Comparison of the Metal Toxicity due to Aluminium and Barium on the Growth Attributes of *Vigna trilobata* (L.) Verde

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Abstract The aim of this study was to investigate the ill effects of Aluminium and Barium on the morphological, biochemical and enzymatic characters of *Vigna trilobata* (L.) Verde which are commonly used in the fireworks industries of this area. After ten days treatment with different concentrations of aluminium and barium (2, 4, 6, 8 & 10mM), the plant exhibited a decline in growth, chlorophyll contents, soluble sugar and protein than in the control. In contrary, the content of free amino acid, proline, leaf nitrate and the activities of enzymes such as catalase and peroxidase were found increased with the increase in the concentration of aluminium and barium while the activity of nitrate reductase were found to be decreased. Comparison of the parameters analyzed between the treated and control reveals that aluminium and barium have seriously affected the *Vigna trilobata* (L.) Verde. Based on this, it was concluded that high concentration of aluminium and barium severely affect the plant growth and result in growth inhibition, decline in physiological and biochemical activities of crop plants.

Keywords Barium, Aluminum, Free Amino Acid, Proline, Catalase and Peroxidase

toxicity is a potential growth limiting factor for plants grown in acid soil in many part of the world [5]. Excess supply of aluminium causes phytotoxic effect [6]. Furthermore, it may cause indirect toxic effects by replacing essential nutrients at cation exchange sites in plants [7]. Barium is phytotoxic to plants at submillimolar (or) millimolar levels and is thought to act through inhibition of K uptake by the roots. The various harmful effects of barium in cereals, include reduction in germination, root-shoot length, changes in the activity of various enzymes and poor grain yield [8]. The effects of barium on plant growth are lethal even as small concentrations can retard growth [9]. This study is aimed to find out the effect of various concentrations of aluminium and barium on *Vigna trilobata* (L.) Verde on the morphometric, pigmental, biochemical and enzymatic characteristics of *Vigna trilobata* (L.) Verde. In the present day context our study is very crucial because the soil in our area remains highly polluted due to aluminium and barium indiscriminately used in fireworks industries which effect most commonly cultivated *Vigna trilobata* (L.) Verde. - the chief crop of locals. Thus there is an urgent necessity to understand the ill effects of aluminium and barium on the crop plants mentioned and the necessary steps to be taken to overcome the problem.

1. Introduction

Exposure to heavy metal toxicity has become a major limiting factor in the growth of crop plants, affecting the sustainability of agricultural production [1]. Large parts of agricultural soil are contaminated with heavy metals by natural and anthropogenic activities. Heavy metals accumulated in excess in plant tissues, cause alteration in various physiological processes such as transpiration, photosynthesis and photosynthetic electron transport, biosynthesis of chlorophyll as well as cell membrane integrity [2]. Aluminium (Al) is not regarded as an essential nutrient, but at low concentrations sometimes increases plant growth or induces other desirable effects [3] [4]. Aluminium

2. Material and Methods

Both control and experimental plants were allowed to grow in soil garden soil. After seven days, the seedling of *Vigna trilobata* (L.) Verde. were treated with various concentrations of aluminium and bariumchloride such as 2, 4, 6, 8 & 10 mM (mM - milimolar) individually. Ten days after that, the morphometric, pigmental, biochemical and enzymatic characters were analyzed.

The morphometric characters such as shoot length and root length were measured by scale and expressed in centimeter which is the unit of measurement length. The leaf area was measured by conventional graphical method by

drawing the outline of the leaf in a graph sheet and counting the small and big squares within it and represented in cm^2 . The fresh weight and dry weight of the seedlings were found out using an electronic balance.

The pigmental characters such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids are estimated by conventional method [10].

Of the biochemical characteristics, the total soluble sugars was measured after centrifugation and further treating the tissue using tri-chloro acetic acid and anthrone as the indicator. The amino acid content was measured by homogenation of leaf tissue in ethanol using ninhydrine as the indicator [11]. The protein content was measured by centrifugation of ground leaf tissue in tri-chloro acetic acid, folin phenol with alkaline copper mixture [12]. The proline was estimated by centrifugation of fresh leaf tissue in sulphosalicylic acid, glacial acetic acid and finally separating with toluene [13].

