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# ROLE OF MEDIUM SUPPLEMENTATION OF GROWTH MODULATORS TO ALLEVIATE CADMIUM-INDUCED OXIDATIVE STRESS IN MAIZE

**Azka Javaid and Abdul Wahid***

**Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan**
***Corresponding author's e-mail: drawahid@uaf.edu.pk**

Cadmium (Cd) toxicity has been a wide-spread phenomenon affecting plant metabolic functions due to excessive production of reactive oxygen species. This study was aimed at medium supplementation of optimized levels of growth modulators viz., salicylic acid (SA, 500 µM), ascorbic acid (AsA, 500 µM), thiourea (TU, 400 µM) and hydrogen peroxide ($H_2O_2$, 100 µM) in improving Cd tolerance in maize (*Zea mays* L. hybrid P-1543) in sand culture under net house conditions. All treatments were applied in the growing medium by dissolving in water. Results revealed that Cd stress (1000 µM) severely reduced the shoot and root dry mass and leaf area per plant and endogenous production of osmoprotectants (soluble sugars, free proline and glycinebetaine), phenolic compounds (soluble phenolics, flavonoids and anthocyanins) and vitamins (ascorbic acid, niacin and riboflavin) in shoot and root tissues indicating that Cd was highly toxic to the metabolic pathways. However, medium supplementation of the selected growth modulators was quite effective in reversing the Cd toxicity effect. Among the medium supplemented compounds, SA and AsA were more effective in reversing the Cd toxicity effect than TU and $H_2O_2$. Presence of tight correlations of growth attributes with metabolites levels substantiated that medium supplementation was greatly effective in orchestrating the endogenous metabolite levels and supporting maize growth under Cd toxicity. A greater effectiveness of the SA, AsA and TU can be attributed to their molecular structure and physiological properties in scavenging ROS and reducing oxidative stress. The results may have great promise and practical implications for growing maize successfully in the marginally Cd contaminated soils.
**Keywords:** Cd toxicity, oxidative damage, ROS, stress alleviation, metabolites accumulation, phenolics.

## INTRODUCTION

Oxidative stress on plants arises due to the production of reactive oxygen species (ROS), which has been identified as a major debacle in accruing the true growth and yield potential of crop plants. It has been estimated that the extent of oxidative damage ranges from as low as 7% to as high as 70% in different plant species (Wahid *et al.*, 2014). The oxidative stress results from an imbalance in production and dousing of ROS, the production being greater than dousing (Wahid *et al.*, 2014). Oxygenic photosynthesis is a major source for the production of ROS in plants (Foyer and Shigeoka, 2011).

In nature, the oxidative stress arises from sub-optimal growth conditions due to abiotic stresses such as salinity (Esfandiari and Gohari, 2017), heat (Santos *et al.*, 2017), water stress (Kar *et al.*, 2012), metal toxicity (Shahid *et al.*, 2014) etc. Likewise, disease incidence causes the hypersensitive response in plants (Etalo *et al.*, 2013). The plants deploy internal machinery to offload the excessively produced ROS, which is quite often related to induction of antioxidative enzymes, which scavenge the ROS and tend to normalize the plant metabolism (AbdElgawad *et al.*, 2017; Santos *et al.*, 2017). In addition to deployment of enzymatic antioxidative arsenal, there is a list of such non-enzymatic agents, the production of which has been quite helpful in rescuing the cells and tissues from the ROS toxicity arising from abiotic stress factors (Hassan *et al.*, 2018; Rehman *et al.*, 2018; Sarwar *et al.*, 2018).

Nonetheless, the oxidative damage on the plants can be managed successfully with the exogenous supply of stress alleviating compounds in different modes including seed pretreatments, foliar spray and medium supplementation, although their supplementation levels may be different for different plant species (Wahid *et al.*, 2017). It is known that pre-sowing seed treatments and foliar spray have proven worthwhile in improving crop productivity by 15-20% in different crop plants by producing critical physiological and biochemical changes (Liu *et al.*, 2014; Mustafa *et al.*, 2018). The medium supplementation of different chemicals and plant extracts has been used on a limited scale but with quite promising results (Perveen *et al.*, 2015; Hussain *et al.*, 2017; Tariq *et al.*, 2018). However, there is still a need to accrue and establish the possible benefits of medium supplementation of different growth enhancing agents on firm footings especially under stressful conditions.

Among different non-essential toxic metals, cadmium (Cd) toxicity is a major one in affecting the plant growth and development throughout the world. The toxicity of Cd ranges from whole plant level to negative effects on physiological functions and disruption of the structure of macromolecules

(Perveen *et al.*, 2011; Wan and Zhang, 2012; Jibril *et al.*, 2017). The studies are imperative on the alleviation of Cd toxicity by the use of ligands and metal chelators when supplemented through growing media. Recently, Perveen *et al.* (2018) have reported the potential of medium supplemental thiourea in improving the membrane stability, osmoprotectants and vitamins synthesis and reducing the Cd toxicity on maize root and shoot tissues. Nevertheless, more studies with exogenous use of compounds of diverse nature will improve our understanding of physiological roles of these medium supplements in combating the oxidative damage and improving Cd tolerance in plants.

From the above account it is clear that Cd-induced oxidative damage is one of the major reasons of hampered plant growth and development. We assume that medium supplementation of different compounds may improve plant growth and reduce Cd toxicity by modulating the internal levels of various metabolites. In this study, an attempt was made to determine the comparative effectiveness of selected levels of medium supplemented salicylic acid (a phenolic), ascorbic acid (a vitamin), thiourea (a synthetic molecule with biological activities) and hydrogen peroxide (an oxidant) in modulating the endogenous levels of metabolites and reducing the Cd toxicity in maize seedlings.

## MATERIALS AND METHODS

***Plant material and experimental details*:** A pot experiment was done on maize (*Zea mays* L.) hybrid P-1543 (Poineer Seeds Pakistan Ltd., Sahiwal) to investigate the comparative efficacy of selected levels of medium supplemented 0.5 mM salicylic acid (SA), 0.5 mM ascorbic acid (AsA), 0.4 mM thiourea (TU) and 0.1 mM hydrogen peroxide ($H_2O_2$) in improving cadmium (Cd) toxicity (1 mM). The levels of all these compounds were optimized by performing preliminary screening experiments under laboratory and greenhouse conditions in the Department of Botany, University of Agriculture, Faisalabad (data not shown). For the current experiments, 7 surface sterilized seeds were sown in pots containing 5 kg of washed river sand, and germination was completed in three days. After thinning, five uniform seedlings were kept per pot and grown for another 10 days.

