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Alleviation of Lead Toxicity Stress in Mustard (*Brassica juncea* L.) Cultivars by 24-Epibrassinolide.

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Abstract
Lead toxicity stress substantially reduced the seedling growth of three cultivars of mustard (*Brassica juncea* L) *viz*, Maya, NRCDR-2 and NRCHB-101. However exogenous application of 24-Epibrassinolide (24-EBL) in a dose dependent manner reduced the impact of lead toxicity on the seedling growth of mustard cultivars. The toxicity alleviation impact of 24-EBL was found to be more on NRCDR-2 and minimal in case of NRCHB-101 cultivars. Supplementation of 24-EBL lowered the MDA content in the seedlings of all the three mustard cultivars indicating reduced membrane peroxidation. The negative of impact of lead toxicity on protein content in mustard seedling was found reduced by 24-EBL application. Further, the toxicity amelioration by 24-EBL was reflected in elevated free proline (osmoprotectant) content. Lead toxicity reduced the activity of catalase and increased the activity of peroxidase, ascorbate peroxidase and superoxide dismutase. 24-EBL feeding caused steep rise in the activities of all the antioxidative enzymes in mustard seedlings growing under toxic levels of lead. The present study clearly demonstrated that 24-EBL remarkably mitigated lead toxicity stress in case of three mustard cultivars by activating antioxidative enzyme activity.

Keywords
24- Epibrassinolide, Lead toxicity, Mustard cultivars.

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INTRODUCTION:
Brassinosteroids (BRs) are a polyhydroxy steroidal phytohormones with significant growth-promoting influence (Vardhini, 2012; Bajguz and Piotrowska-Niczypourk, 2014). Brassinosteroids influence wide spectrum of physiological processes, such as seed germination, plant growth, rhizogenesis, flowering, senescence, abscission, leaf epinasty, pollen tube growth and stem elongation (Rao *et al.,* 2002; Vardhini *et al.,* 2006). Brassinosteroids confers tolerance to various abiotic stresses such as high or low temperature, moisture, drought, salinity, and heavy metal stresses (Divi and Krishna, 2009; Xia *et al*., 2009; Hasan *et al*., 2011; Ahammed *et al.*, 2012; Choudhary *et al.*, 2012*a*, *b*).
During last few decades, the concentration of heavy metals is increasing in soil due to mining smelting, and other industrial activities. Heavy metal contamination in soil could result in inhibition of plant growth and yield reduction. The principle cause

of the prolonged presence of heavy metals in the environment is their non-biodegradable nature. The primary response of plants due to heavy metals stress is the production of reactive oxygen species (ROS) and may cause damage of cell structure. Lead (Pb) is the most common heavy metal contaminant in the environment (Watanabe, 1997). Lead exists naturally in many forms throughout the world. Lead is a common and serious pollutant because of its toxicity. Lead is not only a toxic element but also can be accumulated in plant organs and agricultural products (Burzynski, 1987; Mahmoud and El-Beltagy, 1998), consequently enter human food chain (Wagner, 1993).

The present study was designed to determine the effect of 24-EBL on seedling growth of three cultivars of mustard seedlings under lead toxicity.

MATERIAL AND METHODS:

Chemicals and Plant material

24-epibrassinolide was procured from CID Technologies Inc., Brampton, Ontario, Canada. Seeds of mustard (*Brassica juncea* L.) cultivars were obtained from National Research Centre on Rapeseed-Mustard, Baratpur, Rajasthan, India. The three cultivars of mustard *viz.*, Maya, NRCDR-2 and NRCHB-101 were employed for the investigation. Lead (Pb) in the form of lead nitrate [Pb $(NO_3)_2$] was used for the studies.

Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution from commercially available 4% (w/v) NaClO and washed thoroughly with several changes of sterile distilled water. They were soaked for 30 min either in i) distilled water (control) ii) 2 mM lead and iii) 2 mM lead supplemented with 0.5/1.0/2.0 µM EBL. Twenty seeds from each treatment were placed in each of 90 mm sterile petri dishes layered with Whatman No.1 filter paper. The petri dishes were supplied with 5 ml of respective test solutions. The seeds were allowed to germinate in dark at 20 ±1°C. On the fourth day, 5 seedlings were retained in each petri plate and 3 ml of respective test solutions were added and seedlings were allowed to grow.

