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Amelioration of lead toxicity by 24-epibrassinolide in rose-scented geranium [*Pelargonium graveolens* (L.) Herit], a potential agent for phytoremediation

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Abstract
Effect of 24-epibrassinolide (EBL) on the growth of rose-scented geranium [*Pelargonium graveolens* (L.) Herit] subjected to lead toxicity stress was investigated. Supplementation of EBL substantially alleviated the inhibitory effect of lead on the plant growth. Further, it was observed that EBL feeding negated the deleterious impact of lead toxicity on chlorophyll levels. The studies clearly demonstrated the lead toxicity amelioration capability of epibrassinolide in case of geranium, an aromatic plant with potential for phytoremediation.

Keywords: 24-EBL, lead, toxicity, amelioration, geranium

Introduction
Soil contamination with heavy metals is now a worldwide problem, leading to agricultural losses and hazardous health effects (Singh and Aggarwal, 2005) [1]. Mining, industrial activities, vehicular traffic and agricultural practices contribute to heavy metal contamination of lands, particularly in peri-urban areas (Barman *et al*., 2000) [2]. When heavy metals are present in high levels in the soil and absorbed by the plants, they hamper the growth and productivity of plants. Lead (Pb) is a major heavy metal pollutant in both terrestrial and aquatic ecosystems. Significant increase in the Pb cause sharp decrease in plant productivity (Johnson and Eaton, 1980) [3].

Plant growth regulators play an important role in mitigating the toxic impact of heavy metals on plant growth. Brassinosteroids are now considered as the sixth group of phytohormones in plant kingdom (Rao *et al*., 2002; Clouse, 2011) [4, 5]. Sasse (1997) [6] considered brassinosteroids as plant growth regulators with pleiotropic effects as they influence diverse developemental events such as growth, germination, flowereing, abscission and senescence. Brassinosteroids also confer resistance to plants against biotic and abiotic stresses (Bajguz and Hayat, 2009, Vardhini *et al*., 2010) [7, 8].

Rose scented geranium [*Pelargonium graveolens* (L.) Herit], an important aromatic plant and is the source for the highly valued geraniol and citronellol. In the present study, the effect of 24-epibrassinolide, a bioactive brassinosteroid on the growth of geranium plants subjected to lead toxicity is being investigated.

Materials and Methods
Plant material and Chemicals
Geranium [*Pelargonium graveolens* (L.) Herit] plants were procured from Herbal Garden, Prof. Jayashankar Telangana State Agriculture University, Rajendranagar, Hyderabad and maintained in Botanical Garden, Osmania University, Hyderabad. The bioactive brassinosteroid 24-epibrassinolide (EBL) was purchased from CID tech research Inc, Mississauga, Ontario, Canada.

Growth conditions
Preliminary experiments were conducted employing different concentrations of lead using as lead (II) nitrate [$Pb(NO_3)_2$] and 2mM of lead was selected as metal stress concentration, where growth was considerably but not completely inhibited. Similarly, from a wide range of

concentrations, 2µM concentration of EBL was 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 size plants were sorted out, and one plant each was transplanted to earthen pot containg 10 kg of garden soil and compost in a 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 four treatments maintained for the study were: (i) control (ii) 2µM EBL (iii) 2mM Pb and (iv) 2mM Pb+2µM EBR. 24-epibrassinolide treatment as foliar spray was given on 10th and 25th day. Lead 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.

Growth Parameters

To find out the effect of 24-epibrassinolide on the growth of geranium plants under lead 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 were recorded and expressed in milligrams.

Dry Weight: The plants were dried in the oven at 110^0 C for 24 hours, weight was recorded and expressed in milligrams.

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

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) [9].

Results and Discussion

Lead toxicity considerably reduced the growth of geranium plants. Substantial reduction in height, fresh weight and dry weight observed in plants growing under lead toxicity (Table: 1). However exogenous application of EBL reduced the impact of lead toxicity on the growth of the plants. Epibrassinolide improved the growth of geranium plants exposed to lead toxicity. In an earlier study Swamy and Rao (2008, 2009) [10, 11] obtained substantial increase in the growth of geranium plants due to brassinosteroids application. The impact of lead toxicity was more pronounced on foliage growth as evidenced by reduced leaf area (Table: 3). the decline in leaf area further manifested in overall reduction in plant growth. However, supplementation of epibrassinolide minimized the toxic impact of toxicity and facilitated the restoration of leaf area to a large extent. Such restoration in leaf area in pigeon pea plants challenged with aluminum toxicity was reported by Divyasri *et al* (2016) [12]. In an earlier study Swamy and Rao (2008, 2009) [10, 11] observed enhancement of leaf area in geranium plants due to brassinosteroid application and the present study clearly demonstrated the capability of EBL to improve leaf area even under lead toxicity.

