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ORIGINAL ARTICLE

24-Epibrassinolide Mediated Changes in Photosynthetic pigments and Antioxidative Defence System of Radish Seedlings under Cadmium and Mercury Stress

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The present work was conducted to study the effects of 24-EBL on photosynthetic pigments (total chlorophyll, chl a, chl b, carotenoid, anthocyanin and flavonoid content) and activities of antioxidative enzymes (guaiacol peroxidase, catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, mono-dehydroascorbate reductase, polyphenol oxidase, glutathione peroxidase and glutathione-S- transferase and protein content) in 7-day old seedlings of *Raphanus sativus* exposed to cadmium and mercury toxicity. Findings of present study were revealed that brassinolide was proved beneficial for amelioration of Cd and Hg stress by altering various metabolic processes of plant.

Key words: 24-EBL, antioxidative enzymes, heavy metal, photosynthetic pigments

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Key words: 24-EBL, antioxidative enzymes, heavy metal, photosynthetic pigments

Abbreviations: EBL- Epibrassinolide, POD- Guaiacol peroxidase, CAT- Catalase, SOD- Superoxide dismutase, APOX- Ascorbate peroxidase, GR- Glutathione reductase, DHAR- Dehydroascorbate reductase, MDHAR- Monodehydroascorbate reductase, PPO- Polyphenol oxidase, GST- Glutathione-S-transferase, GPOX- Glutathione peroxidase, Chl- Chlorophyll, h-hours, OD-Optical density.

Industrial revolution and anthropogenic activities have exasperated the metal pollution of environment and it has posed a major threat to mankind by its integration to the food chain which results in the degradation of ecosystem (Chary *et al.*, 2008). Some heavy metals are essential, which are basic for the cellular functions. Though, their concentrations beyond tolerable limits trigger the generation of

reactive oxygen species (ROS). Among heavy metals, Cd and Hg are considered as very toxic to plants and animals. In the soil solution, Cd is present as free hydrated ions or may be complexed by organic or inorganic ligands. Retarded growth and leaf chlorosis are symptoms of high Cd doses in plants. Cd toxicity disturbs the water balance, photosynthetic apparatus and also inhibits the stomatal opening. Activities of various metabolic enzymes are also altered by this metal (Kovalchuk *et al*., 2001).

Mercury is toxic at low concentrations to plants (Chen and Yang, 2012). It damages the photosynthetic membrane, which leads to blockage in oxygen evolution from PSII. Hg affects the cell growth, cell division and gives rise to the production of ROS in the cells. Generally it disturbs the reactivity with sulphydryl group of proteins, cell permeability and competence of ATP binding. It alters the glutathione metabolism, which plays major role in metal homeostasis (Seth *et al*., 2012).

In response to these abiotic stresses, exogenous application of plant hormones combats their deleterious effects. Among various hormones, brassinosteroids (BRs) are natural polyhydroxy steroids, which are ubiquitously occurring phytohormones (Kandelinskaya *et al*., 2007). They have their significant effects on vegetative and reproductive development, seed germination and stress tolerance (Ryu *et al*., 2010). At cellular level, BRs promote photosynthetic capacity, cell elongation and fission (Khripach *et al*., 2000; Yu *et al*., 2004). BRs are reported to play key role as a stress protectant hormone (Kartal *et al*., 2009).

Raphanus sativus is considered to be a hyperaccumulator of heavy metals. This plant is having economic as well as medicinal values (Benzarti *et al*., 2008). Anthropogenic pollution caused due to heavy metals lead to entry of these metals into food chain through plants grown in the polluted environment.

The objective of present study was to examine the stress protective role of 24-epibrassinolide on photosynthetic pigments and antioxidative defence system of *Raphanus sativus* L. exposed to cadmium and mercury.

