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FOLIAR APPLIED 5-AMINOLEVULINIC ACID AMELIORATED THE ADVERSE EFFECTS OF HEAVY METALS (Cd and Pb) BY TRIGGERING ANTIOXIDANT SYSTEM IN TWO VARIETIES OF MUSTARD (*BRASSICA CAMPESTRIS* L.)

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Abstract

Heavy metal (HM) stress has adverse effects on growth of plant and biochemical parameters. The foliar spray of plant growth regulators is a renowned approach to alleviate the negative effects of HM on plants. It is considered that the influence of HM on plant physiological phenomenon can be overcome by the use of amino acids. Therefore, the major objective of present study was to find the role of 5-aminolevulinic acid (ALA) in alleviating the Heavy metals stress in two varieties i.e. Super canola-2018 (V1) & Super Raya-2016 (V2) of mustard. A pot experiment was conducted in Botanical Garden, Department of Botany, University of Gujrat, Gujrat, Pakistan. Total ten treatments were used and each treatment was replicated four times. A Completely Randomized Design (CRD) was used for this experiment. Pb (100µM) and Cd (100µM) were applied in the form of PbCl2 and CdCl2 at the time of sowing to induce heavy metal stress. After two weeks of germination, 5-ALA (50mg/L& 100mg/L) was foliarly applied. Results showed that HMs stress reduced germination by 44%, fresh & dry biomass of root 77% & 73% and fresh & dry biomass of shoot 80% & 79% respectively. Similar trend was also observed for length of shoot (46%) and root (71%). Biochemical parameters like carbohydrates and proteins were reduced by 29% & 33% respectively. However, the foliar spray of 5-ALA (100 mg/L) improved morphological, biochemical and physiological parameters under HMs stress. Biochemical parameters including chl-a 41%, chl-b 13% & carotenoids 27% soluble protein contents by 34% were increased under stressed environment. Antioxidant enzymes activities was higher under HMs stress which was decreased (CAT 76%, POD 40%, SOD 38%) by foliar application of 5-ALA. Adverse effects of HMs were more severe in variety Super canola-2018 than variety Super Raya-2016. It was concluded that 5- Aminolevulinic Acid could be useful to reduce the effect of HM from mustard.

Key words: Brassica, Foliar, 5-amino levulinic acid, Heavy metals, Antioxidants.

Introduction

Heavy metals contamination in the soil has gained increasing attention in recent years due to the toxic effects of metals on plants on the environment (Krystofova *et al*., 2012). Sewage from industrialized water sources and surface are the main sources of heavy metals and trace element (Kurniawan *et al*., 2006). Overall, the problem of heavy metal contamination is of great concern because of its harmful effects on plants (Chen *et al*., 2005; Khan *et al*., 2011). Cd negatively affects yield, growth and development of plants. The harmful indications of Cd on plants includes decreased growth, slowdown of photosynthetic mechanism, negative effects on stomatal apparatus, decline in enzymatic actions, protein degradation and disturbed membrane passage of ions. Lead is harmful as it reduces plants growth, alteration of cell parts, distort ions flow, reductions in chlorophyll, decrease in hormonal biosynthesis and increase of ROS (Shahid *et al*., 2011; Kumar *et al*., 2012). Pb by ROS production causes oxidative stress in plant (Singh *et al*., 2010). Lead pollution is harmful to plants and lead also persists in the environment for long time (Piechalak *et al*., 2003). Negative effect of Pb to plants is less in root biomass as compared to leaves (Siedlecka and Krupa, 2002) reductions in chlorophyll and less seed sprouting rate (Moustakas *et al*., 1994).

According to the Indo-Europeans histories about 300 BC, Mustard was documented as one of the first crops that was cultivated as food and oldest spices. Genus Brassica (Brassicaceae) comprises of more than 30 species (Rakow, 2004). Among these are several important animal fodder, agricultural species, human consumption, condiments, biofuel and oil production, (Bancroft & Schmidt, 2010). Brassica are the source of carbohydrates and tocopherols (Guzman *et al*., 2012). Mustard is one of the most important oil plants in the world, widely cultivated not only for oil but also as leafy vegetables, although it is used as an international herb (Sood, 2010). For vegetable purposes this plant is widely grown in urban areas where sewage is widely used for irrigation. Wild water can easily transfer nutrients to the soil and eventually to plants (Dai *et al*., 2006). As well as it is an important crop for nutrition and oil in many countries. Plant may face heavy metals stress at any stage of life cycle that can affect its physiology and many other developmental processes. Due to heavy metals the growth rate of Mustard varieties was badly affected. It reduced the production and quality of Mustard. Physiological, morphological and biochemical processes were also adversely affected under heavy metals stress (Ali *et al*., 2015).

Plant growth regulators (PGRs) are used to enhance the tolerance ability in plants against heavy metals stress (Ali *et al*., 2018). PGRs can improve plant production by regulating physiological processes. Among many renowned plant hormones, 5-Aminolevulinic acid (ALA) is considered to be very active against the several abiotic stresses in plants. In several studies it has been stated that ALA has been involved in regulating the growth and development of the plant, and has an important role in growth regulation (Akram & Ashraf, 2013). ALA has been well known as growth enhancing and stress-tolerant regulator (Ahmad *et al*., 2018; Bali *et al*., 2018; Jan *et al*., 2018; Handa *et al*., 2018). Foliar application of 5-ALA under heavy metal stress increased plant growth attributes and wheat yield (Al-Thabet, 2006). Foliar spray of 5-Amino-levulinic acid improved plant biomass, green pigment, antioxidant (SOD) and proline contents in pepper under cold stress (Hegedüs *et al*., 2001). According to Wang *et al*., (2004), 5-ALA prompted tolerance of chilling stress by enhancing photosynthetic rate in *Cucumis melo*. Similarly, 5-Amion-levulinic acid under heat stress has improved plant growth characters by reducing Malondihyde content, superoxide radicals (O_2-), and H_2O_2 in cucumber (Zhen *et al*., 2012). Under Salt stress antioxidant enzyme activities and photosynthetic traits has been increased in spinach by foliar application of 5-ALA (Nishihara *et al*., 2003).

