

ALLEVIATION OF ALUMINIUM TOXICITY BY CALCIUM IN *BRASSICA JUNCEA* (L.) CZERNJ & COSSON

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ABSTRACT

The ameliorative effect of Ca on Al toxicity was investigated on a crop plant (*Brassica juncea* L. Czernj & Cosson) that involved an affordable and practical method to contain the toxicity through soil amendments with some salts of Ca, which is available locally and plentifully. It was found that a dose of 1200 $\mu\text{g g}^{-1}$ of Ca as CaCO_3 was sufficient to protect the crop from rhizotoxicity induced at 500 $\mu\text{g g}^{-1}$ of Al. A comparative pot-culture study of different salts of calcium (viz. CaCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was made to find out its most suitable anionic moiety. It was revealed that the salt of CaCO_3 worked the best, as it increased soil pH more than other two salts tested. Our study further revealed that local tube-well water irrigated *Brassica* plants were free from Al-induced toxic effects even at much less of Ca-salt applied to the soil. The findings were also supported by the biochemical analyses of Al and Ca.

Key words: aluminium, aluminium toxicity, *Brassica*, calcium, toxicity alleviation.

Introduction

Phytotoxicity of aluminium (Al^{3+}) is a serious problem limiting crop production on acid soils (Poschenrieder *et al.* 2008; Ma 2007; Liao *et al.* 2006; Ma, 2000). As nearly half of the non-irrigated arable lands in the world are acidic soils, alleviation of Al toxicity was attempted by various workers through application of different chemicals e.g. silicon (Wang *et al.* 2004; Cocker *et al.* 1998; Gu *et al.* 1998), boron (Lenoble *et al.* 1996), rock phosphate (Hu *et al.* 1995) and a few other elements. Calcium compounds are better

known to ameliorate the toxic effects of aluminium. There are a number of experimental evidences in support of the positive role of Ca^{2+} in alleviating Al^{3+} toxicity (Alva *et al.* 1986; Alva and Sumner 1989; Rahman 1992; El-Aggan 1995; Pintro *et al.* 1998; Plieth *et al.* 1999).

The state of Orissa being largely agricultural needs to address the problem of aluminium in the soil. It was tried, if the problem of toxicity could be decreased or avoided by application of some locally available, affordable and usable chemicals to the soil. Keeping these considerations in view,

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experiments with Ca^{2+} compounds and calcium rich local tube-well water were tried on a local oil-seed crop plant, *Brassica juncea* (L.) Czernj & Cosson.

MATERIALS AND METHODS

Soil from nearby agricultural fields was collected, air-dried and ground. With appropriate amendments (equivalent to 90 kg h^{-1} N, 15 kg h^{-1} P, 25 kg h^{-1} K and 3 t h^{-1} Organic manure), the experiments were conducted during the growing season (Nov.-Mar.).

1. Ten day old seedling experiment: Experiment-I

Finely-sieved soil samples containing soil and sand in the ratio 1:2 were used. The plants were grown in 500 gm soil samples taken in perforated polythene bags (Dash *et al.* 1988). Fifty seedlings were studied in each set of five polythene bags, with ten plants per polythene bag. Seven sets of polythene bags were studied to observe the range of toxic effects of Al^{3+} ion ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) on *Brassica*. The sets included a control and soil mixed with different doses of Al from 100 to 1000 $\mu\text{g g}^{-1}$. Observations were taken 10 days after emergence of seedlings. The root lengths of the seedlings were measured. Seedling dry weight was recorded after oven-dried at 80°C for 24 h.

2. Pot culture

Experiments were conducted in earthen pots of about 20 cm diameter with a height of about 25 cm. In each pot 5 kg of soil was taken. The soil when compacted occupied approximately 21 cm height. Seeds of *Brassica* were sown in each pot at 5 seeds /

pot. Each pot was adjusted to an improvised drip irrigation system providing about 25 drops / minute to keep the soil moist all the time. The plants were exposed to 10-12 h of photoperiods (natural day length). After 30 days of growth the seedlings were thinned keeping the best two seedlings per pot. Twenty plants were studied in each set of ten pots with two plants in each, growing up to harvest stage.

