ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Aluminium Uptake by the Seeds of Green Gram (*Phaseolus aureus*) Under Different Chemical Milieu and its Effect on Seed Germination and Plant Growth.

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ABSTRACT

Aluminium uptake by the seeds of green gram (*Phaseolus aureus*) from the solutions of Al^{3+} , as well as, Al^{3+} mixed with different inorganic and organic anions viz., fluoride, phosphate, borate, silicate, malate, tartrate, citrate, malonate or succinate ,has been studied. Effect of inorganic and organic anions on the aluminium uptake by the seeds has been observed. Percentage germination and morphogenic aspects of the plants germinated (in soil) out of intoxicated seeds has also been studied. Results revealed an expression of toxicity by the seeds upon exposure to the aluminium ions under different chemical milieu. Al uptake by the seeds exposed to 0.01M Al^{3+} solution has been found to be 2.16 mg/gm. Inorganic and organic anions (0.01M solution) when present in the milieu along with Al^{3+} were found to inhibit the Al uptake in the range of 0.46 -87.5%. Silicate showed a maximum inhibition of 87.5%. Aluminium intoxicated seeds expressed severe toxicity in germination and plant growth in soil culture. Only 10% germination was seen as compared to that of 100% in the control (non-toxicated) set. Presence of different inorganic and organic anions along with Al^{3+} in the intoxication of seeds, could not exhibit much difference in the seed germination and plant growth as compared to that of Al- only intoxication, except malate ions. The malate had a good amelioration effect on Al toxicity. 70% germination and good plant growth was exhibited by these intoxicated (Al^{3+} **+** malate) seeds. Thus, malate seems to be a good ameliorator of aluminium toxicity.

Keywords: Aluminium toxicity, Aluminium phytotoxicity, Aluminium uptake, Aluminium toxicology, Aluminium toxicity amelioration, Aluminium agrotoxicity.

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ISSN: 0975-8585

INTRODUCTION

Aluminium is the third most abundant element in the earth's crust. It comprises of about 8% of the outer 16 kms of the crust. It is highly reactive metal and hence does not occur free in nature. It occurs chiefly as oxides and aluminosilicates. Though aluminium is one of the most abundant metals in the biosphere, it does not have any useful biological function. It has been rather proved to be toxic to human body [1-14]. Aluminium has been implicated as a potential neurotoxic factor in different pathological conditions [6,7].

Though aluminium is well protected from its bioavailability in nature by the formation of stable complex aluminosilicates, nevertheless, in case of disbalances in ecosystems, such as acid rain or a decrease in soil pH by industrial effluents and consequent water pollution, there may be leaching of aluminium ions and their consequent entry into the flora and fauna through the aquatic systems. This evidently would lead to onward transmission of aluminium ions in the food chains of the ecosystems and hence create toxicity. Aluminium may enter into the body through food, water and air-borne dust particles [9].It may also enter into the food through edible plants grown on aluminium intoxicated soils [15]. Higher bioavailability of aluminium might manifest as ecotoxicity in general.

With the foregoing views in our mind, we have presently studied the aluminium uptake by the seeds of green gram (*Phaseolus aureus*) uder different chemical milieu and its effect on seed germination and plant growth.

MATERIALS AND METHODS

All chemicals used were of A.R. (Analytical Reagent) grade. Aluminium sulphate, $[Al_2(SO_4)_3.16H_2O]$, was used to prepare aluminium ion (Al^{3+}) solution. Sodium tetraborate ($Na_2B_4O_710H_2O$), sodium fluoride (NaF), sodium phosphate (Na_3PO_4), sodium silicate (Na_2SiO_3), di-sodium malonate ($Na_2C_3H_2O_4$), di-sodium succinate ($Na_2C_4H_4O_46H_2O$), tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), di-sodium tartrate ($Na_2C_4H_4O_6$) and di-sodium malate ($Na_2C_4H_4O_5$) were separately used to prepare solutions containing borate ($B_4O_7^{2-}$), fluoride (F^-), phosphate (PO_4^{3-}), silicate (SiO_3^{2-}), malonate ($C_3H_2O_4^{2-}$), succinate ($C_4H_4O_4^{2-}$), citrate ($C_6H_5O_7^{3-}$), tartrate ($C_4H_4O_6^{2-}$) and malate ($C_4H_4O_5^{2-}$) ions respectively. The concentrations of all solutions were 0.01M. All the solutions were prepared in distilled water.