Al-Thabet (2006) applied 5-ALA to wheat plants and noted that it enhanced grain harvest of wheat. Similarly, in barley applications of 5-ALA helped to overcome the effects drought stress (Al-Khateeb *et al*., 2006). Foliar spray of 5-ALA in various crops helped to cope the adverse effects of stresses as, in date palm improved the growth of plants under salt stress (Youssef & Awad, 2008), oil contents in rape seed (Naeem *et al*., 2010), tubers in potato (Zhang *et al*., 2006) and number of leaves in spinach (Nishihara *et al*., 2003). Applications of 5-ALA undergoes numerous physiological changes in plants against salts stress by improving seed sprouting, photosynthetic process and uptake of minerals (Hotta *et al*., 1997; Youssef & Awad, 2008).

In the light of above mentioned literature, it was found that the efficacy of amino acids especially 5-ALA in Mustard (*Brassica campestris*) under heavy metal stress has not been evaluated. That's why, this study was designed to evaluate the role of 5-ALA in Mustard under heavy metal stress in relation to morpho-physiological attributes that can effects crop productivity.

Material and Methods

Field experiments were performed to assess the impact of foliar spray of 5-Aminolevulinic acid (5-ALA) against the Pb and Cd heavy metals stress in Mustard. Experiments were carried out in the Department of Botany, University of Gujrat, Gujrat, Pakistan during 2021-22. Seeds of two varieties of Mustard (Super Canola-2018 and Super Raya-2016) were obtained from the Punjab Seed Corporation Gujranwala, Punjab, Pakistan. Seeds were sown in plastic pots filled with 5 kg sandy loam soil. Eight (8) seeds were sown in each plastic pot and kept 4 plants per pots after germination. Heavy metals treatments were applied at the time of sowing to induce heavy metal stress. Pb and Cd were applied in the form of $PbCl_2$ and $CdCl_2$, respectively. Completely Randomized Design (CRD) was used with four replicates for each treatment. Treatments of the 5-Aminolevulinic acid were applied after two weeks of germination. There were following were the treatments:

T0 = Control
T1= Pb (100µM)
T2 = Cd (100µM)
T3 = Pb (100µM) + Cd (100µM)
T4= Pb (100µM) + 50mg/L ALA
T5 = Cd (100µM) + 50mg/L ALA
T6 = Pb (100µM) + Cd (100µM) +50mg/L ALA
T7 = Pb (100µM) + 100mg/L ALA
T8= Cd (100µM) + 100mg/L ALA
T9 = Pb (100µM) + Cd (100µM) +100mg/L ALA

Heavy metals treatments were applied at the time of sowing and 5-ALA treatments were applied after two weeks of germination.

After 50-60 days of sowing (at vegetative stage) samples of the plants were collected for morphological parameters from each pot and separated into roots and shoots after washing with distilled water. For taking fresh biomass of root and shoot electrical balance was used. For dry weights, samples of root and shoot were kept in the oven at 65°C for three days. Dry biomass was measured by using electrical balance. Root and shoot lengths were measured using a scale. Leaf from each plant was removed and located on a grid paper. The leaf outline was drawn with a pencil on a grid paper. Finally, the leaf area was measured by counting the leaf-covered grids. One of the plants from each replicate was selected and its number of leaves was counted.

The leaf Glycine-betaine contents were determined according to Dustgeer *et al*., (2021). To determine the chlorophyll contents (chlorophyll a, b and carotenoids) Arnon (1949) method was followed. Anthocyanin contents were estimated by the method of Krizek *et al*., (1998). The lipid per oxidation level was found in terms of thiobarbituric acid-reactive substances (TBARS) concentration as defined by Cakmak and Horst (1991) with slight changes.

$$\text{Malondialdehyde per oxidation level (nmol)} = \Delta\ (\text{A 532nm-A 600nm})/1.56\times105$$

The upper 3rd leaf from each replicate was used for determination and extraction of leaf proline in accordance with Bates *et al*., (1973). Superoxide Dismutase (SOD) activity was determined by following the method of Giannopolitis and Ries, (1977). Method of Chance & Maehly (1955) was used to measure the activities of catalase (CAT) and peroxidase (POD).

Statistical analysis

Data for each parameter was analyzed with the help of Analysis of Variance (ANOVA) using Minitab Computer Program to compare mean values. Tukey's test was used to compare mean values for 0 and 5 mg/L of 5-ALA on morphology, biochemical and yield attributes of mustard under Pb and Cd toxicity.

Results

Following results were obtained from this study.

Morphological parameters: Heavy metal stress showed significant results for all the morphological attributes of mustard (Table 1). HM severely decreased the germination rate and maximum decrease was observed in T3 (Pb (100µM) + Cd (100µM) and it was 44 % less than control. Highest germination rate (87.5) was observed in T8 (Cd (100µM) + 100mg/L ALA) that was 11% higher than control (77.5). Heavy metal stress severely decreased the shoot fresh weight and maximum decrease was observed in T3 (Pb (100µM) + Cd (100µM) that was 77% less as compared to control. Highest shoot fresh weight (3.43g) was noted at T8 (Cd (100µM) + 100mg/L ALA) in Super Raya-2016. Foliar application of 5-ALA showed positive effect against Pb and Cd stress and increased the root fresh weight. Maximum increase (0.963g) was observed in T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 and it was 26% higher than control (Fig. 1). With the applications of heavy metals (Pb and Cd) root fresh weight was badly decreased and this decrease was 77% in Super Raya-2016 and 80% in Super canola-2018 as compared to controls. Heavy metal stress decreased the shoot dry weight and maximum (0.101g) decrease was observed in T3 (Pb (100µM) + Cd (100µM) that was 73% compared to control. Maximum shoot dry weight (0.39g) was noted under foliar application of 5-ALA in T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 that was 55% higher. A continuous decreasing trend T5>T4>T3 was observed in shoot dry weight with 50mg/L applications of ALA and T8>T7>T6 with 100mg/L of 5-ALA (Fig. 1). It was noted 5-ALA showed positive response against Pb and Cd stress for root dry weight. Maximum root dry weight (0.494g) was observed in T8 (Cd (100µM) + 100mg/L ALA)of Super Raya-2016 that was 50 % higher than control. Due to heavy metals Pb and Cd stress root dry weight severely decreased and this decrease was 81% in Super Raya-2016 and 80 % in Super canola-2018 as compared to their controls (Fig. 1).

