ORIGINAL ARTICLE

Biochemical and physiological responses in *in vitro* germinated seedlings of *Brassica juncea* exposed to metal stress

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Abstract

Background: Effects of cadmium (Cd) metal on growth, photosynthetic pigments, level of osmolytes and elements, and antioxidant potential of *Brassica juncea* seedlings were evaluated. **Materials and Methods:** Seeds were exposed to different concentrations of metal, i.e., 0, 0.2, 0.4, and 0.6 mM Cd. To investigate the metal influence on biochemical and physiological factors, observations were made on growth (fresh, dry weights, and percent germination), photosynthetic pigments (carotenoid and xanthophyll), osmolytes (proline and glycinebetaine), elements (carbon, hydrogen, nitrogen, and sulfur), antioxidative enzymes, antioxidants, radical scavenging activities, and polyphenols in 7-day old seedlings. **Results:** The findings of this study revealed that retardation was observed in fresh and dry weights and percent germination in comparison to control seedlings. A level of osmolytes and antioxidant potential of *B. juncea* seedlings were found to improve with Cd toxicity. Metal stress altered the level of photosynthetic pigments and ultra-performance liquid chromatography studies showed variations in the polyphenol content in comparison to untreated seedlings. **Conclusion:** The current investigation revealed that the treatment of Cd generated various defense responses in the seedlings of *B. juncea* to overcome the adverse effects of metal due to their hyperaccumulating response.

Key words: Antioxidative defence system, *Brassica juncea,* cadmium stress, osmolytes, photosynthetic pigments, polyphenols

INTRODUCTION

Cadmium (Cd) is one of the highly toxic trace pollutants for all living organisms. It occurs naturally in soils in the form of complexes, but the anthropogenic emissions, mainly due to metallurgical industries, mining activities, intensive use of phosphate fertilizers, and burning of fossil fuels are main sources of soil contamination.[1] It does not bind strongly to organic matter and has relative high mobility in soils. Cd stress leads to a battery of stress symptoms in plants which include chlorosis, necrotic lesions, disturbances in mineral nutrition, carbohydrate metabolism, wilting and thus sturdily inhibit the production of biomass.[2] A reduction of photosynthesis is a common response in plants exposed to Cd as the photosynthetic apparatus is on the whole susceptible to Cd. The risk of uptake of Cd by the crops is followed by its transfer in the food chain that is the matter of elevated concern currently.[3]

Brassica juncea belongs to family *Brassicaceae* and is an important oil crop. Mustard oil is one of the chief edible oils in India. It also possesses medicinal importance. Residual part of seeds is used in fertilizer and also as cattle feed. *B. juncea* L. (Indian mustard) is a fast growing plant that produces a high biomass even in heavy metal polluted soils. Thus, this plant might be a probable candidate for phytofiltration and phytostabilization of heavy metal contamination. So far this plant species has been used in studies of the effects of heavy metals stresses like arsenic[4] on plants. The present experiment was undertaken to investigate a change in the growth, photosynthetic pigment content, level of osmolytes, elements and antioxidative defense system in *B. juncea* treated with Cd to contribute to an understanding of *B. juncea* tolerance mechanism to environmental stress especially heavy metal.

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Received: 02-07-2016
Revised: 13-07-2016
Accepted: 20-07-2016

MATERIALS AND METHODS

Certified and disease-free seeds of *B. juncea* L. cv. "RLC-1" used for this study were grown in the autoclaved Petri dishes lined with Whatman No. 1 filter paper after at 25°C with 16 h photoperiod in a seed germinator. Seeds were treated with 0, 0.2, 0.4, and 0.6 mM Cd doses in the form of $CdCl_2$ solution. Seedlings were harvested on the 7th day for the analysis of following parameters.

