Mitigating Effect of Brassinosteroids on Lead Stress in Potato (*Solanum tuberosum* L.) on Germination Parameter

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Abstract:

Brassinosteroids (BRs) are a plant steroid that acts at the micro level concentration. Homobrassinolide (HBL) effect in two variety of potato plant (kufri lalima and kufri sadabahar) under heavy metal Lead (Pb) stress, investigation focus on finding the optimal concentration against control (C1 and C2)of HBL employing eight different concentrations (0.1,0.5,1.0,2.0,4.0,6.0.8.0,10.00 ppm) through in screening and found out that kufri lalima, the effects of Pb on potato seedling growth and germination parameter were observed and result show that % of germination is high in lower concentrations 0.1 ppm, 0.5 ppm, and 4.0 ppm, while **CVG** is optimum in 1.0 ppm and 2.0 ppm.**VI is** optimum with 4.0 ppm. **EI** is high with lower concentration 0.1 ppm, 0.5ppm, 1.0 ppm and 2.0 ppm. **RSG** is optimum in 1.0 ppm, 2.0 ppm. **SI** is high in higher concentrations 4.0 ppm, 6.0 ppm, 8.0 ppm and 10 ppm, **GI** is optimum in 2.0ppm. In Kufri Sadabahar, the effects of Pb on potato seedling growth and germination parameter were observed and result show that the% of germination is high in 1.0ppm and 6.0ppm while **CVG** is same in 1.0 ppm and 2.0 ppm compared with C1 and C2 and high in 0.1ppm,0.5ppm.**VI** optimum in 2.0 ppm. **EI** is similar in 2.0 ppm concentration compared with C1. **SI** is high in higher concentrations 4.0 ppm, 6.0 ppm. **GI** is optimum in 1.0ppm.

KEYWORDS* - *Brassinosteroids, HBL, Lead, heavy metal, potato.

Introduction:

Potato (*Solanum tuberosum* L.) are the fourth food stuff consumed by mankind because these foods contain carbohydrates (**Tracey, *et al.*, 2018)**.These foods must meet the safety standards set by WHO. Food safety standards set by WHO for heavy metal content are Fe < 0.4 ppm, Pb < 0.2 ppm, Cd < 0.1 ppm, Cr < 0.1 ppm and Zn < 0.35 ppm **(Taberima, 2016; Zeliha, *et al*., 2017).** Many abiotic and biotic processes can cause soil erosion such as water and wind erosion, salinization and accumulation of chemical contaminants. All are serious environmental problems. A soil quality report indicates that the main soil contaminants indicate including strong persistence in the following situations; environmental high toxicity and bioaccumulation, relatively high mobility and presence in significant amount. Accordingly, heavy metals are of particular importance interested in the assessment of soil quality. A great amount of heavy metals and other chemicals, particularly produced by industries, mining, agriculture, during last twenty years, environmental problems have started to be a part of daily life in several countries (**Yadav, *et al*., 2013)**.

Potato is widely grown in India. In the last 20 years, India has had a considerable increase in potato production due to the suitable soil and climatic conditions and the use of highly productive seeds. In India, a total of 53.03 million tonnes of potato from 2.16 million ha/area with an average yield of 24.55 t/ha last year (2022-23). The potato is planted in every region of India; Uttar Pradesh is the major Potato producing State with 31.26% of production share, followed by West Bengal, Bihar, Gujarat and Madhya Pradesh with 23.29 %, 13.22%, 7.43% and 6.20% share respectively. Heavy metals contaminants can be found on the surface and in the tissues of fresh vegetables **(Arif, *et al*., 2011)**. **Tricopoulos (1997)** revealed carcinogenic effects of several heavy metals such as Cadmium (Cd), Iron (Fe),Lead (Pb), Mercury (Hg), Zinc (Zn) and Nickel (Ni).The Ganga basin is among the most heavily populated areas in the world with an average density of 520 persons/ km^2 **(Das, *et al*., 2012)**. The basin of river Ganga, which has very rich heritage, cultural and religious values, drains about 1060,000 km^2 areas and it is the fifth largest in the world. The river system drains about one-fourth of the Indian subcontinents. In India, the river Ganga passes along 29 class I cities, 23 class II cities and approximately 50 towns because of which different types wastes such as industrial, sewage etc. are released into this mighty river eco-system **(Agrawal, *et al*., 2010;Biswas, *et al*., 2015)**.