Root length was significantly affected by the applications of HM and 5-ALA (Table 1). It was found maximum root length (12.25g) was present in Super Raya-2016 with T8 treatment (Cd (100µM) + 100mg/L ALA). Addition of Lead and Cadmium soil medium decreased the root length in both varieties of mustard. It was noted that shoot length of mustard was also significantly affected by HM (Table 1). Maximum shoot length (16.75cm) was observed in T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 as compared to other treatments. It was found that foliar application of 5–ALA showed progressive effects and shoot length was increased in *Brassica* plants under Pb and Cd stress (Fig. 1). Heavy metal (Pb and Cd) stress severely reduced the shoot length (5.55g) in T3 (Pb (100µM) + Cd (100µM) as compared to control. Number of leaves was also affected by HM applications in mustard. Higher number of leaves was counted in T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016. Heavy metal (Pb and Cd) stress severely had deceased the number of leaves with all the treatments of Pb and Cd. Analysis of Variance (ANOVA) depicted that interaction among treatments and varieties was non-significant ($p \geq 0.005$) for most of the morphological attributes (Table 1).

Biochemical parameters: It was noted that biochemical parameters of mustard were significantly affected by heavy metals as well as 5-ALA treatments (Table 2). Different treatments of 5-ALA alleviated the toxic effects of Lead and Cadmium and enhanced the glycine betaine (GB) contents. Higher contents of GB was noted with 100mg/L of 5-ALA as compared to 50mg/L (Fig. 2). It was noted that T5 and T6 treatments had highest value of GB in both Mustard varieties but Super Raya-2016 was superior over Super canola-2018. Analysis of variance (ANOVA) depicted that interaction among treatments and varieties was highly significant ($p \leq 0.005$) for both varieties showed the positive response towards different levels of heavy metals and 5-ALA (Table 2). Heavy metal stress severely decreased the carotenoids contents and maximum decreased was observed in T3 (Pb (100µM) + Cd (100µM) that was 5% as compared to control.

Photosynthetic pigments (chl-a,b and carotenoids) were also adversely affected by HM applications (Table 2). Highest Chl-a contents was calculated at T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 as compared to other treatments. Higher level of 5-ALA (100mg/L) in T7, T8 and T9 showed higher chl-a compared to (50mg/L) of 5-ALA (Fig. 2). Different levels of 5-ALA increased the chlorophyll "b" contents under Pb and Cd stress. Maximum decrease in chl-b was noted in T3 (Pb (100µM) + Cd (100µM) of Super canola-2018 as compared to non-stressed plants. Heavy metal stress severely decreased the anthocyanin contents and maximum decrease was observed in T3 (Pb (100µM) + Cd (100µM) that was 77% less as compared to control. Results showed a non-significant ($p>0.05$) difference between the mean values of anthocyanin for all the treatments of both varieties under stress and 5-ALA applications (Table 2). The highest concentration (33.14) of MDA was observed in T6 of Super canola-2018 which was 57% higher than control (Fig. 2). It was found that foliar application of 5–ALA decreased the MDA contents in *Brassica* plants under Pb and Cd stress. An increasing trend was observed for MDA contents for 5-ALA as compared to 100mg/L foliar application of ALA. A significant ($p<0.05$) difference between the mean values of MDA for all treatments of both varieties under stress and non- stressed conditions was observed. It was found that T8 treatment (Cd (100µM) + 100mg/L ALA) in Super Raya-2016 had the highest carbohydrates contents as compared to all other treatments for both varieties (Fig. 2). Analysis of Variance (ANOVA) depicted that interaction among treatments and varieties was highly significant ($p \leq 0.005$) and both varieties showed the positive response towards different levels of 5-ALA and negative towards heavy metals (Table 2). It was observed that T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 had the highest protein contents as compared to all other treatments in both varieties. Heavy metal stress (Pb and Cd) treatment ((Pb (100µM) + Cd (100µM) reduced the protein contents upto 0.22mg/ml for Super canola-2018 and 0.24mg/ml for Super Raya-2016. It was found that T3 (Pb (100µM) + Cd (100µM) for Super Raya-2016 had the highest proline contents as compared to all other treatments of both varieties. Combined applications of lead and cadmium significantly increased the proline contents in Super Raya-2016. It was observed that under stress conditions proline contents were increased in both mustard varieties (Fig. 2).