2.1. Experiment-II

In this experiment six sets of pots were studied to assess the amelioration effects of Ca^{2+} as CaCO_3 (300-1200 $\mu\text{g g}^{-1}$) on the plants exposed to aluminium. Plants were sampled at flowering (40 - 50 days), siliqua development (65 - 80 days) and harvest (110 - 120 days) stages.

2.2. Experiment-III

This experiment was conducted to find out the most suitable species of Ca^{2+} (CaCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) against Al toxicity. It was done in two batches of 11 sets each arranged in parallel rows - one set irrigated by river water and the other by tube well water. The sampling was as in Experiment. No. II.

2.3. Toxicity parameters:

LECT (Lowest Effective Concentration Tested) refers to the lowest of the tested concentrations that shows a measurable response.

MECT_{50} (Median Effective Concentration Tested) means the concentration that yields a response measured as much as 50% of the control in the experiment.

HECT (Highest Effective Concentration Tested) is that tested concentration, where

the effect is manifested at the maximum without being lethal.

2.4 Analysis of elements

At the thinning stage (30 days after growth), the best plant from the harvested plants of a pot was collected and separated into shoot part (leaves excluded) and root part. The plant parts were washed with deionized water and dried in an oven at 80°C for 72h for the biochemical analyses. Dried plant samples were digested following the method of Blancher *et al.* (1965) using diacid $\text{HNO}_3:\text{HClO}_4$ (3:2 v/v) and diluted to suitable volumes after filtration. This stock solution was used for measurement of Ca and Al by atomic absorption spectrophotometer (ECIL AAS 4129, India)

Statistical Analysis

For all comparisons of results following Al treatment, the means were compared by ANOVA followed by LSD test at 0.05 level, as per procedure given in Gomez and Gomez (1984).

Relative values (RV) were calculated by-
$$\text{RV} = (\text{TV} / \text{CV}) \times 100$$

TV is the value in the Al treatment: CV is the value in control.

RESULTS

Experiment-I showed aerial (purple patches in leaves and collapse of panicles-details not given) and rhizotoxic effects in the plants. However, only the reduction in root length was taken as the measure of toxicity induced by Al. The LECT was found to be $100 \mu\text{g g}^{-1}$ where the percentage of decrease in root length showed no difference

from the control. Dose dependent root length reduction was marked with the MECT_{50} at about $500 \mu\text{g g}^{-1}$ and the HECT at $1000 \mu\text{g g}^{-1}$ (Figure 1).

In Experiment-II, it was observed that there was a Ca dose-dependent amelioration of Al^{3+} toxicity (at MECT_{50}). The ameliorative effects were observed with Ca of $300 \mu\text{g g}^{-1}$ onwards and near total normalcy is restored with a dose of $1200 \mu\text{g g}^{-1}$ Ca (Table 1).

In Experiment III, three different compounds of Ca^{2+} as CaCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were compared with regard to their capacity to reduce the Al-induced toxicity symptoms. It was found out that in all the Ca species, the amelioration was dose-dependent. However, it was quite evident that this alleviating capacity was the best with CaCO_3 followed by $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

The experiment was done in two batches, one with river (tap) water and another with tube-well water. It showed remarkable change in the pattern of amelioration. The batch with tube-well water irrigated plants, showed much better amelioration than the batch with the other type of irrigation (Table 2, 3).

The biochemical analyses revealed accumulation of Al and reduction in the uptake of Ca both in root and shoot part of the Al-treated plants. In the amelioration studies with tap water irrigation, while at $1500 \mu\text{g g}^{-1}$, CaCO_3 significantly reduced the uptake of Al normalizing Ca uptake, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ showed partial response and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ failing to show significant positive co-relation. However, tube well water irrigation could significantly reduce Al uptake, marginally affecting Ca uptake in Al-treated plants and amelioration occurred at much lower dose of Ca with all the three Ca Salts tried (Figure 2)

TABLE – 1. EFFECT OF CALCIUM ON ALUMINIUM INDUCED TOXICITY IN *BRASSICA* GROWTH AND DEVELOPMENTAL PARAMETERS (TAP WATER)