The *In vivo* Nitrate Reductase (NR) activity was assayed according to Jaworski [14] method with modification. Fresh leaf material (100mg) was incubated in scintillation vials containing 5 ml of incubation medium composed of, 100mM KH_2PO_4 KOH buffer pH 7.5, 200mM KNO_3 , 1% (v/v) n-Propanol and 0.1%(v/v) Triton X 100. Incubation was carried out in dark for one hour at room temperature with occasional shaking. Aliquots of 0.5 ml was taken from the vials and analysed for nitrite formed after 1 hour incubation. To 0.5 ml distilled water, 1 ml of 3% sulphanilamide and 1 ml of N-1-N (Naphthyl ethylene-diamine dihydrochloride) were added in quick succession. Fifteen minutes were allowed for colour formation and absorbance was measured at 540 nm. The nitrite was estimated with the help of a standard nitrite curve. The activities of peroxidase and catalase enzyme were estimated from, the leaves of experimental plants weighing about 1 g ground in 5 ml of 100 mM (mM - milimolar) phosphate buffer (pH 6.0) filtered through a three layered cheese cloth and spun at 3000 rpm for 30 minutes. The supernatant obtained was served as the source for crude enzymes such as peroxidase and catalase.

To assay peroxidase activity, the enzyme extract was added to pyrogallol which gets oxidized to a coloured derivative in the presence of hydrogen peroxide (1% (v/v)). The amount of purpurogallin formed during the reaction was

assayed spectroscopically [15]. To 2 ml of pyrogallol phosphate buffer (0.058 M pyrogallol dissolved in 0.1 M phosphate buffer pH 6.0), an aliquot of 0.1 ml of enzyme extract was added. Then, absorbance was set to zero at 420nm. To this, 0.5 ml of H_2O_2 (1% (v/v)) was added. Then, the content was thoroughly mixed and the absorbance was measured using systronics model no. 106 spectrophotometer. The difference in the absorbance at an interval of 20 seconds for a period of 3 minutes was measured. The peroxidase activity was expressed as moles of H_2O_2 reduced per unit enzyme per unit time.

To assay the catalase activity, 3 ml of phosphate buffer was added to 1 ml of H_2O_2 and 1 ml of enzyme extract [16]. The reaction mixture was incubated at 25°C for 1 minute. The reaction was terminated by the addition of 1 ml of H_2SO_4 . The reaction mixture was titrated against 0.01 N KMnO_4 . The end point was the persistence of pink colour at least for 15 seconds. The catalase activity was expressed in micromoles H_2O_2 catalysed.

3. Statistics Analysis

The growth parameters were determined with ten independent replicates. Biochemical characters and enzymatic assay were carried out for five times and the average was considered the data reported as mean $\pm$ SE and with figures in parentheses represent the per cent activity. Statistical analysis (One way ANOVA – Tukey test) was applied using the statistical package, SPSS 16.0.

4. Results

Vigna trilobata (L.) Verde seedlings grown in different concentration of aluminium and barium exhibited inhibition in root and shoot length, leaf area, fresh weight, dry weight, with shoot being affected more than the root. After 10 days of treatment, a significant reduction of 55%, and 32% and 63%, 34% in root growth and shoot growth respectively because of aluminium and barium was noticed under 10Mm concentration (Table 1). Similarly leaf area, fresh weight and dry weight also significantly decreased to tune of 25%, 34%, 41%, and 37%, 52%, and 52% respectively at and 10mM concentration of aluminium and barium respectively.

Table 1. Effect of various concentration of aluminium and barium on the morphometric characteristics of *Vigna trilobata* (L.) Verde (Black gram)

