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Research Article

Enhancing antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate cadmium toxicity

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Abstract

Cadmium (Cd) accumulation is an emerging environmental hazard and has detrimental effects on plant growth and development. Salicylic acid (SA) is a well-known plant growth regulator that can initiate various molecular pathways to ameliorate Cd toxicity. The experiment was executed to scrutinize the mediatory role of SA to accelerate the defensive mechanism of mung bean in response to Cd stress. Mung bean plants were exposed to 0, 5, 10 and 15 mg Cd kg^{-1} of soil. Exogenous application of SA 0, 10^{-6} and 10^{-3} M was added prior flowering. Results exhibited that Cd stress considerably reduced the growth-related attributes i.e. shoot length, root length, fresh and dry biomass, total soluble protein, total amino acids, relative water contents and photosynthetic pigments. Cadmium stress showed a significant increase in antioxidants levels such as peroxidase (POD), ascorbate peroxidase (APX), ascorbic acid (AsA), and catalase (CAT) and promoted the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents. However, exogenously applied SA significantly improved plant biomass and photosynthetic pigments under Cd stress. Moreover, SA improved the defensive system by enhancing antioxidants' activities under the increasing concentration of Cd stress. Furthermore, SA reduced the Cd uptake, membrane damage and, H_2O_2 and MDA accumulation. The

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study's findings concluded that exogenous-applied SA enhanced plant growth, promoted the antioxidant activities, and reduced the oxidative damage in mung bean seedlings under Cd stress.

Keywords: antioxidant machinery; heavy metal stress; mung bean; photosynthesis; reactive oxygen species; salicylic acid

Introduction

The natural environment has frequently acquiring metal pollution from the anthropogenic fonts comprising sewage expulsions, mining manoeuvres, phosphate fertilizers and overflows from metal refining industries and other industries (Yang *et al.*, 2006; Liu *et al.*, 2007; Seleiman *et al.*, 2012; Seleiman *et al.*, 2013; Seleiman *et al.*, 2017; Hassan *et al.*, 2019; Seleiman *et al.*, 2020a; Seleiman *et al.*, 2020b; Hassan *et al.*, 2021; Seleiman *et al.*, 2021a). World health organization categorized the heavy metals pollution as the second most adverse pollution because it has severe threats to human health (Salem *et al.*, 2018). Cadmium is a non-essential heavy metal, readily absorbs by plants that have perilous outcomes on plant growth and confines the crop yield and it also has toxic effects on humans (Yang *et al.*, 2004; Abbas *et al.*, 2017; Aamer *et al.*, 2018; Saleem *et al.*, 2019; Rasheed *et al.*, 2020). Cd is noxious to the plant cell, even at a very minute concentration in leaf greater than 5-10 μg g^{-1} has lethal effects on most plant species (White and Brown, 2010). Furthermore, Cd has damaging effects on the plant metabolism resulting in growth retardation, reducing nutrient uptake, leaf chlorosis and necrosis (Xue *et al.*, 2013), ionic unbalancing, inhibit the stomatal opening and reduce the biomass production *(Barceló and Poschenrieder, 1990; Seleiman et al.*, 2020c).

The efficiency of the photosynthetic system is highly affected due to Cd-induced stress (Vassilev *et al.*, 2005; Ekmekçi *et al.*, 2008) that is affiliated with the inhibition of chlorophyll biosynthesis as well as an alteration in the oxidizing site of PS II. Cd stress degrades the normal functioning of the Calvin cycle by inhibiting the activity of RuBP (Krantev *et al.*, 2008) and degrades the ultra-structure of chloroplast and mitochondria (Šimonová *et al.*, 2007). Cd induces oxidative impairment by assembling the reactive oxygen species (ROS) comprising free hydroxyl ions (OH^-) and super oxide radicals (O_2^-) (Hendry *et al.*, 1992) and thereby reduces the membrane stability and increases the degradation of macromolecules DNA, RNA and protein. It is usually recognized that oxidative mutilation in plants is controlled by the antioxidants (Acar *et al.*, 2001; Türkan *et al.*, 2005). Plants have a scavenging mechanism including enzymatic (i.e. POD, SOD, APX and CAT) and non-enzymatic antioxidants (i.e. anthocyanin and ascorbic acid) against different stress conditions (Al-Ashkar *et al.*, 2020; Hassan *et al.*, 2020; Seleiman *et al.*, 2021a; Seleiman *et al.*, 2021b). They protect the plants from different abiotic stresses, but their activity is reduced due to metal ion interaction (Noriega *et al.*, 2012, Sofy *et al.*, 2020).