Growth parameters

On 7th day, seedling growth was recorded in terms of seedling length, fresh weight and dry weight. The seedlings were carefully removed from petri dishes and the water adhering to them was removed with the help of blotting paper. The length and fresh weights of the seedling were recorded. Seedlings were dried in oven at 110°C for 24 hours and their dry weights were recorded.

Lipid peroxidation.

Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). Seedlings (1.0 g) were homogenized with 3 ml of 0.5% thiobarbituric acid (TBA) in 20% (v/v) trichloroacetic acid. The homogenate was incubated at 95°C for 30 min and the reaction was stopped in ice. The samples were centrifuged at 10 000 × g for 5 min, the absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of malondialdehyde was calculated by using the extinction coefficient of 155mM–1 cm–1.

Free Proline

The amount of proline content was estimated as described by Bates et al (1973). Seedling material (0.5 g) was homogenized with 10 ml of 3 % (w/v) sulfosalicylic acid and the homogenate was filtered through whatman No. 2 filter paper. The supernatant was taken for proline estimation. The reaction mixture was composed of 2 ml of plant extract, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid. The test tubes containing above mixture were heated in a boiling water bath for one hour. The reaction was terminated in an ice bath followed by addition of 4 ml of toluene. The contents were shaken vigorously and then allowed to separate into phases. The chromophase containing upper toluene phase was carefully taken out with the help of a pipette and the absorbance was taken at 520 nm. The amount of proline present was quantified with the help of proline standard graph.

Soluble Proteins

Seedlings were thorouly homogenated in 70% (v/v) ethyl alcohol. Soluble proteins in alcohol homogenate (extract in case of enzyme assay) were precipitated by using 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 5 ml of 1% (w/v) sodium hydroxide and was centrifuged at 4000 rpm for 10 min. The supernatant was used for estimation of proteins by Lowry *et al.* (1951) method.

Antioxidant Enzymes

The fresh seedling material (200 mg) was homogenized with sodium phosphate buffer at pH 7.0 for Catalase, Peroxidase, and Ascorbate peroxidase and at pH 7.8 for Superoxide dismutase activities. The supernatant was used to assess the activity of the enzymes and the protein content in the supernatant was determined according to Lowry *et al* (1951).

Catalase (CAT, EC; 1.11.1.6) activity was assayed by the method of Barber (1980). Enzyme extract (0.5 ml)

was added to 2 ml of hydrogen peroxide and 3.5 ml of phosphate buffer (pH 7.0). The reaction was stopped by adding 10 ml of 2% (v/v) concentrated sulphuric acid, and the residual hydrogen peroxide was titrated against 0.01 M potassium permanganate until a faint purple color persisted for at least 15 sec. The activity of the enzyme was expressed as enzyme units.

Peroxidase (POD, EC; 1.11.1.7) activity was assayed adopting the method of Kar and Mishra (1976). To 0.5 ml of enzyme extract, 2.5 ml of 0.1 M phosphate buffer (pH 7.0), 1.0 ml of 0.01 M pyrogallol and 1.0 ml of 0.005 M H_2O_2 were added. After incubation, the reaction was stopped by adding 1.0 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was quantified by measuring the absorbance at 420 nm. The enzyme activity was expressed in absorbance units.

Ascorbate peroxidase (APX, EC; 1.11.1.11) activity was measured according to the methods described by Nakano and Asada (1981). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 250 mM H_2O_2 and enzyme extract. The activity of APX was measured spectrophotometrically by measuring the rate of ascorbate oxidation at 290 nm for 1 min. The amount of ascorbate was calculated from the extinction coefficient of 2.6 mM−1 cm−1.

