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Studies of Aluminum (Al_2O_3) Stress with biochemical parameters of *Vigna radiata*, L seedling.

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Abstract

Experimental Study of Aluminum (Al_2O_3) Stress on morphology and pigments of *Vigna radiata*, L. seedlings was conducted with the treatment concentrations being 200, 400, 600, 800 and 1000 mg/L for 7 days. The Low concentration has affected the biochemical parameters slightly, but increase in treatment decreased the parameters like Amino Acids (Root), Protein (Shoot) and DNA (Shoot). The Protein content in Root and RNA content in Shoot did not respond to the treatment of Al_2O_3 to mung seedlings. However, there was increased response of Sugar (Root and Shoot), DNA and RNA (Root) contents. Overall, The Al_2O_3 has a little effect on biochemical parameters studied in the seedlings of *Vigna radiata*, L.

Keywords: Aluminum, Mung, Biochemistry, Seedlings, stress.

Introduction

Aluminum (Al) is present in water, soil and air but most of it is incorporated into alumino silicate soil minerals and only very small quantities (at sub micro molar levels) appear in soluble forms capable of influencing biological systems (May and Nordstrom, 1991).

Most plants contain no more than 0.2 mg Al g^{-1} dry mass. However, some plants, known as Al accumulators, may contain over 10 times more Al with-out any injury. Aluminum is not regarded as an essential nutrient, but low concentrations can sometimes increase plant growth or induce other desirable effects [Foy, 1983; Foy and Flemming, 1982; Foy et al., 1978].

Generally, Al interferes with cell division in root tips and lateral roots, increases cell wall rigidity by cross linking pectin, reduces DNA replication by increasing the rigidity of the DNA double helix, fixes phosphorous in less available forms in soils and on root surfaces, decreases root respiration, interferes with enzyme activity governing sugar phosphorylation and the deposition of cell wall polysaccharides and the uptake, transport, and also use of several essential nutrients (Ca, Mg, K, P and Fe) [Foy, 1992].

Aluminum does not affect the seed germination but helps in new root development and seedling establishment [Nosko et al., 1988]. Root growth inhibition was detected 2–4 days after the initiation of seed germination [Bennet et al., 1991].Severa1 reviews on AI toxicity are available [Haug, 1984; Taylor, 1988; Rengel, 1992a]; here we limit our discussion to the sites of Al toxicity in higher plants. Al ions are taken up by plants mostly through the root system, and only small amounts penetrate the leaves. Most authors now agree that generally the active metal up-take processes involve ion-specific carriers with energy expenditure but a specific Al carrier has not yet been found.

The effect of aluminium on plants is complex and can act directly on plant cell processes (Taylor, 1991) or indirectly by interfering with plant nutrition (Roy et al., 1988; Taylor, 1991). Aluminum toxicity and differential Al tolerance in various plant groups were reported in some studies [Anderson, 1988; Foy, 1988; Hai et al., 1989; Rhue, 1979; Roy et al., 1988; Taylor, 1988]. Differential aluminum tolerance to different wheat cultivars were reported by Foy et al, (1967); Slootmaker (1974); Konazak et al., (1976) Aniol and Kaczkowski, (1979). Aniol, (1983), Aniol, (1984) had analyzed the Al tolerance in wheat by breeding.

Keeping a view on the above work, here we have made an attempt to find out the toxicity related stress in mung seedlings treated with $Al_2 O_3$ with biochemical parameters like Amino Acids, Protein, Sugar and Nucleic acids(DNA and RNA)..

Materials and Methods

Test Chemical & Concentration:

The test chemical, Aluminum oxide (Al_2O_3) was used in the seedling stress study was of AR grade and the concentrations selected were 200,400,600,800 and 1000 mg/L of test chemical. The concentrations were chosen basing on our earlier LC 50 study. (Mahapatra et al, 2015)

Experiments were conducted in petriplates (6'') with cotton and blotting paper soaked with different concentrations of Aluminum oxide (Al_2O_3). 15 healthy seeds were used to each petriplate to study the % percentage of germination after 24 to 72 hours.

