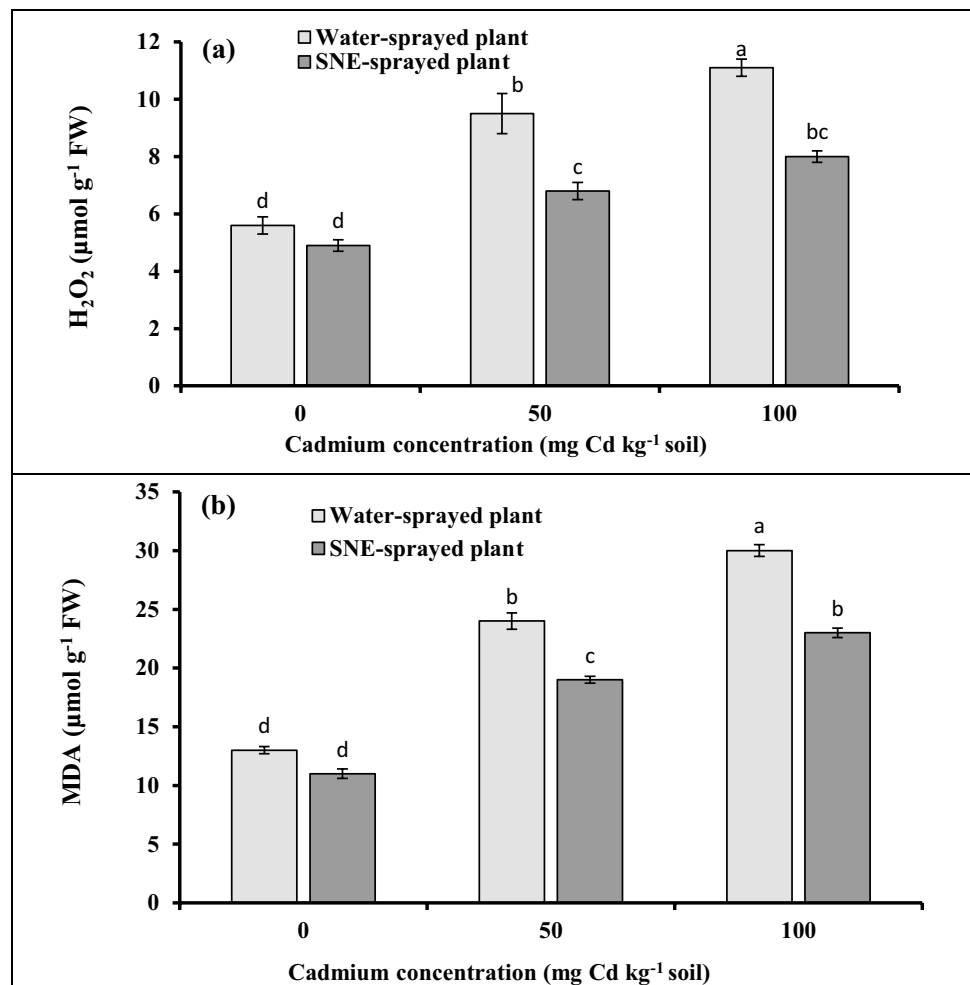


versus the unstressed control sample. Obviously, supplementation of SNE to Cd-treated plants remarkably augmented phenolics (by 26 and 175%) and alkaloid content (by 25 and 68%), respectively, in comparison with the plants subjected to the same levels of Cd stress without SNE spraying (Fig. 3). In addition, SNE foliar spraying to non-stressed plants insignificantly enriched the phenolics content (by 12%) and alkaloid content (by 6%) when compared with that of water-sprayed non-stressed control (Fig. 3). *D. innoxia* plants that were grown under 50 and 100 mg Cd kg<sup>-1</sup> levels displayed an augmented content of GSH (by 67 and 133%), and PCs (by 80 and 200%), respectively, in correspondence with non-stressed control (Fig. 3c, d). Supplement of SNE to Cd-stressed plants displayed a vital role in further exacerbation of GSH content (by 32 and 29%) and PCs (by 50 and 33%), respectively, compared with the plants subjected to 50 and 100 mg Cd kg<sup>-1</sup> only (Fig. 3c, d). SNE spraying treatment to unstressed plants insignificantly decreased the contents of GSH and PCs by 20 and 10%, respectively, against control plants (Fig. 3).