***Treatment application and harvesting*:** The above-mentioned selected levels of the selected compounds (mentioned above) were medium supplemented followed by Cd level. The potted plants were kept under bright sunlight with a ~28/~22±2°C day/night temperature, RH ~45/~58±3% and photosynthetic photo flux density of 900-980 µmol/$m^2$/s. The design of the experiment was completely randomized with factorial arrangement having three replications (each replication constituted one pot). Five seedlings per pot were grown for seven days after abovementioned treatments. Leaf area was measured of intact plants as maximum leaf length × maximum leaf width × 0.68 (correction factor) as described by Carleton and Foote (1965). At harvest, the plant roots were carefully removed from sand in running water. The shoots were separated from the roots, washed and blotted dried. Both the parts were put in the zip lock plastic bags and brought to the laboratory on ice. One portion of the shoot and root from three plants per replication in each treatment were put in paper bags and dried in an oven at 65°C for seven days. The other portion of the shoot and root was preserved in a freezer at -40°C until used for biochemical analysis.

**Tissue Metabolites Measurement Procedures:**

***Oxidative stress parameters*:** For the estimation of $H_2O_2$ concentrations with the method of Velikova *et al.* (2000), the plant tissue was mixed well with trichloroacetic acid (TCA), centrifuged at 12000 × *g* for 15 min. A 0.5 mL of the supernatant was mixed with 0.5 mL each of 10 mM phosphate buffer and 1 M of potassium iodide; mixed well and let stand at room temperature. The absorbance of the complex was taken at 390 nm. For standard curve, a range of standards (0.2 to 1.0 mM) were prepared and run in the same manner. For the measurement of MDA with the protocol of Heath and Packer (1968), 0.1 g of fresh tissue was homogenized with 1 mL of 5% solution of TCA and centrifuged at 12000 × *g* for 15 min. Supernatant (1 mL) was mixed with 1 mL of thiobarbaturic acid, mixed and heated in a water bath at 95°C for 30 min, cooled and again centrifuged at 7500 × *g* for 5 min. The absorbance of the supernatant was taken at 532 and 600 nm. The value obtained at 600 nm was subtracted from that taken at 532 nm to correct the non-specific turbidity. To calculate MDA concentration, the extinction coefficient of 115 mM/cm was used, while 5% TCA was used as blank.

***Osmoprotectants analysis*:** Soluble sugars were determined by reacting with the anthrone reagent (prepared by dissolving 1 g anthrone in 1 L of conc. $H_2SO_4$) with 1 mL of the water extracted sugars sample in a water bath at 95-100ºC for 15 min. The samples were cooled in running water, vortexed and absorbance of the colored complex was taken at 620 nm (Yoshida *et al.*, 1976). The free proline was estimated using the method of Bates *et al.* (1973). A 1 mL of 3% sulphosalicylic acid extract was reacted with I mL acid ninhydrin (prepared by reacting 1.25 g ninhydrin with 30 mL of glacial acetic acid) and 1 mL glacial acetic acid by heating at 100ºC and then the reaction mixture terminated in ice bath. The colored complex aspirated by dissolving in 4 mL of toluene. The absorbance was taken at 520 nm. A standard curve was constructed using the proline. For glycinebetaine (GB) estimation with the method of Grieve and Grattan (1983) the fresh plant material was extracted in 2 N $H_2SO_4$. Then I mL of the sample was transferred to glass tube, cooled at 4ºC and reacted with cold $IKI_2$ (periodide) and rapidly vortexed. These tubes were kept at 4ºC for 16–20 h and centrifuges at 10000 × g. The periodide crystals were dissolved in 9 mL of 1, 2-dichloroethane and voretexed till the crystals were completely dissolved and kept at room temperature for 2–2.5 h. The absorbance of the colored

solution was taken at 365 nm. A standard curve was made to determine the levels of GB in unknown samples.

***Phenolic compounds estimation*:** The amount of total soluble phenolics was estimated with the method of Julkunen-Titto (1985). Fresh (0.1 g) plant sample was extracted with 80% acetone. An aliquot (100 µL) was diluted to 1 mL and reacted with Folin Phenol reagent by vigorous shaking. Immediately after 2.5 mL of $Na_2CO_3$ solution was added and made the final volume to 5 mL and mixed well. The absorbance was taken at 750 nm and tannic acid was used to prepare a standard curve for knowing the amount of soluble phenolics in unknown samples. To measure the flavonoids with the method of Zhishen *et al.* (1999), 0.1 g of the plant material was extracted in 80% acetone. Then to 1 mL of extract was added to 4 mL of water in a 10 mL volumetric flask. After 5 min., 0.6 mL of 5% $NaNO_2$ followed by 0.5 mL of 10% $AlCl_3$ solution were added and mixed. After 1 min, 2 mL of 1 M NaOH was added and made the volume up to 10 mL. The absorbance was taken at 520 nm while using 80% acetone as blank. For the measurement of anthocyanins with the method of Stark and Wray (1989), 0.1 g of fresh material was extracted with 1% acidified methanol and heated in a water bath at 50ºC and filtered the extract. The absorbance of the supernatant was taken at 535 nm while methanol was used as a blank.

***Vitamins analysis*:** for the determination of AsA with the method of Mukherjee and Choudhuri (1983), 0.1 g of the plant material was homogenized with 2 mL of 6% TCA and filtered. A 1 mL of the extract was reacted with 1 mL of the dinitrophenyl hydrazine followed by a drop of 10% thiourea solution (prepared in 70% ethanol). After heating the mixture was cooled in an ice bath for 15 min. While still on ice 5 mL of 80% $H_2SO_4$ was added; mixed and absorbance was taken at 530 nm, A graded series of AsA solution was prepared to make standard curve. For the estimation of niacin with the method of Okwu and Josiah (2006), 0.1 g of fresh plant material was added to 1 mL of 1 N $H_2SO_4$ and shaken well for 20 min and then added 1 drop of ammonia solution and filtered. Then 1 mL of the filtrate was reacted with 0.5 mL of 10% KCN and 0.5 mL of 0.02 N $H_2SO_4$ and shaken. The absorbance of the yellow colored complex was taken at 470 nm. Niacin (Sigma) was used to construct a standard curve. Riboflavin level was measured as described by Okwu and Josiah (2006). A 0.5 g of fresh plant sample was extracted with 10 mL of 50% ethanol and filtered. To 1 mL of extract, 1 mL each of 5% solution of $KMNO_4$ and 30% $H_2O_2$ were added and heated in a water bath at 50ºC for 30 min. When cool, 0.2 mL of 40% $Na_2SO_4$ was added and final volume made up to 5 mL and mixed well. The absorbance of the reaction mixture was taken at 510 nm. Riboflavin (Sigma) was used to prepare a standard curve.

***Statistical analysis*:** Statistix8.0 program was used to carry out analysis of variance of the data recorded for shoot and root dry mass and leaf area per plant, and metabolites to find out significant differences among the stresses and medium supplementation treatments. Least significant difference (LSD) test was used to find out delineate the differences among the treatments (Steel *et al.*, 1996).