S.NO	Growth parametre	Concentration of heavy metals										
		Control	2mM		4mM		6mM		8mM		10mM	
			Al	Ba	Al	Ba	Al	Ba	Al	Ba	Al	Ba
1	Root length (cm)	9.62±0.01 (100) a*	8.67±0.05 (96)	8.1±0.11a* (93)	7.71±0.11 a* (90)	7.5±0.01a* (88)	6.03±0.10a* (82)	5.98±0.02a* (80)	3.57±0.04a* (53)	3.01±0.05a* (46)	2.98±0.02a* (45)	3.84±0.32 a* (37)
2	Shoot length (cm)	18.06±0.02 (100)	17.02±0.01a* (94)	16.79±0.04a* (90)	16.79±0.28 a* (93)	16.02±0.08a* (89)	16.3±0.14a* (90)	15.66±0.11a* (87)	15.67±0.01a* (87)	12.01±0.01a* (67)	12.37±0.19a* (68)	11.37±0.10a* (66)
3	Leaf area	8.99±0.21 (100)	7.91±0.09a* (89)	6.88±0.01a* (84)	7.9±0.13 a* (85)	7.81±0.31a* (80)	5.83±0.02a* (83)	4.97±0.01a* (78)	5.29±0.11a* (76)	3.11±0.01a* (69)	4.14±0.09 * (75)	2.96±0.01a* (63)
4	Total fresh weight (gm)	1.75±0.008 (100)	1.53±0.01a* (88)	1.49±0.01a* (74)	1.45±0.07 a* (83)	1.41±0.07a* (68)	1.27±0.02a* (70)	1.33±0.09a* (54)	1.27±0.02a* (69)	1.001±0.02a* (50)	1.15±0.02a* (66)	0.998±0.11 a* (48)
5	Total dry weight (gm)	0.308±0.1 (100)	0.236±0.01a* (77)	0.211±0.01a* (71)	0.212±0.01 a* (68)	0.20±0.02 * (64)	0.191±0.01a* (62)	0.190±0.03a* (61)	0.191±0.04a* (62)	0.167±0.01a* (53)	0.185±0.07a* (59)	0.181±0.05 a* (48)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE. a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ - Tukey test). a# - refers to non - significant. Al - Aluminium; Ba - Barium; mM - Milimolar; cm- centimeter

The effect of different concentration of aluminium and barium on photosynthetic pigments is depicted in Table. 2. Photosynthetic pigments were significantly decreased with increasing level of aluminium and barium. The reduction of chlorophyll *a*, *b*, total chlorophyll and carotenoids was 52%, 58%, 45% and 61% and 56%, 61%, 64%, and 73% respectively at 10mM aluminium and barium treatment compared to the control. Aluminium and barium also modified the total soluble sugar and protein content in *Vigna trilobata* (L.) Verde leaves (Table. 3). Under the 10mM aluminium and barium concentration the decrease in the total soluble sugar and protein was 53% and 31% and 59% 40% respectively. However the content of the free amino acids, proline and leaf nitrate significantly increased with increasing concentration of aluminium and barium.

Table 2. Effect of various concentrations of aluminium and barium on the pigments content of *Vigna trilobata* (L.) Verde (Black gram)

S. NO	Growth parametre	Concentration of heavy metals											
		Control	2mM		4mM		6mM		8mM		10mM		
			Al	Ba	Al	Ba	Al	Ba	Al	Ba	Al	Ba	
1	Chlorophyll a (mg/g LFW)	3.36±0.01 (100)	3.19±0.1 a* (93)	2.92±0.03 * (88)	2.49±0.01 a* (78)	2.41±0.02 a* (73)	2.30±0.13 a* (65)	2.21±0.11 a* (60)	2.17±0.3 a* (55)	1.98±0.01 a* (52)	1.94±0.03 a* (48)	1.11±0.05 a* (44)	
2	Chlorophyllb (mg/g LFW)	3.98±0.1 (100)	2.67±0.31 a* (94)	2.36±0.31a* (91)	2.44±0.5 a* (71)	2.15±0.05a* (78)	2.31±0.21 a* (62)	2.0±0.03a* (66)	1.99±0.03 a* (52)	1.53±0.09 a* (47)	1.63±0.01 a* (42)	1.18±0.1 a* (39)	
3	Total Chlorophyll content (mg/g LFW)	5.34±0.081 (100)	4.633±0.11 a* (79)	4.6±0.08 a* (78)	4.44±0.04 a* (73)	4.39±0.02a* (69)	4.15±0.12 a* (64)	4.17±0.01a* (65)	3.94±0.06 a* (58)	3.52±0.08 a* (49)	3.83±0.11 a* (55)	3.113±0.01 a* (34)	
4	Total carotinoid content (mg/g LFW)	1.71±0.02 (100)	1.59±0.01 a* (85)	1.51±0.01 a* (80)	1.47±0.02 a* (73)	1.43±0.02a* (71)	1.35±0.01 a* (57)	1.3±0.01a* (53)	1.26±0.01 a* (46)	1.21±0.01 a* (40)	1.17±0.02 a* (39)	1.08±0.02 a* (27)	

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE. a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ - Tukey test). a# - refers to non - significant. Al - Aluminium; Ba - Barium; mM - Milimolar; Al - Aluminium; Ba - Barium; mM - Milimolar; cm- centimeter; mg - milligram; g- gram; LFW - Leaf fresh weight