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| Concentrations In $\mu\text{g g}^{-1}$ In soil | Soil pH | | Shoot Length (cm) | No. of Panicle per Plant \pm S.D. | *No. of Flowers per Panicle \pm S.D. | *No. of Siliquae per Panicle \pm S.D. | *Length of Siliqua \pm S.D. (cm) | *No. of Seeds per Siliqua \pm S.D. | Seed weight In gms. (100nos.) |
|--|------------------|----------------|---------------------------|--|---|--|---|---|--|
| | Before sowing | At maturity | | | | | | | |
| Control | 4.71 | 6.52 | 62.4 \pm 8.5 a | 5.8 \pm 0.4 a | 24.9 \pm 3.2 a | 25.1 \pm 2.4 a | 5.6 \pm 0.3 a | 17.3 \pm 2.3 a | 0.268 \pm 0.03 a |
| Al 500 | 4.68 | 5.03 | 42.7 \pm 3.8 b | 5.4 \pm 0.5 a | 22.5 \pm 2.5 a | 23.7 \pm 2.5 a | 3.4 \pm 0.3 b | 10.0 \pm 1.1 b | 0.151 \pm 0.01 b |
| Ca 300 | 4.80 | 5.26 | 48.5 \pm 5.6 c | 5.2 \pm 0.6 a | 20.8 \pm 1.4 b | 22.6 \pm 2.0 a | 3.3 \pm 0.4 b | 14.3 \pm 1.9 c | 0.176 \pm 0.01 c |
| Ca 600 | 5.19 | 6.37 | 47.9 \pm 4.1 c | 6.0 \pm 0.7 a | 25.6 \pm 2.8 a | 24.8 \pm 1.9 a | 4.0 \pm 0.3 bc | 14.6 \pm 1.7 c | 0.174 \pm 0.02 c |
| Ca 900 | 5.34 | 6.29 | 54.3 \pm 6.6 cd | 5.9 \pm 0.7 a | 25.0 \pm 3.6 a | 24.3 \pm 1.6 a | 4.9 \pm 0.6 ac | 17.4 \pm 2.1 a | 0.229 \pm 0.03 d |
| Ca 1200 | 6.45 | 6.60 | 64.1 \pm 6.7 a | 5.9 \pm 0.6 a | 26.2 \pm 2.3 a | 25.0 \pm 2.0 a | 5.3 \pm 0.5 a | 18.2 \pm 1.2 a | 0.259 \pm 0.04 a |

Any two mean values having a common letter are not significantly different at 5% P level.

*Mean of 20 best samples

BIONATURE : 2010

TABLE – 2. REMEDIAL MEASURES OF ALUMINIUM TOXICITY BY APPLICATION OF CALCIUM WITH THREE DIFFERENT ANIONS IN BRASSICA (TAP WATER)