## RESULTS

***Growth parameters*:** Statistical analysis of data indicated significant differences in the stress treatments and medium supplemented compounds both in the dry mass of shoot and root and leaf area per plant (Table 1). Compared with control, the medium supplementation of SA was the most effective in

**Table 1. Critical value for comparison and mean squares of two way interactions of growth and physiological attributes of maize with the medium supplementation of stress mitigating agents under control and Cd toxicity conditions.**

| Parameters | Shoot | | Root | |
|---|---|---|---|---|
| | CV for comparison | Stress × treatment interaction | CV for comparison | Stress × treatment interaction |
| Dry weight | 0.021 | $0.16\times10^{-3}$** | 0.014 | $0.07\times10^{-3}$** |
| Leaf area per plant | 0.963 | 0.320* | - | - |
| Hydrogen peroxide | 1.144 | 0.451** | 1.509 | 0.785** |
| Malondialdehyde | 1.198 | 0.494** | 1.018 | 0.357** |
| Soluble sugars | 1.408 | 0.684* | 1.244 | 0.533* |
| Free proline | 2.095 | 1.513** | 3.050 | 3.206** |
| Glycinebetaine | 1.650 | 0.938** | 0.880 | 0.267** |
| Soluble phenolics | 5.226 | 9.410* | 5.045 | 8.770* |
| Flavonoids | 1.560 | 0.860** | 0.942 | 0.850** |
| Anthocyanins | 0.048 | $0.81\times10^{-3}$** | 0.045 | $0.70\times10^{-3}$** |
| Ascorbic acid | 1.206 | 0.501** | 0.737 | 0.187** |
| Niacin | 0.759 | 0.197** | 0.456 | 0.072** |
| Riboflavin | 0.389 | 0.052** | 0.596 | 0.123** |

Significant at *, P<0.05 and ** P<0.01

enhancing shoot dry weight both under control condition followed by thiourea and AsA while $H_2O_2$ was ineffective in this regards. However, under Cd stress, the shoot dry weight was highly reduced but all the medium supplements enhanced this attribute although SA was the most effective followed by AsA, TU and $H_2O_2$ (Fig. 1a).

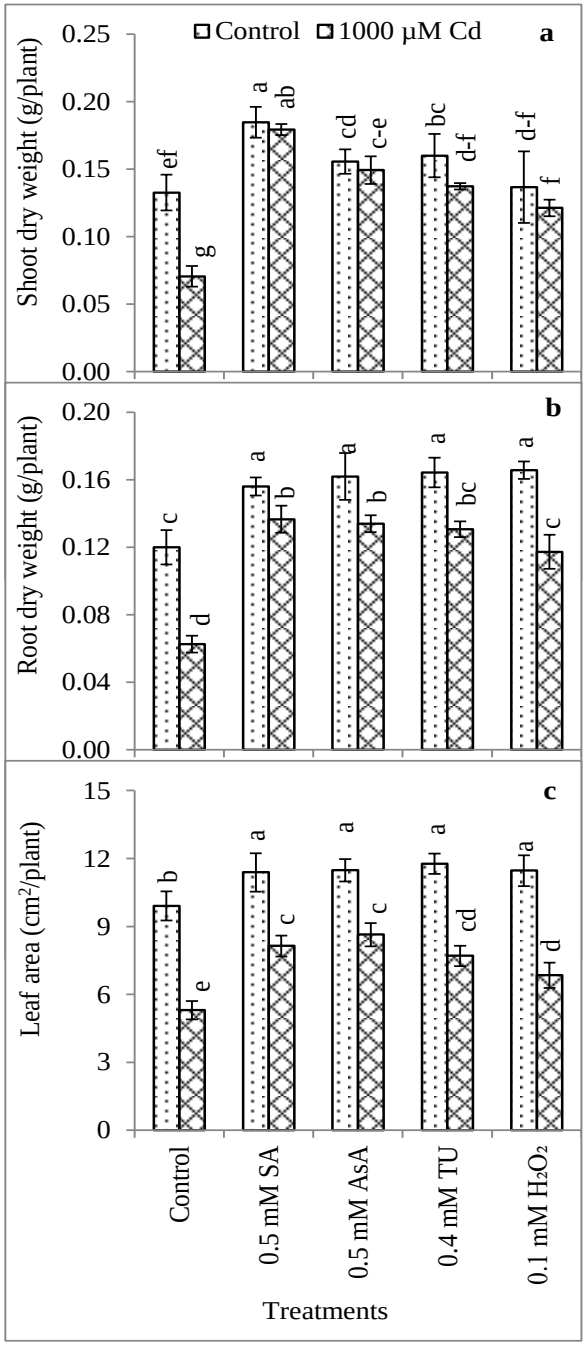


**Figure 1. Growth attributes of maize seedlings with medium supplementation of selected levels of stress alleviating compounds under Cd stress.**

The root dry weight, being low under control condition, was equally improved with the medium supplementation of all the chemicals. However, under Cd stress, the improvement in this attribute was the highest with SA and AsA followed by TU and $H_2O_2$ (Fig. 1b). As regards leaf area per plant, the medium supplemented with SA, AsA and TU indicated a similar improvement while $H_2O_2$ was not much effective. However, under Cd intoxication, although leaf area per plant was not improved much yet TU was more effective followed by SA and AsA while $H_2O_2$ was not effective (Fig. 1c).

**Changes in Tissue Metabolite Levels:**

***Oxidative stress parameters*:** Data showed significant differences in the stress and medium supplementation treatments with significant interaction of these factors for shoot and root $H_2O_2$ and MDA (Table 1). The shoot $H_2O_2$ contents were the lowest under control condition, which indicated some increase with the medium supplementation of all the compounds. However, Cd stress produced a twofold increase in shoot $H_2O_2$ contents while medium supplementation of all the selected compounds substantially reduced the $H_2O_2$ content; TU being relatively more effective followed by $H_2O_2$ (Fig. 2a). As regards root, the $H_2O_2$ level was the lowest in under medium supple of SA but relatively higher under $H_2O_2$ supplementation. However, Cd treatment caused a 2.2-folds increase in $H_2O_2$ in root while all the medium supplemented compounds were almost equally effective in reducing the production of $H_2O_2$, although SA and AsA were relatively more effective (Fig. 2b). As regards MDA contents, under no Cd stress the medium supplementation of SA was the most effective followed by $H_2O_2$ in reducing the shoot MDA contents. However, Cd treatment of maize led to about 2.5 folds greater increase in shoot MDA while among the medium supplemented compound SA was the most effective followed by AsA (Fig. 2c). As for root tissue the MDA content was the lowest under non-medium supplemented plants, which were little higher with all the medium supplements. However, with Cd treatment, the non-supplemented plants indicated the highest MDA content, which declined substantially with all the medium supply, although SA was the most effective followed by TU (Fig. 2d).

***Osmoprotectants accumulation*:** Statistical analysis of data for tissue contents of soluble sugars, free proline and GB indicated significant differences of Cd stress and medium supplementation along significant interaction of these factors (Table 1). As regards soluble sugars, under control condition, the medium supply of SA was the most effective in enhancing in the shoot soluble sugar contents while TU and $H_2O_2$ were not effective at all. However, under Cd stress there was a reduction in the soluble sugar content while medium supplementation of SA and AsA at selected levels were quite effective in enhancing the soluble sugar contents (Fig. 3a).