Nowadays, different hormones are extensively employed to minimize the effect of abiotic and biotic stresses. Salicylic acid is one of the naturally existing signalling molecules and a good hormone for improving plant tolerance counter to diverse biotic and abiotic stresses (Hayat *et al.*, 2010; Hafez *et al.*, 2017). Exogenous application of SA could alleviate the Cd lethality in various crops including barley, maize, soya bean, and wheat. Some organic components and SA promote many physiological processes such as seed germination, flower induction uptake of mineral nutrition and biosynthesis of photosynthetic pigments (Klessing, 2017; Sofy *et al.*, 2020; Uslu *et al.*, 2020). Salicylic acid has a multifaceted role in all metabolic processes most actively involved in regulating enzymatic and non-enzymatic biomolecules under environmental stresses. It has an excellent ability to develop defensive coordination with enzymatic antioxidants. Moreover, it can also produce some non-enzymatic plant products in cellular and subcellular compartments of plant cell to compete with the reactive oxygen species (Singh and Gautam, 2013). SA can inflect the plant response to an inclusive array of oxidative stress by modulating the redox balance, antioxidant responses and scavenging the accumulation of reactive oxygen species (Shi and Zhu, 2008). It has been reported that SA also involves in the regulation of

membrane fluidity, uncoupling the mitochondrial electron transport chain by the activation of glutathione reductase and guaiacol peroxidase (Shao *et al.*, 2010).

Mung bean is a short-duration, drought-tolerant, a leguminous crop with many effective uses and of great economic importance. It is a rich source of protein and also improves soil fertility (Elahi *et al.*, 2004). Therefore, it is highly valuable and sustainable crop across the globe. But it is susceptible to Cd stress (Wahid *et al.*, 2008) and no detailed study was carried out on mung bean against Cd toxicity with exogenous application of SA.

Hence, the current study was executed to explore the ameliorative role of SA (10^{-6} M and 10^{-3} M) on growth, physiochemical mechanisms, and antioxidant system activities in mung bean (*Vigna radiata* L.) grown under Cd stress (0, 5, 10 and 15 mg Cd kg^{-1} of soil).

Materials and Methods

The site and experimental design

The trial was directed to regulate the influence of exogenous-applied salicylic acid on mung bean crop against Cd stress. A pot experiment was conducted at Department of Agronomy, University of Agriculture, Faisalabad. The semiarid climate was prevailed in Faisalabad with a maximum temperature of 36.1 °C and minimum temperature of 20.2 °C, rain fall occurrence of 40 mm, and relative humidity of 41.6% during the period of March- May 2018. The experiment was placed in a completely randomized design (CRD) with factorial arrangement having four replications.

Treatments

The soil was spiked with diverse cadmium concentrations, i.e. 0, 5, 10 and 15 mg Cd kg^{-1} of soil, and left for seven days to equilibrate before sowing. Cadmium chloride $CdCl_2$ (Sigma Aldrich) was used as the source of cadmium. Two salicylic acid concentrations 10^{-6} M and 10^{-3} M were exogenous sprayed while distilling water spray was used as control. The soil analysis is presented in Table (1).

Table 1. Physiochemical properties of experimental soil

Characteristics	Value	Unit
Textural class	Clay loam	-
EC	1.82	dS m^{-1}
Organic matter	0.64	%
pH	7.8	-
Clay	33.4	%
Sand	26.4	%
Silt	41.1	%

Planting material and crop management

Seeds of Mung bean cultivar (NIAB, 2016) were obtained from Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad. Healthy seeds of the same size were purified with a 5% solution of NaClO for 10 min then washed with distilled water three times. The soil was obtained from 1 to 10 cm depth with 1:1 sand and silt composition. The soil samples' physiochemical properties were determined as described by Oweis *et al.* (1999), (Table 1). Soil samples were also subjected to analyze the cumulated heavy metals however, no heavy metals including Cd was found. Plastic pots having a diameter of 24 cm and holes in the bottom for a proper drainage were used in the current study. Each pot was filled with 7 kg of soil. Ten seeds of mung bean were sown in each pot, and thinning was done after emergence to maintain 6 seedlings per pot. The recommended dosage of sulfate of potash (SOP; 3.73 g) and diammonium phosphate (DAP; 7.44 g) was applied at the time

of sowing, irrigation was applied when needed. Remaining all the management practices were kept constant in all the pots.

Plant sampling

Mung bean plants were sampled after ten days of exogenous-applied SA for determination of various growth and physio-chemical parameters. Three plants from each pot were selected to determine the shoot and root fresh weight along with their root and shoot length as well as number of leaves. The same plant samples were dried to determine the root and shoot dry weight. The dried samples were crushed and grounded into a powder to be used for Cd analysis. Likewise, three plants were selected from each pot and reserved at -20 °C for the analysis of diverse physiochemical attributes.

Determination of photosynthetic pigments

Chlorophyll "a" and "b" were resolved by the method of Arnon (1949). A fresh composite sample of plant leaves (0.25 g) was finely chopped into small sections and dipped into 2.5 mL of 80% methanol, and left 24 h at room temperature. The absorbance of sample was noted at OD 645 and OD 663 nm. The final value of chlorophyll 'a' and 'b' was deliberated by the following subsequent formula:

$$chlorophyll\ a = \left((12.7\ (OD663) - 2.69\ (OD645)\right) \times V/1000 \times W$$

$$chlorophyll\ b = \ ((22.9\ (OD645 - 4.68\ (OD663)) \times V/1000 \times W$$

V = volume of methanol (mL); W = plant sample's weight.