MATERIALS AND METHODS

Disease free seeds of *Raphanus sativus* L. var. Pusa chetaki were procured from Punjab Agricultural University, Ludhiana, Punjab. Before soaking in EBL solutions, they were surface sterilized with 0.01% mercuric chloride solution, followed by the repeated washing of sterile double distilled water (DDW). Then these surface sterilized seeds were given treatment of 0, 10^{-11}, 10^{-9}, 10^{-7} M concentrations of 24-EBL for 8 hours, which was purchased from Sigma Aldrich, Ltd., New Delhi. Then they were germinated in *Whatman No.1* filter paper lined glass petriplates containing the 0.25 mM Cd + 0.25 mM Hg metal solution. Cd and Hg were given in the form of cadmium chloride ($CdCl_2$) mercury acetate ($C_4H_6HgO_4$) respectively. Each petriplate was supplied with 3ml of test solution on first day and 2 ml of test solution on alternate days, up to 7 days. Control seedlings were supplied with distilled water only. Each treatment was replicated 3 times. The experiment was conducted under controlled conditions (25°C ± 0.5°C, 16 h

photoperiod). These seedlings were harvested on 7th day to study the following parameters:

Photosynthetic Pigments

Photosynthetic pigments like Chlorophyll content (chl a, chl b and total chl), carotenoids and anthocyanins were determined by Arnon (1949), Maclachlan and Zalik (1963) and Mancinelli (1984) methods respectively.

Chlorophyll content: 1g of fresh plant tissue was homogenized in chilled pestle and mortar using 4ml of 80 % acetone. The crushed material was then subjected to centrifugation using cooling centrifuge (Eltek MP 400 R) for 20 minutes at 13000 rpm at a temperature of 4°C. The supernatant from seedling extract was collected for the analysis of chlorophyll (chl a, chl b and total chl) content. The absorbance of supernatant was taken at 645 and 663 nm by using UV-Visible PC Based Double Beam Spectrophotometer (Systronics 2202). The chlorophylls and carotenoid content were measured by putting the values in following formula:

Total Chlorophyll Content (µg/ml)

= (Absorbance $_{645}$ × 20.2) + (Absorbance $_{663}$ × 8.3) × (V/1000 × W)

Chlorophyll a (µg/ml)

= Absorbance $_{663}$ × (0.058) - (Absorbance $_{645}$) × 0.032

Chlorophyll b (µg/ml)

= Absorbance $_{645}$ × (0.096) - (Absorbance $_{663}$) × 0.01872

Carotenoid content: Supernatant was taken by following the same procedure as used in estimation of chlorophyll. Absorbance was taken at 480 and 510 nm.

= [0.304 (OD 480) - 0.0596 (OD 510)]

Anthocyanin content: 1g of fresh plant tissue was homogenized in 3 ml of acidified methanol (1% HCl). The crushed material was then centrifuged for 20 minutes at 13000 rpm at 4°C. Absorbance of supernatant was taken at 530 nm and 657 nm.

Anthocyanin content = Abs 530 _ 0.25 Abs 657

Total Flavonoid Content: Total Flavonoid content was estimated by the method given by Kim *et al.* (1999). 1 ml of plant extract was added in 4 ml of double-distilled water. 0.3 ml of sodium nitrite ($NaNO_2$) and 0.3 ml of aluminium chloride ($AlCl_3$) were added in it. After 5 minutes of incubation 2 ml of sodium hydroxide (NaOH) was added to it and pink colour appeared. 2.4 ml of distilled water was added in it and absorbance was taken at 510 nm. 1mg/1ml Rutin was used as standard for flavonoid content determination.

Antioxidative Enzymes:

Extraction and assays of antioxidative enzymes

Seedings (1 g) were homogenized in 3 mL of 100 mM potassium phosphate buffer at pH 7.0 using an ice-chilled pestle and mortar. The homogenates were centrifuged at 13,000 rpm for 20 min at 4°C and supernatant used to determine the protein content by Lowry *et al.* (1951) method and activity of antioxidative enzymes [guaiacol peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APOX), polyphenol oxidase (PPO), glutathione peroxidase (GPOX), glutathione-s-transferase (GST), glutathione reductase (GR), dehydroascorbate reductase (DHAR), mono-

dehydroascorbate reductase (MDHAR)]. Activities of these enzymes were determined in different homogenates prepared from treatment of Hg metal, 24- EBL alone and heavy metal supplemented with 24-EBL. Three replicates of each treatment were taken. Calculations were done by following formulae:

Guaiacol peroxidase (POD) was estimated according to Putter (1974). Activity was determined by using the guaiacol as substrate. The reaction mixture consisting of 50 mM phosphate buffer, 20 mM guaiacol solution, enzyme sample and 12.3 mM H_2O_2 solution. The rate of formation of GDHP was followed spectrophotometrically at 436 nm. Activity of enzyme was calculated by using the extinction coefficient of 25 mM^{-1} cm^{-1}. The POD activity was expressed as the amount of enzyme catalyzing the formation of 1 µmol guaiacol dehydrogenation products (GDHP) min^{-1} g^{-1} tissue.

Catalase (CAT) activity was determined according to the method of Aebi (1983). The rate of decomposition of H_2O_2 was followed by decrease in absorbance at 240 nm in a reaction mixture containing K-phosphate buffer (100 mM, pH 7.0), H_2O_2 (150 mM) and enzyme extract. Enzyme activity was determined using the extinction coefficient of 6.93 x 10^{-3} mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was estimated according to Kono (1978). Inhibition of photochemical reduction of nitroblue tetrazolium (NBT) dye due to superoxide radicals, produced by the autooxidation of hydroxylamine hydrochloride. The reaction mixture containing 50 mM sodium carbonate buffer, 24 µM NBT and 0.03% Triton X-100 was taken in the test cuvettes. The reaction was initiated by the addition of 1mM hydroxylamine hydrochloride. After 2 minutes, enzyme extract was added. The percent inhibition at the rate of NBT reduction was recorded as increase in absorbance at 540 nm.

Ascorbate peroxidase (APOX) activity was estimated according to the method of Nakano and Asada (1981) which follow the reduction in absorbance at 290 nm. Reaction mixture containing K-phosphate buffer (100 mM, pH 7.0), ascorbate (5 mM), H_2O_2 (0.5 mM) and enzyme extract. Enzyme activity was determined using the extinction coefficient of 2.8 mM^{-1} cm^{-1}, and was calculated as the amount of enzyme required to oxidize 1 µmol of ascorbate min^{-1} g^{-1} tissue.

Glutathione reductase (GR) activity was measured using the method of Carlberg and Mannervik (1975) followed by the oxidation of NADPH at 340 nm. Reaction mixture containing 1.8 mL K-phosphate buffer (50 mM, pH 7.6), 300 µL EDTA (3 mM), NADPH (0.1 mM), oxidized glutathione (GSSG) (1 mM) and enzyme extract. Enzyme activity was determined using the extinction coefficient of 6.22 mM^{-1} cm^{-1} and was calculated as the amount of enzyme required to oxidize 1 µmol of NADPH min^{-1} g^{-1} tissue.

Dehydroascorbate reductase (DHAR) activity was measured by the method of Dalton *et al.* (1986). The assay mixture consists of 50 mM phosphate buffer, 1.5 mM glutathione reduced, 0.2 mM dehydroascorbate and enzyme extract. Increase in absorbance was recorded at 265 nm and enzyme activity was determined by extinction coefficient 14

mM^{-1} cm^{-1}.

Mono-dehydroascorbate reductase (MDHAR) activity was estimated by Hossain *et al.* (1984) method. Monodehydroascorbate reductase catalyzes the reduction of monodehydroascorbate, which involves the oxidation of NADPH to form ascorbate. Reaction mixture was followed by 50 mM of phosphate buffer, 0.1 mM EDTA, 0.3 mM NADH, 0.25 units ascorbate oxidase and enzymes extract. Enzyme activity was determined by extinction coefficient 6.22 mM^{-1} cm^{-1}. Decrease in absorbance was measured at 340 nm.

2.6.2.8 Polyphenol oxidase (PPO) activity was estimated according to the method given by Kumar and Khan (1982). Reaction mixture contained 0.1M PPB, 0.1M catechol, 0.25 ml of enzyme sample and then the reaction was held for 2 minutes at 25°C. Reaction was accomplished by adding 2.5N H_2SO_4. The absorbance was taken at 495 nm. Enzyme activity was estimated by extinction coefficient 2.9 mM^{-1} cm^{-1}.