Superoxide dismutase (SOD; EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 1.5 ml methionine, 1 ml of NBT, 0.75 ml tri-ton-X-100, 2 mM EDTA, 10 µL of riboflavin and 50 µg of protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

RESULTS & DISCUSSIONS:

Pb toxicity resulted reduction in seedling growth, and their inhibitory effects on seedling growth were ameliorated by the application of 24-epibrassinolide (Table 1). 24-epibrassinolide improved the seedling growth (in dose dependent manner) in terms of seedling length, seedling fresh weight and dry weights in three mustard cultivars under Pb toxicity. Further the impact of 24-EBL on lead stressed seedling growth was higher in NRCDR-02 and lower in NRCHB-101. Brassinosteroids were found to improve seedling growth in *Raphanus sativus* under Cd stress (Anuradha and Rao 2001 and Sharma *et al.,* 2010).

An increase in malondialdehyde content in mustard seedlings grown under Pb stress was observed (Table 2), indicating a high level of lipid peroxidation. However, 24-EBL supplementation lowered malondialdehyde content in the seedlings of three mustard cultivars. In present study MDA content was high in NRCDR-02 and low in NRCHB-101. EBL application resulted in lowered MDA content in *Zea mays* leaves under Mn toxicity (Wang *et al* 2009). BRs also decrease the malondialdehyde content in both control and drought stressed soybean plants (Zhang *et al.,* 2008). Alleviation of zinc stress in radish plants by brassinosteroids was found associated with lowered membrane peroxidation (Ramakrishna and Rao, 2015)

In response to Pb stress mustard seedlings accumulated proline, and supplementation of brassinosteroids further enhanced the proline contents in case of all the three mustard cultivars (Table 2). Among cultivars NRCDR-02 contained more proline than MAYA and NRCHB-101.The application of BRs increases the accumulation of proline and in salt stressed *Cicer arietinum* (Ali *et al.* 2007) and *Vigna radiata* (Hayat *et al.* 2010). Similar results were shown in pigeon pea grown under Al toxicity by Divya Sri *et al* (2016). The increase in the level of proline is a general response to various biotic and abiotic stresses, including heavy metal stress (Schultzendubel and Polle., 2002), to counter the toxicity generated by the stress.

There was a great decline in protein content in the seedlings of three cultivars of mustard growing under lead toxicity (Table 2). NRCDR-02 maintained more protein content than MAYA and NRCHB-101. Exogenous application of 24-epibrassinolide removed the negative impact of Pb on protein content. *Raphanus sativus* seedlings emerged from seeds presoaked in 24-EBL exhibited elevated activity of APX and SOD that eventually resulted in reducing lipid peroxidation, enhanced proline and protein content under Ni exposure (Sharma et al., 2011)

Pb toxicity decreased the CAT activity in mustard seedlings and addition of BRs increased its activity (Table 3). Pb toxicity increased the POD activity in mustard seedlings (Table 3). An increase in POD activity is a common response to oxidative and abiotic stresses. However, it was observed that brassinosteroids applied to Pb stressed mustard seedlings reduced the POD activity in all three cultivars of mustard. In present study reduced levels of POD was in higher NRCDR-02. A similar decrease in CAT activity and increase in POD activity was reported in wheat leaves under heavy metal stress (Panda et al., 2003).

APX activity showed an increase with heavy metal stress (Table 3). 24-EBL supplementation further enhanced the activity in three cultivars mustard seedlings. The activity was higher in NRCDR-02 and lower in NRCHB-101 cultivars. A similar increase in APX activity due to EBL application to the radish seedlings growing under zinc toxicity stress was observed by Ramakrishna and Rao (2012).

Pb stress increased the SOD activity of mustard seedlings (Table 3). The supplementation of 24-epibrassinolide resulted in further enhancement of SOD enzyme activity in three mustard cultivars. NRCDR-2 showed high SOD activity among three cultivars. Similarly, increased SOD activity in *Arabidopsis* in response to heavy metal toxicity caused by BRs application has been also reported by Cao et al. (2005).

Table -1: Effect of 24- epibrassinolide on seedling growth of mustard under lead toxicity.