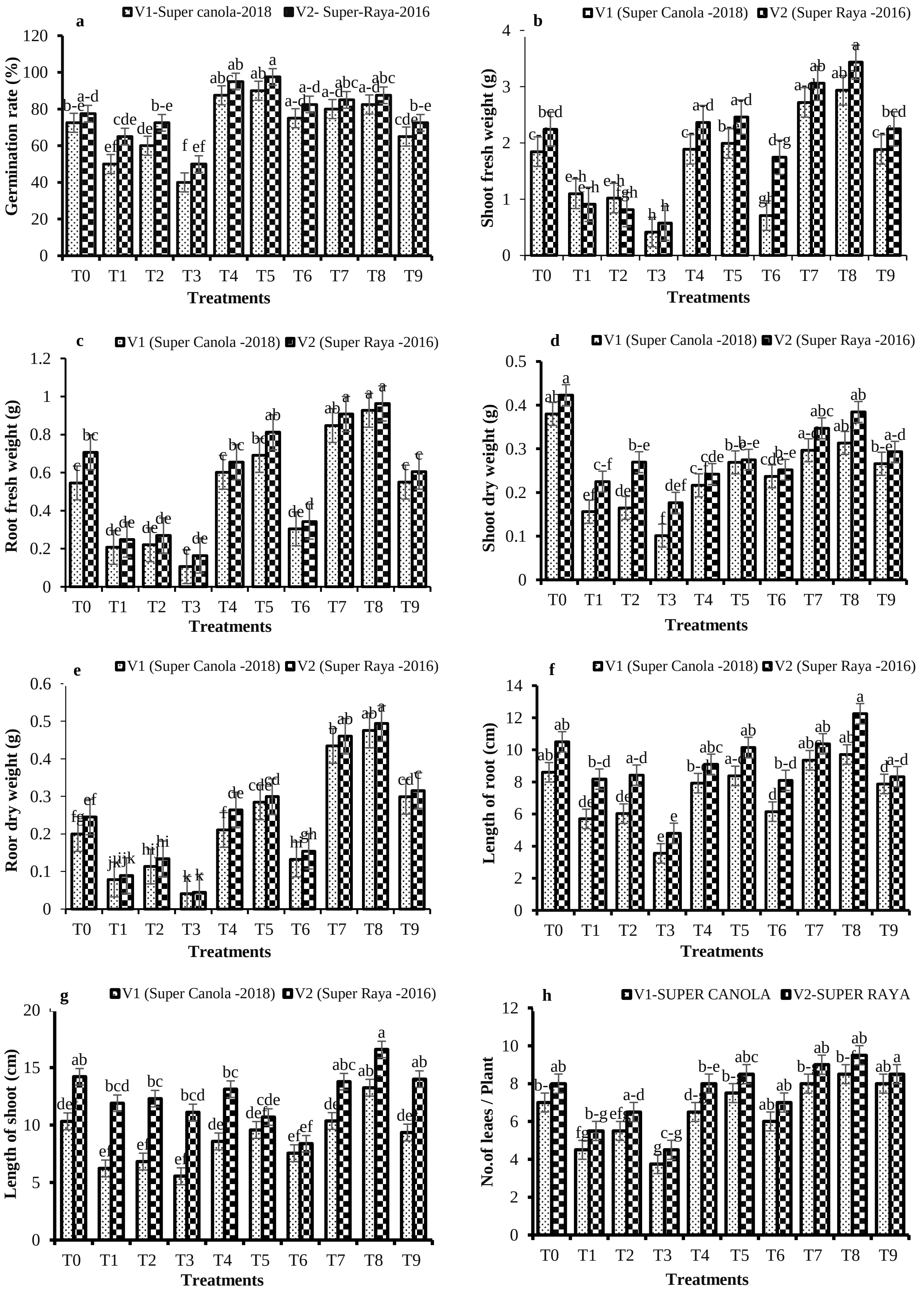


Fig. 1. Effect of different levels of 5-Amino-levulinic acid on germination rate, shoot and root fresh and dry weight, length of root and shoot and No. of leaves/plant of two mustard varieties grown under HM stress.

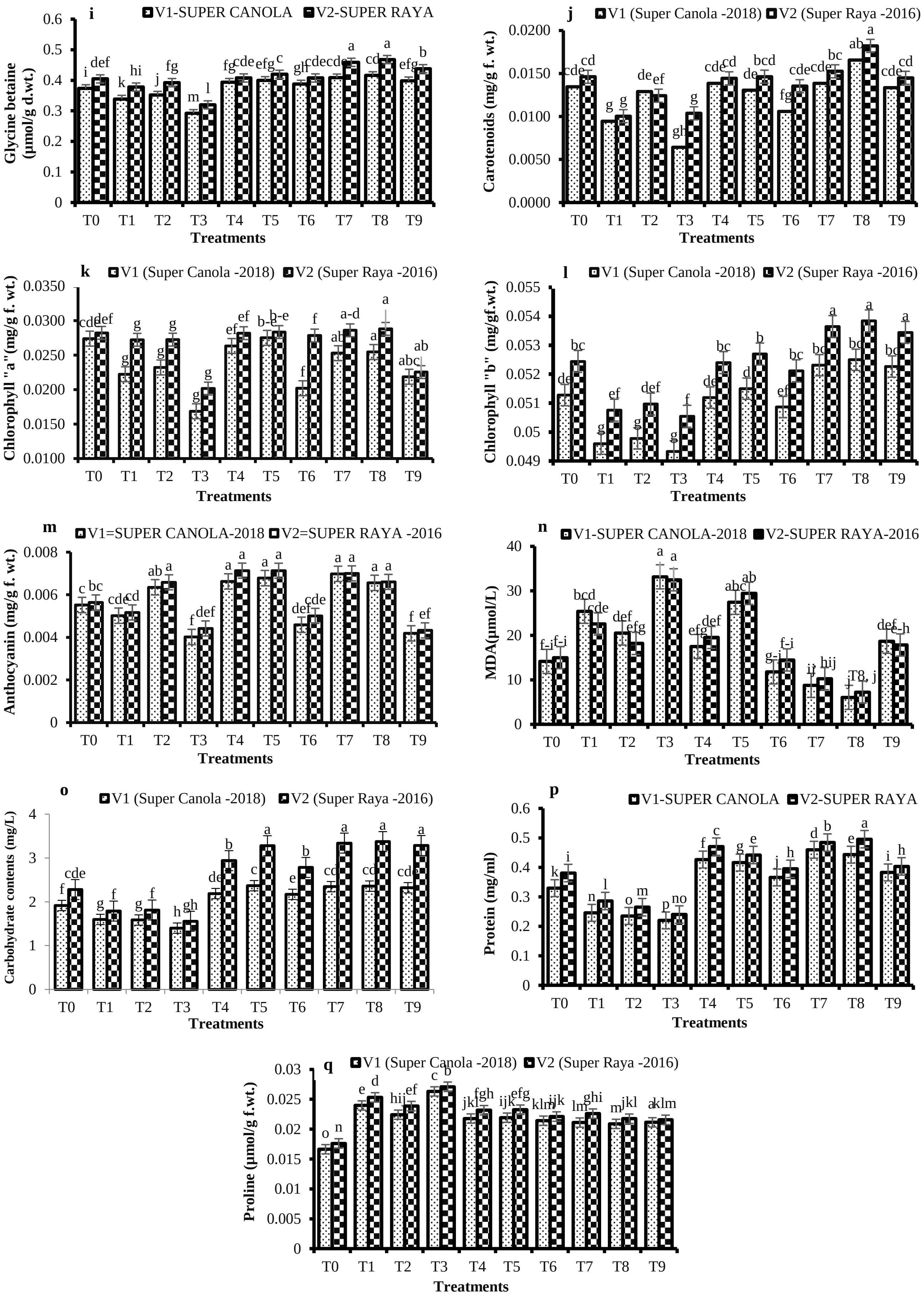


Fig. 2. Effect of different levels of 5-Amino-levulinic acid on activity of GB, photosynthetic pigments, Anthocyanin, MDA, carbohydrates, protein and proline of two mustard varieties grown under HM stress conditions.