In present-day, Industrial effluents and sewage entering the water bodies are one of the prime sources of environmental toxicity, which endangers aquatic biota and deteriorates water quality **(Sinha, *et al*., 2012; Sinha, *et al*., 2016)**.Higher concentrations of heavy metals can form harmful complex compounds, which critically effect different biological functions **(Rajbanshi, 2009)**.Heavy metals distribution in the sediments and sewer river confluence points of river Ganga in Varanasi Mirzapur region were also studied by **(Prasad, *et al*., 1989; Sharma, *et al*., 1992)** studied the heavy metal pollution of River Ganga in the Mirzapur region and came to the conclusion that the river was polluted. **Chaturvedi and Pandey (2006)** analyzed physicochemical parameters as well as few toxic metals of river Ganga at Vindhyachal Ghats of Varanasi. **Gupta *et al*. (2009)** studied the occurrence and bioaccumulation of several heavy metals (Cu, Cr, Cd, Pb, Zn) in the river water, sediment and the muscles of two catfish species procured from the river Ganga at Allahabad.

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According to them the order of occurrence of different heavy metals to be Zn > Pb > Cu > Cr > Cd, respectively. The heavy metals analysis in sediment indicated that among the five heavy metals, Zn was maximally accumulated followed by Pb, Cr, Cu, and Cd. The impact of effluents discharged in Ganga through various sources on chemical composition, energy transformation rate and level of heavy metals was studied at Kanpur and Varanasi by **Kumar *et al*. (2009**).Geo-morphologic facies and granulometric analyses suggest that the sand and silt are the principle constituents of the sediment of the river Ghaghara. The deposited sediment particles at the bottom of the river are non-cohesive and unconsolidated, in an effort to undergo weathering, transportation, and sliding **(Singh, *et al*., 1973).**

Ghaghara River is a lifeline for the people of the districts of Bahraich, Gonda, Faizabad, Azamgarh, Mau Nath Bhanjan, Gorakhpur, Deoria, Ballia, and Saran in north eastern part of Uttar Pradesh and a small part of western Bihar. It is a major left bank tributary of the river Ganga. The total length of the river is about 1080 km and basin area is about 1,27,950 km^2, whereas the average discharge is 2990 m^3/s. Out of the total catchment area, India has only 57,647 km^2, which is 45% of the total area, heavy metals (Cu, Cr, Cd, Pb and Zn) showed higher values in the effluents, at the discharged point and considerable improvement was observed below the discharge zone investigated geochemical environment of the river sediment in the middle stretch of the river Ganga at Ghazipur, Buxar and Ballia urban centres.

The soil of the Ghaghara river basin is alluvial and is exceedingly fertile because of the large quantity of soil transported with the water of the flood. Shifting of the river channel can be seen at many locations within the stretch, it is a specific feature of the river Ghaghara. Cd is a by-product of Zn and Pb mining and smelting, and more mobile in the aquatic environments than most of the other metals and also bio-accumulative. In similar studies for the sediment of the river Ganga it was found that the value of Ni was 42 mg/kg at Ghazipur, 59 mg/kg at Buxar, and 52 mg/kg at Ballia, the river Ganga sediment quality using the same method, Cd concentration was 0.72 mg/kg at Ghazipur and 0.62 mg/kg at Buxar, which was slightly lower, the average estimate of Zn was recorded as 97 mg/kg at Ghazipur urban station, 109 mg/kg at Buxar and 104 mg/kg at Ballia for the stream Ganga, the average estimate of Pb was recorded as 19 mg/kg at Ghazipur, at Buxar it was recorded as 19 mg/kg, while at Ballia the average estimate of Pb was recorded as 21 mg/kg. Comparative values of Pb concentration of the river Ghaghara, The value of Pb was recorded highest at Ayodhya in the study area. This is presumably because of the presence of small scale battery fabricating units at this site **(Singh, *et al*., 2013a).**