Measurements of fresh weight, dry weight, and percentage germination of 7-day seedlings of *B. juncea* were taken. Carotenoid content was estimated by the method given by Maclachlan and Zalik,[5] xanthophylls content was measured by following the method of Lawrence,[6] proline content was analyzed by Bates *et al*.[7] method, and glycine betaine content was measured by Grieve and Grattan[8] method. The percentage of carbon, hydrogen, nitrogen, and sulfur (CHNS) in 7-day old plants were determined with the help of CHNS Analyzer (Elementar Vario ELIII). Activities of antioxidative enzymes were determined by the standard methods of Putter[9] for guaiacol peroxidase (POD), Nakano and Asada[10] for ascorbate peroxidase (APOX), Kumar and Khan[11] method for polyphenol oxidase (PPO), Habig *et al*.[12] method for glutathione-S-transferase (GST), and Flohé and Günzler[13] method for glutathione peroxidase (GPOX) activity. Tocopherol and glutathione content were estimated by the method of Martinek[14] and Sedlak and Lindsay,[15] respectively. 2,2-diphenylpicrylhydrazyl (DPPH) assay was analyzed by following the method of Blois,[16] 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay by the method of Re *et al*.,[17] molybdate ion reduction assay in accordance with the method of Prieto *et al*.,[18] and reducing power assay was performed by the method given by Oyaizu.[19] Qualitative estimation of polyphenols was carried out by ultra-performance liquid chromatography.

Statistical Analysis

Each experiment was conducted in three replicates. Data were expressed in mean ± standard error. To check the statistical significant difference between the treatments, one-way ANOVA was carried out using Assistat version 7.7 beta.

RESULTS

With increasing metal concentration, fresh weight was found to reduce and in 0.6 mM Cd-treated seedlings, maximum reduction (0.9 g) was observed. Reduction in dry weight was found from 0.2 to 0.6 mM Cd, i.e., 0.93 to 0.76 g, respectively. Percent germination of 7-day old seedlings was decreased from control seedlings to 0.6 mM Cd-stressed seedlings, i.e., from 95% to 87.33%, respectively [Table 1].

Carotenoid content was found to decrease with the Cd metal treatment with respect to control (7.17 mg/g FW). The results revealed that 1.78-folds decline in carotenoid content (4.03 mg/g FW) was recorded in the seedlings in exposure to 0.6 mM Cd stress. Similarly, at 0.4 mM Cd treatment raised the level of carotenoid from 4.9 (0.2 mM Cd) to 5.8 mg/g FW and further reduced it to 4.03 mg/g FW [Figure 1]. A sharp increase in xanthophylls content was seen in 7-day old seedlings of *B. juncea*. The level of xanthophylls increased 1.85-folds in 0.6 mM Cd-treated seedlings (8.07 mg/g DW) against control (4.35 mg/g DW) [Figure 2].

Table 1: Effect of Cd on fresh weight, dry weight and percentage germination of 7-day old seedlings of *Brassica juncea*

Treatments (mM)	Fresh weight (g)	Dry weight (g)	Percentage germination
0.0	1.35±0.06[a]	1.01±0.01[a]	95±0.58[a]
0.2	1.23±0.03[a]	0.93±0.05[a,b]	91±0.58[b]
0.4	0.98±0.03[b]	0.85±0.03[b,c]	89.67±0.33[b,c]
0.6	0.9±0.04[b]	0.76±0.02[c]	87.33±0.88[c]

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher LSD *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on various growth parameters.
SE: Standard error, LSD: Least significant difference,
Cd: Cadmium

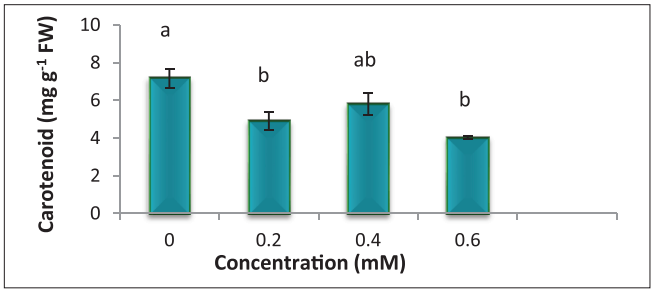

Figure 1: Effect of cadmium on carotenoid content (mg/g FW) in 7-day seedlings of *Brassica juncea.*