### 3.5 *S. nigrum* Leaf Extract Reduced Cd Stress-Induced Oxidative Injury in *D. innoxia* Plants

*D. innoxia* plants grown under 50 and 100 mg Cd kg<sup>-1</sup> levels exhibited an increased level of H<sub>2</sub>O<sub>2</sub> by 66 and 98%, and MDA by 85 and 131%, respectively, when compared with non-stressed control (Fig. 4a). Application of SNE to Cd-stressed plants had a pivotal role in curtailing H<sub>2</sub>O<sub>2</sub> level (by 28 and 27%, respectively) and MDA (by 21 and 23%, respectively) compared with the plants subjected to 50 and 100 mg Cd kg<sup>-1</sup> only (Fig. 4a, b). Under normal conditions *D. innoxia* plants sprayed with SNE displayed decreasing in the level of MDA and H<sub>2</sub>O<sub>2</sub> by 13 and 15%, respectively, compared with that of untreated control (Fig. 4).

**Fig. 4** Impact of Cd stress, foliar application with *Solanum nigrum* extract and their interactions on MDA ( $\mu\text{mol g}^{-1}$  FW) (a) and H<sub>2</sub>O<sub>2</sub> ( $\mu\text{mol g}^{-1}$  FW) (b) in *Datura innoxia* plants. Water and *Solanum nigrum* leaf extract (SNE) indicate the plants sprayed with distilled water, *Solanum nigrum* extract, respectively. Bars donate averages and standard errors of four independent replicates ( $n = 4$ ). Different alphabetical letters designate significant differences among the treatments at  $P < 0.05$ , based on LSD test. FW: fresh weight





### 3.6 *S. nigrum* Leaf Extract Altered Lignin Content and Its Mediated Enzyme (Peroxidase, POX) Activity of Cd-Exposed *D. innoxia* Plants