| Concentrations In $\mu\text{g g}^{-1}$ In soil | Soil pH | | Shoot Length (cm) | No. of Panicle per Plant \pm S.D. | *No. of Flowers per Panicle \pm S.D. | *No. of Siliquae per Panicle \pm S.D. | *Length of Siliqua \pm S.D. (cm) | *No. of Seeds per Siliqua \pm S.D. | Seed weight In gms. (100nos.) |
|--|------------------|----------------|---------------------------|--|---|--|---|---|--|
| | Before sowing | At maturity | | | | | | | |
| Control | 4.69 | 6.41 | 61.8 \pm 6.9 a | 5.3 \pm 0.5 a | 27.0 \pm 2.9 a | 24.7 \pm 1.5 a | 5.2 \pm 0.4 a | 14.7 \pm 1.8 a | 0.259 \pm 0.03 a |
| Calcium Carbonate | Al 500 | 4.66 | 40.4 \pm 4.3 b | 5.4 \pm 0.5 a | 15.4 \pm 1.8 b | 20.6 \pm 2.4 a | 5.1 \pm 0.4 a | 9.4 \pm 0.9 b | 0.154 \pm 0.01 b |
| | Ca 500 | 4.76 | 43.3 \pm 4.8 b | 5.0 \pm 0.4 a | 15.9 \pm 1.3 b | 21.3 \pm 2.7 a | 4.9 \pm 0.5 a | 9.9 \pm 1.4 b | 0.172 \pm 0.02 b |
| | Ca 1000 | 5.41 | 57.0 \pm 6.1 a | 5.2 \pm 0.6 a | 22.7 \pm 2.2 c | 24.1 \pm 1.9 a | 4.7 \pm 0.4 b | 12.4 \pm 1.6 c | 0.225 \pm 0.02 c |
| | Ca 1500 | 6.77 | 60.8 \pm 5.2 a | 4.9 \pm 0.3 a | 27.1 \pm 3.2 a | 24.8 \pm 2.6 a | 5.0 \pm 0.7 a | 14.9 \pm 2.0 a | 0.251 \pm 0.02 a |
| Calcium Sulphate | Ca 500 | 4.63 | 43.9 \pm 3.6 b | 4.7 \pm 0.3 b | 15.8 \pm 1.6 b | 19.2 \pm 1.1 b | 4.7 \pm 0.5 b | 8.5 \pm 1.0 b | 0.155 \pm 0.01 b |
| | Ca 1000 | 4.50 | 46.2 \pm 5.6 bc | 5.0 \pm 0.4 a | 20.4 \pm 2.0 c | 19.5 \pm 2.3 b | 4.6 \pm 0.3 b | 9.4 \pm 1.4 b | 0.182 \pm 0.03 b |
| | Ca 1500 | 4.42 | 51.1 \pm 5.3 c | 5.2 \pm 0.3 a | 20.9 \pm 2.3 c | 23.9 \pm 2.1 a | 5.1 \pm 0.4 a | 11.2 \pm 1.7 c | 0.191 \pm 0.02 bc |
| Calcium Chloride | Ca 500 | 4.58 | 39.3 \pm 4.4 b | 5.1 \pm 0.2 a | 16.6 \pm 1.8 b | 16.2 \pm 1.7 c | 5.0 \pm 0.3 a | 8.8 \pm 1.0 b | 0.160 \pm 0.01 b |
| | Ca 1000 | 4.51 | 44.5 \pm 4.6 bc | 3.3 \pm 0.3 c | 15.7 \pm 1.1 b | 16.8 \pm 2.0 c | 5.0 \pm 0.6 a | 9.3 \pm 1.6 b | 0.158 \pm 0.01 b |
| | Ca 1500 | 4.33 | 44.6 \pm 4.9 bc | 3.2 \pm 0.2 c | 17.1 \pm 2.0 b | 18.3 \pm 1.4 c | 4.8 \pm 0.2 b | 9.1 \pm 1.4 b | 0.179 \pm 0.01 b |

Any two mean values having a common letter are not significantly different at 5% P level.

*Mean of 20 best samples

ALLEVIATION OF ALUMINIUM TOXICITY BY CALCIUM IN BRASSICA JUNCEA (L.)

TABLE – 3. REMEDIAL MEASURES OF ALUMINIUM TOXICITY BY APPLICATION OF CALCIUM WITH THREE DIFFERENT ANIONS IN BRASSICA (TUBE WELL WATER)