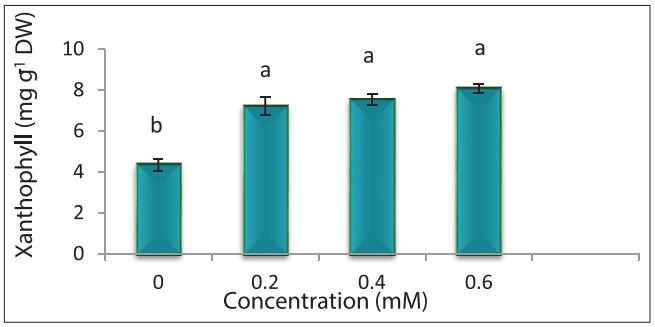

Figure 2: Effect of cadmium on xanthophyll content (mg/g FW) in 7-day seedlings of *Brassica juncea*. Bars presented in mean ± standard error. Different letters (a, b, c and d) within various concentrations of cadmium (Cd) (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher least significant difference *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on pigments

With increasing metal concentration, proline content was noticed to enhance from control to 0.6 mM [Figure 3]. A maximum content was found in the seedlings treated with 0.6 mM Cd (22.61 µmol/g FW), followed by 0.4 mM Cd (22.31 µmol/g FW) and 0.2 mM Cd (18.46 µmol/g FW) in comparison to control seedlings (15.86 µmol/g FW). Similarly, a significant increase in glycine-betaine was noticed in 0.6 mM Cd-stressed seedlings (15.04 µmol/g FW) as compared to control (9.58 µmol/g FW) [Figure 4]. The increase in carbon and hydrogen values was recorded in the seedlings exposed to Cd. The highest values of C and H were 62.68% and 9.98%, respectively [Table 2]. The percentage of S also enhanced in dose-dependent manner. Decrease in N percentage was found with Cd treatment in 7-day old seedlings. The highest value was found in control (4.61%) and lowest value was recorded as 2.13% in 0.6 mM Cd-treated seedlings.

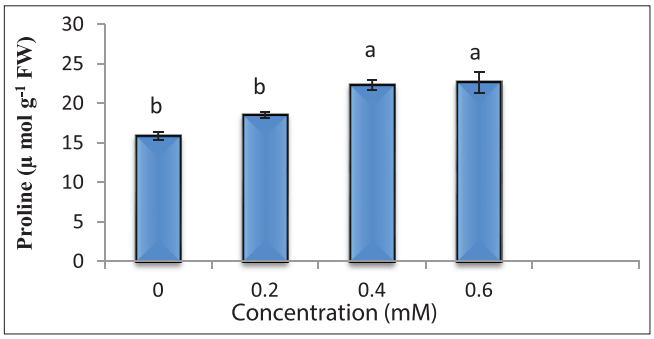

Figure 3: Effect of cadmium on proline content (µmol/g FW) in 7-day seedlings of *Brassica juncea*.

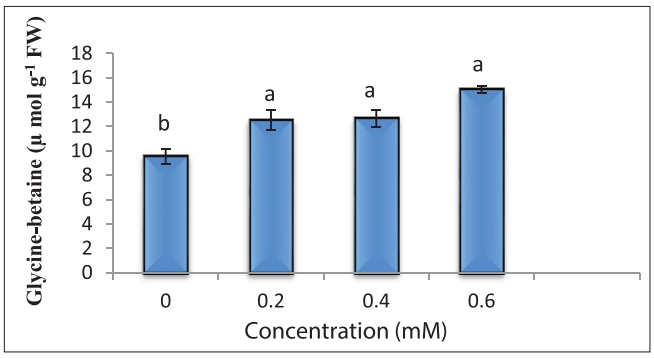

Figure 4: Effect of cadmium (Cd) metal on glycine-betaine content (µmol/g FW) in 7-day seedlings of *Brassica juncea*. Bars presented in mean ± standard error. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher least significant difference *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on osmoprotectants

