**International Journal of Botany Studies**
**ISSN: 2455-541X, Impact Factor: RJIF 5.12**
**www.botanyjournals.com**
**Volume 2; Issue 4; July 2017; Page No. 54-59**

# Effect of 24-epibrassinolide on growth and metabolism of rose-scented geranium [*Pelargonium graveolens* (L.) Herit] under cadmium toxicity

*** S Seeta Ram Rao, K Raghu**
Department of Botany, Osmania University, Hyderabad, Telangana, India

## Abstract

Effect of exogenous application of 24-epibrassinolide on the growth, metabolite content and antioxidant mechanism in geranium [*Pelargonium graveolens* (L.) Herit] plants growing under cadmium toxicity stress was investigated. 24-EBL reduced the deleterious impact of cadmium toxicity on the plant growth. Foliar application of 24-EBL accounted for restoration of pigment levels and improvement of carbohydrate, nucleic acid and protein contents in geranium plants under cadmium stress. The deleterious impact of cadmium on membrane integrity (measured in the levels of MDA, the product of lipid peroxidation) was found negated by 24-EBL. The ameliorative impact of 24-EBL on cadmium toxicity stress was found associated with elevated levels of proline and enhanced activities of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase and ascorbate peroxidase.

**Keywords:** brassinosteroids, cadmium toxicity, metabolism, antioxidant enzymes

## 1. Introduction

Increasing heavy metal (HM) concentrations in the soil have become a significant problem in the modern industrialized world due to several anthropogenic activities. Heavy metals (HMs) are no biodegradable and have long biological half-lives, thus, once entered in food chain, their concentrations keep on increasing through biomagnification (Thakur *et al*., 2016) [38]. HMs at toxic levels have the capability to interact with several vital cellular biomolecules such as nuclear proteins and DNA, leading to excessive production of reactive oxygen species (ROS). This would inflict serious morphological, metabolic, and physiological anomalies in plants. In response, plants are equipped with a repertoire of mechanisms to counteract heavy metal (HM) toxicity (Emamverdian *et al*., 2015) [14]. This green technology that involved “tolerant plants” has been utilized to clean up soil and ground water from heavy metals and other toxic organic compounds. Rose scented geranium [*Pelargonium graveolens* (L.) Herit], an important aromatic plant, is source for the highly valued geraniol and citronellol. Vegetative propagation is the only method for its cultivation (Swamy and Rao, 2006) [37].

Brassinosteroids are a new type of phytohormones with significant growth promoting influence (Clouse and Sasse, 1998) [10]. Brassinosteroids regulate various aspects of plant growth and development, including cell elongation, photomorphogenesis, xylem differentiation, seedgermination, leaf bending and epinasty, proton pump activation, regulation of gene expression, nucleic acid and protein synthesisand photosynthesis (Rao *et al*., 2002; Clouse, 2011; Yang *et al*., 2011) [11, 42]. BRs can reduce negative impacts of various abiotic environmental stresses induced by drought, high temperature, chilling, salinity and heavy metals (Vardhini *et al*., 2010; Vriet *et al*., 2012) [39, 40]. The present study was undertaken to identify the impact of 24-epibrassinolide, a bioactive brassinosteroid on growth and metabolism of rose scented geranium subjected to cadmium toxicity stress.

## 2. Materials and Methods

### 2.1 Plant material and Chemicals

Geranium [*Pelargonium graveolens* (L.) Herit] plants were procured from CSIR-CIMAP Research center, Bangalore and maintained in Botanical Garden, Osmania University, Hyderabad, India. 24-epibrassinolide (EBL) purchased from CID tech research Inc, Mississauga, Ontario, Canada was employed for the studies.

### 2.2 Growth conditions

Preliminary experiments were conducted employing different concentrations of cadmium using as cadmium chloride monohydrate ($CdCl_2.H_2O$) and 1mM of cadmium was selected as metal stress concentration where growth was considerably but not completely inhibited. Similarly, from a wide range of concentrations, 1µM and 2µM concentrations of EBL were chosen as hormone concentration where substantial growth promotion was observed.