The root tissue exhibited lower soluble sugars contents under

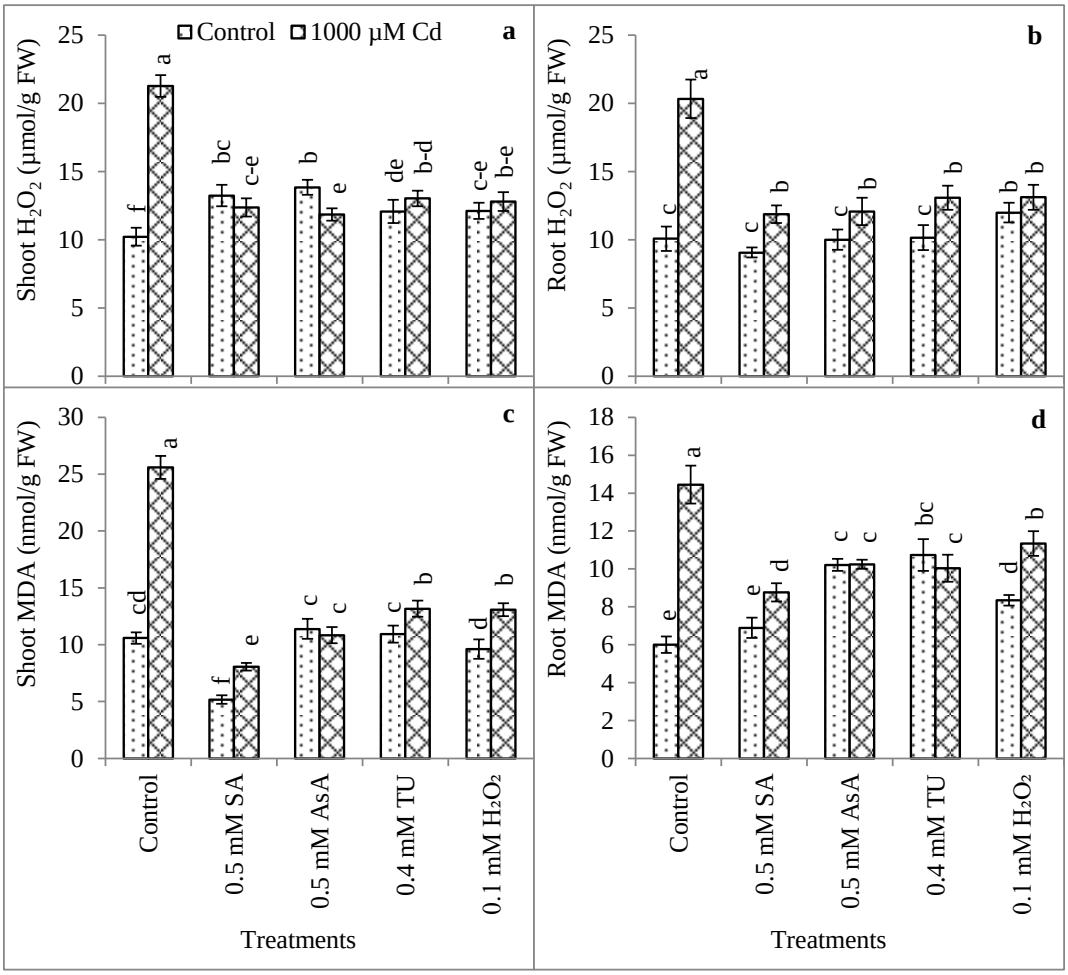


**Figure 2. Changes in the tissue levels of hydrogen peroxide ($H_2O_2$) and malondaldehyde (MDA) in Cd-intoxicated maize seedlings with the medium supplementation of selected levels of stress alleviating compounds.**

which was improved by 2.5 times with medium supply of SA control while medium supplementation of AsA was the most effective in improving this attribute. Contrarily under Cd stress, medium supply of TU and SA were the most effective in improving the root soluble sugar contents (Fig. 3b). As regards free proline accumulation, shoot free proline was lower under no medium supplementation treatments while under Cd stress both SA and AsA manifested about twofold greater accumulation of free proline followed by $H_2O_2$ and TU (Fig. 3c). Root free proline accumulation did not differ much with or without medium supplementation treatments. However, under Cd stress the root free proline content displayed a twofold increase with SA and AsA while 1.5 folds increase with TU and $H_2O_2$ when compared with no-medium supplementation (Fig. 3d). Under no-Cd treatment, medium supplementation with AsA was the most effective in improving shoot GB followed $H_2O_2$ compared with other compounds. However, Cd intoxication reduced the shoot GB, and by two times with AsA, TU and $H_2O_2$ (Fig, 3e). As regards root GB, under no Cd toxicity but with medium supplementation the increase in root GB was in the order SA = AsA > $H_2O_2$ > TU. On the other hand, the Cd induced reduction in root GB was improved by about two times with SA and AsA but by ~1.5 times with $H_2O_2$ and TU (Fig. 3f).

***Phenolics accumulation*:** Data indicated significant difference in and the interactions of the medium supplemented compounds for soluble phenolics, flavonoids and anthocyanons accumulation in shoot as well as root tissue (Table 1). Except for SA under control condition, which showed greater shoot soluble phenolics, all other supplemental compounds did not differ from each other. However, under Cd stress there was more than two times higher accumulation of shoot soluble phenolics with SA and AsA followed by TU and $H_2O_2$ (Fig. 4a).