The activity of POD enzyme was enhanced from control (4.67 UA/mg protein) to 0.6 mM Cd-treated seedlings (7.81 UA/mg protein). Increase in activity was observed from 4.67 to 5.39 and 5.94 UA/mg protein at 0.2 and 0.4 mM Cd, respectively. Similarly, with increasing the doses of Cd, APOX activity was also increased. Maximum activity of enzyme was found in 0.6 mM Cd-treated seedlings (15.86 UA/mg protein). PPO activity enhanced from control to 0.2 mM Cd (from 5.39 to 6.07 UA/mg protein) and 0.4 mM treated seedlings (from 5.39 to 8.13 UA/mg protein) treated seedlings. Cd stress also increased the activity of GST enzyme from control (4.32 UA/mg protein) to 0.6 mM Cd-stressed seedlings (7.28 UA/mg protein) by 1.68-folds increase in enzyme activity. Similarly, highest GPOX activity was noticed at 0.6 mM Cd-treated seedlings, i.e., 5.83 UA/mg protein [Table 3].

Further increase in tocopherol content was found from 7.64 to 8.3 mg/g FW, i.e., from control to 0.4 mM Cd treatment. Maximum tocopherol was possessed by the seedlings treated with 0.6 mM Cd (9.22 mg/g FW). A sharp rise in glutathione level was reported in 7-day old seedlings of *B. juncea* during stress [Table 4].

In 7-day old seedlings, percentage inhibition of DPPH radical was found to increase with increasing Cd doses [Table 5] as compared to control seedlings (70.99%). DPPH scavenging capacity was noticed maximum at 0.4 mM Cd-treated seedlings (86.08%). A continuous increase in reduction of molybdate ion was observed from 46.36 to 61.7% at 0.2 mM Cd and from 46.36 to 76.85% at 0.6 mM Cd treatment. Cd toxicity caused increase in the reduction of ferric ions. At 0.2 mM Cd stress, maximum decrease (82.71%) in FRAP ions was found as compared to 0.4 mM (62.67%) and 0.6 mM Cd-treated seedlings (72.56%). It was observed that increase in scavenging of ABTS radical was found with the Cd stress. Rise in the scavenging capacity was noted from 56.65 to 68.75% at 0.2 mM Cd, from 56.65 to 79.14% at 0.4 mM Cd, and from 56.65 to 74.5% at

Table 2: Effect of Cd on carbon, hydrogen, nitrogen and sulphur content in 7-day old seedlings of *Brassica juncea*

Treatments (mM)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
0.0	32.79±1.7^{a,b}	4.99±0.03^{b}	4.61±0.5^{a}	0.20±0.05^{b}
0.2	29.97±2.3^{a,b}	4.59±0.07^{b}	3.86±0.01^{b}	0.28±0.001^{b}
0.4	37.23±4.1^{b}	9.98±0.5^{a}	3.6±0.1^{a,b}	0.31±0.01^{b}
0.6	62.68±1.5^{a}	5.64±1.0^{b}	2.13±0.1^{c}	0.67±0.02^{a}

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher LSD *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on elemental analysis. SE: Standard error, LSD: Least significant difference, Cd: Cadmium

Table 3: Effect of Cd on specific activities of POD, APOX, PPO, GST and GPOX in 7-day old seedlings of *B. juncea*

Treatments (mM)	POD (UA/mg protein)	APOX (UA/mg protein)	PPO (UA/mg protein)	GST (UA/mg protein)	GPOX (UA/mg protein)
0.0	4.67±0.14^{c}	11.23±0.28^{c}	5.39±0.58^{b}	4.32±0.2^{c}	3.77±0.17^{c}
0.2	5.39±0.09^{b}	12.37±0.42^{b,c}	6.07±0.22^{b}	4.89±0.42^{b,c}	4.55±0.19^{b}
0.4	5.94±0.18^{b}	13.78±0.27^{b}	8.13±0.11^{a}	6.26±0.33^{a,b}	4.65±0.76^{a,b}
0.6	7.81±0.13^{a}	15.86±0.23^{a}	7.11±0.45^{b}	7.28±0.48^{a}	5.83±0.57^{a}