Lead (Pb) is one of the most established metals known to man and the greater part of its compounds is noxious in nature. It is found on the earth's crust in an average concentration of 0.1 mg/kg. Pb binds with sulfide ores of Zn, Cu and Pb which is obtained as a by-product during the processing of these ores and is discharged in the surface water through paints, solders, pipes, building material, gasoline, etc. Higher levels of Pb pose eventual threat to fisheries resources, whereas, in plants, it initially results in enhanced growth, but from a concentration of 5 mg/kg onwards, this is counteracted by severe growth retardation, discoloration, and morphological abnormalities.

Brassinosteroids (BRs) are steroidal phytohormones that regulate various physiological processes, such as root development and stress tolerance; Seed germination is a physiological phenomenon, which is controlled at the cellular and molecular level by various growth hormones. Gibberellic acid is the primary hormonal factor responsible for inducing the activity and/or synthesis of α-amylase during seed germination. Application of different analogous of BRs improved seed germination under normal as well as stressed conditions. **Ahmad *et al.* (2012)** concluded on their study on tomato seed germination under phenanthrene stress that seeds treated with different concentrations of 24-epiBL improved seed germination which was consistent with those of nickel and chromium stressed seed germination in (*Brassica juncea* L.). It was concluded that GPA-1 (the alpha subunit of G protein) in *Arabidopsis* indirectly operated on GA pathway to control germination was directly mediated by BRs.

In potato (*Solanum tuberosum* L.) tubers, BRs induced susceptibility towards potato late blight fungus (*Phytophthora infestans* L.) by stimulating mycelial growth and intensity of spore formation of the virus, this induction by BRs was suggested due to weakening of immune status of plant tissues, which was evident by inhibition of wound healing and repair **(Bamnolker, *et al*., 2012)**. **Bajguz (2000)** reported stimulatory effect of BR treatments on PC synthesis in green alga (*Chlorella vulgaris*) cells exposed to lead, which was different for different BRs and maximum effect was by 24-epiBL followed by 28-homoBL, castasterone, 24-epiCS and 28-homoCS.The role of 24-epiBL in improved growth of 100 mg kg−1 (NaCl+Cu) in *Cucumis sativus* L.By up regulation in the antioxidant activities **(Fariduddin, *et al*., 2013)**.

Materials and methods:

The experimental plant is potato (S*olanum tuberosum* L.*)* and two variety of potato have been selected is kufri lalima (red variety) and kufri sadabahar (white variety), which are popular in Uttar Pradesh. The set of experiment were conducted in triplicate at S M M Town P G

College, Botany department, plant physiology lab, Ballia, Uttar Pradesh. The potato seeds were collected from local market of Ballia. For pre-treatment potato seeds were washed thoroughly with tap water and then with de-ionized water to remove any soil and dirt particles. Then the potato seeds were partially dried in air and cut carefully with single healthy eyes areas with the help of stainless steel knife. Then freshly cut potato tubers are soaked in different concentration of BRs overnight. The Stock Solution (10ppm) of 28-homobrassinolide (Double, Godrej Agrovet Ltd, Vikroli, Mumbai) was prepared different concentrations of BRs, i.e. 0.1, 0.5, 1.0, 2.0, 4.0 , 6.0, 8.0, 10.0 ppm against control C1(only with lead) and C2 (without lead). Soaked potatoes were sown in the soil mixture preprated in 3:1 ratio of sand and vermicompost. The plants were grown in rectangular pots (30×15×12), each pot contained 2 kg of soil. The heavy metal Pb $(C_2H_3O_2)_2$ was mixed thoroughly with the soil (0.8g/kg). Five seeds were sown in each pot and each treatment maintains in triplicate. All pots were watered as per requirement to the soil capacity.