The control set was kept with Al_2O_3 free environment. In each concentration of $Al_2 O_3$, three replicate were taken to find out the % of germination of seeds. The seed germinator (Remi , C-6) was used in experimentation with 25±2^o C temperature 90% humidity and 12 hours light cycle exposure.

Test Organism: The prime pulse seed *Vigna radiata*,L. var.PDM 139, Samart commonly used in eastern state of India, particularly Odisha State has been chosen for study. Healthy seeds of radiation were obtained from OUAT Extension Centre, Ratnapur, Ganjam for the experimentation.

Parameters Evaluated: The seedling parameters studied were root length, shoot length. R/S ratio of the seedlings after treatment and seedling growth period of 7 days. In Biochemical studies, Amino acids, Protein, DNA and RNA, Carbohydrates contents of shoot and root of 7 day old seedlings were analyzed.

1 .Estimation of Protein: 200mg of material (shoot/root) of each treated and control sets were grinded separately in mortar and pestle with 80% Acetone. The homogenate was centrifuged at 10000 x g for 20 minutes. The supernatant was discarded. To the residue 4 ml of 1 N, NaOH was added and kept in refrigerator overnight. Then it was kept in boiling water bath for 20 minutes, cooled and centrifuged at 3000 x g for 10 minutes. The supernatant was collected for protein estimation following the method of Lowry *et al.*, (1951). Bovine serum albumin was used as the reference standard.

2. Estimation of Amino Acid: 100 mg of material (shoot/ root) of each treated and control sets were grinded separately in mortar and pestle with 70% ethanol. The homogenate was centrifuged at 3000 x g for 10 minutes and the supernatant was collected in test tube. The residue was again treated with 70% alcohol and centrifuged. The supernatant was collected and mixed with the previous supernatant. Both the supernatant together was made to a volume of 10 ml by adding 70% alcohol. The Amino Acid content was determined by Ninhydrin method of Moorie and Stein (1948). Glycine was used as Amino Acid standard.

3. Estimation of soluble sugar: 100 mg of plant material (shoot/ root) of each sets including control was taken and grinded in mortar and pestle separately with 70% alcohol (ethyl alcohol). The homogenate was centrifuged at 3000xg for 10 minutes and the supernatant was collected in test tubes. The residue was again treated with 70% ethanol and centrifuged and the two supernatants were pooled together. The ethanol was evaporated and made to 1 ml volume. It was then diluted to 10 ml with distilled water. The sugar content of aqueous extract was measured by adding Anthrone reagent (Yoshida *et al.*, 1972). A standard curve was prepared by taking Maltose.

4. Estimation of DNA: After collection of the supernatant for estimation of soluble sugar or Amino Acid, the residue was taken to estimate Nucleic Acid. To the residue 1 ml of 5% Trichloro Acetic Acid was added and kept in refrigerator overnight. The next day it was centrifuged at 5000 x g for 10 minutes, the supernatant was discarded and the residue is used for DNA estimation by adding 5 ml of 10% TCA. It was boiled in water bath, and then cooled, centrifuged and from the supernatant the DNA content was estimated

by adding Diphenyl amine following the method of Schmeider (1957) using Calf thymus DNa as standard.

5. Estimation of RNA: The supernatant collected for DNA estimation was also taken for estimation of RNA content by adding 3 ml orcinol reagent to 1 ml of supernatant following the method of Schmeider (1957).

Results

The results obtained after estimation of biomolecules like Protein, Amino Acids, Sugar, DNA and RNA expressed in terms of mg/g fresh tissue (Shoot and Root) of mung bean seedlings are given in Fig. No. 1-10.