Fresh and healthy geranium plant cuttings of 14 cm in height and having three top leaves (other leaves were carefully excised) were transplanted into nursery covers filled with garden soil and maintained at an adequate moisture level for 20 days for rooting. On the 20th day, uniform sized plants were sorted out, and one plant each was transplanted to earthen pot containg 10 kg of garden soil and compost in 10:1 ratio. Plants were maintained in a glass house at 25/18°C day/night temperature under natural day length and watered thrice weekly. The six treatments maintained for the study were: (i) control (ii) 1µM EBL (iii) 2µM EBL (iv) 1mM Cd (metal stressed control) (v) 1mM Cd +1µM EBL. (vi) 1mM Cd +2µM EBL. 24-epibrassinolide treatment as foliar spray was given on 10th and 25th day. Cadmium was applied to the soil as solution throughout the growth period at 5 day intervals. The plants were allowed to grow for 60 days. On 60th day growth of the plants was recorded.

### 2.3 Growth studies

To find out the effect of 24-epibrassinolide on the growth of geranium plants under cadmium stress, the growth parameters recorded were plant height, fresh weight and dry weight. 60 day old plants were uprooted carefully ensuring safety of roots from pots and dipped in bucket full of water to remove the adhering soil particles and the plants were blotted dry and employed for recording growth parameters.

Plant height: The height of the plant was measured (before uprooting) from the soil surface to the tip of the apical leaf using a scale and expressed in centimeters.

Fresh weight: The fresh weight of the plants (shoot and root) was recorded and expressed in grams.

Dry weight: The plants (shoot and root) were dried in the oven at $110^0$ C for 24 hours, weight was recorded and expressed in grams.

Leaf area: Total leaf area of the plant was recorded employing Leaf Area Meter (CI-203, CID Inc. Vancouver, Washington-USA).

Root area: The root area was recoded employing WhinRhizo Root Scanner (XLRHIZO 2012a, Regent Instruments Inc, and Canada).

Leaf material from different treatments was thoroughly homogenized in 70% (v/v) ethyl alcohol and homogenate was stored in deep freezer (at 20ºC) for the estimation of nucleic acids, soluble proteins and carbohydrate fractions. For the extraction pigments, assaying enzymes and estimation of free proline and MDA content fresh leaf material was used.

### 2.4 Extraction and estimation of chlorophyll pigments

Chlorophyll pigments were extracted from fresh leaf material in 80% (v/v) acetone and estimated according to the method of Arnon (1949) [4].

### 2.5 Carbohydrate Fraction

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars (Yoshida *et al.*, 1976) [43] and reducing sugars (Nelson, 1944) [27]. The residue was used for the estimation of starch (McCready *et al*., 1950) [24]. The non-reducing sugar content was calculated by adopting the formulae given by Loomis and Shull (1937) [22].

### 2.6 Nucleic Acids

DNA and RNA fractions in the ethyl alcohol homogenate were separated (Ogur and Rosen, 1950) [28]. DNA was estimated by employing diphenylamine reagent (Burton, 1968) [9] and RNA was quantified by using orcinol reagent (Schneider, 1957) [35].

### 2.7 Soluble Proteins

Soluble proteins in alcoholic homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry *et al* was used for the quantification of proteins.

### 2.8 Lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content following the method of Heath and Packer (1968) [19]. Plant material (1.0 g) was homogenized with 3 ml of 0.5% thiobarbituric acid (TBA) in 20% (w/v) trichloroacetic acid. The homogenate was incubated at 95 ºC 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 the value for the non-specific absorbance at 600 nm was subtracted. The absorbance coefficient of MDA was calculated by using the extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

### 2.9 Free Proline

The amount of proline content was estimated as described by Bates *et al*. (1973) [7]. Plant 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. To 2 ml of plant extract, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added. 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 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.