Glutathione-s-transferase activity (GST) was measured according to the method described by Habig *et al.* (1974). The reaction was carried out in a total reaction mixture of PPB (0.2 M), GSH (20 mM), CDNB (20 mM) and enzyme sample. Activity of enzyme was determined by extinction coefficient 9.6 mM^{-1} cm^{-1}.The change in absorbance at 340 nm was recorded.

Glutathione peroxidase (GPOX) activity was analyzed according to the method of Flohe and Gunzlar (1984). In 1 ml of reaction mixture 50 mM PPB, 0.5 mM EDTA, 0.15 mM NADPH and 0.15 mM H_2O_2 was added. Then enzyme extract was added in it. Decrease in absorbance due to oxidation of NADPH was measured after 1 minute at 340 nm. Enzyme activity was calculated by extinction coefficient 6.22 mM^{-1} cm^{-1}.

RESULTS

In the present study, levels of photosynthetic pigments were found to inhibit in the seedlings exposed to metal stress as copared to control (Table 1). Further EBL enhanced the total chl, chl a, carotenoid and anthocyanin content. But no particular trend was observed in chl b and flavonoid content with EBL supplementation. 10^{-7} M enhanced the total chl (mg/ml) and anthocyanin content (mg/g) maximum (39.86 ± 5.6 and 1.612 ± 0.003). Chl a content was found maximum with 10^{-11} M along with metal treatment (18.033 ± 0.6) and for carotenoid content 10^{-9} M EBL was proved most effective (11.36 ± 0.24).

An increase in protein content, specific activities of POD, SOD and APOX was observed in metal treated seedlings (Table 2). Specific activities of these enzymes (µmole UA mg $protein^{-1}$) further enhanced with EBL. 10^{-7} M EBL was proved as most effective in increasing protein content (mg/g FW) and activity of POD (9.812 **±** 0.30 and 4.497 **±** 0.04 respectively). Whereas 10^{-11} M EBL stimulated the activities of SOD and APOX to the maximum (4.629 ± 0.8 and 26.25 ± 0.65). Activity of CAT was inhibited by metal stress and then increased with EBL supplementation. 10^{-11} M EBL maximum enhance CAT activity (11.19 ± 0.79).

Activities of GR, MDHAR, PPO and GPOX were found to enhance with metal as well as EBL treatment (Table 3). Specific activity of DHAR was decreased

with metal stress, however further increase was reported with EBL. A significant increase in activities was observed in GR and DHAR with the treatment of hormone. 10^{-9} M maximum enhanced the GR and MDHAR activities (15.65 ± 1.35 and 7.78 ± 1.50 respectively), whereas 10^{-7} M proved as most effective for increasing DHAR (37.72 ± 3.95) and GPOX activities (0.793 ± 0.01). 10^{-11} M EBL stimulated the specific activity of PPO to the maximum (33.6 ± 2.9).

Table 1. Effect of different concentrations of 24-EBL on Photosynthetic Pigments of 7 days old seedlings of *Raphanus sativus* L. under Hg stress.