Table 1. Mean squares (MS) of ANOVA for various morphological of two varieties of mustard (*Brassica campestris* L.) when subjected to different levels of 5-Amino- levulinic acid under lead and cadmium stress.

Sources	df	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Root length	Shoot length	Leaf area	No. of leaves/ Plant
Variety	1	2.2465***	4.4462***	0.047483***	0.010695***	57.3927***	296.065***	24.8645***	49.6125***
Treatments	9	6.1969***	25.429***	0.048553***	0.174586***	29.7288***	35.946***	72.4254***	13.1236***
Var×Treat	9	0.2572ns	0.4160***	0.001941ns	0.000457ns	0.9778ns	6.005ns	0.7197*	0.5569ns
Total	60	0.1840	0.0435	0.002621	0.000389	3.9887	9.593	1.9857	0.8042
Error	79								

Table 2. Mean squares (MS) of ANOVA for various biochemical parameters of two varieties of mustard (*Brassica campestris* L.) when subjected to different levels of 5-Amino- levulinic acid under lead and cadmium stress.

Sources	df	Glycine betaine	Carotenoids	Chl-a	Ch-b	Antho-cyanin	MDA	Carbo-hydrates
Variety	1	0.022278***	0.000042***	0.000000 ns	0.000030***	1.253ns	892.525***	2.37852***
Treatments	9	0.012727***	0.000052***	0.000000***	0.000011***	533.899***	52.910***	7.66198***
Var × Treat	9	0.000347***	0.000003***	0.000000ns	0.000000ns	8.028ns	11.956*	0.25187***
Total	60	0.000023	0.000001	0.000000	0.000389	6.659	4.453	0.00447
Error	79							

Table 3. Mean squares (MS) of ANOVA for the data of proteins, CAT, POD, SOD, electrolyte leakage and proline of two varieties of mustard (*Brassica campestris* L.) when subjected to different levels of 5-Amino-levulinic acid under lead and cadmium stress.

Sources	df	Proteins	CAT	POD	SOD	Electrolyte leakage	Proline
Variety	1	0.022680***	0.001496***	0.011435***	0.012532***	1393.6***	0.000020***
Treatments	9	0.067190***	0.179577***	0.081210***	0.083675***	21582.5***	0.000116***
Var × Treat	9	0.000301***	0.000251***	0.000715***	0.001980ns	50.7***	0.000091***
Total	60	0.000010	0.000029	0.000124	0.001408	18.5	0.000000
Error	79						

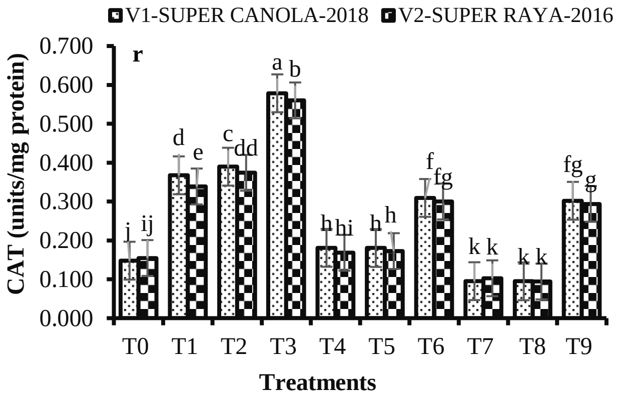
Antioxidant activities: Heavy metal stress had highly significant effect in mustard plants (Table 3). It was noted that HM stress increased the CAT, POD and SOD activities and maximum increase was observed in combined treatment of Pb (100µM) + Cd (100µM). Lowest vales for antioxidant activities were observed under foliar application of 5-ALA in T8 (Cd (100µM) + 100mg/L ALA) of Super Raya-2016 (Fig. 3). A continuous increasing trend T1<T2<T3 of CAT activity with 50mg/L of 5-ALA was observed. Results showed significant ($p<0.05$) changes between the mean values of CAT activity of all treatments in both varieties under stress and non-stressed conditions (Table 3). Heavy metal stress increased the POD activities and maximum increase was observed in T3 (Pb (100µM) + Cd (100µM) that was 65% higher as compared to control. Heavy metal stress increased the SOD activities and maximum (0.461) increase was observed in T3 (Pb (100µM) + Cd (100µM) which was 71% higher as compared to control. It was observed that foliar application of 5–ALA under heavy metal stress decreased the SOD activity in *Brassica* plants (Fig. 3).

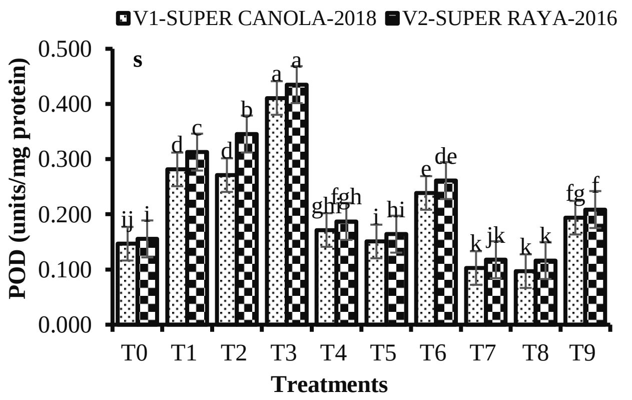
Discussion

In the present study, foliar application of 5-ALA enhanced the morphological and biochemical attributes of mustard under HM stress. These findings are in line with previous studies as Naeem *et al*., (2010) observed foliar application of 5-ALA enhanced growth and physiological parameters in oilseed rape. Similarly, Al-Thabet (2006) observed that foliar spray of 5-aminolevulinic acid showed remarkable results to enhance the growth characteristics of wheat when applied under stress conditions.