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher LSD post-hoc test, $P \leq 0.05$) and signify the effect of Cd metal on enzyme activities. SE: Standard error, LSD: Least significant difference, Cd: Cadmium, POD: Guaiacol peroxidase, APOX: Ascorbate peroxidase, PPO: Polyphenol oxidase, GST: Glutathione-S-transferase, GPOX: Glutathione peroxidase

Table 4: Effect of Cd on tocopherol and glutathione content in 7-day old seedlings of *Brassica juncea*

Treatments (mM)	Tocopherol (mg/g FW)	Glutathione (mg/g FW)
0.0	7.64±0.35^{a,b}	10.82±0.31^{c}
0.2	7.07±0.44^{b}	14.03±0.37^{b}
0.4	8.3±0.37^{a,b}	15.89±0.65^{a,b}
0.6	9.22±0.33^{a}	17.18±0.47^{a}

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher LSD *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on antioxidants. SE: Standard error, LSD: Least significant difference, Cd: Cadmium

0.6 mM Cd treatment [Table 6]. Polyphenols such as gallic acid, catechin, chlorogenic acid, caffeic acid, coumaric acid, ellagic acid, quercetin, and kaempferol were observed in 7-day old seedlings of *B. juncea* seedlings. At 0.2 mM additional polyphenols such as epicatechin, umbelliferone, and rutin were expressed in comparison to control. With increasing Cd stress (0.4 and 0.6 mM), the activity of epicatechin and rutin, respectively, were noticed against untreated seedlings [Figures 5-8 and Table 6].

DISCUSSION

Uptake of Cd at toxic level causes mineral deficiency, desiccation and cellular metabolic disturbances in plants. Present investigation revealed the decrease in reduction in fresh and dry biomass and percent germination of *B. juncea* seedlings exposed to Cd stress. The increased uptake and accumulation of heavy metal in plants cause osmotic shift, metabolic alterations and also ROS induced damages.[20] Cd-induced restricted uptake of water hampers turgor mediated wall extensibility that reduces cell division.[21] Cd mediated collective influence of these factors caused an inhibition in fresh and dry biomass. These observations are supported by the findings of Kalaikandhan *et al*.,[22] where Cu and Zn toxicity inhibited the growth of *Sesuvium portulacastrum* plants. Accumulation of ROS due to metal stress imbalances the PSII by degrading D1 protein, and therefore, causes decline in carotenoid content.[23] *Triticum aestivum* plants when subjected to Cu and Zn toxicity revealed the decrease in carotenoids level.[24] Whereas, increase in xanthophyll content was reported in *B. juncea* seedlings under Cd stress. This might be due to the fact that xanthophylls act as antioxidant and protect the photosynthetic system of plants against oxidative stress.[25] Accumulation of osmolytes hydrate the enzymes to restore their activity, neutralize reactive oxygen/nitrogen species and help in the protection of membranes and subcellular structures.[26] Besides, in maintaining cellular redox status and stress-induced tolerance, osmolytes, antioxidant enzymes and antioxidants are major players.[20] Although the enhanced activity of antioxidant enzymes and that of osmolytes apparently stabilize the membranes to prevent water loss, protects metabolic machinery and support nutrient uptake to the plants.[27] These compatible solutes act as reservoir for the elements such as carbon and hydrogen as they are the important structural elements of carbohydrates and proteins, which play an important role in stress amelioration.[28] In the present investigation, antioxidant potential of *B. juncea* was found to improve in terms of antioxidative enzyme activities, level of antioxidants and free radicals scavenging capacity. Enhanced activities of antioxidative enzymes cause rise in detoxification efficiency to a greater extent, which provides better resistance to plants against heavy metal induced oxidative stress.[29] Similarly, various antioxidants such as GSH, tocopherol, and polyphenols play a key role in the antioxidant system and trigger the scavenging of free radicals generated during stress.[30] It is also reported by Singh and Malik[31] that phenolic compounds possess strong antioxidant activity as they are oxidized by peroxidase and contribute in scavenging H_2O_2 in plants like *Brassica* treated with heavy metal.