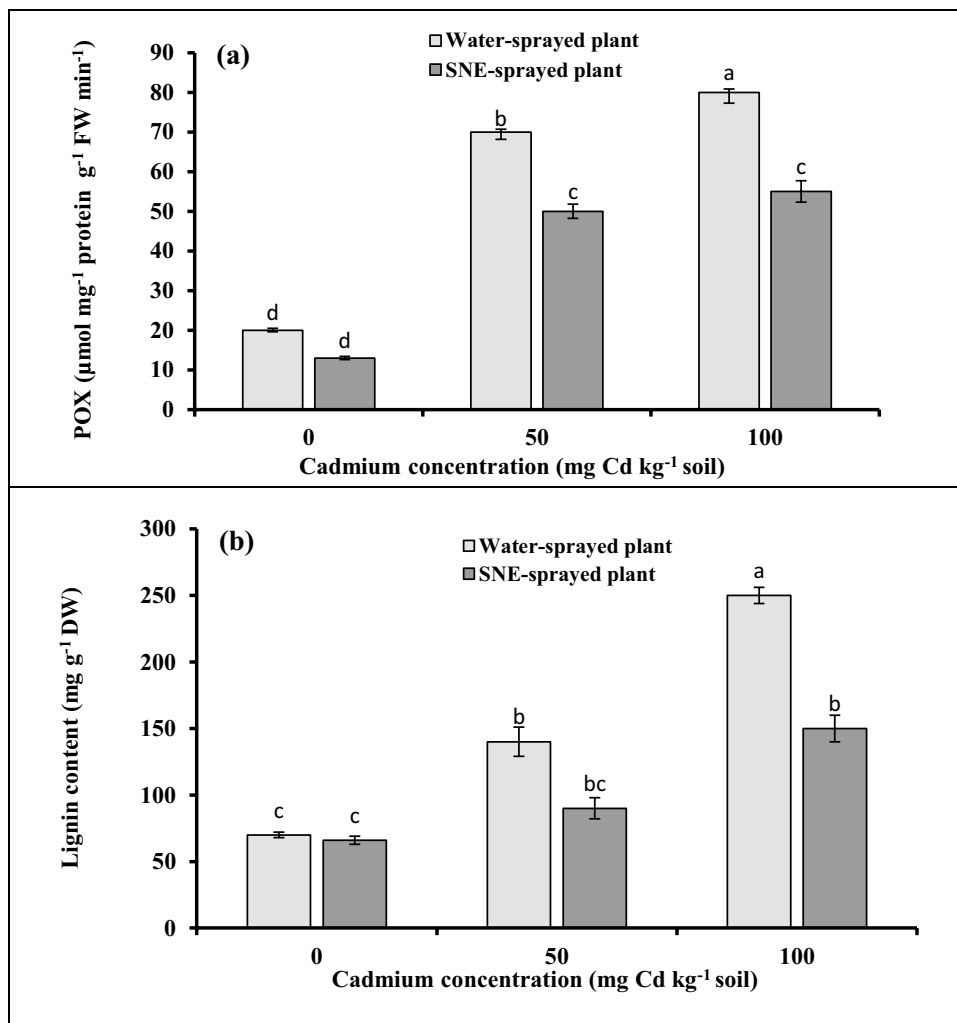
*D. innoxia* plants that were grown under 50 and 100 mg Cd kg<sup>-1</sup> levels displayed an augmented content of lignin by 100 and 257%, respectively, in correspondence with non-Cd treated control (Fig. 5a). Application of SNE to Cd-stressed plants shouldered a pivotal role in minimizing the level of lignin (by 36 and 40%, respectively) compared with the plants subjected to 50 and 100 mg Cd kg<sup>-1</sup> only (Fig. 5a). Besides, *D. innoxia* plants exposed to 50 and 100 mg Cd kg<sup>-1</sup> levels elevated the activity of POX by 250 and 300%, respectively, against non-Cd treated control (Fig. 4b). However, SNE application downregulated the activity of POX by 29 and 31% in Cd-exposed plants (50 and 100 mg Cd kg<sup>-1</sup>, respectively) versus stressed plants only (Fig. 5b). Under normal conditions, *D. innoxia* plants delivered SNE decreased lignin content insignificantly and

POX activity significantly by 6 and 35%, respectively, in comparison with that of untreated control (Fig. 5).

### 3.7 *S. nigrum* Leaf Extract Altered Cadmium Content and Its Translocation Factor of Cd-Exposed *D. innoxia* Plants

Data presented in Table 3 showed that the increase in the soil cadmium level caused an increase in cadmium content of root and leaves tissues. Lower amount of cadmium was recorded in leaves than root. Application of SNE significantly decreased cadmium content of root and leaves tissues in plants exposed to 50 and 100 mg Cd kg<sup>-1</sup> by 34.5 and 56.9%, and 11.5 and 67.9%, respectively, when compared with the plants subjected to the same levels of Cd stress without SNE spraying. The translocation of Cd from roots to leaves *D. innoxia* plants was expressed by the translocation factor (TF) (Table 3). In plants subjected to 50 and 100 mg Cd kg<sup>-1</sup>, translocation factor increased from 0.651 to 0.795. Surprisingly, plants received SNE exhibited the lowest TF.

**Fig. 5** Impact of Cd stress, foliar application with *Solanum nigrum* extract and their interactions on Lignin content (mg g<sup>-1</sup> DW) (a) and Activity of peroxidase (POX;  $\mu\text{mol mg}^{-1}$  protein g<sup>-1</sup> FW min<sup>-1</sup>) (b) in *Datura innoxia* plants. Water and *Solanum nigrum* leaf extract (SNE) indicate the plants sprayed with distilled water, *Solanum nigrum* extract, respectively. Bars donate averages and standard errors of four independent replicates ( $n = 4$ ). Different alphabetical letters designate significant differences among the treatments at  $P < 0.05$ , based on LSD test.



**Table 3** Impact of cadmium stress, foliar application with *Solanum nigrum* (*S. nigrum*) extract and their interactions on cadmium root and leaves content and translocation factor (TF) in *Datura innoxia* (*D. innoxia*) plants.