Determination of electrolyte leakage

A fresh sample of plant leaves (0.3 g) was added to a glass tube and dipped with 20 mL of distilled water. Tubes were retained at room temperature for three h then assayed for EC_1 using an EC meter. The same samples were left for 24 h and then placed in a boiler for 50 min at 90 °C and analyzed for EC_2 (Yan *et al.*, 1996). The ultimate value of electrolyte leakage was determined via the following formula:

$$EC\% = (\frac{EC1}{EC2}) \times 100$$

Determination of RWC

Relative water contents were measured by the technique of Turner (1981). Fresh leaves of equal size were plucked and their fresh weight (FW) was calculated. Afterwards, leaves were dipped in distilled water and left over for a night and their turgid weight (TW) was determined. The same leaf samples were dehydrated in an oven at 90 °C for 2 h to determine the dry weight (DW), and finally the RWC was evaluated by given below formula:

$$RWC\% = (FW - DW \div TW - DW) \times 100$$

Determination of soluble protein

Soluble protein in the cell was examined by the technique of Bradford (1976). Leaf sample (0.5 g) was grounded under cold condition by adding 5 mL of potassium phosphate buffer (50 mM) then centrifuged at 15,000 rpm at 4 °C. The supernatant was collected in a glass tube and 3 mL of Bradford reagent was poured in the tube and left for 15 min at 37 °C. The absorbance was checked at wavelength 595 nm by using a spectrophotometer (Model 6305, Jenway, Staffordshire, United Kingdom).

Determination of free amino acid

Moore and Stein (1954) technique was used to analyze the free amino acid in the plant cell. The leaf sample (0.5 g) was homogenized by adding 5 mL of potassium phosphate buffer (50 mM). The plant extract was separated at 15,000 rpm at 4 °C and plant extract was treated with one mL of 2% ninhydrin and one mL of 10% pyridine in a glass tube. Then tubes containing the mixture was heated at 90 °C for 30 min. After

heating distilled water was added to these test tubes and maintained volume up to 20 mL. The absorbance was determined at 570 nm by using a spectrophotometer.

Determination of hydrogen peroxide

H_2O_2 content was examined by the technique of Velikova et al. (2000). A fresh leaf sample (0.5 g) was standardized in 5 mL of 5% trichloroacetic acid. Then centrifuged the crud at 10,000 rpm for 15 min at 4 °C, one mL of supernatant, one mL of 1 M KI, and 100 µl of potassium phosphate buffer was poured in a test tube and hydrogen peroxide content was checked at 390 nm on a spectrophotometer.

Determination of MDA

Malondialdehyde was scrutinized by the scheme of Cakmak and Horst (1991). The plant sample (0.5 g) was finely grounded in 5 mL of 5% TCA to get the supernatant under cold conditions. Then, one mL of supernatant, one mL of 0.5% TCA, and one mL of 20% TBA (thiobarbituric acid) was mixed in the test tube and sited tubes in the water bath at 90 °C for 30 min. The malondialdehyde was figured out at a wavelength of 532 nm and 600 nm via a spectrophotometer.

Activity of antioxidants

The activity of CAT and POD was examined as described by Chance and Maehly (1955). The activity of ascorbate peroxidase was analyzed by following the procedure of Asada (1987) and the activity of ascorbic acid was figured out by following the way of Mukherjee and Choudhuri (1983) with minor amendments. Fresh plant material (leaves) (0.5 g) was completely grounded in 5 mL of potassium phosphate buffer (50mM) pH (7.0) under ice-cold conditions and centrifuged at 15,000 rpm and 4 °C made the supernatant and stocked it at -20 °C for determination of antioxidants activities.

For determining CAT activity, a 2.5 mL potassium phosphate buffer was treated with 100 µl of H2O2 (5.6 mM), then added 100 µl of plant sample and CAT activity was checked at 240 nm via a spectrophotometer. Peroxidase reaction contains one mL of potassium buffer, 100 µl of (180 mM) guaiacol, 100 µl of (180 mM) H_2O_2 and then add 100 µl of plant sample and checked the peroxidase activity at 470 nm by using a spectrophotometer. The ascorbate peroxidase (APX) reaction comprises 700 µl of potassium phosphate buffer (50 mM), 100 µl of ascorbic acid, 100 µl of (5.1 mM) H_2O_2 and then added 100 µl of plant sample. Enzyme activity was checked at a wavelength of 290 nm via a spectrophotometer. The ASA activity was analyzed by treating the 2 mL of plant sample with DTC reagent (0.5 mL). Then the reaction mixture was left for 3 h at room temperature; after that, chilled H_2SO_4 was added slowly and left the mixture for 30 min at room temperature, then subjected to check the AsA contents at 520 nm via a spectrophotometer.

Determination of Cd contents

Accumulated Cd contents in plant tissue were analyzed by following the technique of Hsu and Kao (2003). A dried plant sample (0.5 g) was added in a conical flask and treated with (1:2) of concentrated (HCL and HNO_3) and the reaction mixture was left for 3 h at room temperature. After that conical flask containing the reaction mixture was placed on a hot plate and heated until the brown fumes wiped out and a clear solution was obtained. This solution was then filtered into a small plastic bottle and diluted to 25 mL by adding the distal water. The final value of Cd content was determined by using atomic absorption spectrophotometry.

Statistical analysis

Data was subjected for statistical analysis using software STATISTIX 8.1 for ANOVA and the least significance difference (LSD) test will be used to check the significance of treatment means at a 5% probability level.