Seeds of green gram (*Phaseolus aureus*) were procured from the local market and washed thoroughly with Rozar solution (fungicide, prepared by dissolving 10 mg in 200 mL of distilled water) and followed by distilled water.

Experiments

One gram of green gram seeds were weight out in separate sets. The seeds in each set were placed in 250 mL beakers. The seeds were treated with different solutions as follows:

(i) 1 gm seeds + 50 mL distilled water (blank set)
(ii) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL distilled water.
(iii) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL borate (0.01M) sol^n.
(iv) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL fluoride (0.01M) sol^n.
(v) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL phosphate (0.01M) sol^n.
(vi) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL silicate (0.01M) sol^n.
(vii) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL malonate (0.01M) sol^n.
(viii) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL succinate (0.01M) sol^n.
(ix) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL citrate (0.01M) sol^n.
(x) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL tartrate (0.01M) sol^n.
(xi) 1 gm seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL malate (0.01M) sol^n.

Thus, a total of 11 sets including one blank set were set up. The beakers were covered with watch glass and left for 24 hours. After 24 hours deep soaking, the seeds were separated out from the solution in each set and washed with distilled water. The seeds were dried at room temperature. The seeds were then transferred into 250 mL conical flask in separate sets. 10 mL of 1M HNO_3 was added to each flask and boiled for 8-10 minutes, where upon the seeds got decomposed and a dry residue was left out. The dry residue were extracted with distilled water and transferred to 100 mL volumetric flasks and made upto the mark with distilled water. The aluminium content of the solutions were determined spectrophotometrically using Eriochrome cyanine R reagent[16].

In another experiment, 10 seeds of green gram were separately placed in 250 mL beakers. The seeds were treated with different solutions as follows:

(i) 10 seeds + 50 mL distilled water (blank set)
(ii) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL distilled water.
(iii) 10 seeds + 25 mL Al^{3+} (0.01 M) sol^n + 25 mL borate (0.01M) sol^n.
(iv) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL fluoride (0.01M) sol^n.
(vi) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL phosphate (0.01M) sol^n.
(vii) 10 seeds + 25 mL Al^{3+} (0.01M) soln + 25 mL malonate (0.01M) sol^n.
(viii) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL succinate (0.01M) sol^n.
(ix) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL citrate (0.01M) sol^n.
(x) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL tartrate (0.01M) sol^n.
(xi) 10 seeds + 25 mL Al^{3+} (0.01M) sol^n + 25 mL malate (0.01M) sol^n.

Thus, a total of 11 sets including one blank set were set up. The beakers were covered with watch glass and left for 24 hours. After 24 hours deep soaking, the seeds were separated out from the solution in each set, and washed with tap water. The seeds were sown in 4 Kg of soil in earthen pots in a number of sets. The pots were left in a place where proper sun light would be available to them during the day hours. From time to time, proper amount of tap water was added to the pots for providing proper moisture. The germination and growth pattern were observed for 15 days. At the end, the shoot length and number of leaves of each plant in each case were recorded. Mean shoot length and number of leaves were calculated

ISSN: 0975-8585

out. All experiments were carried out in three replicates and mean values were calculated out and reported.

RESULTS AND DISCUSSION

Aluminium uptake by the seeds of green gram exposed to different chemical milieu is recorded in Table-1. *In-vivo* germination and morphogenic characteristics of plants born out of seeds exposed to different chemical milieu are recorded in Table-2. Relative effect of different anions on the aluminium uptake by the seeds is shown in Fig.1. Percentage germination (*in-vivo*) of seeds intoxicated by different chemical milieu is shown in Fig.2. Relative growth pattern of plants germinating out of intoxicating seeds is shown in Fig.3.

Toxicity of aluminium would primarily be decided by its uptake by the seeds and its effect on germination as well as morphological aspects of plants comming out of germinated seeds. A study of Table-1 suggests that the seeds of green gram (*Phaseolus aureus*) uptake aluminium from the milieu (0.01M Al^{3+}) to the extent of 2.16 mg/gm. This amount of aluminium showed definite toxicity because only 10% germination was shown *in-vivo* (Table-2), as compared to 100% germination in control, in the soil. Presence of other inorganic and organic anions in the milieu, (in addition to Al^{3+}) had shown different effect on the aluminium uptake. Silicate (SiO_3^{2-}) showed the highest inhibition (87.5%) of Al uptake. In presence of silicate only 0.27 mg/gm Al^{3+} was taken up. Fluoride, borate and phosphate showed only a slightly lower uptake compared to that in case of pure Al^{3+}. The uptake inhibition by these inorganic anions has been in the range of 37.5 – 39.8%.