| Concentrations In $\mu\text{g g}^{-1}$ In soil | Soil pH | | Shoot Length (cm) | No. of Panicle per Plant \pm S.D. | *No. of Flowers per Panicle \pm S.D. | *No. of Siliquae per Panicle \pm S.D. | *Length of Siliqua \pm S.D. (cm) | *No. of Seeds per Siliqua \pm S.D. | Seed weight In gms. (100nos.) | |
|--|------------------|----------------|---------------------------|--|---|--|---|---|--|---------------------|
| | Before sowing | At maturity | | | | | | | | |
| Control | 4.64 | 6.69 | 64.5 \pm 6.9 a | 4.7 \pm 0.2 a | 23.0 \pm 3.1 a | 24.4 \pm 2.6 a | 4.9 \pm 0.4 a | 17.7 \pm 1.9 a | 0.284 \pm 0.02 a | |
| Calcium Carbonate | Al 500 | 4.71 | 50.7 \pm 5.3 b | 4.2 \pm 0.4 a | 21.6 \pm 2.6 a | 17.5 \pm 2.0 b | 4.7 \pm 0.5 a | 14.2 \pm 1.6 b | 0.230 \pm 0.02 b | |
| | Ca 500 | 5.17 | 54.1 \pm 5.4 b | 4.6 \pm 0.4 a | 22.9 \pm 2.1 a | 24.1 \pm 2.7 a | 4.7 \pm 0.6 a | 16.3 \pm 1.1 a | 0.274 \pm 0.03 a | |
| | Ca 1000 | 5.96 | 61.4 \pm 7.2 a | 4.8 \pm 0.6 a | 24.4 \pm 2.1 a | 24.6 \pm 2.5 a | 4.6 \pm 0.5 a | 17.1 \pm 1.4 a | 0.286 \pm 0.04 a | |
| | Ca 1500 | 6.84 | 63.6 \pm 6.0 a | 4.6 \pm 0.5 a | 24.3 \pm 2.8 a | 25.0 \pm 3.1 a | 4.5 \pm 0.4 a | 17.8 \pm 1.6 a | 0.286 \pm 0.03 a | |
| | Ca 500 | 4.62 | 6.46 | 60.2 \pm 4.5 a | 4.2 \pm 0.3 a | 23.2 \pm 2.3 a | 22.7 \pm 2.5 a | 4.9 \pm 0.5 a | 15.8 \pm 1.4 ab | 0.261 \pm 0.02 a |
| Calcium Sulphate | Ca 1000 | 4.51 | 6.41 | 60.1 \pm 5.8 a | 4.5 \pm 0.4 a | 21.5 \pm 1.5 a | 22.5 \pm 2.1 a | 4.2 \pm 0.3 a | 16.3 \pm 1.3 a | 0.270 \pm 0.03 a |
| | Ca 1500 | 4.39 | 6.40 | 64.5 \pm 6.7 a | 4.4 \pm 0.4 a | 24.1 \pm 2.0 a | 24.6 \pm 2.7 a | 4.5 \pm 0.4 a | 17.2 \pm 1.5 a | 0.282 \pm 0.03 a |
| | Ca 500 | 4.55 | 6.38 | 51.2 \pm 4.8 b | 4.1 \pm 0.4 a | 22.2 \pm 2.7 a | 24.0 \pm 1.8 a | 4.4 \pm 0.4 a | 13.8 \pm 1.4 b | 0.249 \pm 0.02 ab |
| Calcium Chloride | Ca 1000 | 4.48 | 6.35 | 50.8 \pm 5.6 b | 4.4 \pm 0.2 a | 22.9 \pm 2.7 a | 24.0 \pm 2.6 a | 4.5 \pm 0.5 a | 14.9 \pm 1.2 b | 0.251 \pm 0.02 ab |
| | Ca 1500 | 4.29 | 6.24 | 57.4 \pm 4.7 b | 4.4 \pm 0.3 a | 22.7 \pm 2.4 a | 23.9 \pm 1.7 a | 5.0 \pm 0.6 a | 16.4 \pm 1.8 a | 0.254 \pm 0.02 ab |

Any two mean values having a common letter are not significantly different at 5% P level.

*Mean of 20 best samples

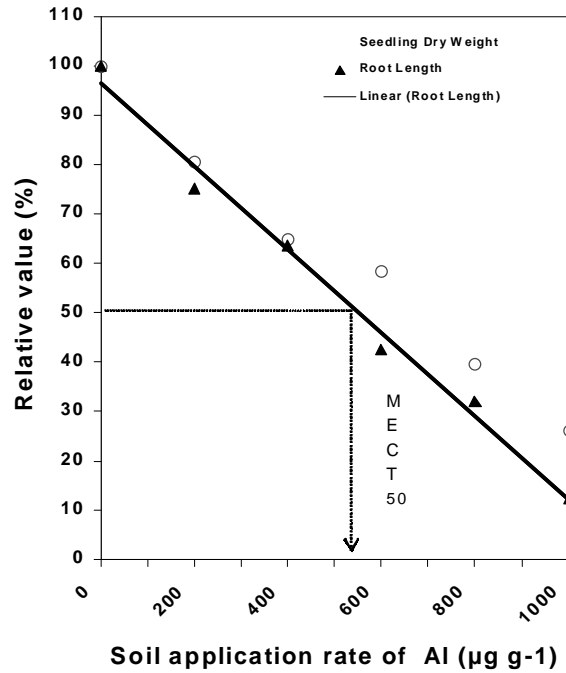


Fig. 1- Effect of Al (pH 4.66) on the seedling dry weight and root length (cm) of *Brassica juncea* (L.) Czernj & Cosson (10 days old). Relative values are values of Al-treated plant relative to the control. Values are means of 50 seedlings.