S. No.	Treatments	Total Chl Content (mg/ml)	Chl a Content (mg/ml)	Chl b Content (mg/ml)	Carotenoid Content (mg/g)	Anthocyanin Content (mg/g)	Flavonoid Content (µg/ml)
1.	0 (Control)	36.3 ± 1.4	23.9 ± 1.19	14.97 ± 1.0	5.4 ± 0.23	0.72 ± 0.01	176.1 ± 9.9
2.	10^{-11} M EBL	17.31 ± 3.3	4.93 ± 0.2	9.13 ± 0.68	4.19 ± 0.05	0.83 ± 0.006	134 ± 5.8
3.	10^{-9} M EBL	13.75 ± 1.06	4.9 ± 0.8	6.37 ± 0.3	5.99 ± 1.2	0.94 ± 0.06	131.2 ± 3.5
4.	10^{-7} M EBL	27.47 ± 4.8	8.73 ± 0.4	13.1 ± 2.8	4.64 ± 1.11	0.89 ± 0.01	141.4 ± 2.7
		F-Ratio $_{(df\ 3,8)}$ = 22.14*, HSD = 14.05	**F-Ratio $_{(df\ 3,8)}$ = 137.88*, HSD = 3.49**	**F-Ratio $_{(df\ 3,8)}$ = 3.98, HSD = 8.9**	**F-Ratio $_{(df\ 3,8)}$ = 33.39*, HSD =0.48**	**F-Ratio $_{(df\ 3,8)}$ = 3.69, HSD = 0.15**	**F-Ratio $_{(df\ 3,8)}$ = 11.24*, HSD = 27.93**
5.	0.25 mM Cd + 0.25 mM Hg	23.2 ± 4.1	14.13 ± 0.78	18.87 ± 1.0	4.72 ± 1.03	0.58 ± 0.01	134.3 ± 11.57
6.	10^{-11} M EBL + 0.25 mM Cd + 0.25 mM Hg	23.13 ± 1.6	18.033 ± 0.6	10.87 ± 0.7	10.9 ± 1.51	0.97 ± 0.02	101.9 ± 1.973
7.	10^{-9} M EBL + 0.25 mM Cd + 0.25 mM Hg	33.9 ± 3.7	13.37 ± 1.7	12.3 ± 0.6	11.36 ± 0.24	1.07 ± 0.03	112.8 ± 2.56
8.	10^{-7} M EBL + 0.25 mM Cd + 0.25 mM Hg	39.86 ± 5.6	15.03 ± 1.6	17.7 ± 0.4	9.41 ± 0.14	1.612 ± 0.0003	118.1 ± 11.71
		F-Ratio $_{(df\ 3,8)}$ = 4.243*, HSD = 19.36	**F-Ratio $_{(df\ 3,8)}$ = 2.159, HSD = 9.7**	**F-Ratio $_{(df\ 3,8)}$ = 16.38*, HSD = 5.1**	**F-Ratio $_{(df\ 3,8)}$ = 0.61, HSD = 3.68**	**F-Ratio $_{(df\ 3,8)}$ = 106.47*, HSD = 0.09**	**F-Ratio $_{(df\ 3,8)}$ = 2.594, HSD = 37.99**

Table 2. Effect of different concentrations of 24-EBL on Protein Content and Antioxidative Enzyme activities of 7 days old seedlings of Raphanus sativus L. under Hg stress.

S. No.	Treatments	Protein Content (mg/g FW)	POD (µmole UA mg protein^{-1})	CAT (µmole UA mg protein^{-1})	SOD (µmole UA mg protein^{-1})	APOX (µmole UA mg protein^{-1})
1.	0 (Control)	7.97 ± 0.48	1.09 ± 0.06	6.70 ± 0.8	2.64 ± 0.3	14.95 ± 0.25
2.	10^{-11} M EBL	7.43 ± 0.46	0.16 ± 0.03	9.23 ± 0.41	3.23 ± 0.2	17.75 ± 0.05
3.	10^{-9} M EBL	9.06 ± 0.28	0.2 ± 0.009	6.82 ± 0.6	2.13 ± 0.13	11.13 ± 0.3
4.	10^{-7} M EBL	8.22 ± 0.33	0.38 ± 0.002	4.95 ± 0.3	3.15 ± 0.04	13.7 ± 0.8
		F-Ratio $_{(df\ 3,8)}$ = 2.92, HSD = 1.80	**F-Ratio $_{(df\ 3,8)}$ = 88.07*, HSD = 0.21**	**F-Ratio $_{(df\ 3,8)}$ = 15.14*, HSD = 2.05**	**F-Ratio $_{(df\ 3,8)}$ = 5.117*, HSD = 1.02**	**F-Ratio $_{(df\ 3,8)}$ = 37.53*, HSD = 2.03**
5.	0.25 mM Hg + 0.25 mM Cd	8.947 ± 0.37	2.58 ± 0.04	4.56 ± 0.31	3.495 ± 0.2	23.1 ± 1.8
6.	10^{-11} M EBL + 0.25 mM Cd + 0.25 mM Hg	9.596 ± 0.72	2.50 ± 0.01	11.19 ± 0.79	4.629 ± 0.8	26.25 ± 0.65
7.	10^{-9} M EBL + 0.25 mM Cd +0.25 mM Hg	9.763 ± 0.37	3.30 ± 0.08	6.42 ± 0.21	3.103 ± 0.04	25.23 ± 0.73
8.	10^{-7} M EBL + 0.25 mM Cd +0.25 mM Hg	9.812 ± 0.30	4.497 ± 0.04	5.74 ± 0.4	3.539 ± 0.4	22.35 ± 0.25
		F-Ratio $_{(df\ 3,8)}$ = 3.590, HSD = 2.136	**F-Ratio $_{(df\ 3,8)}$ = 8.28*, HSD = 0.186**	**F-Ratio $_{(df\ 3,8)}$ = 23.756*, HSD = 2.70**	**F-Ratio $_{(df\ 3,8)}$ = 1.415, HSD = 2.49**	**F-Ratio $_{(df\ 3,8)}$ = 45.129*, HSD = 3.025**