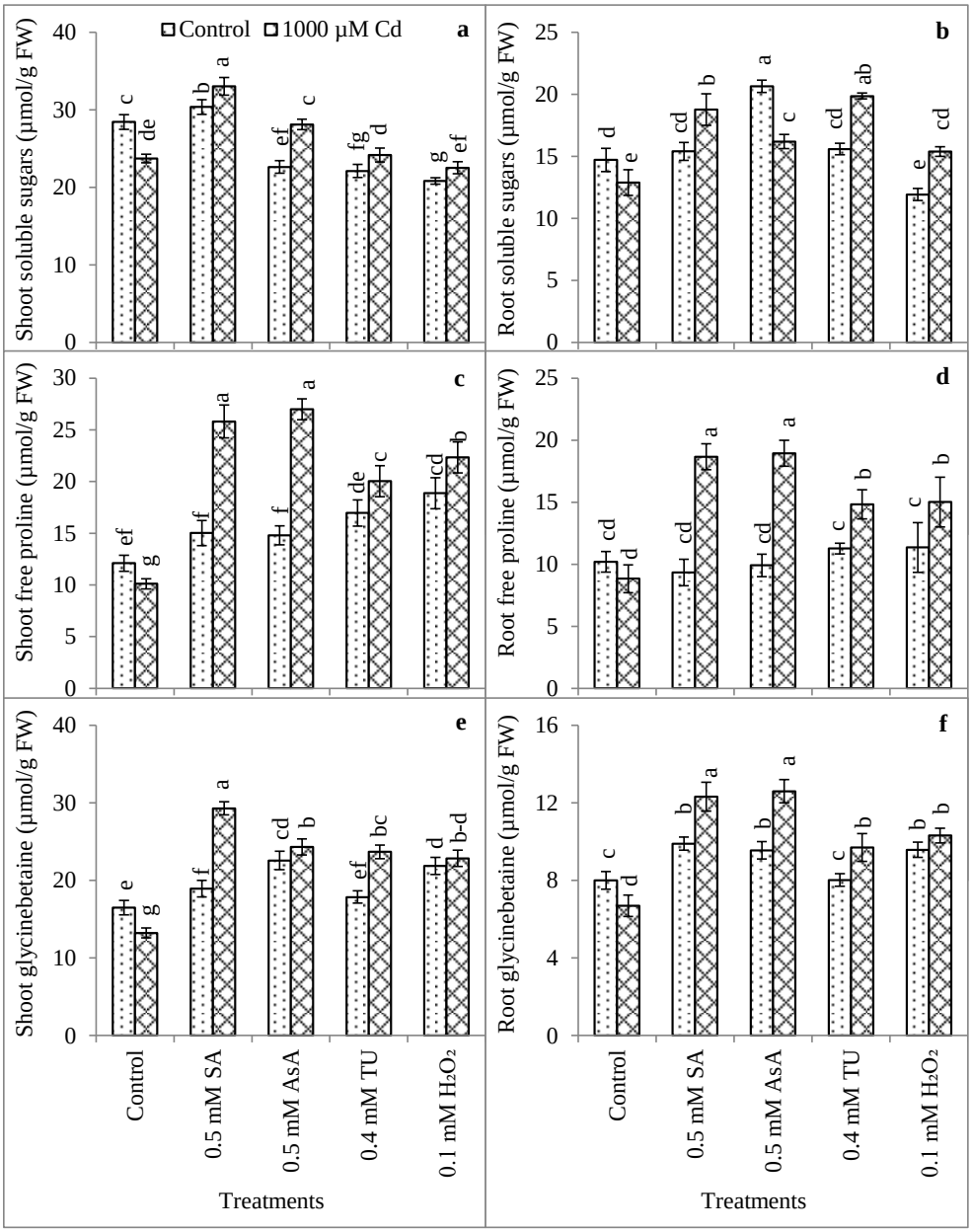


**Figure 3. Changes in the tissue levels of hydrogen peroxide ($H_2O_2$) and malondaldehyde (MDA) in Cd-stressed maize seedlings with the medium supplementation of selected levels of stress alleviating compounds.**

As for root soluble phenolics, the data revealed that under no Cd stress, except for an increase in soluble phenolics under SA supplementation, there was no difference in control and rest of the medium supplementations. Conversely under Cd toxicity, medium supplementation with SA and AsA led to a greater soluble phenolics accumulation followed by TU and $H_2O_2$ (Fig. 4b). Under no Cd toxicity the shoot flavonoids kept low irrespective of the medium supplementation treatments. However, under Cd stress, the shoot flavonoids were the highest with medium supply of SA and AsA but low with TU and $H_2O_2$ (Fig. 4c). The root flavonoid contents remained low with or without medium supplementations under no Cd stress. However, under Cd toxicity, SA followed by AsA exhibited the greatest increase in the root flavonoids followed by TU and $H_2O_2$ (Fig. 4d). Under no Cd treatment the shoot anthocyanins contents were the highest with medium supplementation of SA followed by AsA. On the other hand under Cd stress, highest shoot anthocyanins were

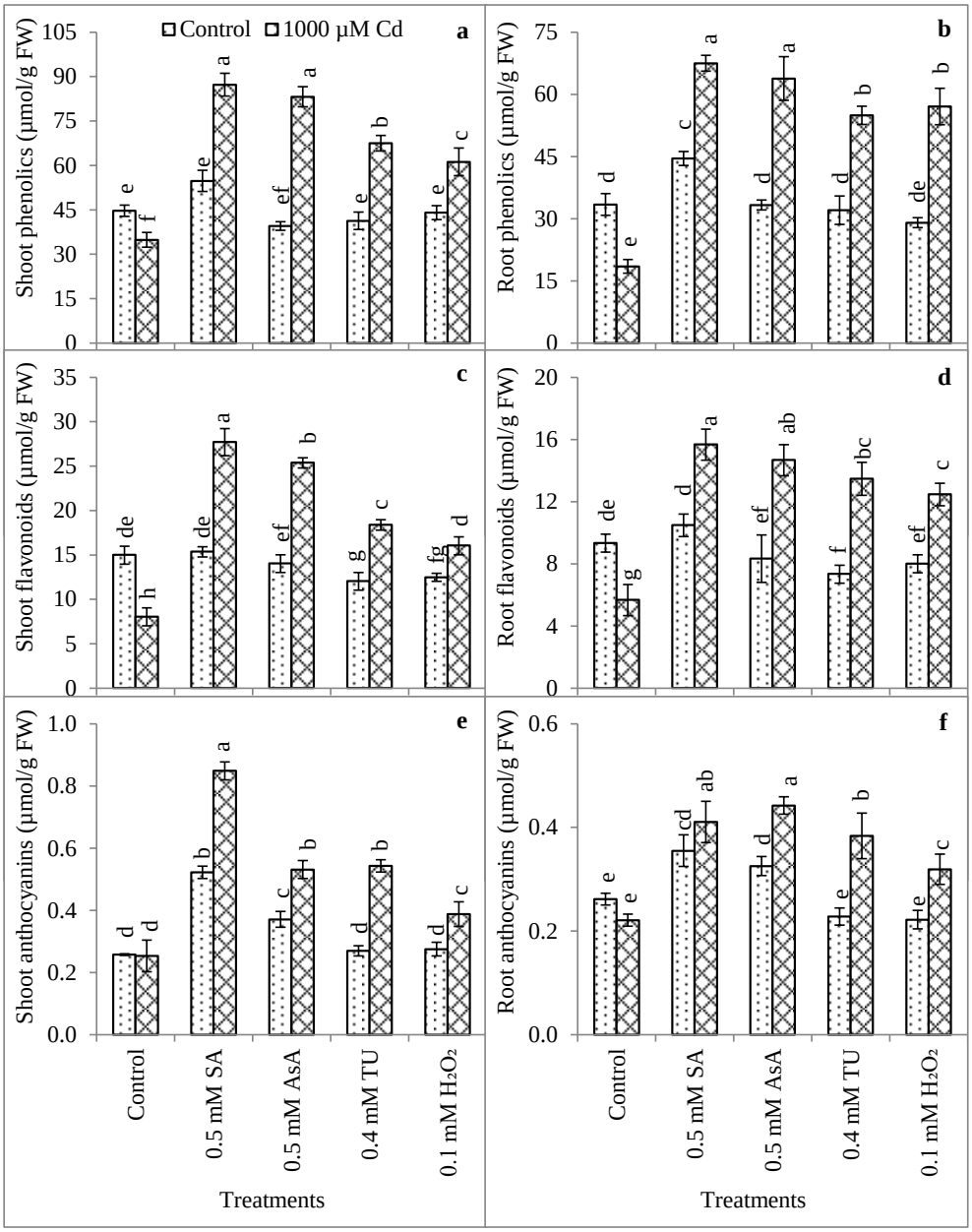


**Figure 4. Variations in the tissue levels of soluble phenolics, flavonoids and anthocyanins contents in Cd-treated maize seedlings with the medium supplementation of selected levels of stress lessening compounds.**

analyzed in SA supplementation (three times higher than the Cd treated controls) while in the rest of the treatments the anthocyanins contents were quite low in concentration (Fig. 4e). In root tissue under no Cd stress, the anthocyanins contents were significantly higher with medium supply of SA followed by AsA. However, under Cd stress, medium supplementation caused generally greater shoot anthocyanins being the highest with SA and AsA supplementation followed by TU and $H_2O_2$ (Fig. 4f).