Cultivars	Treatments	Seedling length (cm)	Seedling FW(mg)	Seedling DW(mg)
	Control	12.2±0.5	1.30±0.06	0.128±0.005
	2mM Pb	4.8±0.1	0.96±0.10	0.098±0.002
MAYA	Pb+0.5 µM EBL	7.9±0.4	1.02±0.09	0.103±0.011
	Pb+1 µM EBL	10.6±0.6	1.06±0.08	0.114±0.009
	Pb+2 µM EBL	11.7±0.7	1.17±0.11	0.120±0.007
	Control	13.8±0.6	1.56±0.16	0.152±0.022
	2mM Pb	6.2±0.2	1.27±0.09	0.121±0.010
NRCDR-02	Pb+0.5 µM EBL	11.2±0.6	1.36±0.18	0.129±0.032
	Pb+1 µM EBL	12.5±0.8	1.43±0.27	0.142±0.021
	Pb+2 µM EBL	13.3±1.0	1.55±0.33	0.149±0.019
	Control	14.2±0.9	1.53±0.31	0.148±0.018
	2mM Pb	5.5±0.2	1.06±0.09	0.107±0.013
NRCHB-101	Pb+0.5 µM EBL	10.5±0.6	1.11±0.32	0.112±0.026
	Pb+1 µM EBL	11.4±0.9	1.17±0.34	0.124±0.021
	Pb+2 µM EBL	13.4±1.0	1.27±0.29	0.131±0.011

The data presented above are Mean ± S.E. (n=5). EBL=24-epibrassinolide; Pb= Lead.

Table-2: Effect of 24- epibrassinolide on Lipid peroxidation, free proline content and Soluble protein concentration of mustard plants under lead toxicity.

Cultivars	Treatments	MDA (µmol $min^{-1}g^{-1}$ FW)	Free Proline (mg g^{-1} FW)	Soluble Proteins (mg g^{-1}fw)
	Control	19.52±1.23	4.29±0.42	5.62±0.55
	2mM Pb	24.82±1.35	5.91±0.47	4.23±0.32
MAYA	Pb+0.5 µM EBL	23.13±1.09	6.44±0.29	4.53±0.62
	Pb+1 µM EBL	21.68±1.11	7.08±0.38	4.75±0.57
	Pb+2 µM EBL	19.87±1.16	7.35±0.27	5.36±0.63
	Control	21.60±1.35	5.12±0.25	6.18±0.45
	2mM Pb	27.68±1.06	7.28±0.82	4.82±0.55
NRCDR-02	Pb+0.5 µM EBL	24.22±0.97	7.94±0.75	5.18±0.38
	Pb+1 µM EBL	22.79±1.02	8.89±0.29	5.50±0.57
	Pb+2 µM EBL	21.56±1.09	9.34±0.35	6.17±0.91
	Control	14.56±1.22	3.62±0.56	4.60±0.43
	2mM Pb	19.16±1.25	4.88±0.64	3.35±0.58
NRCHB-101	Pb+0.5 µM EBL	17.99±1.07	5.27±0.72	3.58±0.33
	Pb+1 µM EBL	16.84±1.21	5.80±0.55	3.75±0.29
	Pb+2 µM EBL	15.56±1.01	6.0±0.43	4.22±0.48

The data presented above are Mean ± S.E. (n=5). EBL=24-epibrassinolide; Pb= Lead.

Table-3: Effect of 24- epibrassinolide on the activities of antioxidative enzymes in mustard seedlings under lead toxicity