Treatments	Cd content ( $\mu\text{g plant}^{-1}$ DW)		TF
	Root	Leaves	
Control	0.123 $\pm$ 0.010e	0.014 $\pm$ 0.001d	0.114 $\pm$ 0.010e
SNE	0.044 $\pm$ 0.001f	nd	---
50 mg Cd kg <sup>-1</sup> soil	20.32 $\pm$ 0.23c	13.230 $\pm$ 0.24b	0.651 $\pm$ 0.032b
50 mg Cd kg <sup>-1</sup> soil + SNE	13.43 $\pm$ 0.44d	5.810 $\pm$ 0.32c	0.433 $\pm$ 0.021c
100 mg Cd kg <sup>-1</sup> soil	60.09 $\pm$ 2.02a	47.760 $\pm$ 0.65a	0.795 $\pm$ 0.014a
100 mg Cd kg <sup>-1</sup> soil + SNE	53.110 $\pm$ 1.01b	15.360 $\pm$ 0.55b	0.289 $\pm$ 0.012d

Each value donates average  $\pm$  standard error of four independent replicates ( $n = 4$ ). a, b, c different letters within the same row designate significant divergent at  $P < 0.05$  among the treatments, based on LSD test. SNE, *Solanum nigrum* leaf extract; DW, dry weight;  $\mu\text{g}$ , microgram; kg, kilogram; Cd, cadmium; nd, not detected

## 4 Discussion

Heavy metal tolerance is established as a complicated process, so looking for another approach encompassing the exploiting of metal-hyperaccumulators extracts as naturally pervasive to metalliferous soils species (Hagemeyer 2004) may provide some promising perceptions to a higher potential of detoxification capacity of plants (Shanmugaraj et al. 2019). The detrimental influence of high Cd level in *D. innoxia* performance can be cleared to decrease the area of leaves, fresh, dry weight, and length of shoot and root of *D. innoxia* plants grown under Cd stress. The growth reduction influence of Cd on *D. innoxia* plants may be due to different factors; among them are oxidative burst, nutrients deficiency, and water balance disturbance (Sarwar et al. 2010). Moreover, cell elongation, cell division, and an amplification may be repressed by Cd stress as demonstrated by El Rasafi et al. (2020).

Utilizing natural plant extracts has been registered in the literature to underpin the growth of various plants such as wheat (Tomar et al. 2015), and venca (Abeed et al. 2021). Stimulating impacts of SNE were noticeable owing to the existence of citric acid (CA), ascorbic acid (AsA) and proline (as antioxidants) and some micro and macronutrients (Fe, iron; Zn, zinc; Mg, magnesium) in the plant extract (Table 1). Study of Zeng et al. (2021) on castor bean proved that foliar sprayed citric acid alleviated lead (Pb) stress via improving growth, as well as fresh and dry biomass. Also, a study of Zhang et al. (2019) on Cd-stressed maize plants revealed that AsA application induced growth improvement via stimulation in photosynthetic pigments, amino acids, and protein, which might have enhanced cell enlargement and growth of plant (Aziz et al. 2018). Study of Zouari et al.

(2016) indicated that proline enhanced growth rate of date palm (*Phoenix dactylifera* L.) under Cd stress. In addition to Mahdieh et al. (2018) who pointed that Zn as a micronutrient caused an increment in the growth parameters (root and shoot lengths, dry and fresh weights, and number of leaves) of *Phaseolus vulgaris* plants. Wu et al. (2020) stated that Zn foliar sprayed enhanced the photosynthesis, tissue biomass of wheat under Cd stress.

Photosynthetic pigments and carotenoids are fundamental for photosynthesis in the plant (Abeed et al. 2020; Ding et al. 2021). The drop in photosynthetic pigments may be ascribed to reduction in leaf area which shoulders light capturing and photosynthesis achieving (Xu et al. 2009). Moreover, diminutions in photosynthetic pigments resulted from chlorophyll destruction by triggering activity of chlorophyllase and chlorophyll degrading enzymes under environmental stress circumstances (Vernon and Selly 1966; Sayyari et al. 2013). The protective effect SNE on photosynthetic pigment and carotenoids could be owing to SNE with a high incidence of AsA, Mg, Ca (calcium), Zn and Fe ions (Table 1), which directly influence photosynthetic pigments since Fe and Ca activate the chlorophyll biosynthesis, whereas ascorbic acid acts as an antioxidant. It was also established to be conjugated with several biological activities in the plant as an enzyme catalyst and as a donor/receptor in electron transport (Abdel-Hafeez et al. 2019). Zhang et al. (2019) also continued the results of AsA-mediated protection of chlorophyll versus environmental stresses. Hence, it is shown that foliar spraying with AsA withstand the negative effects of Cd on photosynthesis and retained pigments biosynthesis and related components.