Results exhibited that Pb and Cd toxicity significantly reduced the morphological parameters of Brassica plants that probably was due to the imbalance created in the water level and lead to disturbed uptake of the nutrients which in return inhibits the cell division of the root tip cells (Sharma & Dubey, 2005). Reduction in morphological parameters has been found in various crops under Cd stress as in *Juncus effuses* by Rivetta *et al*., (1997) and radish by Najeeb *et al*., (2011). However, when plants were treated with foliar application of 5-Amino-levulinic acid, it expressively improved the morphology of root and shoot of *Brassica* plants under the Pb and Cd stress. Development in morphology of *Brassica* with 5-amino-levulinic acid might be due to the fact that ALA is a key precursor in the biosynthesis of porphyrins as in chlorophyll and activation of antioxidant systems (APX, POD and CAT) to scavenge the reactive oxygen species like H_2O_2 (Ali *et al*., 2013). It is well studied that 5-ALA played vital role to cope the plant under various stresses in different crops (Kosar *et al*., 2015; Ahmad *et al*., 2017; Air *et al*., 2018).

Higher accumulation of Glycine betaine (GB) and proline during this study in mustard plants under HMs stress was due to its significant role in protecting membranes and proteins, along with various important stress resistant enzymes (*Banu et al*., 2010). There are many reports in the literature about the role of 5-ALA as osmoprotectants including proline and glycine contents under the stress conditions. In contrast, Akram *et al*., (2012) noted that there was no significant results of foliar application of 5- ALA on proline and GB concentration in the sunflower under saline environment. It was noted that heavy metal stress decreased the physiological parameters (chl-a, chl-b & carotenoids). The decrease in photosynthetic characteristics might be due to damage of functional units of photosynthesis and lower efficiency of transportation of water from roots to shoots of plants due to heavy metal toxicity that damaged the chloroplast and protein complex and inhibit the electron transport chain (Mohanty *et al*., 1989; Vassilev *et al*., 1995; Shakoor *et al*., 2014). Furthermore, breakdown of chlorophyll might be due to an increase in chlorphyllase activity under HMs stress (Farid *et al*., 2018).





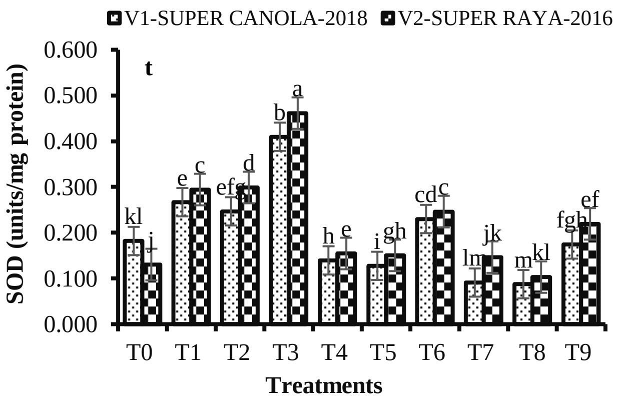


Fig. 3. Effect of different levels of 5-Amino-levulinic acid on the activity of Antioxidant activity of two mustard varieties grown under HM stress conditions.

MDA contents under Cd and Pb stress was higher as compared to non-stressed plants. Higher MDA concentration indicates the level of membrane lipid peroxidation that is an important physiological indicator (Noctor *et al*., 2015). HM stress can cause production of ROS in plants that directly indicate stress levels (Gill & Tuteja, 2010). Plants increase the levels of their endogenous enzymes (SOD, POD and CAT) to deal with the oxidative damage under stress. Treatments with 5-ALA reduced the MDA contents, indicating that 5-ALA might control the reactive oxygen species production that can prevent membrane lipid peroxidation under stressed conditions. Application of 5-ALA with 100 and 400 µM concentrations of Pb decreased reactive oxygen species contents in roots and leaves of *Brassica napus* (Ali *et al*., 2014). It was found that heavy metal stress decreased the carbohydrates contents that might be due to some metabolic changes of carbohydrate synthesis that inhibit hexokinase and phosphofructokinase, because of their high affinity for the free electron pairs in cysteine -SH groups, which are essential in enzyme function (Maria *et al*., 2013). Foliar spray of 5-ALA in response to HM stress increased carbohydrates contents might be by sustaining enzyme functioning (Hotta *et al*., 1998).

High level of Pb and Cd caused severe reduction in soluble protein contents probably was due to manufacturing disorder of protein machinery or might be some enzyme has been stopped working under stress and it can stimulate enzyme activity of plant cells that results in breakdown of the protein (Cobbett & Goldsborough, 2002). It might also be due to decrease in the production of new proteins due to lethal effects on enzymes and organelles associated with protein synthesis (Hall, 2002; John *et al*., 2009). While the plants with foliar spray of 5-ALA, protein concentrations increased as compared to stress plants and due to detoxification mechanism in response to stress destroying. Exogenous 5-ALA applications improved the soluble proteins, perhaps ABA stimulated the expression of genes encoding stress-responsive genes. During the HM stress in plants, the expression of Cd-binding protein can be persuaded consequential to minimize Cd toxicity (Cobbett & Goldsborough, 2002; Hall, 2002).

Conclusion

It was concluded from the above studies that 5-Aminolevulinic Acid can be useful to minimize the effects of heavy metal stress from mustard. Application of that 5-Aminolevulinic Acid helped to enhance the morphological and physiological attributes in mustard under stress conditions.

References

Ahmad, P., M.A. Ahanger, D. Egamberdieva, P. Alam, M.N. Alyemeni and M. Ashraf. 2018. Modification of osmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea seedlings under mercury (Hg) toxicity. *J. Plant Growth Regul.*, 37(1): 309-322.