In case of protein content (Fig No.1 and 2) , the shoots of the treated seedlings showed a decreasing trend with the treatment where as root did not respond at low concentration of Al , but was slightly elevated at highest concentration of Al (1000 mg/L).The Amino acid content in root(Fig. No.3) of the mung seedlings showed a decreasing trend with the increase in Al treatment, but shoot (Fig. No.4) amino acids decreased upto 400mg/L Al and then an irregular trend of increase followed by decrease again.

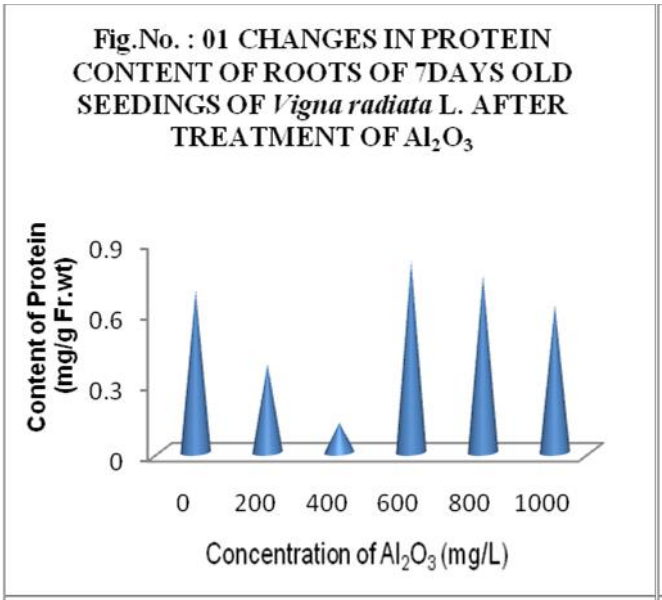


Fig.No. : 01 CHANGES IN PROTEIN CONTENT OF ROOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

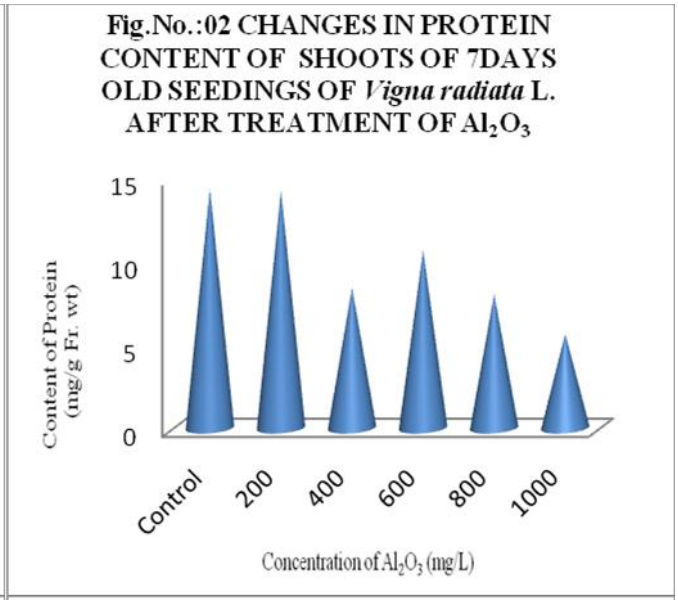


Fig.No.:02 CHANGES IN PROTEIN CONTENT OF SHOOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