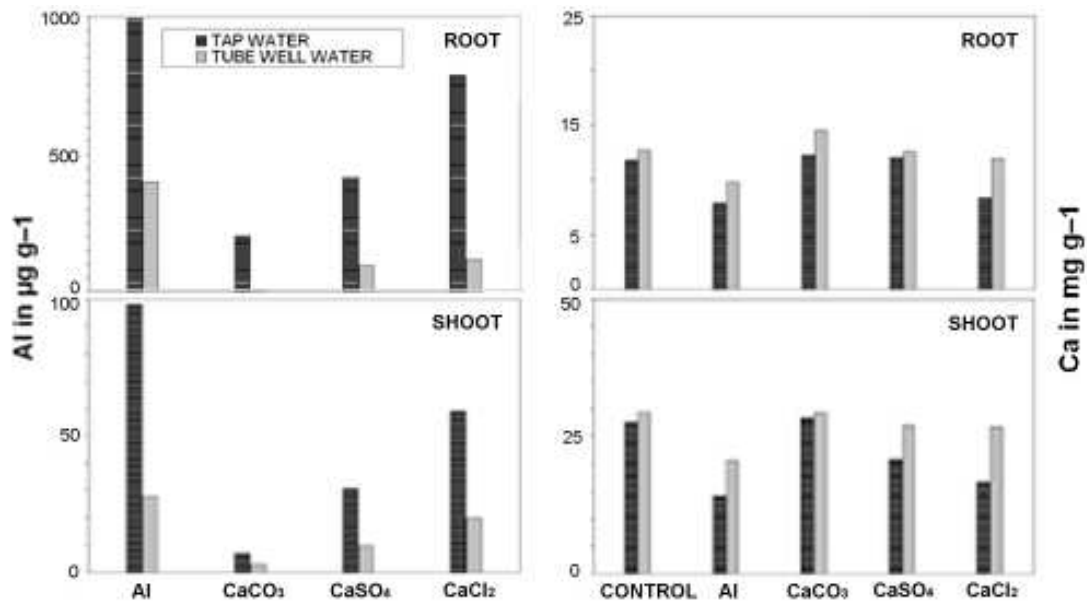


Fig. 2 - Al and Ca contents of root and shoot parts of 30 days old *Brassica* plants exposed to Al stress showing differential amelioration by three different salts of calcium (CaCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in tap water and tube well water irrigation. Values are means of 10 replicates.

DISCUSSION

The toxicity of Aluminium (Al) is the major growth-limiting factor for plants in strongly acidic soils and mine spoils (Foy 1984). Al-Ca interaction is well known in plants as in-plant (Jones *et al.* 1998; Ramos-Diaz *et al.* 2007) and/or soil-plant interactions. Many authors have cited the efficacy of Ca^{2+} in reducing Al^{3+} toxicity in many crop plants (Alva *et al.* 1986; Andersson 1988; Foy 1992; Brady *et al.* 1993.). Wright *et al.* (1987) found that exchangeable Ca, soil Ca saturation percentage, and total soil solution Ca were positively correlated with snap bean (*Phaseolus vulgaris* L.) root and shoot growth in acid soil, while soil Al saturation percentage, total soil solution aluminium and extractable aluminium were negatively correlated with growth. They found that the ratio of Ca:Al in the soil solution was more closely related to growth than the concentration of any single element in the soil solution.

Brassica juncea (L.) Czernj & Cosson illustrated different aerial and rhizotoxic symptoms by different doses of Al mixed with the soil. Based on the rhizotoxicity data the dose of $500 \mu\text{g g}^{-1}$ (MECT_{50}) of Al was taken as the reference dose for subsequent experiments. Lance and Pearson (1969) showed that reduced Ca uptake was the first externally observable symptom of Al damage on cotton seedling roots. Blockage of Ca channel by Al observed in *Amaranthus* (Rangel and Elliot 1992) also operates here (Figure 2).