The screening experiment had been carried out to check germination and growth parameters, which are as follows-

(1) Germinability (%G) = Total No. of Seeds Germinated 100 x Total No. of Seeds Sown.
(2) Coefficient of Velocity of Germination (**CVG**)**-Kotowski (1926)** CVG=Sum of n x 100/ Sum of (nt); Where, n= No. of seeds emerged on the day, t = time or days of soaking.
(3)Vigour Index (VI) = Germination% x seedling length **(Abdul-Baki and Anderson, 1973)**

(4) Emergence Index (**EI**) was calculated by the formula of by **Baskin (1969).**
EI = (n1/dn1) + (n2/dn2) + (n3/dn3)………. nx /dnx)
Where, n = No. of seeds emerged on the day 1st, 2nd, 3rd …nth day.
dn = No. of days from the day of sowing.
dnx = No. of days to the final count.
(5) Relative Seed Germination (**RSG**) RSG = No. of seeds germinated in the extract x100
No. of seeds germinated in the control

(6) Seedling Index (**SI**) =Seedling growth (SG) = $\frac{\text{Total seedling Growth length in cm}}{\text{No. of germinated seeds}}$

(7) Growth Index (GI) = % seed germination x % root elongation/100 **(Tam and Tiquia, 1994).**

Results and Discussion:

Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Seedling Growth Parameters of Kufri Lalima Variety:

The effects of heavy metal lead (Pb) on potato seedling germination parameter were observed and compared with C1 (with Pb) and C2 (without Pb) under homobrassinolide. The result in variety Kufri Lalima showed that the **% of germination (%G)** with lower concentrations of BRs 0.1 ppm and 0.5 ppm (93.33%) is similar to C1 and C2, while 1.0 ppm and 2.0 ppm showed maximum 100% germination (Table-1). **Coefficient of velocity of germination (CVG)** is optimum in 1.0 ppm and 2.0 ppm (35.71) as compared to C1 and C2 (32.69) (Table-1). **Vigour index (VI) is** optimum with 4.0 ppm (3453.21) as compared to C1 and C2 while 0.1 ppm and 0.50 ppm showed near the value (Table-1). **Emergence Index (EI)** is high with lower concentration 0.1 ppm (5.29), 1.0 ppm (5.13) and 2.0 ppm(5.01) is compared with C1 (4.23) that is similar to 6.0 ppm concentration (Table-1). **Relative seed germination (RSG)** is optimum in 1.0 ppm, 2.0 ppm (107.14) compared with C1and C2 (100) (Table-2). The **seedling index (SI)** is high in concentrations 0.1 ppm (2.76),4.0ppm(2.64),6.0 ppm (2.61),8.0 ppm(2.83) and 10 ppm(3.40) compared with C1 (2.50) and C2(2.42). **Growth index (GI)** is optimum in 2.0ppm (3750.00) compared with C1.In C2 Growth index higher in low concentration (Table-2), respectively.

Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Seedling Growth Parameters of Kufri Sadabahar Variety:

The effects of heavy metal lead (Pb) in potato seedling Kufri Sadabahar were observed and compared with C1 (with Pb) and C2 (without Pb) under homobrassinolide. The result showed that the **% of germination** in concentrations of 1.0 ppm, and 6.0 ppm(93.33%) which is similar to C1 and C2 (Table-3).**Coefficient of velocity(CVG)** is same in 1.0 ppm and 2.0 ppm (32.69) compared with C1 and C2 (32.69) (Table-3).**Vigour index (VI)** optimum in 2.0 ppm (2919.76) compared with C1 (2426.58) and C2 (3079.89) while1.0 ppm (2893.23) and 6.0 ppm (2706.57) showed value this is high in C1 (Table-3). **Emergence Index (EI)** is near about similar in 2.0 ppm (4.07) concentration compared with C2 (4.09) and low C1 (4.62) (Table- 3). **Relative seed germination (RSG)** is similar in 1.0 ppm compared with C1and C2 (100) (Table-4). The **Seedling index (SI)** is high in higher concentrations 2.0 ppm (2.76), 4.0 ppm (3.4),6.0 ppm (3.4) and compared with C1 (1.85) and C2 (2.35) (Table-4). **Growth index (GI)** is same in 1.0ppm (6666.00) compared with C1 (6666.00) (Table-4) respectively.