Table 3. Effect of various concentrations of aluminium and barium on the biochemical characteristic of *Vigna trilobata* (L.) Verde (Black gram)

S. NO	Growth parametre	Concentration of heavy metals										
		Control	2mM		4mM		6mM		8mM		10mM	
			Al	Ba	Al	Ba	Al	Ba	Al	Ba	Al	Ba
1	Total soluble sugar (mg/g LFW)	6.46±0.1 (100)	5.94±0.01 a* (92)	5.89±0.1 a* (89)	5.82±0.03 a* (80)	5.76±0.09 a* (72)	5.15±0.21 a* (68)	5.15±0.3 a* (68)	4.9±0.05 a* (58)	4.68±0.05 a* (50)	4.37±0.31a* (47)	4.13±0.31 a* (41)
2	Total soluble protein (mg/g LFW)	27.79±0.1 (100)	27.21±0.41a* (95)	26.87±0.4 a* (90)	26.63±0.21 a* (92)	24.91±0.11a* (81)	25.43±0.14a* (85)	21.88±0.16 a*(70)	22.78±0.01a* (79)	21.03±0.21 a* (65)	21.23±0.02 a* (69)	20.65±0.01 a* (60)
3	Total free amino acid (mg/g LFW)	0.34±0.1 (100)	0.98±0.3 a* (165)	0.81±0.04a* (156)	1.74±0.02 a* (256)	1.70±0.1 a* (250)	2.45±0.16a* (321)	2.14±0.1 a* (318)	2.99±0.01 a* (385)	2.92±0.31 a* (380)	4.01±0.02 a* (410)	2.99±0.1 a* (390)
4	Proline content (mg/g LFW)	0.9±0.10 (100)	0.992±0.1 a* (155)	1.11±0.1 a* (166)	1.451±0.13 a* (213)	1.921±0.11a* (210)	2.43±0.1 a* (290)	2.39±0.14 a* (285)	3.03±0.31 a* (364)	2.534±0.11 a* (356)	3.13±0.2 a* (385)	3.11±0.13 a* (380)
5	Leaf nitrate (mg/g LWF)	1.34±0.10 (100)	2.73±0.04a* (145)	2.87±0.19 a* (156)	3.11±0.02 a* (228)	3.09±0.02a* (212)	3.16±0.4 a* (275)	3.45±0.1 a* (258)	3.59±0.04 a* (364)	4.04±0.09 a* (393)	4.98±0.3 a* (401)	4.34±0.02 a* (411)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE. a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ - Tukey test). a# - refers to non - significant. Al - Aluminium; Ba - Barium; mM - Milimolar; Al - Aluminium; Ba - Barium; mM - Milimolar; cm- centimeter; mg - milligram; g- gram; LFW - Leaf fresh weight

The result of the present study shows that, *in vivo* NR activity of the leaves was significantly inhibited to 24%, 26% at 10mM concentration of aluminium and barium. There was a tremendous increase rise in catalase and peroxidase activity with significant increase in different aluminium and barium treatments (Table. 4). The increase was about 190%, 175%; 31%, 33 at 6mM respectively.

Table 4. Effect of various concentrations of aluminium and barium on the enzyme activities of *Vigna trilobata* (L.) Verde (Black gram)

S. NO	Growth parametre	Concentration of heavy metals										
		Control	2mM		4mM		6mM		8mM		10mM	
			Al	Ba	Al	Ba	Al	Ba	Al	Ba	Al	Ba
1	In vivo NR activity (μ mole/g FW)	24.16±0.05 (100)	23.25±0.1 a* (90)	22.01±0.13a* (87)	21.49±0.11 a* (80)	21.99±0.21 a* (87)	19.96±0.05a* (87)	19.99±0.13a* (79)	18.31±0.05a* (77)	18.94±0.01 a* (75)	17.46±0.11 a* (76)	17.46±0.1 a* (74)
2	Catalase activity (μ mole/g FW)	0.013±0.1 (100)	0.094±0.1 a* (183)	0.09±0.3 a* (190)	0.162±0.02 a* (250)	0.173±0.01 a* (263)	0.288±0.13a* (290)	0.22±0.2 a* (275)	0.3±0.17a* (365)	0.37±0.11 a* (388)	0.411±0.01 a* (401)	0.39±0.03a* (381)
3	Peroxidase activity (μ mole/g FW)	1.83±0.013 (100)	5.01±0.24a* (123)	5.5±0.04a* (125)	6.18±0.04 a* (128)	7.18±0.14 a* (129)	11.25±0.01a* (131)	14.25±0.02a* (133)	17.31±0.01a* (138)	15.3±0.02 a* (134)	19.44±0.11 a* (141)	16.44±0.1 a* (136)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE. a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ - Tukey test). a# - refers to non - significant. Al - Aluminium; Ba - Barium; mM - Milimolar; Al - Aluminium; Ba - Barium; mM - Milimolar; cm- centimeter; mg - milligram; g- gram; LFW - Leaf fresh weight