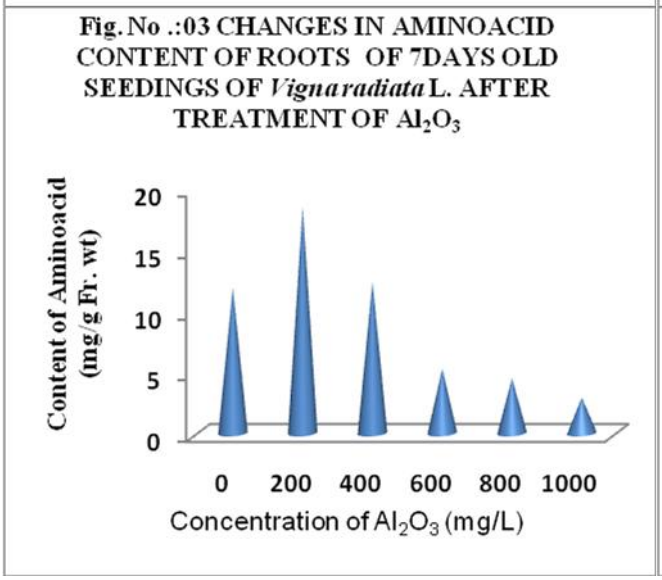


Fig. No .:03 CHANGES IN AMINOACID CONTENT OF ROOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

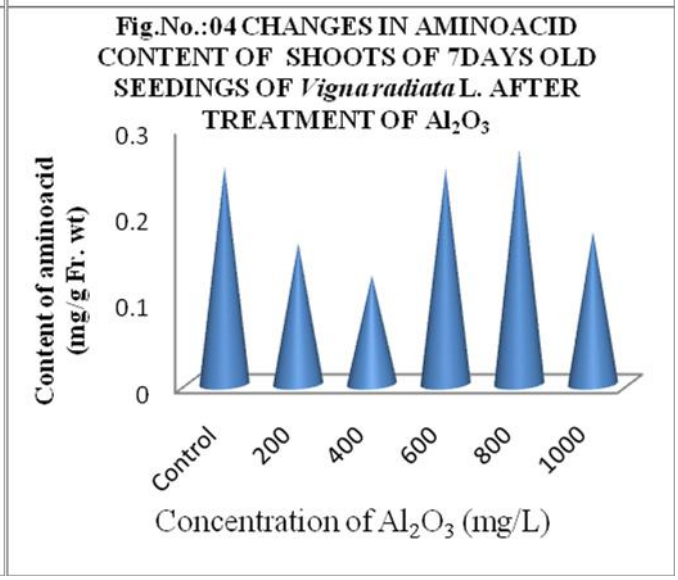


Fig.No.:04 CHANGES IN AMINOACID CONTENT OF SHOOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

In the study of Sugar content (Fig. No.5 and 6), both shoot and root showed an increase in Sugar content at different Al concentration than control values but the effect was very low.

In case of DNA content, the roots (Fig. No.7) of treated seedling showed no response of any change in content in comparison to control values but, in case of Shoot, first there was a increase and followed by a decreasing values (Fig. No.8).The response of RNA was a many fold increase than control values in case of root (Fig. No.9) but, shoots of the seedlings showed an irregular trend.

Fig .No.: 05 CHANGES IN SUGAR CONTENT OF ROOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

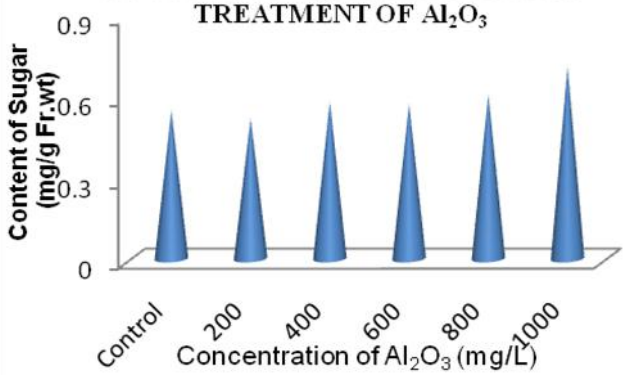


Fig.No.:06 CHANGES IN SUGAR CONTENT OF SHOOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