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Table-1 Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Growth Parameters of Kufri Lalima Variety-

Treatments	**% G**	**CVG**	**VI**	**EI**
C1	93.33	32.69±5.22	3266.55	4.23
C2	93.33	32.69±5.22	3173.22	7.44
0.10	93.33	32.67±5.22	3249.76	5.29
0.50	93.33	32.67±5.22	3170.03	5.18
1.00	100.00	35.71±0.00	3075.01	5.13
2.00	100.00	35.71±0.00	3100.00	5.01
4.00	93.33	32.69±5.22	3453.21	4.12
6.00	86.33	30.94±8.24	2935.22	4.23
8.00	80.00	26.66±0.00	2720.00	2.76
10.00	33.33	11.9 ±4.12	566.61	1.49

GP%: germination percentage, CVG: Coefficient of velocity, VI: Vigour index, EI: Emergence Index

Table-2 - Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Growth Parameters of Kufri Lalima variety-

Treatment	**RSG**		**SI**	**GI**
C1	100		2.50	3499.87
C2	100		2.42	3499.87
0.1	92.85		2.76	2166.50
0.5	92.85		2.15	2166.50
1.00	107.14		2.08	2500.00
2.00	107.14		2.06	3750.00
4.00	100.00		2.64	3499.87
6.00	92.85		2.61	2158.25
8.00	85.71		2.83	2000.00
10.00	35.71		3.40	416.62

RSG: Relative seed germination, SI: Seedling Index, GI: Growth index

Fig- Different conc. Of seedling growth in kufri lalima variety.

Table-3 Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Growth Parameters of Kufri Sadabahar Variety—

Treatments	%G	CVG	VI	EI
C1	93.33	32.69±5.22	2426.58	4.62
C2	93.33	32.69±5.22	3079.89	4.09
0.10	80.00	31.93±7.22	2589.45	3.98
0.50	80.00	31.93±7.22	2679.00	3.81
1.00	93.33	32.69±5.22	2893.23	3.90
2.00	86.66	32.69±5.22	2919.76	4.07
4.00	80.00	31.93±3.08	2760.00	3.94
6.00	93.33	32.69±5.22	2706.57	3.70
8.00	80.00	27.93±3.08	2000.00	2.94
10.00	60.00	21.42±7.22	1860.00	2.31

GP%: germination percentage, CVG: Coefficient of velocity, VI: Vigour index, EI: Emergence Index

Table-4 Effects of Brassinosteroids to Mitigate Lead (Pb) Stress on Germination and Growth Parameters of Kufri Sadabahar Variety-

Treatment	RSG		SI	GI
C1	100		1.99	6666.00
C2	100		2.35	6666.00
0.1	92.85		1.98	5776.75
0.5	92.85		2.83	5776.75
1.00	92.85		2.21	6666.00
2.00	92.85		2.76	5776.75
4.00	85.71		3.40	4666.50
6.00	85.71		3.40	4000.00
8.00	64.28		2.08	2877.37
10.00	42.85		2.07	2777.38