Among the diverse plant responses to Cd stress is generation of compatible osmoprotectants. This aids cells to decline oxidative injury produced by ROS in response to high Cd stress level via protecting sub-cellular cells and also displaying osmotic adjustment (Abeed and Dawood 2020). Documented drops in soluble sugars are linked to a decrease in leaves photosynthetic pigments that resulted in suppression of photosynthetic activity and employment of carbohydrates into another sink.

In this concern, Yaghoubian et al. (2016) who documented that Cd stress declines photosynthetic pigment and total carbohydrates due to photosystem II higher sensitivity. The decrease of soluble proteins in *D. innoxia* plants was demonstrated by Dawood and Abeed (2020) which was inhibited by the buildup of proline and total amino-N. Moreover, Hussain et al. (2020) deduced this reduction as a result of the disturbance in nitrogen metabolism or nitrate absorption inhibition. On the other hand, spraying with SNE enhances the ability of Cd-stressed *D. innoxia* plants to intensify levels of soluble sugars and soluble proteins. These findings designated that SNE may have participated in osmotolerance of Cd-exposed *D. innoxia* plants by stimulation of

carbohydrates production in response to Zn, which has an important role in activation of the enzymes that are responsible for carbohydrates biosynthesis and transformation (Gheshlaghi et al. 2019). The existence of Ca in the Cd-hyperaccumulator extract may increase protein synthesis or decrease protein degradation in Cd-treated plants.

There is a close relationship between the accumulation of amino acids, proline and potency of plants to survive against Cd stress. Proline acts for osmotic adjustment and donates the stability of the subcellular structures to scavenge ROS and to neutralize cellular redox potential in addition to its role as a signaling molecule and source of energy (Szabados and Savouré 2010). Results of this study witnessed highly significant increases in total free amino acids and proline contents in Cd-exposed plants. Comparable results were detected by Yılmaz and Parlak (2011). The rehabilitation from the noxious effect of Cd stress has manifested as a result of the existence of enriched content of ascorbic acid and proline in the extract of *S. nigrum* plant and this increase in amino acids could be ascribed to ascorbic acid suppressed the rise of the ROS (Shalata and Neumann 2001). Ascorbic acid plays as a detoxification agent to destructive damage induced by free radicals (Asada 1999). Perveen et al. (2016) documented that alfalfa leaf extract conferred Cd resistance of wheat plant by increasing leaf free proline and total free amino acids thus increased growth and yield of plants under Cd stress.

Alkaloids and Phenolics act as non-enzymatic antioxidants that enhance the antioxidant capacity of a cell to scavenge ROS liberated under Cd stress (Gill and Tuteja 2010). Alkaloids metabolites are not essential for the completion of life cycle of the plant; rather frequently function as signal molecules, or chemical defenses against stress conditions (Vernay et al. 2008). Also, phenols serve as a substrate for many antioxidant enzymes, so, it mitigates Cd stress injuries. Evaluation the content of alkaloids and total phenolic compounds in this investigation denotes the non-enzymatic level of ROS scavenging, and we noticed that the levels of both alkaloids and phenolic compounds were increased in the shoots of *D. innoxia* plants supplied with moderate Cd level ( $50 \text{ mg kg}^{-1}$ ) confirming the hypothesis of the external constraints (e.g. HMs) which restrict the rate of dry matter accumulation may elicit the alkaloids manufacture (Vernay et al. 2008). It has also been postulated that secondary metabolism may be an integral part of the plant capacity to adjust metabolic processes to survive and reproduce in abiotic stress involving HMs existence. Our findings are parallel with the finding in *Narcissus tazetta* (Soleimani et al. 2020), *Papaver somniferum* (Lachman et al. 2006) and *Brassica juncea* (Kaur et al. 2018). Furthermore, Manquían-Cerda et al. (2016) who reported that the content of phenolic compounds significantly increased in  $100 \mu\text{M}$  Cd-stressed blueberry (*Vaccinium corymbosum* L.) leaves. While under high Cd level ( $100 \text{ mg}$