Table 1: Mean aluminium uptake by the seeds exposed to different chemical milieu.

S. No.	Milieu	Al uptake by seeds (mg/gm)	Percentage inhibition of Al uptake
1	Control	—	—
2	Al^{3+}	2.16	—
3	Al^{3+} + borate	1.30	39.81
4	Al^{3+} + fluoride	1.32	38.88
5	Al^{3+} + phosphate	1.35	37.5
6	Al^{3+} + silicate	0.27	87.5
7	Al^{3+} + malonate	2.10	2.77
8	Al^{3+} + succinate	2.15	0.46
9	Al^{3+} + citrate	2.13	1.38
10	Al^{3+} + tartarate	1.89	12.5
11	Al^{3+} + malate	1.27	41.20

ISSN: 0975-8585

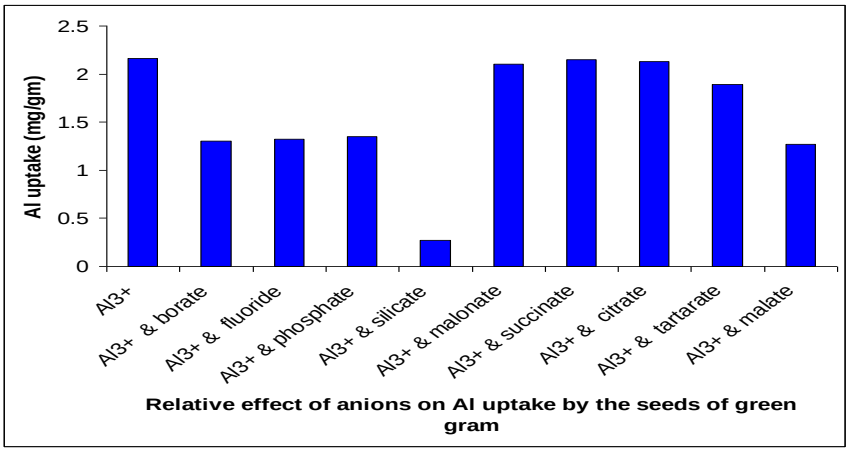

Figure 1

Table 2: *In- vivo* germination and morphogenic characteristics (Mean values) of plants born out of seeds exposed to different chemical milieu.

S. No.	Milieu	Percentage germination	Shoot Length (cm)	No. of leaves
1	Control	100	8.76	42
2	Al^{3+}	10	4	4
3	Al^{3+} + borate	20	8	8
4	Al^{3+} + fluoride	30	1	6
5	Al^{3+} + phosphate	40	4.25	14
6	Al^{3+} + silicate	50	4.4	14
7	Al^{3+} + malonate	0	-	-
8	Al^{3+} + succinate	10	2	2
9	Al^{3+} + citrate	10	2	5
10	Al^{3+} + tartrate	20	2	4
11	Al^{3+} + malate	70	6	31

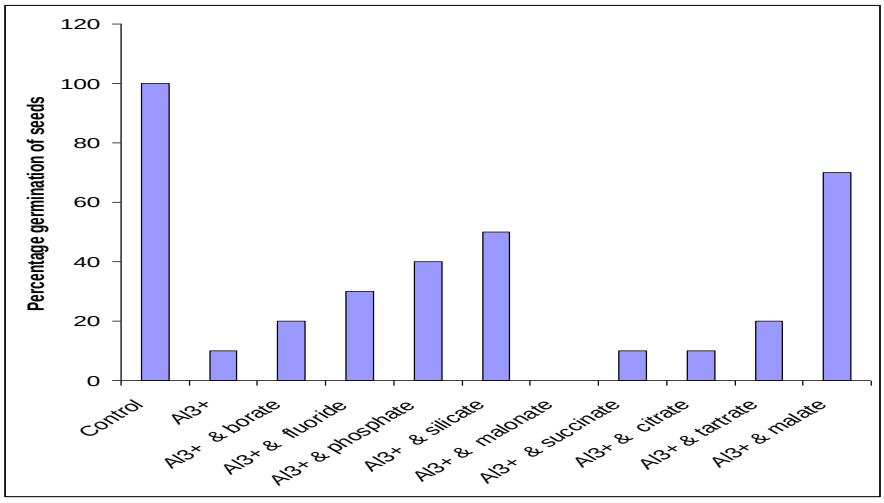

Figure 2: Percentage germination (*in-vivo*) of seeds of green gram exposed to different chemical milieu.