Results

Growth attributes

Cadmium induced stress markedly reduced the related growth parameters (Table 2). Root and shoot length decreased significantly under different Cd concentrations however, the maximum reduction was observed at15 mg Cd kg^{-1} of soil. Shoot and root length were reduced by 34% and 40% respectively at higher Cd concentration (15 mg Cd kg^{-1}) as related to control treatment. Similarly, exposure of plants to Cd also diminished the fresh and dry biomass of different plant tissues, nonetheless, the maximum reduction in root FW (56%), shoot FW (51%), root dry mass (62%) and shoot dry mass (21%) was recorded at 15 mg Cd kg^{-1}as compared to control (Table 2). However, exogenous-applied SA prevented the plant growth from damaging effects of Cd stress and considerably amended all related growth parameters. Exogenous applied SA (10^{-3} M) improved the shoot and root length by (9% and 39%), shoot and root fresh biomass (43%, 38%), and dry biomass of shoot and root (33%, 71%) respectively under 15 mg Cd kg^{-1} level responding to its control (Table 2).

Relative water contents and electrolyte leakage

Relative water contents markedly decreased under Cd stress. Conversely, a significant increase in the electrolyte leakage was recorded under different concentrations of Cd (Figure 1). The highest value of electrolyte leakage was noted 69%; while, water content was recorded as 46% under 15 mg Cd kg^{-1} of soil. Exogenous application of SA improved the relative water contents by 23% and stables the ionic homeostasis by preventing membrane damage.

Photosynthetic pigments

The increasing concentration of Cd gradually decreased the chlorophyll contents in mung bean seedlings (Figure 2). The decrease in chlorophyll a and b was recorded as 24% and 27% respectively under a higher Cd level alone. On the other hand, exogenous SA improved the photosynthetic pigments under Cd-induced stress. Chlorophyll a and chlorophyll b were increased in response to the exogenous application of SA even under a higher level of Cd stress. An escalation in chlorophyll a (5%) and chlorophyll b (4%) was recorded at 10^{-3} M of exogenous-applied SA as compared to the water spray (Figure 2).

Table 2. Changes in growth attributes of mung bean (*Vigna radiata* L.) under different treatments of salicylic acid (SA) and cadmium (Cd)

		Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves
Cd_0	SA_0	25.17c±0.56	5.30c±0.30	4.68c±0.22	1.02c±0.02	0.62cd±0.03	0.25e±0.05	10.50b±0.5
	SA_1	26.45b±0.64	5.95b±0.05	5.50b±0.30	1.18b±0.02	0.75b±0.02	0.34b±0.02	12.20a±0.82
	SA_2	27.77a±0.68	8.92a±0.37	6.41a±1.00	1.33a±0.04	0.93a±0.04	0.41a±0.007	13.0a±0.70
Cd_1	SA_0	22.00e±0.55	4.60e±0.1	4.03d±0.22	0.74f±0.01	0.65c±0.03	0.25e±0.005	8.50def±0.86
	SA_1	23.35 d±0.35	4.87fg±0.40	4.59c±0.30	0.88d±0.02	0.74b±0.02	0.30cd±0.08	9.50bcd±0.5
	SA_2	25.17c±0.85	5.77b±0.22	4.67c±0.11	1.00c±0.01	0.77b±0.04	0.38a±0.01	10.25bc±0.43
Cd_2	SA_0	18.25fg ± 04	3.87g±0.08	3.25e±0.08	0.54h±0.01	0.57de±0.03	0.26e±0.01	8.00fg±0.40
	SA_1	21.62e±0.73	4.20f±0.21	3.58de±0.23	0.65fg±0.02	0.63c±0.02	0.27de±0.01	8.25efg±0.24
	SA_2	23.02d±0.35	4.97cd±0.10	4.02d±0.08	0.82d±0.03	0.74b±0.03	0.32bc±0.01	9.25cde±0.47
Cd_3	SA_0	15.50h±0.55	3.15h±0.11	2.30f±0.47	0.45i±0.01	0.49f±0.03	0.15g±0.01	6.00h±0.40
	SA_1	17.67g±0.72	3.77g±0.08	3.17e±0.65	0.55h±0.01	0.56e±0.03	0.21f±0.01	7.25g±0.47
	SA_2	18.62f±0.41	4.72de±0.08	3.28e±0.21	0.62g±0.01	0.65c±0.02	0.26e±0.01	8.00fg±0.40
LSD at 5%		0.713**	0.357**	0.480 **	0.045*	0.053**	0.031**	$1.14^{N.S}$

Different letters within each column indicate significant difference at 5% probability level. ** = highly significant; *, significant; NS= non-significant.