### 2.10 Antioxidant enzymes

Fresh leaf material (200 mg) was homogenized with sodium phosphate buffer at pH 7.0 for catalase, peroxidase and ascorbate peroxidase activities and at pH 7.8 for superoxide dismutase activity. The supernatant was used to assay the activity of the enzymes.

Catalase (CAT, EC; 1.11.1.6) activity was assayed by the method of Barber (1980) [6]. 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 $KMnO_4$ until a faint purple color persisted for at least 15 sec. The activity of the enzyme is expressed as enzyme units.

Peroxidase (POD, EC; 1.11.1.7) activity was assayed adopting the method of Kar and Mishra (1976) [20]. 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 estimated by measuring the absorbance at 420 nm. The enzyme activity is expressed in absorbance units.

Superoxide dismutase (SOD, E.C; 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) of Beauchamp and Fridovich (1971) [8]. Three ml of reaction mixture contained 40 mM phosphate buffer (PH=7.8), 13 mM methionine, 75 µM nitroblue tetrazolium, 0.1 mM EDTA, 0.1 ml of enzyme extract and 2 µM riboflavin. Riboflavin was added at the end. After mixing the contents, test tubes were shaken and placed 30 cm below light source consisting of two 15 watt fluorescent tubes. The reaction was started by switching on the lights. The reaction was allowed to take place for 30 minutes and was stopped by switching off the lights. A tube with protein kept in the dark served as blank, while the control tube was without the enzyme and kept in the light. The absorbance was measured at 540 nm. The activity of superoxide dismutase is the measure of NBT reduction in light without protein minus NBT reduction in light with protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

Ascorbate peroxidase (APX; E.C 1.11.1.11) APX activity was assayed by the method of Nakano and Asada (1981) [26]. The reaction mixture contained 1.5 ml of 50 mM sodium phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 ml of 0.5 mM ascorbic acid, 0.5 ml 0.5 mM $H_2O_2$ and 0.5 ml of enzyme sample. The activity was recorded as the decrease in absorbance at 290 nm for 1 minute and the amount of ascorbate oxidized was calculated from the extinction coefficient of 2.6 $mM^{-1}cm^{-1}$.

## 3. Results and Discussion

Cadmium toxicity stress drastically reduced the growth of geranium plants (Table.1, 2, 3).

**Table 1:** Effect of brassinosteroids on the shoot growth of geranium plants under cadmium toxicity stress

| Treatments | Plant Height (cm) | Shoot FW (gm) | Shoot DW (gm) |
|---|---|---|---|
| Control | 58.21±2.62 | 63.12±2.52 | 7.93±0.72 |
| 1µM EBL | 67.42±3.02 | 70.76±4.10 | 8.22±0.53 |
| 2µM EBL | 75.85±1.41 | 84.02±2.43 | 10.15±1.21 |
| 1mM Cd | 40.34±1.52 | 47.01±4.33 | 4.01±1.75 |
| 1mM Cd+1µM EBL | 48.02±3.52 | 59.72±3.35 | 6.91±0.48 |
| 1mM Cd+2µM EBL | 57.09±2.86 | 64.35±3.12 | 7.55±0.17 |

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

**Table 2:** Effect of brassinosteroids on root growth of geranium plants under cadmium toxicity stress

| Treatments | Root FW (gm) | Root DW (gm) | Root Area ($cm^2$) |
|---|---|---|---|
| Control | 4.99±0.52 | 0.497±0.03 | 75.830±6.05 |
| 1µM EBL | 5.17±0.14 | 0.553±0.12 | 84.371±8.52 |
| 2µM EBL | 6.09±0.32 | 0.827±0.08 | 96.752±9.78 |
| 1mM Cd | 2.96±0.04 | 0.284±0.01 | 53.784±9.75 |
| 1mM Cd+1µM EBL | 3.02±0.52 | 0.306±0.06 | 68.532±7.58 |
| 1mM Cd+2µM EBL | 4.35±0.39 | 0.505±0.07 | 76.512±9.02 |