Lund (1970) found that Ca reduced the detrimental effects of Al in nutrient solutions. Franco and Munns (1982) reported that increasing Ca (CaCl_2) concentrations from 8 to $80 \mu\text{g g}^{-1}$ suppressed Al toxicity in bean. In our experiment the induced effects of Al^{3+} at $500 \mu\text{g g}^{-1}$ in *Brassica juncea* (L.) Czernj & Cosson were ameliorated by a dose of $1200 \mu\text{g g}^{-1}$ of Ca (CaCO_3).

A comparative study of the efficacy of the different compounds of Ca indicated similar trend of amelioration, but with differential dose action, the order of efficacy being $\text{CaCO}_3 > \text{CaSO}_4 \cdot 2\text{H}_2\text{O} > \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. At lower pH the availability of Al to the plant is more (Tan *et al.* 1993) and the dose of Ca needed to compensate the action is more. This is clearly reflected in the differences of pH values associated with the three different solutions of the Ca compounds in our experiments (Table 2).

Parallel study with river (tap) water and the tube-well water irrigation (Figure 2; Table 2 and 3) makes interesting finding in which all the three Ca-compounds have similar results in the later case. This can be explained by the high percentage of the basic nutrients and the alkalinity of the tube-well water (pH 7.52, Total alkalinity as CaCO_3 464.00 mgL^{-1} , calcium 44.00 mgL^{-1} , magnesium 49.08 mgL^{-1}). Thus, it is suggested that use of CaCO_3 in the form of locally available mineral lime in the soil along with the tube-well watering may be tried to reduce the toxicity of Al^{3+} species in the soil.