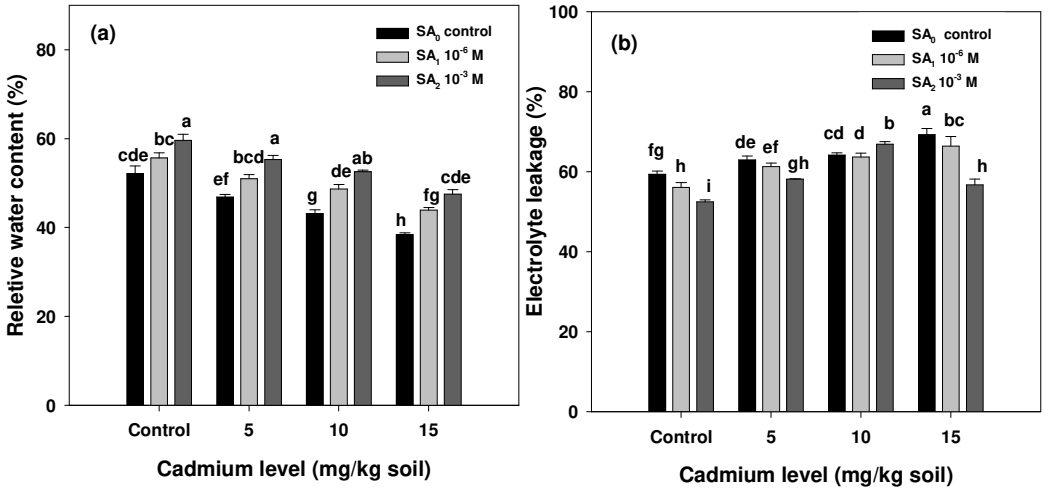

Figure 1. Effects of salicylic acid (SA) and cadmium (Cd) treatments on **(a)** relative water content, and **(b)** electrolyte leakage in the leaves of mung bean
Error bars represent the standard error at a 5% probability level under the LSD test.

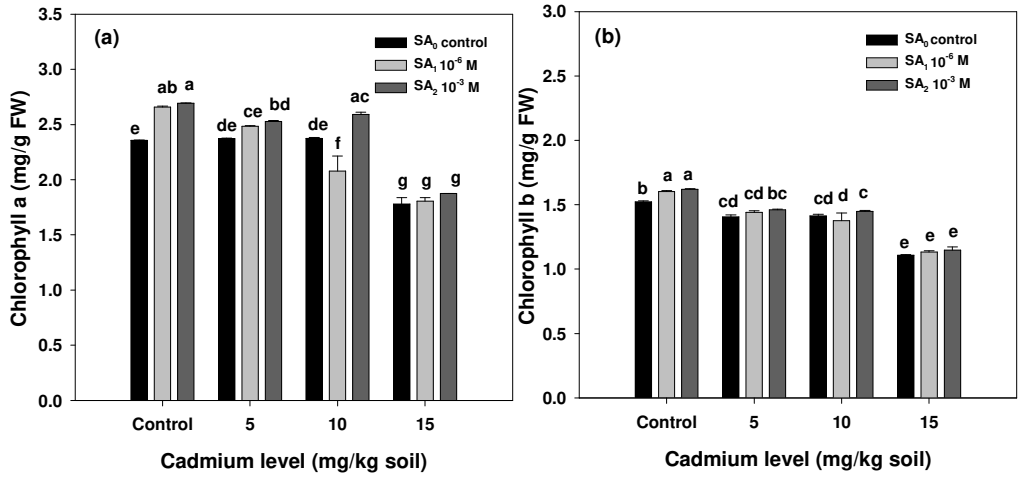

Figure 2. Effects salicylic acid (SA) and cadmium (Cd) treatments on photosynthetic pigments (a) chlorophyll a, and (b) chlorophyll b in the leaves of mung bean
Error bars represent the value of standard error at a 5% probability level under the LSD test.

Total soluble protein and free amino acids

Cadmium stress exhibited a substantial reduction in the total soluble proteins and free amino acids (Figure 3). A sharp reduction in TSP 55% and free amino acids 49% was found under 15 mg Cd kg^{-1} of soil without exogenous-applied SA. However, exogenous application of SA boosted the total soluble protein under control conditions even a gradual increase was observed at the different concentrations of Cd. Exogenous application of SA 10^{-3} M upturn the total soluble protein by 39% under 15 mg Cd kg^{-1} of soil as compared to water spray but it had no significant effects on total free amino acids (Figure 3).

Lipid peroxidation

Membrane damage is analyzed in terms of MDA and H_2O_2 and they are used as a key to membrane damage in the biological system. The H_2O_2 and MDA contents were elevated under increasing concentrations of Cd (Figure 3). The higher concentration of H_2O_2 (14.25 μmol) and MDA (6.90 nmol) were observed under

15 mg Cd kg^{-1} of soil without foliar-applied SA. A minimum concentration of H_2O_2 (9.49 µmol) and MDA (2.59 nmol) was noticed under control conditions. Moreover, foliar application of SA at a concentration of 10^{-3} M significantly reduced the MDA and H_2O_2 contents by 28% and 23% respectively at the 15 mg Cd kg^{-1} of soil as compared to water spray (Figure 3).