***Vitamins biosynthesis*:** Results revealed significant differences in the levels of AsA, niacin and riboflavin under Cd stress and medium supplementation of various compounds (Table 1). Under no Cd toxicity the shoot AsA level was the highest in the plants supplemented with AsA while its level was almost similar to control in rest of the medium supplementation treatments. Under Cd stress, however, the shoot AsA was the lowest in the non-medium supplemented plants while the highest with the medium supplementation of SA and AsA (Fig. 5a).

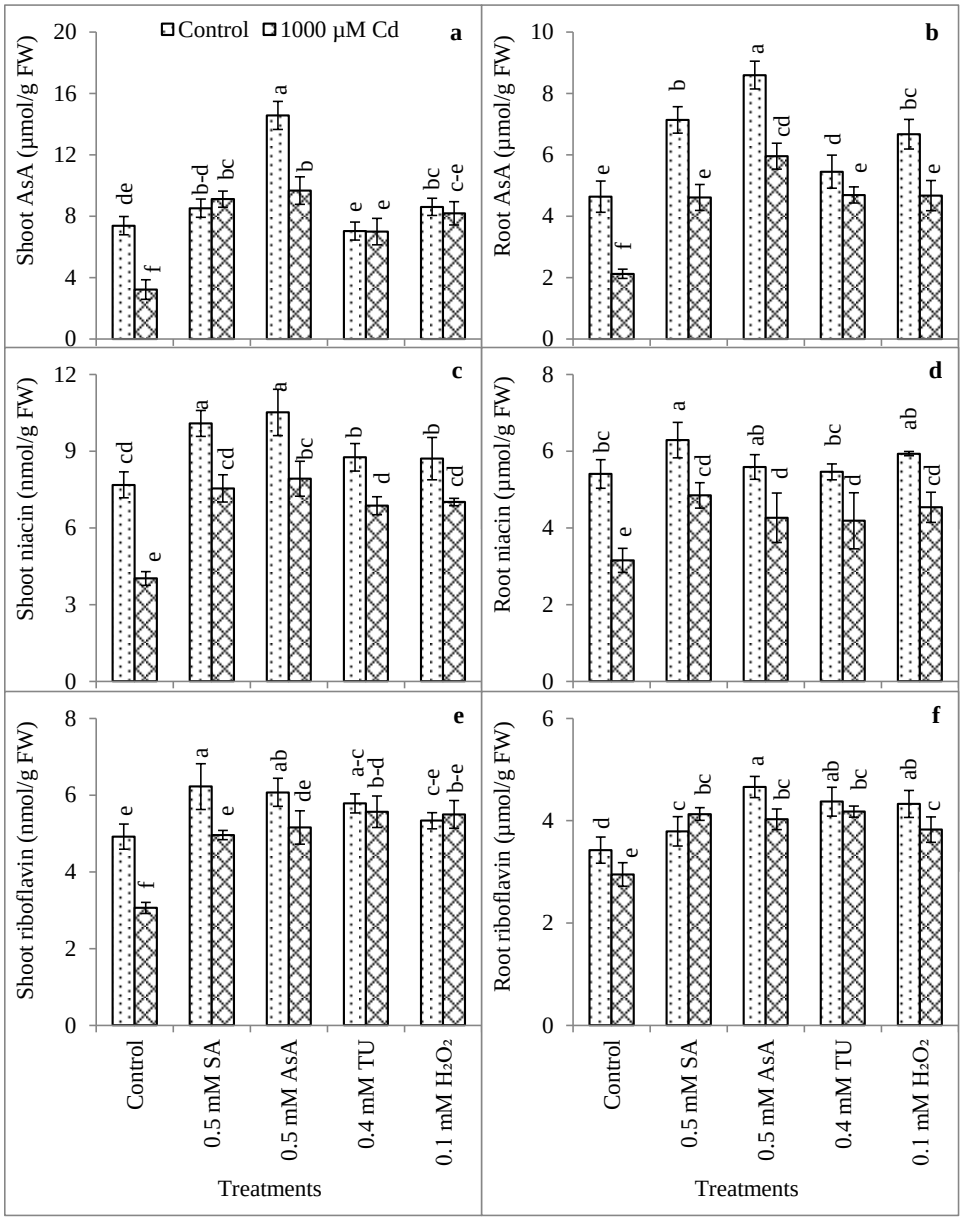


**Figure 5 Variations in the tissue levels of ascorbic acid, niacin and riboflavin in the shoot and root of Cd-treated maize seedlings with the medium supplementation of selected levels of stress mitigating compounds.**

In root tissue the medium supplementation with AsA led to a highest accumulation of AsA followed SA and $H_2O_2$. The trend of AsA accumulation was higher noticed under Cd stress (Fig. 5b). As regards niacin contents, under control (no Cd stress), the shoot niacin was the highest with medium supplementation of AsA and SA followed by TU and $H_2O_2$. However, under Cd stress, shoot niacin, being lowest in untreated plants, accumulated it highly in SA and AsA treated plants (Fig. 5c). As regards root niacin contents, under no-Cd treatment, the medium supplementation of compounds did not show difference from the control plants. However, under Cd stress, medium supplementation with SA and $H_2O_2$ were comparatively more effective as compared to other treatments (Fig. 5d). Under control (no Cd) condition the shoot riboflavin contents were almost similar with the medium supplementation of SA, AsA and TU. However, under Cd stress the shoot riboflavin was the lowest in non-medium supplemented plants but was the highest in TU supplemented plants followed by $H_2O_2$ and AsA supplements (Fig. 5e). As for root riboflavin contents, under control (no Cd stress)