CONCLUSION

From the present investigations, it is concluded that heavy metal stress alters the physiological and biochemical processes resulting in altered metabolism, and therefore, retards growth

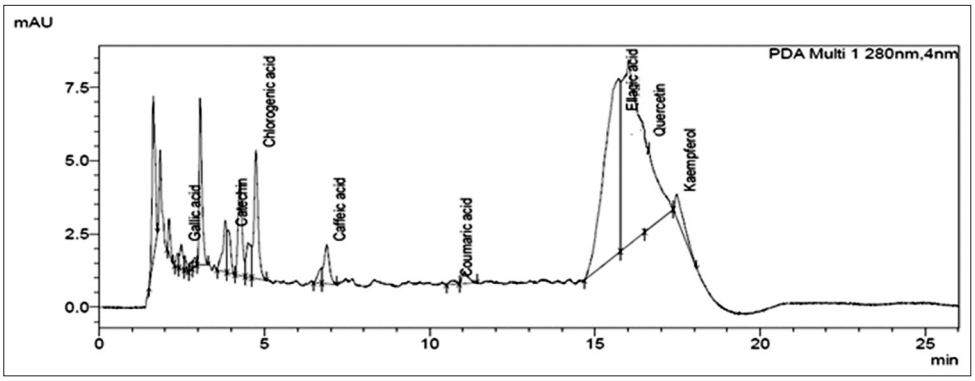

Figure 5: Analysis of polyphenols in 7-day control seedings of *Brassica juncea*

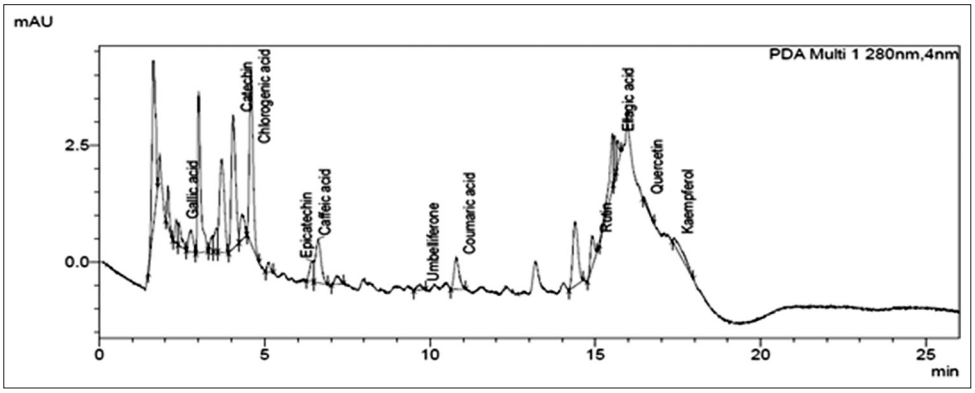

Figure 6: Analysis of polyphenols in 0.2 mM cadmium treated 7-day old seedlings of *Brassica juncea*

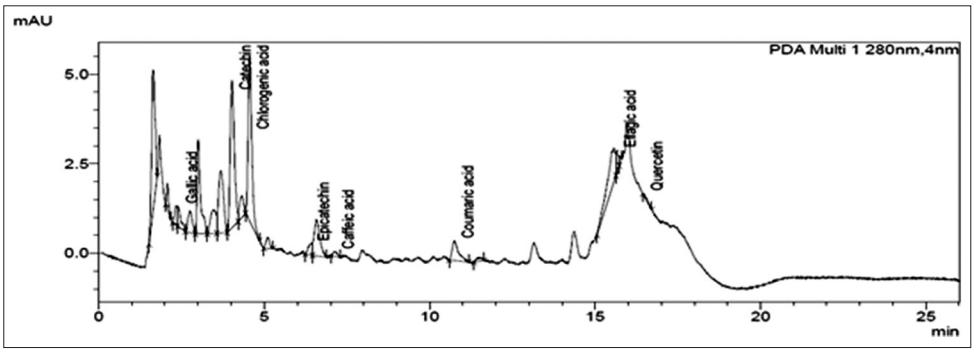

Figure 7: Analysis of polyphenols in 0.4 mM cadmium treated 7-day seedlings of *Brassica juncea*