5. Discussion

In the present study, the different concentration of aluminium and barium caused a significant reduction in root length, shoot length, leaf area, fresh weight and dry weight of the plant with increased metal concentration. Similar report was observed by Minakshiet al. [17] in *Ocimum tenuifolium* under heavy stress. Sevugaperumal et al. [18] reported that higher concentration of aluminium reduced the root length, shoot length, leaf area, fresh weight and dry weight in *Vigna radiata* L. falling in the line with the findings of Mahapatra, et al. [19] in *Vigna radiata* L. According to Shafiq et al. [20] reduced root length is due to reductions in both new cell

formation and cell elongation in the extension region of the root. The reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress [21].

The photosynthetic process is related to biomass accumulation, which in turn, relies upon the pigment level. The chlorophyll content, which is an indicator of the photosynthetic activity of plants, showed a remarkable reduction in aluminium and barium treated plants. The decrease in these plant pigments may be due to cellular disorganization under heavy metal toxicity which causes agglutination of chloroplast [22]. Reduced Leaf chlorophyll and carotenoid contents with the application of aluminium

and barium in the present study is in accordance with the earlier reports of Boddi *et al.* [23][24][25] [26]. The decrease in chlorophyll content was also reported in sunflower [27] and in almond [28].

The decrease in total soluble sugar content coincides with the photosynthetic inhibition or stimulation of respiration rate [29]. Sarma *et al.* [30] reported that the reduction of sugar content may be the result of the minimum uptake of phosphate. Reduction in protein level observed in the present study may be attributed to the decrease in protein synthesis or due to the denaturation of the enzymes involved in the synthesis of proteins [31]. The reduction in soluble protein level with associated increase in free amino acids under stress may be attributed both to the disruption in protein synthesis [32] as well as to the enhanced proteolysis [33]. Reduced protein content may be due to either inhibition of protein synthesis by Orcutt and Nilsen, [34] or unavailability of essential component amino acids [35] or inhibition of amino acids mobilization to the site of protein synthesis [36]. There was a linear increase in free proline accumulation with increasing metal concentrations in the leaf of *Abelmoschus*, *Brassica* and *Amaranthus*. Proline accumulation is considered as an adaptive response to stress [37]. Accumulation of proline has frequently been used as a biochemical marker for water stress in plants [38]. Proline accumulation is considered a protective device for the plants to preserve water, which is necessary to tide over any internal water deficit situation [39]. The leaf nitrate content was analysed and found to be more in all the heavy metal treated plants. The accumulation of leaf nitrate content was found to be associated with a reduction in *in vivo* nitrate reductase (NR) activity. Similar increase in leaf nitrate content, reduction in *in vivo* NR activities with increased in concentration of cadmium chloride on *Vigna radiata* has been reported earlier [40]. The increased peroxidase activity caused a major impact on the chlorophyll degradation [41]. Catalase is another antioxidant scavenging enzyme. Catalase is special type of peroxidative enzyme which catalyses the degradation of H_2O_2 which is a natural metabolite toxic to plants [42]. The mechanism involved for reducing toxicity may be due to chelating of the metal ions by the ligands, which include organic acids, amino acids, peptides or polypeptides [43].

6. Conclusions

Conclusively, our results show that aluminium and barium at high concentration exceeding 2mM decreased the growth, chlorophyll content, protein and sugar content but increased the content of proline, free amino acid & leaf nitrate. Our result suggest that the presence of aluminium and barium in low concentration is good for *Vigna trilobata* (L.) Verde growth but in high concentration make the soil toxic to the crop plants and result in growth inhibition, structural damage, decline in physiological and biochemical activities of crop plants.

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