Cultivars	Treatments	CAT (Umg^{1} protein min^{-1})	POD (U mg $^{-1}$ protein min^{-1})	APX (µmol ASA mg^{-1} protein min^{-1})	SOD (U mg^{-1} protein min^{-1})
	Control	6.85±0.55	2.82±0.43	4.46±0.26	3.96±0.21
	2mM Pb	4.46±0.44	4.37±0.34	5.31±0.42	5.05±0.17
MAYA	Pb+0.5 µM EBL	5.10±0.78	5.19±0.55	5.86±0.25	5.81±0.31
	Pb+1 µM EBL	5.82±0.88	5.96±0.46	6.34±0.32	6.11±0.19
	Pb+2 µM EBL	6.39±0.67	6.47±0.38	6.82±0.27	6.88±0.24
	Control	6.29±0.54	3.36±0.65	6.34±0.28	4.63±0.33
	2mM Pb	4.34±0.87	5.23±0.44	7.61±0.46	5.96±0.24
NRCDR-02	Pb+0.5 µM EBL	5.06±0.96	6.31±0.24	8.48±0.33	6.86±0.15
	Pb+1 µM EBL	5.69±0.57	7.19±0.33	9.18±0.38	7.26±0.23
	Pb+2 µM EBL	6.27±0.64	7.82±0.54	9.87±0.42	8.16±0.32
	Control	7.84±0.62	2.39±0.27	3.73±0.35	3.12±0.22
	2mM Pb	4.96±0.92	3.67±0.29	4.42±0.45	3.93±0.28
NRCHB-101	Pb+0.5 µM EBL	5.59±0.74	4.32±0.51	4.86±0.48	4.51±0.32
	Pb+1 µM EBL	6.40±0.64	4.95±0.41	5.22±0.24	4.69±0.25
	Pb+2 µM EBL	7.09±0.78	5.38±0.28	5.62±±0.22	5.30±0.34

The data presented above are Mean ± S.E. (n=5). EBL=24-epibrassinolide; Pb= Lead

CONCLUSION:

24-epibrassinolide markedly lowered the impact of lead toxicity in three mustard cultivars. EBL application reduced the impact of lead toxicity on membrane peroxidation as evidenced by lowered MDA content. Application of 24-epibrassinolide significantly increased the activities of antioxidative enzymes (CAT, POD, APX and SOD) and proline (osmolyte) in Pb stressed mustard seedlings. The result of the present investigation clearly demonstrated the ameliorative effect of EBL on Pb toxicity imposed growth inhibition in all three mustard cultivars.

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REFERENCES:

1. Vardhini BV. Application of brassinolide mitigates saline stress of certain metabolites of sorghum grown in Karaikal. *J. Phytol.* 4, 1–3. (2012)
2. Bajguz A and Piotrowska-Niczyporuk A. Interactive effect of brassinosteroids and cytokinins on growth, chlorophyll, monosaccharide and protein content in the green alga *Chlorella vulgaris* (*Trebouxiophyceae*). *PlantPhysiol. Biochem.* 80C, 176–183. (2014)
3. Rao SSR, Vardhini BVV, Sujatha E and Anuradha S. Brassinosteroids – A new class of phytohormones. Curr. Sci *82*, 1239–1245. (2002)
4. Vardhini BV, Anuradha S and Rao SSR. Brassinosteroids-New Class of Plant Hormone with Potential to Improve Crop Productivity. *Indian Journal of Plant Physiology* **11**, 1-12. (2006)
5. Divi UK and Krishna P. Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *N Biotechnol* 26, 131–136. (2009)
6. Xia XJ, Zhang Y, Wu JX, Wang JT, Zhou YH, Shi K, Yu YL and Yu JQ. Brassinosteroids promote metabolism of pesticides in cucumber. *J Agric Food Chem* 57, 8406–8413. (2009)
7. Hasan SA, Hayat S and Ahmad A. Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. *Chemosphere* 84, 1446–1451. (2011)
8. Ahammed GJ, Yuan HL, Ogweno JO, Zhou YH, Xia XJ, Mao WH, Shi K and Yu JQ. Brassinosteroid alleviates phenanthrene and pyrene phytotoxicity by increasing detoxification activity and photosynthesis in tomato. *Chemosphere* 86, 546–555. (2012)
9. Choudhary SP, Oral V, Bhardwaj R, Yu JQ and Tran LS. Interactions of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus*. *J Exp Bot* 63, 5659–5675. (2012a)
10. Choudhary SP, Yu JQ, Yamaguchi-Shinozaki K, Shinozaki K and Tran LS. Benefits of brassinosteroid crosstalk. *Trends Plant Sci* 17, 594–605. (2012b)
11. Watanabe MA. Phytoremediation on the brink of commercialization. *Environ Sci Technol* 31, 182A–186A. (1997)
12. Burzynski M. The uptake and transpiration of water and the accumulation of lead by plants growing on lead chloride solutions. *Acta Societatis Botanicorum Poloniae* 56, 271-280. (1987)