$\text{kg}^{-1}$ ), *D. innoxia* plant showed considerable depletion in the content of alkaloids and phenolics indicating that Cd contamination can alter the chemical composition of *D. innoxia* leaves, thus, affecting the quality, efficacy, and safety of natural plant derivatives produced by medicinal species. The lack of capacity to produce these molecules (alkaloids and phenolics) in leaves under high Cd level suggests the loss of biosynthetic components that may be due to an inactivation of enzymes or a redirection of metabolic functions to maintain growth (Vernay et al. 2008). Supplementation of SNE exacerbated content of alkaloids and phenolics significantly in moderate Cd-stressed plants and re-nourished alkaloids and phenolics content of leaves in highly Cd-stressed plants, thereby participating adjustment of osmotic status or an increase in plant hormone activities. Thus, the over-accumulation of plant secondary metabolite acts as a plant adaptive mechanism in response to Cd stress (Ashraf et al. 2018). These results are in harmony with that of Howladar (2014) and Khalofah et al. (2020) who indicated that foliar application with moringa leaf extract significantly augmented the content of total phenols and the antioxidant potential in shoots of *Phaseolus vulgaris* and *Lepidium sativum* plants when compared to plants grown under Cd stress.

One of mechanisms to metal detoxification is by conjugation or chelation and compartmentalizes them in the vacuole away from the cytosolic environment. In plant cell, GSH and PCs shoulder the coordinate of Cd by binding it with the thiol group. In the current study, GSH and PCs display similar responses to Cd treatments (both increased), probably due to their co-regulation. High glutathione levels facilitate phytochelatin synthesis by the activation of the enzyme phytochelatin synthase thereby sequestration of heavy metal phytochelatin conjugates in the vacuole. Several investigations indicated that exacerbated GSH content is associated with capability of plants to counter Cd-induced oxidative stress (Hossain et al. 2010). SNE enhanced their content for efficient Cd-detoxification, and the subsequent tolerance of Cd toxicity. These results may be ascribed to biochemical components denoted in the extract such as proline and citric acid. Similar results of Xu et al. (2009) proved that proline application serves in attenuating Cd toxicity by detoxifying ROS, boosting the glutathione level and protecting the activity of antioxidative enzymes in Cd-stressed plants. Al Mahmud et al. (2018) showed that citric acid confer Cd stress tolerance via enhancing the pool of ascorbate (AsA) and glutathione (GSH) and increasing the phytochelatin (PCs) content. Furthermore, GSH can play a key role in ROS detoxification and altering cellular redox status of protein via the AsA-GSH pathway in plants this pathway was activated by natural amended ascorbic in SNE (Table 1).

In the current study, *D. innoxia* plants grown under different Cd levels displayed higher content of hydrogen peroxide and malonaldehyde. Production of malonaldehyde in plant

amplified under elevated Cd stress levels as a result of the plant losing its ability to hamper ROS whilst hydrogen peroxide posed membrane injury by the generation of hydroxyl radical (OH<sup>•</sup>) and lipid peroxidation (Das and Roychoudhury 2014). Addition of SNE to Cd-stressed *D. innoxia* plant registered the ability to counteract the noxious impacts mediated by Cd stress through lessening MDA and H<sub>2</sub>O<sub>2</sub> hyperaccumulation. These results may be ascribed to biochemical components denoted in the extract such as ascorbic acid, proline and organic acids viz. citric acid (Table 1) that shoulders an important role in declining ROS levels in the Cd stressed plants via activation of antioxidant enzymes (Eissa and Abeed 2019). These findings are in line with Weijie et al. (2021). AsA was linked to ROS metabolism protecting plant tissues from detrimental oxidative injury by serving as reductant thus decreased free radicals level in cell (Zhang et al. 2019). Citric acid has been reported in iron-generated stress, aluminum detoxification, and tolerance towards heavy metal (Gao et al. 2010; Faraz et al. 2020).