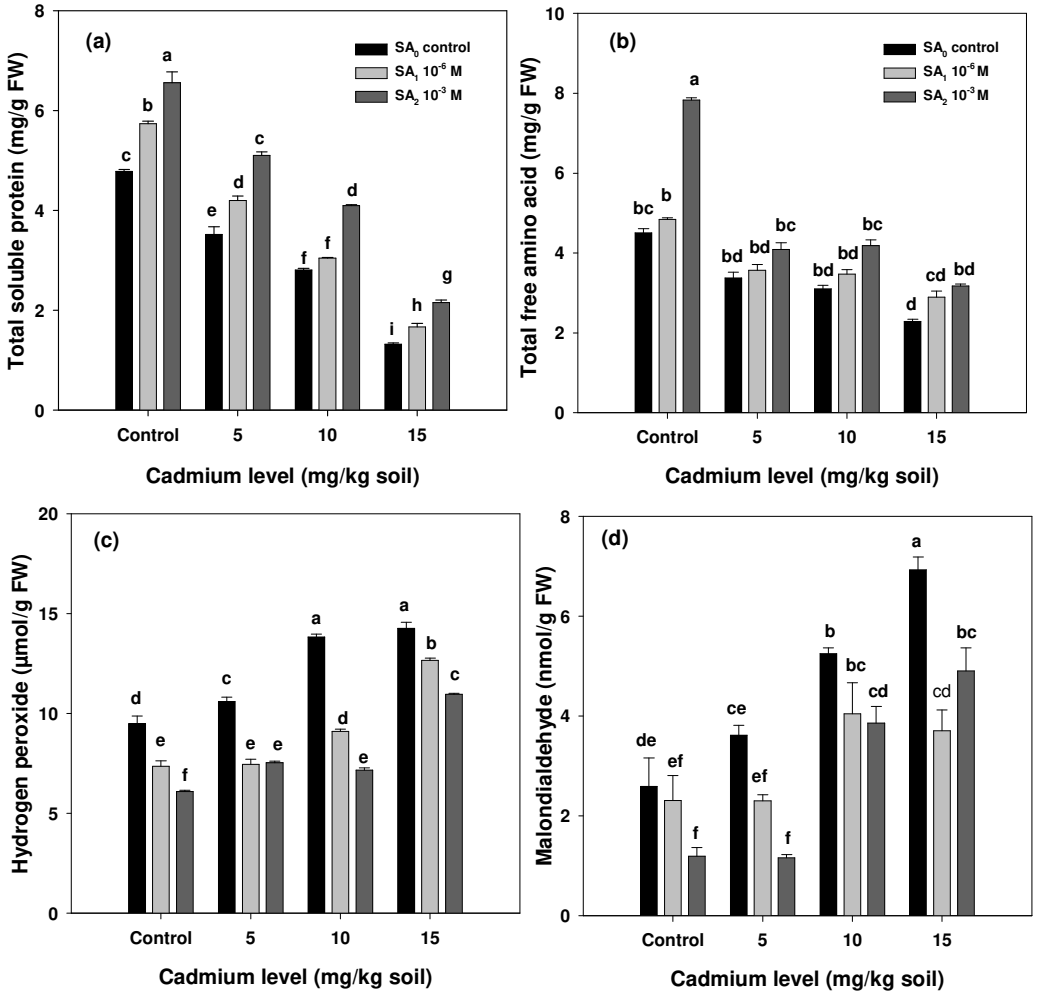

Figure 3. Effects of salicylic acid (SA) and cadmium (Cd) treatments on contents of (**a**) total soluble protein, (**b**) total free amino acid, (**c**) hydrogen peroxide, and (**d**) malondialdehyde of mung bean
Error bars represent the standard error at a 5% probability level under the LSD test.

Antioxidants

The results regarding the enzymatic antioxidant activities including CAT, POD, APX and AsA have been shown in Figure 4. Results revealed that Cd-induced stress considerably increased the antioxidant enzyme activities related to the control treatment. However, folia- applied SA induced the additive effects in antioxidants under stress and control conditions but it has no significant effects on CAT activity. The activities of POD, APX and AsA were improved by 48%, 22% and 18%, respectively at 15 mg Cd kg^{-1} of soil in counter to the 10^{-3} M of foliar-applied SA as compared to control treatment (water spray).