13. Mahmoud WH and El-Beltagy A. Isolation, Identification and potential use of lead reduction from heavy metal polluted soil. *Menufiya J Agric Res* 23, 1461-1473. (1998)
14. Wagner GJ. Accumulation of heavy metals in crop plants and its consequences to human health. *Adv Agron* 51, 173-177. (1993)
15. Heath RL and Packer L. Photoperoxidation in isolated chloroplasts kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 12, 189-198. (1968)
16. Bates L, Waldren RP and Teare ID. Rapid Determination of Free Proline for Water-Stress Studies. *Plant and Soil* 39, 205-207. (1973)
17. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with folin-phenol reagent. *J Biol Chem* 193, 265-275. (1951)
18. Barber JM. Catalase and peroxidase in primary leaves during development and senescence. Z. Pflanzen Physiol 97, 135–144. (1980)
19. Kar M and Mishra D. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol* 57, 315-319. (1976)
20. Nakano Y and Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22, 867-880. (1981)
21. Beauchamp C and Fridovich I. Superoxide dismutase improved assay and an assay applicable to acrylamide gels. *Anal Biochem* 44, 276-287. (1971)
22. Anuradha S and Rao SSS. The effect of brassinosteroids on radish (*Rahanus sativus* L.) seedlings growing under cadmium stress. *Plant soil environ* 53(11), 465-472. (2007)
23. Sharma I, Pati PK and Bhardwaj R. Regulation of growth and antioxidant enzyme activities by 28-homobrassinolide in seedlings of *Raphanus sativus* L. under cadmium stress. *Indian J Biochem Biophys* 47, 172–177. (2010)
24. Wang C, Zhang SH, Wang PF, Hou J, Zhang WJ, Li W and Lin ZP. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere* 75, 1468-1476. (2009)
25. Zhang M, Zhai Z, Tian X, Duan L and Li Z. Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). *Plant Growth Regul* 56, 257-264. (2008)
26. Ramakrishna and Rao. Foliar application of brassinosteroids alleviates adverse effects of zinc toxicity in radish (*Rahanus sativus* L.) plants. *Protoplasma* 252(2), 665-677. (2015)
27. Ali B, Hayat S and Ahmad A. 28-homobrassinolide ameliorates the saline stress in *Cicer arietinum* L. *Environ Exp Bot* 59, 217-223. (2007)
28. Hayat S, Hasan SA, Yusuf M, Hayat Q and Ahmad A. Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. *Environ exp Bot* 69, 105- 112. (2010)
29. Divya Sri N, Madhan M, Mahesh K, Raghu K and Rao SSR. Amelioration of aluminium toxicity in pigeon pea [*Cajanus cajan* (L.) Millsp.] Plant by 24-epibrassinolide. *American Journal of Plant Sciences* 7, 1618-1628. (2016)
30. Schultzendubel A and Polle A. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. *J Exp Bot* 53, 1351–1365. (2002)
31. Sharma I, Pati PK and Bhardwaj R. Effect of 24-epibrassinolide on oxidative stress markers induced by nickel-ion in *Raphanus sativus* L. *Acta Physiol Plant* 33, 1723–1735. (2011)
32. Panda SK, Chaudhary I and Khan MH. Heavy metal induced lipid peroxidation and affect antioxidants in wheat leaves. *Biol Plantarum* 46: 289–294. (2003)
33. Ramakrishna and Rao. 24- Epibrassinolide alleviated zinc- induced oxidative stress in radish (*Rahanus sativus* L.) seedlings by enhancing antioxidative system. *Plant Growth Regul* 68, 249-259. (2012)
34. Cao S, Xu Q, Cao Y, Qian K, An K, Zhu Y, Binzeng H, Zhao H and Kuai B. Loss of function mutations in DET2 gene lead to an enhanced resistance to oxidative stress in *Arabidopsis. Physiol Plant* 123, 57–66. (2005)