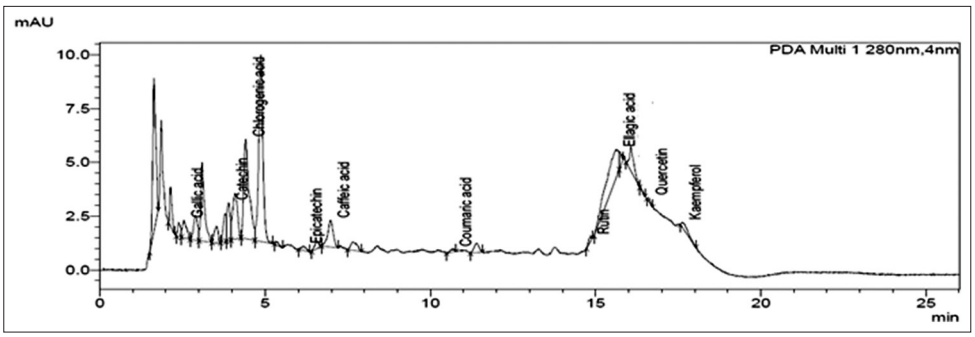

Figure 8: Analysis of polyphenols in 0.6 mM cadmium treated 7-day seedlings of *Brassica juncea*

in *B. juncea*. Plants employ different strategies to cope with stress including excess production and accumulation of organic osmolytes, selective uptake of ions and increased expression of the antioxidative defense system.

Table 5: Effect of Cd on scavenging activities of DPPH, FRAP, molybdate ion and ABTS in 7-day old seedlings of *Brassica juncea*

Treatments (mM)	DPPH (%)	FRAP (%)	Molybdateion (%)	ABTS (%)
0.0	70.99±4.16^{b}	53.7±2.44^{c}	46.36±2.27^{b}	56.65±6.2^{b}
0.2	81.26±1.34^{a,b}	82.71±3.75^{a}	61.7±4.31^{ab}	68.75±3.19^{a,b}
0.4	86.08±2.06^{a}	62.67±4.42^{b,c}	56.28±5.97^{b}	79.14±2.74^{a}
0.6	82.99±1.64^{a}	72.56±1.56^{a,b}	76.85±2.32^{a}	74.5±2.63^{ab}

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mM) are significantly different (Fisher LSD *post-hoc* test, $P \leq 0.05$) and signify the effect of Cd metal on antioxidant assays. SE: Standard error, LSD: Least significant difference, Cd: Cadmium, DPPH: 2,2-diphenylpicrylhydrazyl, FRAP: Ferric reducing ability of plasma, ABTS: 3-ethylbenzothiazoline-6-sulfonic acid

Table 6: Concentrations of phenolic compounds in 7-day old *Brassica juncea* seedlings treated with Cd stress

Polyphenolic compound	Percentage			
	Control	0.2 mM Cd	0.4 mM Cd	0.6 mM Cd
Gallic acid	0.390	0.211	0.240	0.484
Catechin	4.194	10.639	14.989	5.030
Chlorogenic acid	4.950	3.679	4.638	6.772
Caffeic acid	1.075	0.774	0.088	1.017
Coumaric acid	0.058	0.243	0.244	0.041
Ellagic acid	43.462	0.964	0.588	8.759
Quercetin	9.807	0.088	0.082	0.123
Kaempferol	6.601	1.958	-	1.770
Epicatechin	-	0.951	0.703	0.539
Umbelliferone	-	0.260	-	-
Rutin	-	1.319	-	0.376

Cd: Cadmium

ACKNOWLEDGMENT

Authors are thankful to Department of Botanical and Environmental Sciences, Guru Nanak Dev University - Amritsar (India) for providing laboratory facilities for this work.