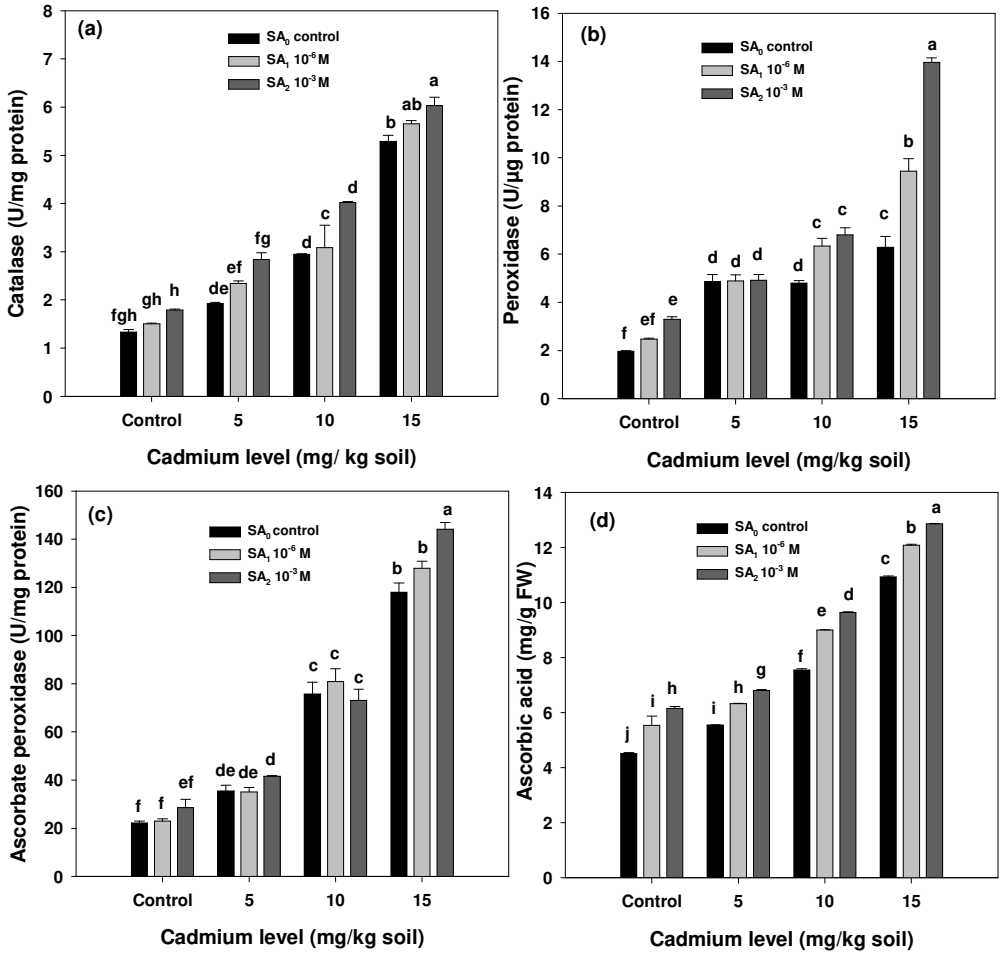

Figure 4. Effects salicylic acid (SA) and cadmium (Cd) treatments on antioxidant enzyme activities **(a)** catalase, **(b)** peroxidase, **(c)** ascorbate peroxidase and **(d)** ascorbic acid of mung bean
Error bars represent the standard error at a 5% probability level under the LSD test.

Cadmium contents in shoot and root

As expected, Cd uptake was observed higher in roots as compared to shoot of mung bean seedlings (Figure 5). Without exogenous applied SA, Cd uptake in shoots and roots was recorded as 36% and 50% respectively at maximum Cd level (15 mg Cd kg^{-1} of soil) as relates to the control treatment. Although both levels of foliar-applied SA significantly reduced the Cd uptake under all stress levels, the application of SA at 10^{-3} M level considerably suppressed the Cd uptake in shoot and root under 15 mg Cd kg^{-1} of soil as compared to respective control.

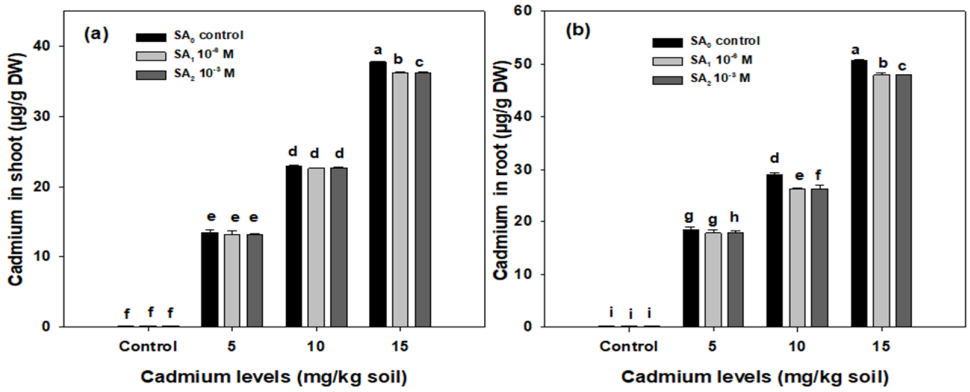

Figure 5. Effects of salicylic acid (SA) and cadmium (Cd) treatments on Cd uptake **(a)** Cd content in shoot **(b),** and Cd content in root of mung bean
Error bars represent the standard error at a 5% probability level under the LSD test.

Discussion

Cadmium induced stress significantly reduced the growth by influencing the dry and fresh biomass of mung bean plants. In the current study, the substantial reduction was recorded at 15 mg Cd kg^{-1} of soil in all related growth parameters (Table 2). Previous studies showed that suppression in related growth traits might be due fact that Cd toxicity inhibits the cell elongation and expansion before the cell division starts. Root growth is highly impaired on contact to the Cd in soil (Shakirova *et al.*, 2007), which ultimately affects the root growth and lessen the root hairs. In addition, it is observed that Cd stress inhibits the water uptake and disturbed cellular homeostasis. This study showed that treatment of SA significantly promoted plant growth against Cd toxicity (Table 2). Plant growth also significantly improved in Cd-free plants in comeback with the foliar application of SA. Outcomes of this study can be related to previous studies where researchers identified that SA ameliorated the harmful effects of Cd and showed positive effects on mung bean growth which may be accompanied by maximum induction of indole acetic acid (IAA) activity that promote the rapid cell division in the apical meristematic region (Shakirova *et al.*, 2007). In this study SA proved as an imminent molecule that enhances the plant growth under the high level of Cd-induced stress.