**Table 2. Pearson's correlation coefficient (*r*) of some growth attributes with some physiological attributes of maize hybrids under medium supplementation of thiourea subjected to control and Cd stress conditions.**

| Variable | Shoot dry weight | | Leaf area per plant | | Root dry weight | |
|---|---|---|---|---|---|---|
| | Control | Cd stress | Control | Cd stress | Control | Cd stress |
| Hydrogen peroxide | 0.587ns | -0.879* | 0.736ns | -0.903* | 0.254ns | -0.988** |
| Malondialdehyde | -0.748ns | -0.964** | -0.114ns | -0.917* | 0.744ns | -0.969** |
| Soluble sugars | 0.442ns | 0.779ns | -0.546ns | 0.626ns | 0.113ns | 0.822ns |
| Free proline | 0.056ns | 0.926* | 0.777ns | 0.933* | 0.313ns | 0.938* |
| Glycinebetaine | -0.083ns | 0.977** | 0.564ns | 0.879* | 0.521ns | 0.906* |
| Soluble phenolics | 0.657ns | 0.990** | -0.095ns | 0.969** | -0.402ns | 0.979** |
| Flavonoids | 0.272ns | 0.984** | -0.578ns | 0.946** | -0.459ns | 0.992** |
| Anthocyanins | 0.868ns | 0.944* | 0.280ns | 0.769ns | -0.017ns | 0.946* |
| Ascorbic acid | -0.037ns | 0.905* | 0.259ns | 0.915* | 0.618ns | 0.918* |
| Niacin | 0.616ns | 0.915* | 0.627ns | 0.944* | 0.370ns | 0.895* |
| Riboflavin | 0.659ns | 0.901* | 0.649ns | 0.895* | 0.849ns | 0.989** |

Significant at *, P<0.05; **, P<0.01 and ns, P>0.05

condition, the value of this attribute was the highest with medium supplementation of AsA, TU and $H_2O_2$. However, under Cd stress maximum root riboflavin was analyzed with medium supply of SA, AsA and TU (Fig. 5f).

***Correlations*:** To validate the possible association of different metabolite levels with the growth attributes (shoot and root dry mass and leaf area per plant), the Pearson's correlation coefficients were computed. Under control conditions shoot dry mass, leaf area per plant and root dry weight were not correlated with any of the metabolites. However, under Cd stress, all the growth parameters were significantly correlated with the metabolites except no correlation of soluble sugars with any of the growth attributes and no correlation of leaf area with anthocyanins contents (Table 2).

## DISCUSSION

In this study, it was noted that Cd stress adversely affected the growth of maize, while the medium supplementation of the selected levels of compounds minimized the adverse effect of Cd, although their effectiveness was variable (Fig. 1). It has been reported that Cd stress applied to plant roots competes with the calcium uptake at Ca channels, competitively taken up and causes toxicity to the cytoplasmic structures (Wahid *et al.*, 2009; Choppala *et al.*, 2013). In this research we noticed that there was a reduction in the dry weight of shoot and root as well as leaf area per plant, while the medium supplementation of SA, AsA, TU and $H_2O_2$ helped the plants to diminish the toxic effect of Cd as noticed from markedly greater dry weight of shoot and root and leaf area per plant of maize (Fig. 1). The major effect of Cd as toxic metal is the production of ROS, leading to the disruption of lipid bilayer in the membranes. This can be measured in terms of MDA contents as a consequence of peroxidation of membrane lipids (Shahid *et al.*, 2014; Kumar *et al.*, 2018). So, a linear relationship exists between the ROS and MDA content (Tian *et al.*, 2012). Our data suggested that due to Cd toxicity, both $H_2O_2$ and MDA were produced in quite high concentrations (Fig. 2), but the medium supplementation of compounds used in this study have definitive roles in rescuing the plants from Cd damage.

Various metabolites produced endogenously protect the membranes and improve stress tolerance. Major ones include osmoprotectants, phenolics and vitamins. All these compounds have their specific modes of action (Emamverdian *et al.*, 2015; Belkadhi *et al.*, 2016; Tariq *et al.*, 2018). In view of the fact that membrane damage is a major effect of heavy metal toxicity, it was deemed appropriate to find out the possible mechanistic basis of medium supplemented compounds in lessening the adverse effect of Cd on the shoot and root tissues. The matter of specific interest for us was to find out changes in all these compounds with the medium supplementations. Our results suggested that with Cd intoxication, there was an enormous reduction in the levels of soluble sugars, free proline and GB (Fig. 3), decline in the levels of soluble phenolics and anthocyanins among the flavonoids (Fig. 4) and also reduced the production of AsA, niacin and riboflavin among the vitamins (Fig. 5). This indicated that Cd was entirely toxic to metabolic pathways probably inhibiting the enzymatic activities (Hassan *et al.*, 2013). However, medium supplementation of the selected compound improved their tissues concentrations to a great extent. Among the medium supplemented compounds, SA was most effective followed by AsA and TU while $H_2O_2$ was the least effective (Fig. 3-5). It was quite evident from the results that improved growth of maize with the medium supplementation was due to concerted action of the endogenously synthesized metabolites, which included protection of cellular membranes (by osmoprotectants), scavenging of ROS (phenolic compounds) and sustained metabolic activities (vitamins). So, these results indicated that Cd toxicity was effectively minimized by the exogenous application of different growth enhancing compounds.

To further substantiate the above findings the correlations were drawn between the changes in growth attributes and metabolite levels under control and Cd toxicity conditions (Steel *et al.*, 1996). The data revealed that under control conditions none of the growth attributes indicated any relationship with any of metabolic parameters. However, under Cd toxicity MDA and $H_2O_2$ were negatively while the rest of the attributes including osmoprotactants, phenolics and vitamins were positively related to shoot and root dry weight and leaf area per plant. These data confirmed that the medium supplementation have effective role not only in mitigating the adverse effect of Cd but also normalizing the metabolic functions in maize. Among the medium supplemented compounds, SA followed by AsA were the most effective. Thus, the medium supplementation of the chemical used in the present research emerged as an effective tool in growing maize at least in marginally cadmium contamination. Greater effectiveness of the SA, AsA and TU are assignable to their molecular and physiological properties in scavenging ROS.

***Conclusion*:** The Cd-intoxication of maize seedlings caused oxidative damage on the plant growth attributes especially disrupting the metabolic functions as revealed from much reduced levels of osmoprotectants, phenolics and vitamins while medium supplementations competently improved the endogenous levels of all these metabolites. Among the medium supplemented compounds, SA and AsA were more effective by virtue of their prominent metabolic role as effective ROS scavengers in plants. The results carry great implications for successfully growing maize in marginally Cd-contaminated soils.

## REFERENCES


AbdElgawad, H., G. Zinta, M.M. Hegab, R. Pandey, H. Asard and W. Abuelsoud. 2017. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front. Plant Sci. 7:276.

Belkadhi, A., W. Djebali, H. Hédiji and W. Chaïbi. 2016. Cadmium stress tolerance in plants: A key role of endogenous and exogenous salicylic acid. Plant Sci. Today 3:48-54.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.

Carleton, M. and K. Foote.1965. A comparison of methods for estimating total leaf area of barley plants. Crop Sci. 5:602-603.

Choppala, G., Saifullah, N. Bolan, S. Bibi, M. Iqbal, Z. Rengel, A. Kunhikrishnan, N. Ashwath and Y.S. Ok. 2014. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. Crit. Rev. Plant Sci. 33:374-391.