Table 3. Effect of different concentrations of 24-EBL on Antioxidative Enzyme activities of 7 days old seedlings of *Raphanus sativus* L. under Hg stress.

S. No.	Treatments	GR (µmole UA mg protein^{-1})	DHAR (µmole UA mg protein^{-1})	MDHAR (µmole UA mg protein^{-1})	PPO (µmole UA mg protein^{-1})	GST (µmole UA mg protein^{-1})	GPOX (µmole UA mg protein^{-1})
1.	0 (Control)	2.75 ± 0.56	23.02 ± 4.09	4.83 ± 0.6	20.1 ± 1.3	1.43 ± 0.05	0.53 ± 0.05
2.	10^{-11} M EBL	10.51 ± 1.58	20.95 ± 3.38	7.54 ± 0.72	38.1 ± 4	0.74 ± 0.09	0.64 ± 0.01
3.	10^{-9} M EBL	5.72 ± 0.81	17.97 ± 1.06	9.76 ± 0.88	23.8 ± 0.2	0.33 ± 0.03	0.56 ± 0.05
4.	10^{-7} M EBL	10.09 ± 1.31	23.15 ± 1.35	6.85 ± 1.02	34.7 ± 2.3	0.85 ± 0.08	0.79 ± 0.12
		F-Ratio $_{(df\ 3,8)}$ = 10.57*, HSD = 5.45	**F-Ratio $_{(df\ 3,8)}$ = 1.63, HSD = 13.33**	**F-Ratio $_{(df\ 3,8)}$ = 3.75, HSD = 6.39**	**F-Ratio $_{(df\ 3,8)}$ = 26.07*, HSD = 7.62**	**F-Ratio $_{(df\ 3,8)}$ = 12.70*, HSD = 0.59**	**F-Ratio $_{(df\ 3,8)}$ = 2.66, HSD = 0.34**
5.	0.25 mM Cd + 0.25 mM Hg	5.38 ± 1.62	22.76 ± 3.70	5.95 ± 0.34	29.05 ± 3.7	1.377 ± 0.07	0.59 ± 0.06
6.	10^{-11} M EBL + 0.25 mM Cd + 0.25 mM Hg	13.37 ± 2.3	34.81 ± 1.89	6.46 ± 0.86	33.6 ± 2.9	0.673 ± 0.011	0.637 ± 0.01
7.	10^{-9} M EBL + 0.25 mM Cd + 0.25 mM Hg	15.65 ± 1.35	29.93 ± 2.56	7.78 ± 1.50	31.4 ± 2.8	1.24 ± 0.1	0.563 ± 0.05
8.	10^{-7} M EBL + 0.25 mM Cd + 0.25 mM Hg	13.77 ± 2.44	37.72 ± 3.95	7.39 ± 1.77	31.05 ± 1.05	0.83 ± 0.02	0.793 ± 0.01
		F-Ratio $_{(df\ 3,8)}$ = 6.85*, HSD = 7.07	**F-Ratio $_{(df\ 3,8)}$ = 5.36*, HSD = 11.43**	**F-Ratio $_{(df\ 3,8)}$ = 11.77*, HSD =2.55**	**F-Ratio $_{(df\ 3,8)}$ = 4.048, HSD = 0.774**	**F-Ratio $_{(df\ 3,8)}$ = 10.163*, HSD = 0.472**	**F-Ratio $_{(df\ 3,8)}$ = 1.905, HSD = 0.34**