RSG: Relative seed germination, SI: Seedling Index, GI: Growth index

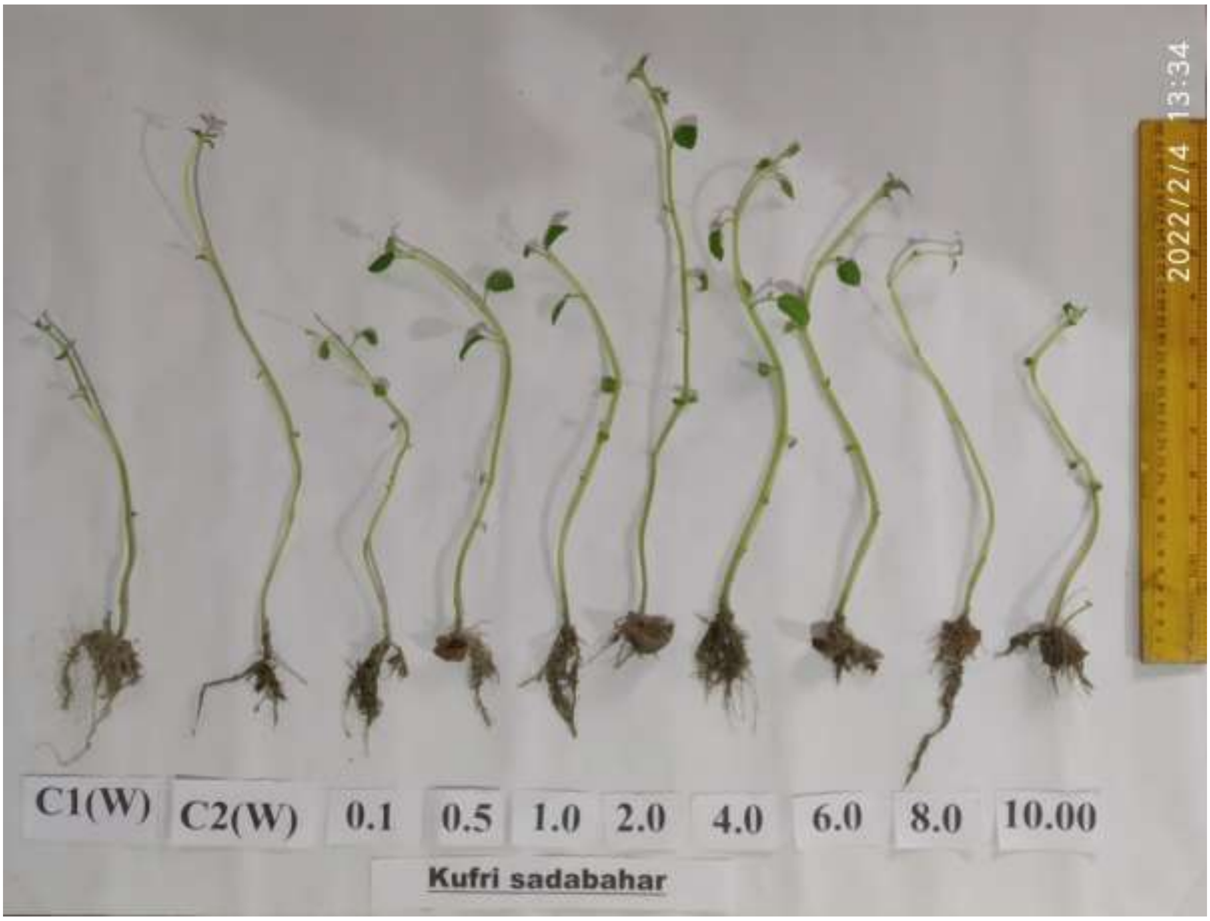

Fig- different conc. of seedling growth in kufri sadabahar variety.

It is well documented that Brassinosteroids promote seed germination. The treatment of the seeds of *Eucalyptus camaldulemis* **(Sasse *et al.*, 1995)** and *Lepidium satives* **(Jones-Held *et al*, 1996)** with BL improved % germination. Similarly, BRs promoted seedling germination in case of rice **(Dong *et al*, 1989 b)**, *Orabanahal minor* (**Takeuchi *et al*, 1997), *B.napus*** (Chang and Cai, 1988), wheat **(Sairam *et al*, 1996;Haya't *et al.*, 2003)**, tomato **(Vardhini and Rao, 2000)**, tomato **(Shagun *et al*, 2011 b)** and mung bean**(Kshitij *et al*, 2011)**. Brassinolide (BL), EBL and HBL promoted seed germination in groundnut seeds **(Vardhini and Rao, 1997)**. Study showed that brassinolide (BL) affects potato seedling that the effects of BL on root development from stem fragments explants as well as on primary root development are dose-dependent 0, 0.01, 0.1, 1, 10, and 100 μg/L BL and that BL application alleviates salt stress on potato seedling by improving root activity, root/shoot ratio and antioxidative capacity in shoots and maintaining K+/Na+ homeostasis in potato shoots and roots by **(Yueqing, *et al*., 2016).**