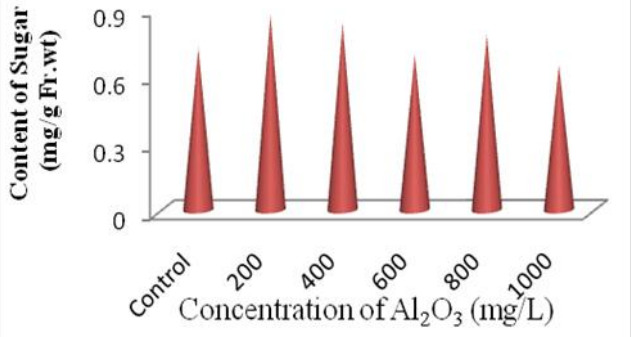


Fig.No.:07CHANGES IN DNA CONTENT OF ROOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

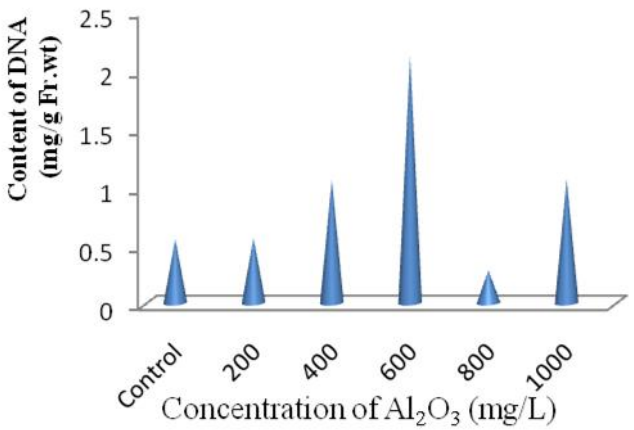


Fig.No.:08CHANGES IN DNA CONTENT OF SHOOTS7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

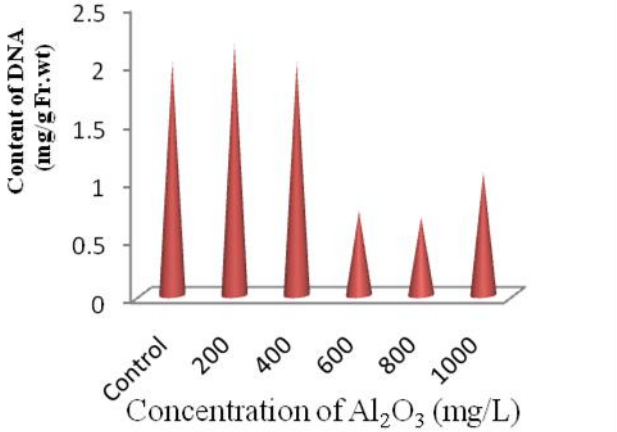


Fig.No.:09CHANGES IN RNA CONTENT OF ROOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3

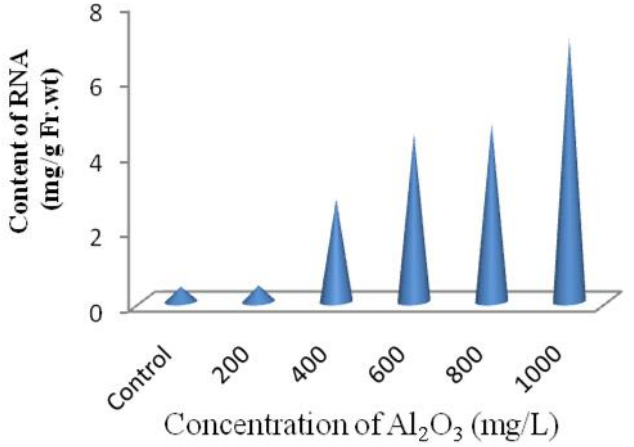
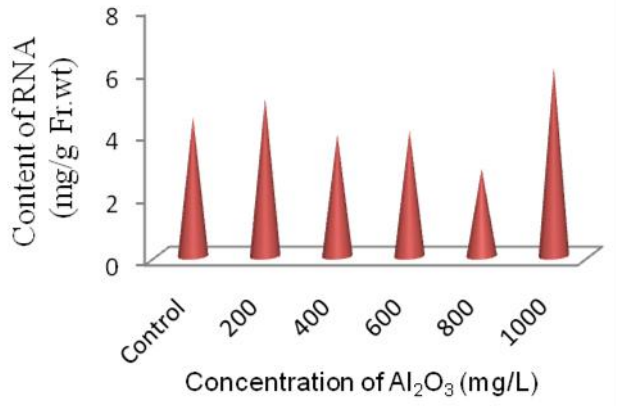