Ahmad, R., S. Ali, F. Hannan, M. Rizwan, M. Iqbal, Z. Hassa and F. Abbas. 2017. Promotive role of 5-aminolevulinic acid on chromium-induced morphological, photosynthetic, and oxidative changes in cauliflower (*Brassica oleracea* botrytis L.). *Environ. Sci. Pollut. Res.*, 24(9): 8814-8824.

Air, T., N.A. Akram, S. Kausar, N. Farid, M. Ashraf and F.A. AL-Qurainy. 2018. 5-Aminolevulinic acid induces regulation in growth, yield and physio-biochemical characteristics of wheat under water stress. *Sains Malays,* 47: 661-670.

Akram, N.A., M. Ashraf and F. Al-Qurainy. 2012. Aminolevulinic acid-induced regulation in some key physiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus* L.) under saline regimes. *Hort. Sci.,* 142: 143-148.

Akram, N.A. and M. Ashraf. 2013. Regulation in plant stress tolerance by a potential plant growth regulator, 5-aminolevulinic acid. *J. Plant Growth Regul.,* 32(3): 663-679.

Ali, B., C.R. Huang, Z.Y. Qi, S. Ali, M.K. Daud, X.X. Geng and W.J. Zhou. 2013. 5-Aminolevulinic acid ameliorates cadmium-induced morphological, biochemical, and ultrastructural changes in seedlings of oilseed rape. *Environ. Sci. Pollut. Res.,* 20(10): 7256-7267.

Ali, B., X., Xu, R.A. Gill, S. Yang, S. Ali, M. Tahir and W. Zhou. 2014. Promotive role of 5-aminolevulinic acid on mineral nutrients and antioxidative defense system under lead toxicity in *Brassica napus. Ind. Crops Prod.,* 52: 617-626.

Ali, B., R.A. Gill, S. Yang, M.B. Gill, M.A. Farooq, D. Liu and W. Zhou. 2015. Regulation of cadmium-induced proteomic and metabolic changes by 5-aminolevulinic acid in leaves of *Brassica napus* L. *PLoS One,* 10(4): 223-228.

Ali, S., M. Rizwan, A. Zaid, M.S. Arif, T. Yasmeen, A. Hussain and G.H. Abbasi. 2018. 5-Aminolevulinic acid-induced heavy metal stress tolerance and underlying mechanisms in plants. *J. Plant Growth Regul.,* 37(4): 1423-1436.

Al-Khateeb, S.A., R. Okawara, A.A. Al-Khateebi and I.A. Al-Abdoulhady. 2006. Effects of 5-aminolevulinic acid (5-ALA) on fruit yield and quality of date palm CV, Khalas. *Arab Gulf J. Sci. Res.,* 24: 7-11.

Al-Thabet, S.S. 2006. Promotive effect of 5-aminolevulinic acid on growth and yield of wheat grown under dry conditions. *J. Agron.,* 5:45-49.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. *Plant Physiol.,* 24(1): 1-5.

Bali, S., P. Kaur, P.K. Kohli, P. Ohri, A.K. Thukral, R. Bhardwaj, L. Wijaya, M.N. Alyemeni and P. Ahmad. 2018. Jasmonic acid induced changes in physio-biochemical attributes and ascorbate-glutathione pathway in *Lycopersicon esculentum* under lead stress at different growth stages. *Sci. Total Environ.,* 645: 1344-1360.

Banu, M.N.A., M.A. Hoque, M. Watanabe-Sugimoto, M.M.I. Uraji, K. Matsuoka and Y. Murata. 2010. Proline and Glycinebetaine ameliorated NaCl stress via scavenging of hydrogen peroxide and methylglyoxal but not superoxide or nitric oxide in tobacco cultured cells. *Biosci. Biotechnol. Bioch.,* 12170-12175.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil,* 39(1): 205-207.

Cakmak, I. and W.J. Horst. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant,* 83(3): 463-468.

Chance, B. and A.C. Maehly. 1955. Assay of Catalase and Peroxidase. *Methods Enzym.,* 2:764-775.

Cobbett, C. and P. Goldsbrough. 2002. Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.,* 53(1): 159-182.

Dai, J.Y., C.H., E.N. Ling, J.F. Zhao and M.A Na. 2006. Characteristics of sewage sludge and distribution of heavy metal in plants with amendment of sewage sludge. *J. Environ. Sci. Stud.,* 18(6): 1094-1100.

Dustgeer, Z. M.F. Seleiman, I. Khan, M.U. Chattha, E.F. Ali, B.A. Alhammad, R.S. Jalal , Y. Refay and M.U. Hassan. 2021. Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. *Not. Bot. Hort. Cluj-Napoca,* 49(1): 12248.

Farid, M., S. Ali, M., Rizwan, Q. Ali, R. Saeed, T. Nasir and T. Ahmad. 2018. Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. *Ecotoxicol. Environ. Safety,* 151: 255-265.

Giannopolitis, C.N. and S.K. Ries.1977. Superoxide dismutase: I. Occurrence in higher plants. *Plant Physiol.,* 59(2): 309-314.

Gill, S.S. and N. Tuteja. 2011. Cadmium stress tolerance in crop plants: Probing the role of sulfur. *Plant Signal. Beha.,* 6(2): 215-222.

Guzman, I., G.G. Yousef and A.F. Brown. 2012. Simultaneous extraction and quantitation of carotenoids, chlorophylls, and tocopherols in Brassica vegetables. *J. Agric. Food Chem.,* 60(29): 7238-7244.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot*., 53: 1-11.

Handa, N., S.K, Kohli, A, Sharma, A.K, Thukral, R. Bhardwaj, M.N. Alyemeni, L. Wijaya and P. Ahmad. 2018. Selenium ameliorates chromium toxicity through modifications in pigment system, antioxidative capacity, osmotic system, and metal chelators in *Brassica juncea* seedlings. *S. Afr. J. Bot.,* 119: 1-10.

Hegedüs, A., S. Erdei and G. Horváth. 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Sci.,* 160(6):1085-1093.

Hotta, Y., T. Tanaka, B.S. Luo, Y. Takeuchi and M. Konnai. 1998. Improvement of cold resistance in rice seedlings by 5-aminolevulinic acid. *J. Pestic. Sci.,* 23: 29-33.