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

**Table 3:** Effect of brassinosteroids on the foliage growth of geranium plants under cadmium toxicity stress

| Treatments | No of Leaves (Per plant) | Total leaf area ($cm^2$) |
|---|---|---|
| Control | 35.15±2.52 | 1201.76±10.25 |
| 1µM EBL | 48.35±1.74 | 1331.44±21.30 |
| 2µM EBL | 55.02±2.33 | 1514.21±17.26 |
| 1mM Cd | 28.43±1.14 | 843.16±14.62 |
| 1mM Cd+1µM EBL | 31.19±1.21 | 978.72±23.01 |
| 1mM Cd+2µM EBL | 33.72±1.09 | 1127.36±21.85 |

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

There was substantial decrease in shoot, root as well as foliage growth due to cadmium toxicity. However exogenous application of 24-epibrassinolide in a dose dependent manner alleviated the toxic impact of cadmium on the growth of the plants. Earlier Swamy and Rao (2009) [36] reported the enhancement of the growth and herbage yield of geranium due to brassinosteroid application. In the present study 24-EBL was found to offset the deleterious impact of cadmium on root growth of geranium plants. The results of the study are in consistent with the observations made by Divya sri *et al* (2016) [12] who reported the restoration of the root growth due to 24-EBL application of pigeon pea plants growing under elevated levels of aluminium. One of the significant finding of the study is that exogenous application of 24-EBL (at 2µM concentration) completely eliminated the negative influence on foliage growth, the prime criteria of economic yield.

Geranium plants growing under cadmium fortified substratum showed the symptoms of chlorosis which was reflected in lowered pigment levels (Table.4)

**Table 4:** Effect of brassinosteroids on photosynthetic pigments of geranium plants under cadmium toxicity stress

| Treatments | Chlorophyll –a (mg $g^{-1}$ FW) | Chlorophyll –b (mg $g^{-1}$ FW) | Total Chlorophylls (mg $g^{-1}$ FW) |
|---|---|---|---|
| Control | 0.814±0.036 | 0.381±0.054 | 1.195±0.012 |
| 1µM EBL | 0.897±0.014 | 0.413±0.095 | 1.311±0.004 |
| 2µM EBL | 0.954±0.005 | 0.495±0.042 | 1.449±0.025 |
| 1mM Cd | 0.674±0.016 | 0.307±0.068 | 0.981±0.008 |
| 1mM Cd+1µM EBL | 0.781±0.075 | 0.341±0.062 | 1.122±0.073 |
| 1mM Cd+2µM EBL | 0.804±0.052 | 0.375±0.016 | 1.180±0.049 |

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

The treatment of geranium plants with EBL considerably increased the content of photosynthetic pigments and counteracted the damaging effect generated by Cd stress. Plants from brassinosteroids-alone treatments also showed increased levels photosynthetic pigments. It is suggested that brassinosteroids could prevent loss of photosynthetic pigments either by activating specific genes responsible for chlorophyll synthesis or by reducing the chlorophyll degradation (Hayat *et al*. 2011) [18]. Anuradha and Rao (2009) [3] found the restoration of pigment levels in radish plants challenged with cadmium stress due to the foliar application of 24-epibrassinolide. Similar observations were made by Ramakrishna and Rao (2015) [32] in case of radish plants under zinc stress.

Cadmium toxicity stress sharply lowered the carbohydrates levels in geranium plants (Table.5).