We found lignin content was highly abundant and its biosynthetic peroxidase activity was triggered in Cd-exposed plants. Cd is recognized to motivate oxidative stress as a signaling molecule; H<sub>2</sub>O<sub>2</sub> elicits secondary reactions, such as an induced peroxidase activity, which contributes to boosted lignification (Finger-Teixeira et al. 2010). Lignification results in decrement of the cell wall extensibility which might limit cell enlargement as evidenced by reduced growth and stunted *D. innoxia* plants grown under Cd stress.

Lignin is cell wall material that highly deposited as a mechanical barrier against external stressor, e.g. HMs, preventing metal entrance (stress lignin) (Bruce and West 1989). Addition of SNE to Cd-stressed *D. innoxia* plants showed the ability to reduce POX activity and subsequent adequate lignification regulated from high inducible rate (restricting cell growth) to relatively low or moderate level to the extent that permits cell elongation and growth. Normal lignin deposition accompanied with reduced POX activity under SNE application may be mostly attributed to the represented ascorbic acid in the extract (Table 1). Peroxidase has been proposed to play a vital role in the lignification of cell walls which can oxidize phenolics to phenoxy radicals, whereas AsA has a regulatory role in the oxidation of phenolics. The oxidation is completely suppressed by a low AsA concentration. AsA compete phenolics as a substrate result in oxidation of AsA into dehydroascorbic acid rather than production of phenoxy radicals (Takahama 1993). These radicals, formed via phenolics oxidation, can bind to cell wall leading to lignification or formation of cross-links in walls, but this would be impossible as long as any AsA was present in cell walls (Takahama 1993).

The lower content of Cd in leaves than roots jointed with absence of toxicity symptoms in SNE treated Cd-stressed plants could rationalize the importance of Cd root-retention

mechanism established by *D. innoxia* plants in order to protect aerial parts versus the toxic influence of Cd. Similarly, Guo et al. (2015) reported that Cd ions were mainly retained in the roots of Thuya plants (*Platycladus orientalis*) and juniper (*Juniperus chinensis*) submitted to Cd stress, and therefore, small amounts of Cd were transported to the leaves.

Also, the current study revealed that SNE application reduced the Cd content in roots and leaves of Cd-stressed *D. innoxia* plants that witnessed by the apparent plant liveliness compared with the SNE non-treated Cd-stressed plants. These results may be maily ascribed to proline denoted in the extract. Hence, similar results of Zouari et al. (2016) proved that proline application not only serves in reduced Cd uptake by date palm roots but also reinforced its exclusion, and therefore, exogenous proline could constitute a barrier against Cd absorption the matter that emphasized herein by the decrease of Cd translocation recorded in SNE-treated plants.

## 5 Conclusions

Using plant extracts derived from plants that are highly tolerant to heavy metal toxicity can be accounted as a satisfactory approach for a healthy future. *Solanum nigrum* leaf extract herein accomplished beneficial role in enhancing the performance of plants grown under cadmium toxicity via improving morphological attributes, photosynthetic pigments, osmo-metabolic compounds, cell extensibility accomplished by adequate lignification in terms of low lignin content and downregulated pexoidase activity, and non-enzymatic antioxidants that imparted its medicinal properties/quality as well as curtailed oxidative stress and reduced foliar cadmium content. Accordingly, it could be employed in the agricultural sector under cadmium stress conditions and as an opportune way in cadmium stress green mitigation in plants. To the best of the authors' knowledge, this is the first investigation clarifying the impact of *Solanum nigrum* leaf extract on physio-biochemical performance of *Datura innoxia* under Cd stress and additional proteomic reports can provide information on the influence of *Solanum nigrum* leaf extract on plant metabolism under cadmium stress to ensure the safety and sustainability; thus, it can be utilized *Solanum nigrum* leaf extract as integrated practice when metal-contaminated regions were exploited for the agriculture of the multipurpose plants.

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## Declarations

**Conflict of Interest** The authors declare no competing interests.

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