Geranium plants growing under lead toxicity stress showed chlorosis of leaves which was found reflected in lowered chlorophyll content (Table: 2). The content of carotenoids was not much altered due to lead toxicity. EBL feeding to plants growing under elevated lead levels resulted in offsetting the deleterious effects of lead on the chlorophyll content. There was considerable improvement in chlorophyll content in geranium plants receiving EBL supplementation. The positive impact of EBL on chlorophyll content was observed in unstressed as well as lead stressed plants. Such an increase in photosynthetic pigments due to brassinosteroid application was reported in case of radish plants growing under zinc toxicity (Ramakrishna and Rao, 2015) [13]. The increase of photosynthetic pigments in geranium plants under lead toxicity as conferred by epibrassinolide might have improved the rate of photosynthesis leading to stimulation of overall growth. In fact the positive impact of brassinosteroids on photosynthesis was reported in case of radish plants subjected to cadmium stress (Anuradha and Rao, 2009) [14]. The results of the present study clearly demonstrated the alleviation of lead toxicity stress on chlorophyll levels of geranium plants.

Phytoremediation is a novel concept where plants are employed to remove heavy metals from soil. It is clean, ecofriendly, inexpensive and rapid method with immense scope to reclamate heavy metal contaminated soil. Fast growing, high biomass accumulating plants with the ability to tolerate high content of metals in their body are chosen for phytoremediation. However the problem of heavy metal contamination remains the same or even aggravated if edible plants are employed as agents of phytoremediation. Use of edible plants for phytoremediation is not available option. Aromatic plants hold great promise as agents for phytoremediation. As economic products of these plants are volatile substances, the heavy metals which accumulate in foliage or other vegetable parts do not enter into the ultimate economic produce during steam distillation. Heavy metals do not enter the food chain through phytoremediation by aromatic plants (Gupta *et al*., 2013) [15]. Further the growth promoting nature of brassinosteroids coupled with heavy metal tolerance induction can be profitably employed for assisted phytoextraction. The use of geranium plants not only provides a fragment solution to heavy metal toxicity but also adds aesthetic value.

Table 1: Effect of 24-epibrassinolide on plant height, fresh and dry weight of geranium plants.

Treatments	Plant Height (cm)	Fresh Weight (gm)	Dry Weight (gm)
Control	61.35±1.24	70.57±1.03	16.90±0.18
2 µM Ebl	65.45±0.74	83.94±3.18	21.45±2.35
2 mM Pb	48.70±1.39	46.93±1.20	8.91±1.24
2 mM Pb +2 µM Ebl	62.15±0.54	57.31±1.85	14.03±1.02

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

Table 2: Effect of 24-epibrassinolide on photosynthetic pigments of geranium plants.

Treatments	Chlorophyll 'a' (µg/gm FW)	Chlorophyll 'b' (µg/gm FW)	Total Chlorophyll (µg/gm FW)	Carotenoids (µg/gm FW)
Control	743±2	1296±4	2100±2	1045±3
2 µM Ebl	902±1	1318±7	2293±2	1058±5
2 mM Pb	587±3	964±2	1976±4	989±1
2 mM Pb + 2 µM Ebl	681±8	1097±8	1998±1	1003±8

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

Table 3: Effect of 24-epibrassinolide on foliage growth of geranium plants.

Treatments	Area (cm^2)	Length (cm)	Width (cm)	Perimeter (cm)
Control	45.77±1.39	7±0.29	10.14±0.19	34.05±2.54
2 µM Ebl	49.70±2.26	7.85±1.44	9.80±0.02	34.95±10.64
2 mM Pb	28.15±0.24	5.85±0.04	6.97±0.09	23.45±1.14
2 mM Pb + 2 µM Ebl	38.17±3.15	7.13±0.02	8.18±0.34	33.25±0.04

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

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