**Table 5:** Effect of brassinosteroids on content of carbohydrate fraction of geranium plants under cadmium toxicity stress

| Treatments | Reducing sugars (mg $g^{-1}$ FW) | Non- reducing sugars (mg $g^{-1}$ FW) | Starch (mg $g^{-1}$ FW) |
|---|---|---|---|
| Control | 2.241±0.261 | 4.31±0.13 | 5.97±0.47 |
| 1µM EBL | 2.617±0.024 | 4.85±0.18 | 7.49±0.86 |
| 2µM EBL | 3.604±0.035 | 6.71±0.09 | 8.74±0.12 |
| 1mM Cd | 1.613±0.091 | 2.77±0.52 | 4.17±0.27 |
| 1mM Cd+1µM EBL | 2.041±0.076 | 4.25±0.22 | 4.86±0.15 |
| 1mM Cd+2µM EBL | 2.343±0.083 | 4.64±0.41 | 6.03±0.72 |

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

Foliar feeding of 24-EBL found to set aside the Cd stress impact on the levels of various components of carbohydrate pool. The enhanced levels of carbohydrates might be due to increased photosynthesis as conferred by 24-EBL. In fact activation of photosynthesis in radish plants under cadmium stress due to brassinosteroids was reported by Anuradha and Rao (2009) [3].

Sharp decline in the contents of DNA and RNA were observed in Cd-stressed geranium plants as compared to unstressed plants (Table.6).

**Table 6:** Effect of brassinosteroids on nucleic acid and protein contents of geranium plants under cadmium toxicity stress

| Treatments | DNA (µg $g^{-1}$ FW) | RNA (µg $g^{-1}$ FW) | Soluble proteins (mg $g^{-1}$ FW) |
|---|---|---|---|
| Control | 0.614±0.015 | 0.819±0.019 | 3.97±0.52 |
| 1µM EBL | 0.635±0.017 | 0.847±0.041 | 4.31±0.31 |
| 2µM EBL | 0.672±0.065 | 0.907±0.006 | 5.26±0.29 |
| 1mM Cd | 0.512±0.007 | 0.705±0.091 | 2.99±0.37 |
| 1mM Cd+1µM EBL | 0.598±0.034 | 0.774±0.014 | 3.78±0.54 |
| 1mM Cd+2µM EBL | 0.609±0.026 | 0.804±0.032 | 4.02±0.35 |

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

However, 24-EBL feeding dose dependently reduced the toxic impact of Cd on the nucleic acid levels in the geranium plants. BRs application markedly increased the DNA and RNA content in maize plants under salinity stress (El-Khallal *et al*., 2004) [13]. The ameliorative influence of BRs on salinity stress induced growth inhibition in rice plants was linked to elevated levels of nucleic acids (Anuradha and Rao., 2003) [1].

Geranium plants raised on cadmium enriched substratum showed, lowered soluble proteins as compared to their counterparts growing on metal stress free conditions (Table.7)

**Table 7:** Effect of brassinosteroids on MDA levels and free proline content of geranium plants under cadmium toxicity stress

| Treatments | MDA (µmol $g^{-1}$FW) | Free proline (mg $g^{-1}$ FW) |
|---|---|---|
| Control | 47.25±3.12 | 2.36±0.52 |
| 1µM EBL | 42.17±1.95 | 2.85±0.42 |
| 2µM EBL | 35.28±2.36 | 3.42±0.96 |
| 1mM Cd | 52.19±2.75 | 3.11±0.37 |
| 1mM Cd+1µM EBL | 46.37±1.82 | 3.87±0.28 |
| 1mM Cd+2µM EBL | 39.31±4.01 | 4.12±0.53 |

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

it was observed that foliar application of 24-EBL reduced the deleterious impact of cadmium on protein content in geranium plants. The enhanced the protein content in radish seedlings under zinc stress due to brassinosteroid application was attributed to *de novo* protein synthesis and reduced protein carbonylation (Ramakrishna and Rao, 2012) [32].

Excess production of reactive oxygen species (ROS) is a common phenomenon of heavy metal stress in plants, which causes oxidative damage to macro molecules, leading to irreparable metabolic dysfunction and cell death (Yadav, 2010) [41]. In geranium plants challenged with excess Cd, oxidative alterations to lipids occurred as evidenced by increased levels of MDA (a final product of lipid peroxidation) (Table. 8).