The present study was designed to evaluate the effect of Pb and combine effect of Pb with brassinolide (BL) on the growth parameter in potato seedling. Pb suppressed the seedling growth of the potato, however potato seedling treatment with BL mitigated the adverse effects of Lead Pb and stimulated the growth of potato seedling growth promotion was associated with enhanced rate of seedling growth in potato seedling plants under Pb stress. The BL treatment also enhanced glutathione reductase activity under stress, from the results it can be concluded that BL could play a positive role in the alleviation of oxidative stress caused by ROS by enhancing antioxidant defense system, thus improving the tolerance of potato seedling plants to Pb stress, Similarly work reported by **(Anuradha S. and Eeta ram rao., 2011)** in Radish (*Raphanus sativus* L.). **Sharma and Bhardwaj (2007)** observed an improvement in the shoot emergence and plant biomass production under Cu stress when mustard seeds were soaked for 8 hours in EBL (10^{-7},10^{-9} and 10^{-11} M) solutions, EBL blocked Cu metal uptake and accumulation in these plants, Lead stress negatively affects germination and seedling growth of mustard. Brassinosteroids improved initial plant growth and development under lead stress BRs decreased lead toxicity mediated through antioxidative enzyme regulation. The effects of 24-epibrassinolide depend on the form of application and concentration used. **(Tássia Fernanda Santos Neri Soares *et al.*, 2020)** tolerance indices and seedling vigor index of L. leucocephala for individuals and combined metal treatment also decreased with increase in concentration of Pb and Cd treatment as compared to control. The more reduction in seedling tolerance indices of L. leucocephala was recorded for combined metal treatment. This reduction was prominent for Pb+Cd at 100 ppm as compared to control treatments **(Muhammad Kabir *et al.*, 2018)**.**Anuradha and Rao (2007)** studied that pre-treatment of 24-epiBL diminish the toxic effects of Pb and increased the relative growth rate in radish seedlings by enhancing the antioxidant enzyme activities. Mitigation of Pb was studied in tomato plants as a result of 24-epiBL mediated increased antioxidant activities **(Rady and Osman, 2012).** Role of 28-homobrassinolide on growth parameters such as plant height, root growth, leaf area, leaf number, leaf weight, fresh and dry weights of *Trigonella foenu-graecum* L. plants subjected to lead toxicity was studied by **(Swamy, *et al*., 2014)**. **Serna *et al*. (2015)** carried out the study to analyze the effects of brassinosteroids on alleviating salt stress in lettuce plants; samples that were separated into shoot and root after 5 days of brassinosteroids application and reduction in weight due to NaCl was observed. 28-homoBL diminishes the effects of salt stress by regulating the efficiency of photosynthesis and seed yield in *Brassica juncea* L. **(Alyemeni, *et al*., 2013)**. Application of 28-homoBL enhanced the superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase activities in salt stressed plants of *Brassica juncea* L.cv *RLC 1*, which was found to provide tolerance against NaCl salt stress **(Kaur, *et al*., 2015)**.

Lead as well as without lead the **% germination** is same and lower concentration of BRs do not mitigate on improve the germination .While higher concentration 1.00 and 2.00ppm try to mitigate effect of lead and improve the germination up maximum (100%). Lead as well as without lead the **CVG** is same and lower concentration of BRs do not mitigate on improve the CVG .While higher concentration 1.00 and 2.00ppm try to mitigate effect of lead and improve the CVG maximum (100%).Lead as well as without lead the **VI** is same and lower concentration of BRs do not mitigate on improve the VI. While in 4.0ppm try to mitigate effect of lead and improve VI. In **EI** is high in lower concentration and also down in higher concentration which combat effect of BRs. **SI** are same result show in both variety high concentration mitigate and improve the SI. Lead as well as without lead the **GI** lower concentration of BRs do not mitigate on improve the GI.

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