REFERENCES

1. Tran TA, Popova LP. Functions and toxicity of cadmium in plants: Recent advances and future prospects. Turk J Bot 2013;37:1-13.
2. Villiers F, Ducruix C, Hugouvieux V, Jarno N, Ezan E, Garin J, *et al*. Investigating the plant response to cadmium exposure by proteomic and metabolomic approaches. Proteomics 2011;11:1650-63.
3. Dias MC, Pinto G, Correia C, Moutinho-Pereira J, Silva S, Santos C. Photosynthetic parameters of *Ulmus minor* plantlets affected by irradiance during acclimatization. Biol Plant 2012. DOI: 10.1007/s10535-012-0234-8.
4. Abate E, Hussien S, Laing M, Mengistu F. Aluminium toxicity tolerance in cereals: Mechanisms, genetic control and breeding methods. Afr J Agric Res 2013;8:711-22.
5. MacLachlan S, Zalik S. Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. Can J Bot 1963;41:1053-60.
6. Lawrence JF. Determination of total xanthophyll and marigold oleoresin. J Assoc Off Anal Chem 1990;2:970-5.
7. Bates LS, Waldren RP, Tear ID. Rapid determination of free proline for water stress studies. Plant Soil 1973;39:205-7.
8. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 1983;70:303-7.
9. Putter J. Peroxidase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Vol. 2. New York: Verlag Chemie, Weinhan; 1974. p. 685-90.
10. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific-peroxidase in spinach chloroplasts. Plant Cell Physiol 1981;22:867-80.
11. Kumar KB, Khan PA. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Indian J Exp Bot 1982;20:412-6.
12. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic

acid formation. J Biol Chem 1974;249:7130-9.
13. Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114-21.
14. Martinek RG. Method for the determination of Vitamin E (total tocopherols) in serum. Clin Chem 1964;10:1078-86.
15. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26(9-10):1231-7.
18. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E. Anal Biochem 1999;269:337-41.
19. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 1986;44:307-15.
20. Giri J. Glycinebetaine and abiotic stress tolerance in plants. Plant Signal Behav 2011;6:1746-51.
21. Marshner P. Marschner's Mineral Nutrition of Miglier Plants. 3rd ed. London: Academic Press UK; 2012.
22. Kalaikandhan R, Vijayarengan P, Sivasankar R, Mathivanan S. The effect of copper and zinc on the morphological parameters of *Sesuvium portulacastrum* L. Int J Curr Res Acad Rev 2014;2:105-20.
23. Trebst A, Depka B, Holländer-Czytko H. A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. FEBS Lett 2002;516:156-60.
24. Kumar A, Prasad MN, Sytar O. Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. Chemosphere 2012;89:1056-65.
25. Milborrow BV. The pathway of biosynthesis of abscisic acid in vascular plants: A review of the present state of knowledge of ABA biosynthesis. J Exp Bot 2001;52:1145-64.
26. Kavi Kishor PB, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KR, *et al.* Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr Sci 2005;88:424-38.
27. Irfan M, Ahmad A, Hayat S. Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. Saudi J Biol Sci 2014;21:125-31.
28. Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K, *et al*. Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*. Proc Natl Acad Sci U S A 2009;106:17588-93.
29. Mohamed AA, Castagna A, Ranieri A, Sanità di Toppi L. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiol Biochem 2012;57:15-22.
30. Andre CM, Yvan L, Daniele E. Dietary antioxidants and oxidative stress from a human and plant perspective: A review. Curr Nutr Food Sci 2010;6:2-12.
31. Singh Y, Malik CP. Phenols and their antioxidant activity in *Brassica juncea* seedlings growing under $HgCl_2$ stress. J Microbiol Biotechnol Res 2011;1:124-30.

Source of Support: Nil. **Conflict of Interest:** None declared.