ISSN: 0975-8585

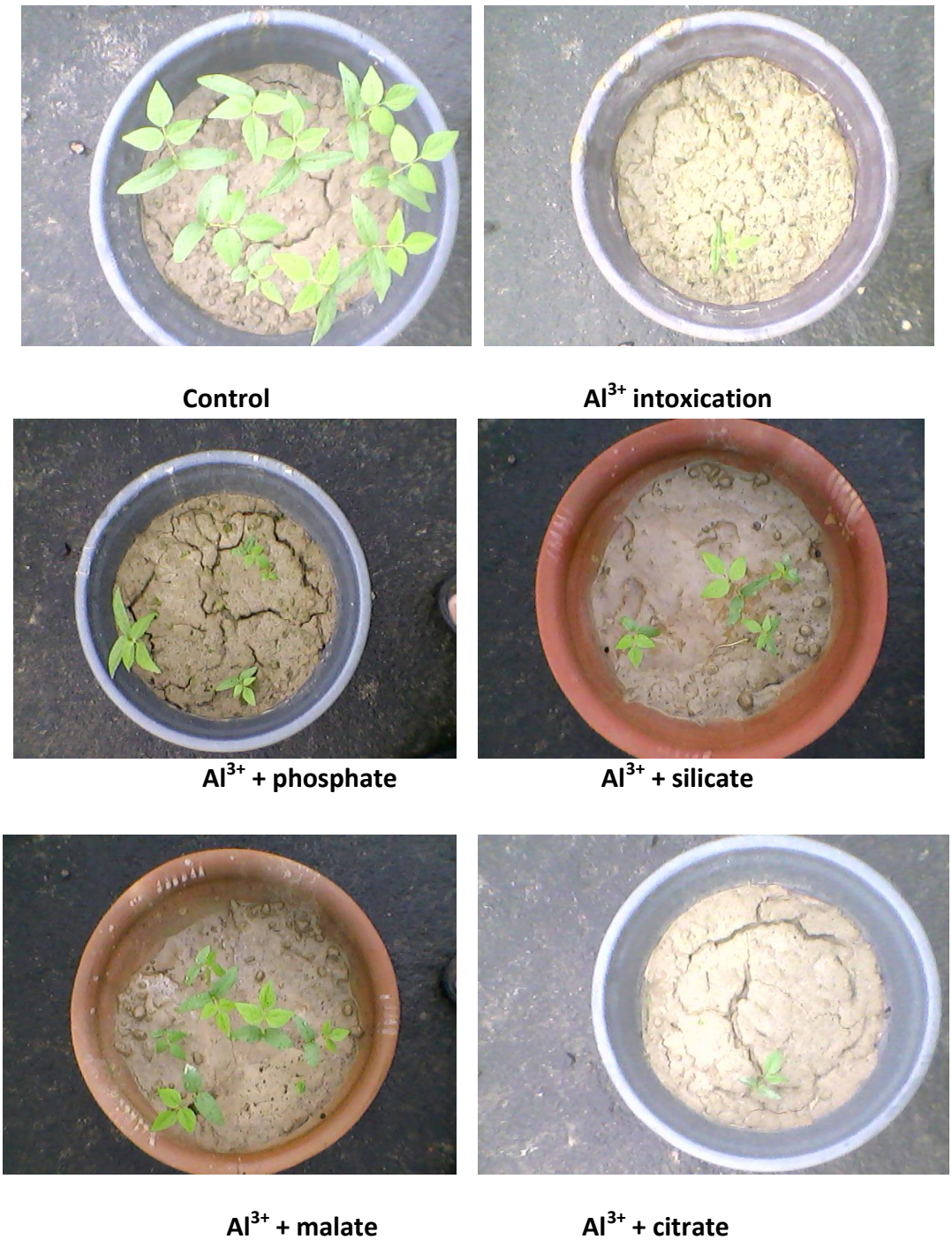

Figure 3: Relative growth pattern of plants germinating out of intoxicating seeds