REFERENCES

- Alva, A. K., Asher, C. J. & Edwards, D. G. (1986). The role of Calcium in alleviating aluminum toxicity (subterranean clover; soybean). *Austr. J. of Agri. Res.* **37**: 375-382.
- Alva, A. K. & Sumner, M. E. (1989). Alleviation of aluminum toxicity to soybeans by phosphogypsum or calcium sulfate in dilute nutrient solutions. *Soil Sci.* **147**: 278-285.
- Andersson, M. (1988). Toxicity and tolerance of aluminium in vascular plants – a literature review. *Water, Air and Soil Poll.* **39**: 439-462.
- Blancher, R. W., Rehm, G. & Coldwell, A. C. (1965). Determination of sulphur in plant material by digestion with nitric acid and perchloric acid. *Soil Sci. Soc. Am. Proc.* **29**: 71-72.
- Brady, D. J., Edwards, D. G., Asher, C. J. & Blamey, F. C. P. (1993). Calcium amelioration of aluminium toxicity effects on root hair development in soybean (*Glycin max* L.) Merr. *New Phytol.* **123(3)**: 531-538.
- Cocker, K. M., Evans, D. E. & Hodson, M. J. (1998). The amelioration of aluminium toxicity by silicon in wheat (*Triticum aestivum* L.): malate exudation as evidence for an in planta mechanism. *Planta* **204**: 318-323.
- Dash, S., Panda, K. K. & Panda, B. B. (1988). Biomonitoring of low levels of mercurial derivatives in water and soil by *Allium* micro nucleus assay. *Mutat. Res.* **203**: 11-21.
- El Aggan, W. H. (1995). The role of calcium in alleviation of aluminium toxicity on *Azolla mexicana*. *Alexandria J. Agric. Res.* **40**: 175-189.
- Foy, C. D. (1984). Physiological effects of hydrogen, aluminum and manganese toxicities in acid soils', in F. Adams (ed.), *Soil acidity and liming* (Second Edn.), *Soil Sci. Soc. Am., Amer. Soc. Agron. and Crop Sci. Soc. Am.* Madison, Wisconsin, pp. 57-97.
- Foy, C. D. (1992). Soil chemical factors limiting plant root growth. *Adv. Soil Sci.* **19**: 97-149.
- Franco, A. A. & Munns, D. N. (1982). Acidity and aluminum restraints on nodulation, nitrogen fixation, and growth of *Phaseolus vulgaris* in nutrient solution. *Soil Sci. Am. J.* **46**: 296-301.
- Gomez, K. A. & Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research*, John Wiley & Sons, New York, 680 pp.
- Gu, MingHua, Koyama, H., Hara, T. & Gu, MH. (1998). Effects of silicon supply on amelioration of aluminum injury and chemical forms of aluminum in rice plants. *Jap. J. Soil Sci. Plant Nutr.* **69**: 498-505.
- Hu, HongQing, Huang, QiaoYun, Li, XueYuan, Xu, FengLin, Hu, HQ., Huang, QY, Li, XY & Xu, FL. (1995). The deminishing effect of phosphate rock application on aluminium toxicity in acid soil. *Scien. Agric. Sinica.* **28**: 51-57.
- Jones D. L, Kochian L. V. & Gilroy S. (1998). Aluminum induces a decrease in cytosolic calcium concentration in BY-2 tobacco cell cultures. *Plant Physiol.* **116**: 81-89.
- Lance, J. C. & Pearson, R. W. (1969). Effect of low concentrations of aluminum on growth and water and nutrient uptake by cotton roots. *Soil Sci. Soc. Am. Proc.* **33**: 95-98.
- Lenoble, M. E., Blevins, D. G., Sharp, R. E. & Cumbie, B. G. (1996). Prevention of aluminium toxicity with supplemental boron. I. Maintenance of root elongation and cellular structure. *Plant Cell Environ.* **19**: 1132-1142.
- Liao H, Wan H, Shaff J, Wang X, Yan X & Kochian L. V. (2006). Phosphorus and aluminum interactions in soybean in relation to aluminum tolerance. Exudation of specific organic acids from different regions of the intact root system. *Plant Physiol.* **141(2)**: 674-84.
- Lund, Z. F. (1970). The effect of calcium and its relation to several cations in soybean root growth. *Soil Sci. Soc. Am. Proc.* **34**: 456-459.
- Ma, J. F. (2000). Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol.* **41**: 383-390.
- Ma J. F. (2007). Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. *Int Rev Cytol.* **264**: 225-52.
- Pintro, J., Calba, H., Fallavier, P. & Barloy, J. (1998). Effects of different calcium and sulfate concentrations in nutrient solutions on ionic strength values, aluminium activity and root growth of maize plants. *J. Plant Nutr.* **21**: 2381-2387.
- Plieth, C., Sattelmacher, B., Hansen, U. P. & Knight, M. R. (1999). Low pH mediated elevations in cytosolic calcium are inhibited by aluminium: a potential mechanism for aluminum toxicity. *Plant J.* **18**: 643-650.
- Poschenrieder, C, Gunsé, B, Corrales, I. & Barceló, J. (2008). A glance into aluminum toxicity and resistance in plants. *Sci Total Environ.* **400(1-3)**: 356-68.
- Rahman, S. (1992). Amelioration of aluminium toxicity in *Eucalyptus camaldulensis* seedlings by high calcium in nutrient solution. *Bangl. J. of Soil Sci.* **23**: 51-60.

- Ramos-Diaz, A., Brito-Argaez, L., Munnik, T. & Hernandez-Sotomayor, S. M. T. (2007). Aluminum inhibits phosphatidic acid formation by blocking the phospholipase C pathway. *Planta* **225**: 393-401.
- Rengel, Z. & Elliott, D. C. (1992). Mechanism of aluminum inhibition of net $^{45}\text{Ca}^{2+}$ uptake by *Amaranthus* protoplasts. *Plant Physiol.* **98**: 632-638.
- Tan, K., Keltjens, W. G. & Findenegg, G. R. (1993). Aluminum toxicity in sorghum genotypes as influenced by solution acidity. *Soil Sci. Plant Nutr.* **39**: 291-298.
- Wang Y., Stass, A. & Horst, W. J. (2004). Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize. *Plant Physiol.* **136(3)**: 3762-70.
- Wright, R. J., Baligar, V. C. & Wright, S. F. (1987). The influence of acid soil factors on the growth of snap beans in major appalachian soils. *Commun. Soil Sci. Plant Anal.* **18**: 1235-1252.