Hotta, Y., T. Tanaka, H. Takaoka, Y. Takeuchi and M. Konnai. 1997. New physiological effects of 5-aminolevulinic acid in plants: the increase of photosynthesis, chlorophyll content, and plant growth. *Biosci. Biotechnol. Biochem.,* 61: 2025-2028.

Jan, S., M.N. Alyemeni, L. Wijaya, P. Alam, K.H. Siddique and P. Ahmad. 2018. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in *Pisum sativum* L. seedlings. *BMC Plant Biol.,* 18(1): 1-18.

John, R., P. Ahmad, K. Gadgil and S. Sharma. 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *Int. J. Plant Prod.,* 3(3): 65-76.

Khan, S., M.A. Khan and S. Rehman. 2011. Lead and cadmium contamination of different roadside soils and plants in Peshawar City, Pakistan. *Pedosphere,* 21(3): 351-357.

Kosar, F., N.A. Akram and M. Ashraf. 2015. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. *S. Afr. J. Bot.,* 96: 71-77.

Krizek, D.T. and R.M.B. Mirecki. 1998. Inhibitory effects of ambient levels of solar UV-A and UVB radiation on growth of cv. New Red Fire lettuce. *Physiol. Plant,* 103(1): 1-7.

Krystofova, O., O. Zitka, S. Krizkova, D., Hynek, V. Shestivska, V. Adam and P. Babula. 2012. Accumulation of cadmium by transgenic tobacco plants (*Nicotiana tabacum* L.) carrying yeast metallothionein gene revealed by electrochemistry. *Int. J. Electrochem. Sci.,* 7: 886-907.

Kumar, A., M.N.V. Prasad and O. Sytar 2012. Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. *Chemosphere,* 89(9): 1056-1065.

Kurniawan, T.A., G.Y. Chan, W.H. Lo and S. Babel. 2006. Physico–chemical treatment techniques for wastewater laden with heavy metals. *Chem. Eng. J.,* 118(1-2): 83-98.

Maria, S., M. Puschenreiter and A.R. Rivelli. 2013. Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. *Plant Soil Environ.,* 59(6): 254-261.

Mohanty, N., I. Vass and S. Demeter 1989. Impairment of photosystem 2 activity at the level of secondary quinone electron acceptor in chloroplasts treated with cobalt, nickel and zinc ions. *Plant Physiol.,* 76: 386-390.

Moustakas M., T. Lanaras, L. Symeonidis and S. Karataglis. 1994. Growth and some photosynthetic characteristics of field grown *Avena sativa* under copper and Pb stress. *Photosynthetica,* 30: 389-396.

Naeem, M.S., Z.L. Jin, G.L. Wan, D. Liu, H.B. Liu, K. Yoneyama and W.J. Zhou. 2010. 5-Aminolevulinic acid improves photosynthetic gas exchange capacity and ion uptake under salinity stress in oilseed rape (*Brassica napus* L.). *Plant and Soil,* 332: 405-415.

Najeeb, U., G. Jilani, M.S. Naeem and W. Zhou. 2011. Calcium invigorates the cadmium-stressed *Brassica napus* L. plants by strengthening their photosynthetic system. *Environ. Sci. Poll. Res.,* 18(9): 1478-1486.

Nishihara, E., K. Kondo, M.M. Parvez, K. Takahashi, K. Watanabe and K. Tanaka. 2003. Role of 5-aminolevulinic acid (ALA) on active oxygen scavenging system in NaCl-treated spinach (*Spinacia oleracea*). *J. Plant Physio.,* 160: 1085-1091.

Noctor, G., C. Lelarge-Trouverie and A. Mhamdi.2015. The metabolomics of oxidative stress. *Phytochem.,* 112: 33-53.

Piechalak, A., B. Tomaszewska and D. Barałkiewicz. 2003. Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. *Phytochem.,* 64(7): 1239-1251.

Rakow, G. 2004. Species origin and economic importance of *Brassica*. Springer, Berlin, Heidelberg. pp. 3-11.

Rivetta, A., N. Negrini and M. Cocucci. 1997. Involvement of Ca^{2+}-calmodulin in Cd^{2+} toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination. *Plant, Cell and Environ.,* 20(5): 600-608.

Schmidt, R. and I. Bancroft. 2011. Genetics and genomics of the brassicaceae. Springer, New York. pp. 677.

Shahid, M., E. Pinelli, B. Pourrut, J. Silvestre and C. Dumat. 2011. Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. *Ecotoxic. Environ. Safety,* 74: 78-84.

Shakoor, M.B., S., Ali, A. Hameed, M. Farid, S. Hussain, T. Yasmeen, U. Najeeb, S.A. Bharwana and G.H. Abbasi. 2014. Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb induced morphological and biochemical damages. *Ecotoxic. Environ. Safety,* 109: 38-47.

Sharma, P. and R.S. Dubey, 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.,* 17(1): 35-52.

Siedlecka, A. and Z. Krupa, 2002. Functions of enzymes in heavy metal treated plants. In: Prasad, M.N.V., and S. Kazimierz. Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer, Netherlands. pp. 314-317.

Singh, R., R.D. Tripathi, S. Dwivedi, A. Kumar, P.K. Trivedi and D. Chakrabarty. 2010. Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. *Biores. Tech.,* 101: 3025-3032.

Sood, S.K. 2010. Healing herbs: Traditional medications for wounds, sores and bones. Pointer Publishers, Jaipur. pp. 421-423.

Vassilev, A., A. Perez-Sanz, B. Semane, R. Carleer and J. Vangronsveld. 2005. Cadmium accumulation and tolerance of two Salix genotypes hydroponically grown in presence of cadmium. *J. Plant Nutr.,* 28(12): 2159-2177.

Youssef, T. and M.A. Awad. 2008. Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. *J. Plant Grow. Regul.,* 27: 1-9.

Zhang, Z.J., H.Z. Li, W.J. Zhou, Y. Takeuchiand and K. Yoneyama, 2006. Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers *In vitro*. *J. Plant Grow. Regul.,* 49: 27-34.

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