Among the chelating organic anions, only malate and tartrate showed some appreciable inhibition of Al uptake. The inhibition by malate was 41.2% where as that by tartrate was 12.5%. In fact malate inhibited almost to the extent of that by fluoride, phosphate and borate. The malonate, succinate and citrate, on the other hand, have failed to suppress the aluminium uptake by the seeds to any appreciable extent. The inhibition has only been 0.4% to 2.7%.

ISSN: 0975-8585

A study of Table-2 suggests that under *In-vivo* conditions, the Al^{3+} + malate treated seeds show the best germination, 70% as compared to 100% that of control. Silicate, though is a good supressure of aluminium uptake, somehow could not germinate more than 50% when Al^{3+} + SiO_3^{2-} treated seeds were sown in soil. Fluoride, borate and phosphate though controlled the Al uptake but this control did not reflect in the increase of germination. Only 20-40% germination was seen when these intoxicated seeds were sown in the soil. Succinate and citrate could not inhibit Al uptake by the seeds to any appreciable extent and *in-vivo* germination percentage of the corresponding seeds was also very poor (0-10%) and is almost as that of (10%) pure Al^{3+} treated seeds.

The morphogenic characteristics of the plants germinated out of the intoxicated seeds did not show any uniformity and trend, as compared to the inhibition of Al uptake by anions , except in case of malate. The shoot length (6 cm) and number of leaves (31) in case of Al^{3+} and malate intoxicated seeds almost matched to that of shoot length (8.76 cm) and number of leaves (42) of those in pure control (pure non-toxicated seeds). Thus, it seems only the malate is capable enough to cope up and tackle the Al-toxicity to appreciable extent in case of green gram seeds. The silicate is only effective in inhibiting Al uptake but somehow fails in affectivity on seed germination and plant growth. The plants germinated out of seeds, treated with Al^{3+} and other inorganic and organic anions, viz., fluoride, phosphate, borate , silicate , citrate , tartrate , malonate or succinate showed a very poor germination and growth.

Metal detoxification by chelating agents has been useful method for controlling metal toxicity. Presently, we have studied simple chelating organic acids as inhibitors of aluminium uptake by the seeds. These simple organic acids might either inhibit the metal uptake by chelating aluminium ions or themselves chelate out preferably with some useful metal of seeds and become toxic to the seeds on one hand and help out aluminium uptake by the useful, metal holding chelators of seeds which have now been set free by the release of their useful metals, thus creating more and more toxicity, on the other hand.

Presently, it is seen that the aluminium toxicity could be countered effectively only by the malate ions. This is evident from the fact that malate could germinate upto 70% as compared to only 10% by pure Al^{3+} intoxication. Malate might be ameliorating aluminium toxicity by the formation of aluminium malate chelate inside the seed after uptake. Other chelating agents like citrate, tartrate etc. might not the screening the uptaken Al by chelation. Silicate, though a good preventor of Al uptake and thus a good restrictor of bioavailability of Al, but is, somehow, unable to prevent the expression of Al toxicity. It is also likely that the silicate, when present in the milieu as silicate ion, might be itself intoxicating the seeds (silicate toxicity) and hence, resulting in poor germination and growth pattern.

ISSN: 0975-8585

CONCLUSION

Our present study suggests that Al^{3+} is highly toxic to the seed germination and plant growth of green gram (*Phaseolus aureus*). Only 10% germination as compared to 100% that of control is observed,The toxicity appears due to the uptake of Al^{3+} (present in the milieu) by the seeds. Out of many inorganic and organic anions only malate is able to counter/ameliorate Al toxicity, to some extent.

In view of the above, the edible plants, grown in the soils containing large Al^{3+} concentration, might lead to Al toxicity transmission (through these edible plants) to the animals and humans. This, in turn, might lead to nurotoxicity. As such, soils must be tested for Al^{3+} content before agriculture. Aluminium leaching factors such as, acid rain and discharge of acidic effluents to the soil, should be properly monitored before the cultivation of edible plants in such regions.

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