Esfandiari, E. and G. Gohari. 2017. Response of ROS-scavenging systems to salinity stress in two different wheat (*Triticum aestivum* L.) cultivars. Not. Bot. Hort. Agrobot. Cluj-Napoca 45:286-291.

Etalo, D.W., I.J.E. Stulemeijer, H.P. van Esse, R.C.H. de Vos, H.J. Bouwmeester and M.H.A.J. Joosten. 2013. System-wide hypersensitive response-associated transcriptome and metabolome reprogramming in tomato. Plant Physiol. 162:1599-1617.

Foyer, C.H. and S. Shigeoka. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol. 155:93-100.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. Plant Soil 70:303-307.

Hassan, W., M. Akmal, I. Muhammad, M. Younas, K.R. Zahaid and F. Ali. 2013. Response of soil microbial biomass and enzymes activity to cadmium (Cd) toxicity under different soil textures and incubation times. Aust. J. Crop. Sci. 7:674-680.

Hassan, F.A.S., E.F. Ali and K.H. Alamer. 2018. Exogenous application of polyamines alleviates water stress-induced oxidative stress of *Rosa damascena* Miller var. *trigintipetala* Dieck. S. Afr. J. Bot. 116:96-102.

Hussain, I., M.A. Ashraf, R. Rasheed, M. Iqbal, M. Ibrahim, T. Zahid, S. Thind and F. Saeed. 2017. Cadmium-induced perturbations in growth, oxidative defense system, catalase gene expression and fruit quality in tomato. Intl. J. Agric. Biol. 19:61-68.

Emamverdian, A., Y. Ding, F. Mokhberdoran and Y. Xie. 2015. Heavy metal stress and some mechanisms of plant defense response. Sci. World J. 2015:756120.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. Arch. Biochem. Biophys. 125:189-198.

Jibril, S.A., S.A. Hassan, C.F. Ishak and P.E.M. Wahab. 2017. Cadmium toxicity affects phytochemicals and nutrient elements composition of lettuce (*Lactuca sativa* L.). Adv. Agric. Article ID 1236830.

Julkunen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. Agric. Food Chem. 33:213-217.

Kar, R.K. 2011. Plant responses to water stress: Role of reactive oxygen species, Plant Signal. Behav. 6:1741-1745.

Kumar, R., R. Gopal and Y.K. Sharma. 2018. Influence of cadmium and phosphorus enhance absorption and membrane damage in wheat seedlings grown in nutrient medium. J. Plant Nutr. 41:793-805.

Liu, H., J. Zhu, H. Ding, L. Wang, Z. Sun, P. Liu, T. Li and H. Du. 2018. Foliar spray of growth regulators significantly increases trans-1,4-polyisoprene production from *Eucommia ulmoides* Oliver short-rotation coppice. Indust. Crops Prod. 113:383-390.

Mukherjee, S.P. and M.A. Choudhuri. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant. 58:166-170.

Mustafa, A., R. Ahmad, M. Farooq and A. Wahid. 2018. Effect of seed size and seed priming on stand establishment, wheat productivity and profitability under different tillage systems. Intl. J. Agric. Biol. 20:1710-1716.

Okwu, D.E. and C. Josiah. 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol. 5:357-361.

Perveen, A., A. Wahid and F. Javed. 2011. Varietal differences in spring and autumn sown maize (*Zea mays*) for tolerance against cadmium toxicity. Intl. J. Agric. Biol. 13:909-915.

Perveen, A., A. Wahid, S. Mahmood, I. Hussain and R. Rasheed. 2015. Possible mechanism of medium-supplemented thiourea in improving growth, gas exchange, and photosynthetic pigments in cadmium-stressed maize (*Zea mays*). Braz. J. Bot. 38:71-79.

Perveen, A., I. Hussain, R. Rasheed, S. Mahmood and A. Wahid. 2018. Potential of thiourea in modifying membrane stability, osmoprotectants, vitamins and antioxidants levels under cadmium stress in maize. Intl. J. Agric. Biol. 20:2866-2874.

Rehman, M.Z.U., M. Rizwan, A. Hussain, M. Saqib, S. Ali, M.I. Sohail, M. Shafiq and F. Hafeez. 2018. Alleviation of cadmium (Cd) toxicity and minimizing its uptake in wheat (*Triticum aestivum*) by using organic carbon sources in Cd-spiked soil. Environ. Pollut. 241:557-565.

Santos, M.M., E.E.D.E. Borges, G.D. Ataide and G.A. de Souza. 2017. Germination of seeds of *Melanoxylon brauna* Schott. under heat stress: Production of reactive oxygen species and antioxidant activity. Forests 8:405-417.

Sarwar, M., M.F. Saleem, N. Ullah, M. Rizwan, S. Ali, M.R. Shahid, S.A. Alamri, M.N. Alyemeni and P. Ahmad. 2018. Exogenously applied growth regulators protect the cotton crop from heat-induced injury by modulating plant defense mechanism. Sci. Rep. 8:17086.

Shahid, M., B. Pourrut, C. Dumat, M. Nadeem, M. Aslam and E. Pinelli. 2014. Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. Rev. Environ. Contam. Toxicol. 232:1-44.

Stark, D. and V. Wray. 1989. Anthocyanins. In: J.B. Harborne (ed.), Methods in Plant Biology, Plant Phenolics, Vol. I. Academic Press/Harcourt Brace Jovanovich, London; pp. 32-356.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1996. Principles and Procedures of Statistics: A biometrical approach, 3rd Ed. McGraw Hill Co., New York, USA.

Tian, Z.G., F. Wang, W.N. Zhang, C.M. Liu and X.M. Zhao. 2012. Antioxidant mechanism and lipid peroxidation patterns in leaves and petals of marigold in response to drought stress. Hortic. Environ. Biotechnol. 53:183-192.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci. 151:59-66.

Wahid, A., M. Arshad and M. Farooq. 2009. Cadmium phytotoxicity: responses, mechanisms and mitigation strategies. In: E. Lichtfouse (ed.), Advances in Sustainable Agriculture - Book Series, Vol 1. Springer, The Netherlands; pp.371-403.

Wahid, A., M. Farooq and K.H.M. Siddique. 2014. Implications of oxidative stress for crop growth and productivity. In: M. Pessarakli (ed.), Handbook of Plant and Crop Physiology, 3rd Ed. Taylor and Francis Press, New York, USA; pp.549-556.

Wahid, A., S.M.A. Basra and M. Farooq. 2017. Thiourea: A molecule with immense biological significance for plants. Intl. J. Agric. Biol. 19:911-920.

Wan, L. and H. Zhang. 2012. Cadmium toxicity: Effects on cytoskeleton, vesicular trafficking and cell wall construction. Plant Signal. Behav. 7:345-348.

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez. 1976. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute (IRRI), Los Banos, The Philippines.

Zhishen, J., T. Mengcheng and W. Jinming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64:555-559.