Data shown are Mean ± SE. Each treatment consisted of three replicates.

* Statistically significant differences from control at P ≤0.05.

DISCUSSION

Heavy metal toxicity has become a major focus due to the increased environmental pollution. These lead to detrimental biological effects due to their non-biodegradable nature (Jaleel *et al*., 2009). After accumulating in the soil, they decrease soil fertility, induce toxicity in plants and infect the food chains (Kleizaith *et al*., 2004).

In the present study, level of photosynthetic pigments was decreased during metal stress and further EBL supplementation enhanced their level. It was reported by Rady (2011) that EBL stimulates the antioxidant system and which further protect the photosynthetic machinery. Reduction of chlorophyll content in stress conditions might be because heavy metals block the supply of Fe and Mg, which is required for the synthesis of chlorophyll.

Plant growth depends upon the synthesis of proteins and nucleic acids. BRs take part in the processes of transcription and translation, thus stimulate growth of plant tissue. It is reported by Bajguz (2000) that the improvement in growth of plant and high activities of RNA and DNA polymerase is manifested by increase in protein, DNA and RNA contents. High activities of these enzymes may be due to the regulation of gene expression by BRs and thus growth of the plants may also be induced by this hormone. These studies also revealed the stress-protective roles of BRs. 24-EBL and 28-HBL were found to enhance the growth of mung bean seedlings

by escalating the photosynthesis rate and activity of carbonic anhydrase under Al stress (Ali *et al*., 2007). BRs regulate cell elongation and cell division by stimulating the activities of the cell wall loosening enzymes, which further induce the synthesis of cell wall and membrane materials (Khripach *et al*., 2000).

In the present study, activities of enzymes were altered by BRs under metal stress condition. These reports are in coherence with the findings of Pietrini *et al*., (2003), in *Phragmites australis* under Cd metal stress. Previous reports also showed that BRs treatment modified antioxidant enzyme activity in stressed radish, maize, mustard and wheat plants (Sharma *et al*., 2007, 2010, 2011). BRs can alter the proteins and activity of other enzymes within the membrane by affecting either protein activity or conformation by direct interactions of protein-sterol (Lindsey *et al*., 2003). Generation of ROS during metal stress might enhance the oxidative degradation of phospholipids, resulting in disruption of cell membranes permeability, which can be overcome by antioxidative enzymes. Increase in activities of these antioxidative enzymes is a common response during stress conditions. SOD dismutates superoxide radical ($O_2^{\cdot-}$) into H_2O_2 and which is removed by CAT in the peroxisomes. APOX also contribute in the removal of H_2O_2 in the chloroplast by playing major role in the ascorbate-glutathione antioxidant cycle (Foyer *et al*., 1997). Activity of GR maintains the glutathione pool in the reduced state, which further reduces dehydroscorbate to ascorbate. SOD, CAT and POD enzymes provide the endogenous defence to the plant cells. (Mittler *et al*., 2004). Besides this, alteration in the different physiological processes of the plants depends upon the type of plants, hormones and their mode of treatment (Fariduddin *et al*., 2008).

CONCLUSION

Combination of Cd and Hg metals altered the photosynthetic machinery and various metabolic processes of radish seedlings, which is overcome by the treatment of steroid hormone 24-EBL. Oxidative stress produced by these heavy metals is ameliorated through activating the antioxidative mechanism of radish by 24-EBL.

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