Fig.No.:10 CHANGES IN RNA CONTENT OF SHOOTS OF 7DAYS OLD SEEDINGS OF *Vigna radiata* L. AFTER TREATMENT OF Al_2O_3



Discussion

The common responses of shoots to Al include: cellular and ultra structural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot bio-mass (Thornton et al., 1986).

Al apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko et al., 1988).Many trivalent cations are toxic to plants and, because Al toxicity is largely restricted to acid conditions, it is generally assumed that $Al_{3'}$ is the major phototoxic species. Some researchers have considered the interaction between Al and the membranes of root cells (Grauer and Horst, 1992; Kinraide et al., 1992), and this approach makes sense because regardless of what is happening in the surrounding solution, it is this interaction that will ultimately determine the degree of stress.

The root apex (root cap, meristem, and elongation zone) accumulates more Al and attracts greater physical damage than the mature root tissues In general; many plant species are resistant or can be tolerant to certain amounts of metals. This is probably achieved through trapping of these metals with metal-binding proteins. Many of the biochemical effects of Al on plants is probably associated with the alteration of root membrane structure and function (Foy, 1992).

Inhibition of root and shoot growth is a visible symptom of Al toxicity. The earliest symptoms concern roots. Shoots in contrast to the situation observed for Mn toxicity are less affected (Chang et al., 1999) Young seedlings are more susceptible than older plants. Al apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko et al., 1988).This review discusses recent information on aluminum toxicity with an emphasis on plant response to Al stress. Al is reported to interfere with cell division in root tips and lateral roots, increase cell wall rigidity by cross linking pectins, reduce DNA replication Although productions of phytochelatins confers heavy metal tolerance in plants (Cobbet,2000), however, phytochelatins do not con-tribute to Al tolerance, most likely because they do not bind Al effectively (Larsen et al.,1996). Al tends to bind to the phosphate or carboxyl groups rather than to -SH groups characteristic for chelatins (Gunsé et al., 1997). However, Snowden et al. (1995) and Wu et al. (2000) suggested that plant metallo thionein-like protein and phyto chelatins may play a role in Al tolerance Although aluminum has been shown to be age nontoxic metal, the molecular mechanism of Al toxicity to plants is not well understood. Al is a complicated ion in terms of chemical form and exerts a divergent biological function. Al interferes with cell division in root tips and lateral roots, increases cell wall rigidity by cross linking pectins, reduces DNA replication by increasing the rigidity of the DNA double helix, fixes phosphorous in less available forms in soils and on root surfaces, decreases root respiration, interferes with enzyme activity governing sugar phosphorylation and the deposition of cell wall polysaccharides, and the uptake, transport, and also use of several essential nutrients like Ca, Mg, K, P and Fe (Foy ,1992). Aluminum does not affect the seed germination but helps in new root development and seedling establishment (Nosko, 1988). Root growth inhibition was detected 2–4 days after the initiation of seed germination (Bennet et al., 1991).

The Low concentration has affected the biochemical parameters slightly, but increase in treatment decreased the parameters like Amino Acids (Root), Protein (Shoot) and DNA (Shoot). The Protein content in Root and RNA content in shoot did not respond to the treatment of Al_2O_3 to mung seedlings. However, there was increased response of Sugar (Root and Shoot), DNA and RNA (Root) contents. Overall, The Al_2O_3 has a little effect on biochemical parameters studied in the seedlings of *Vigna radiata*, L.

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