**Table 8:** Effect of brassinosteroids on the activity of antioxidant enzymes of geranium plants under cadmium toxicity stress

| Treatments | CAT (U $mg^{-1}$ protein $min^{-1}$) | POD (U $mg^{-1}$ protein $min^{-1}$) | SOD (U $mg^{-1}$ protein $min^{-1}$) | APX (µmol ASA $mg^{-1}$ protein $min^{-1}$) |
|---|---|---|---|---|
| Control | 22.14±0.17 | 0.521±0.051 | 1.617±0.20 | 4.17±0.51 |
| 1µM EBL | 27.17±0.51 | 0.649±0.007 | 1.768±0.35 | 5.04±0.13 |
| 2µM EBL | 34.95±0.14 | 0.704±0.004 | 2.276±0.84 | 5.91±0.74 |
| 1mM Cd | 30.93±0.55 | 0.614±0.025 | 2.653±0.11 | 5.93±0.22 |
| 1mM Cd+1µM EBL | 33.37±0.31 | 0.715±0.003 | 2.951±0.05 | 6.45±0.18 |
| 1mM Cd+2µM EBL | 42.65±0.24 | 0.748±0.021 | 3.421±0.44 | 6.72±0.13 |

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

However, 24-EBL treatment to such plants helped to prevent the damping impact of cadmium as evidenced by the lowered MDA content indicating the positive role of 24-EBL in maintance membrane integrity. The results of the present study are in tune with the observations made in case of brassinosteroid treated radish plants under zinc stress (Ramakrishna and Rao, 2013) [31].

Geranium plants subjected to Cd toxicity exhibited enhanced levels of free proline. Exogenous application of 24-EBL caused further increase in proline content in geranium plants growing under excess Cd levels. To increase plant tolerance to abiotic stresses plants accumulate compounds of low molecular mass such as proline. Proline as an osmoprotectant increase the activity of many enzymes and stabilize protein integrity. Proline contributes to maintenance of the redox balance, can regulate development, and is a component of metabolic signaling networks controlling mitochondrial functions, stress relief and development (Lipiec *et al*., 2013) [21]. Proline accumulates in response to abiotic stress and scavenge ROS and is considered as important non-enzymatic antioxidant (Michalak, 2006; Ashraf and Foolad, 2007) [25, 5]. Brassinosteroids enhanced the levels of free proline content in chick pea under cadmium stress (Hasan *et al*., 2008) [17]. The alleviation of zinc toxicity in radish by BRs was attributed to enhanced free proline content (Ramakrishna, B. and Rao, 2012) [32].

Plants have developed different detoxification strategies to copewith excessive ROS under heavy metal stress. To protect the cells against oxidative damage, superoxide dismutase constitutes the first line of defense to funnel superoxide radicals into $H_2O_2$ and $O_2$. Subsequently, $H_2O_2$ is eliminated by conversion to water by the action of catalase and peroxidases (Gill and Tuteja, 2010) [15]. An increase in the activities of SOD, CAT, POD and APX in geranium plants subjected to Cd stress was observed in the study. Foliar application of EBL further increased the activities of all the antioxidative enzymes in Cd stressed geranium plants. Activation of enzymatic antioxidant mechanism by brassinosteroid was reported in case of radish seedlings under cadmium toxicity (Anuradha and Rao, 2007) [1] and radish plants under zinc toxicity (Ramakrishna and Rao, 2015) [32].

## 4. Conclusions

The present study clearly revealed the cadmium toxicity stress amelioration by 24-epibrassinolide. The metal stress alleviation as conferred by 24-EBL was found associated with activation of antioxidant enzyme system as well as accumulation of proline, an important non-enzymatic antioxidant. The potential of aromatic plants for phytoremediation is recently recognized (Roodi *et al*, 2012; Gupta *et al*, 2013) [34, 16]. The present investigation demonstrated the importance of brassinosteroids in improving the performance of rose-scented geranium in metal stressed soils.

## 5. Acknowledgement

The financial assistance from University Grants Commission, New Delhi, India is gratefully acknowledged.

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