Our results demonstrated the degradation of photosynthetic pigments chlorophyll a and b Cd's increasing concentration (Figure 2). One of the visible symptoms of pigment loss is the chlorosis of plant leaves that might be due to the reticence of the chlorophyll biosynthesis (Pietrini *et al.*, 2003). Induction of Cd stress displaces the ultra-structure of chloroplast mainly grana length (Souza *et al.*, 2005).The main component of chlorophyll Mg^{2+} also disturbs under Cd stress and it ultimately affects the activity of PS II that becomes the reason for changing the redox balance across the Z scheme. Earlier findings have exposed that deterioration and depletion of photosynthetic pigments in contradiction of HM stress that could direct the conflict in the electron transport chain of PS I and PS II, also ensuing the assembly of ROS.

Exogenous application of SA significantly ameliorates the Cd-induced stress for the photosynthetic pigments. This is an expression that SA alleviates the adverse effect of MDA and ROS affected by Cr stress (Gill *et al.*, 2016). Proteins are multipurpose molecules that perform various functions in living cells, but their activity is degraded under heavy metal stress (Goldberg 2003). In the current study, it is clear that the total soluble proteins and free amino acids declined under increasing Cd levels and were elevated by the SA treatment (Figure 3). This reduction may be due to inadequate functioning of the plasma membrane, metal ions also inhibit the binding of cysteine-rich, induce changes at nucleotide-binding site leading to affect the chain of amino acids that ultimately inhibit protein synthesis. There are diverse findings of the functioning of proteins

in cells under biotic stress, similar findings were observed in *Vigna radiata* under salinity and SA treatment (Akhtar *et al.*, 2013).

Increasing concentration of Cd significantly enhance the cellular damage as shown in (Figure 1). An increasing level of electrolyte leakage may be possible due to changes in structure of plasma membrane, ionic transport across membrane also the changes in cellular homeostasis. Exposure of the mung bean plants to Cd stress results in an elevated level of ROS that leads towards membrane damage and the production of free radicals (Anjum *et al.*, 2011). In the current study, we examined that exposure to the Cd stress brings the oxidative disruption by the production of ROS estimated by the analysis of MDA and H_2O_2 contents in leaves (Figure 3). By-products of H_2O_2, free radicals and hydroxyl ions alter the regular circuit of the electron transport chain, ultimately disturb the redox potential at the final electron acceptor of the mitochondrial membrane (Laspina *et al.*, 2005; Guo *et al.*, 2013). Further, foliar-applied SA competed for the MDA and H_2O_2 contents under Cd stress that was proposed to be a membrane stabilizer because of its aptitude to act as an antioxidant scavenger against ROS (Gill *et al.*, 2016).

Plants have a natural scavenging system against abiotic stresses called as the enzymatic and non-enzymatic antioxidants that fight against the ROS (Nagamiya *et al.*, 2007). Exogenous applied SA significantly enhanced the defense system of mung bean plants by enhancing antioxidants activity (CAT, POD, APX and AsA) under increasing Cd levels (Figure 4). Antagonistic results of SA were observed in this study as MDA and H_2O_2 contents decreased by the application of SA. However, exogenous SA increased the activities of antioxidants under Cd stress, so it is proved as an important defensive molecule. Our findings delineated that antioxidant enzyme activities are directly or indirectly regulated through SA application; thus, this molecule acts as a stabilizer against Cd stress. The possible explanation about SA against ROS may enhance the regulation of NADPH loops in a specific cellular compartment to produce positive signals against abiotic stresses (Mittler *et al.*, 2004). As expected that Cd contents were more in roots as compared to shoot (Figure 5) which provides clear evidence the roots are the partisan site of Cd accumulation (Bezrukova *et al.*, 2011). However, foliar-applied SA helped to reduce the Cd uptake in mung bean plants (Figure 5) SA act as a barrier for Cd uptake in the root zone that could be facilitated by the upturn of citrate efflux in roots of the SA treated plants (Yang *et al.*, 2003).

Conclusions

This study revealed that Cd stress significantly decreased the growth of mung bean seedlings mainly by altering physiological and biochemical processes. Meanwhile, SA considerably improved the plant growth and antioxidant defense system against Cd suggesting that SA detoxifies the Cd-induced stress, thus it is involved in the modulation of the metabolic process. Improvement in morphological, physiological and biochemical processes of mung bean against Cd stress was approached by foliar application of 10^{-3}M of SA. Furthermore, comprehensive experimentation relating latest molecular practices are required to fix the defined mechanism underlying SA-induced Cd stress tolerance.

Authors' Contributions

Conceptualization: IK, MC, MS, RJ, FM, WI and MH; Formal analysis: FH, EA, MS, BA, RR, OU and WI; Investigation: IK and WI; Methodology: IK, MC, MS, RJOU, FH, FM, WI and MH; Writing - original draft: IK, WI, FM, MS and MH; Writing - review and editing: MS, OU, RR, RJ, BA, FH, EA and MC; All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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