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ALUMINUM (AL^{+3}) INDUCED GROWTH INHIBITION IN MAIZE (*ZEA MAYS L*)

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ABSTRACT

The aim of the study was to see the effect of Aluminum (Al) toxicity on the growth of Maize plants. Plants were treated with different concentrations of Aluminum (1.5mM, 3.5mM, 5.5mM, 7.5mM, and 9.5mM) for over a period of 15 days and 30 days. In the present study the effect of Al on root growth, shoot growth, leaf growth, fresh weight, dry weight of total plants, Relative Growth Index(RGI), % phytotoxicity and Chlorophyll content in Maize plants were studied. Growth of Maize was adversely affected in terms of root, shoot, leaf growth, fresh weight and dry weight of total plants at 7.5mM and 9.5mM concentrations of Al. Reduction of growth was noted in both 15^{th} and 30^{th} day old plants. In contrast % phytotoxicity increased. Root growth was more affected than the shoot growth by the Al toxicity. Chlorophyll content was gradually reduced with Al treatments in both 15 and 30 day old plants. Maximum reduction of Chlorophyll content was noted at 9.5mM Al exposure.

KEYWORDS: Fresh weight, dry weight, Relative Growth Index (RGI), % phytotoxicity and Chlorophyll content.

INTRODUCTION

Intense industrial activity and urbanization in recent times especially in developing countries have led to serious environmental pollution resulting in a large number and variety of contaminated sites which became a threat to the local ecosystems. In all these natural resources such as soil, water, air, and vegetation are adversely affected by these pollutants. Al toxicity is directly related to acidic soils and substantially limits crop yield. Aluminum is one of the most abundant elements in the earth's crust, and toxic for many plants when the concentration is greater than 2–3 ppm with a soil pH < 5.5]. Acidic soils are phytotoxic as a result of nutritional disorders deficiencies or unavailability of essential nutrient such as Calcium, Magnesium, Molybdenum, Phosphorus and toxicity of Aluminum, Manganese and Hydrogen activity (Foy *et al.*, 1978; Foy, 1984; Carver and Own by, 1995; Jayasundara *et al.*, 1998) Al toxicity is considered the most important growth limiting factor for plants in acidic soils (Foy *et al.*, 1978; Foy, 1984; Carver and Own by, 1995; Jayasundara *et al.*, 1998). The primary response to Aluminum stress occurs in the roots (Foy *et al.*, 1978; Foy, 1984; Taylor, 1988, Jayasundara *et al.*, 1998.). Aluminum injured roots are stubby and brittle. Root tips and lateral roots thicken and turn brown the root system as a whole is affected, with many stubby lateral roots and no fine branching such roots are inefficient in absorbing nutrients and water (Foy *et al.*, 1978). The main symptom of Al toxicity is rapid inhibition of root growth. A number of mechanisms may cause this including Al interactions within the cell wall, the plasma membrane, or the root symplasm (Taylor, 1988; Marschner 1991; Horit 1995, Kochian 1995).

Maize constitute a staple food in many regions of the world belongs to *Poaceae* (Gramineae) family. Maize can be grown on a wide variety of soils, but performs best on well drained, well aerated, deep warm loams and silt loams containing adequate organic matter and well supplied with available nutrients and grown successfully on soils with a pH of 6.0 – 7.0. In a 100 gram Maize kernels contain 86 calories and a good source of the B vitamins, thiamin, niacin, pantothenic acid (B5), folate, vitamin A, vitamin C, iron, Mg, and K. Maize is increasingly used as feedstock for the production of Ethanol fuel and it is widely used in Germany as a feedstock for biogas plant. In this study root growth, shoot growth, leaf size, width, RGI, % phtotoxicity, chlorophyll content of 15^{th} day and 30^{th} day of Maize plants under different concentrations of Aluminum were assayed.

MATERIALS AND METHODS

Preparation of soil for experiment

The soil was collected from a local nursery. The soil was air dried and sieved through 2mm sieve to discard the non soil particles. Earthen pots of 18 cm diameter and 22cm height were selected for growing the plants. Each pot was filled with 2.5kg of air dried soil.

Growth conditions

The seeds of Maize were collected randomly from the research field of Agricultural University, Karimnagar and Telangana, India. Seeds were surface sterilized with 0.001M mercuric chloride for two minutes and thoroughly washed with water several times. Ten sterilized seeds were sown in each pot. All the pots were watered to field capacity daily. Plants were thinned to a maximum of three seedlings per pot after a week of germination. The seven day old plants were treated with five different concentrations of Al solution i.e.: 1.5mM, 3.5mM, 5.5mM, 7.5mM and 9.5mM taken as Aluminum sulphate. Different concentrations of Aluminum solution (300 ml) were given once in two days to the field capacity, total of ten such doses were given during the experimental period. Plants treated with water served as control. Two doses of soil application of NPK in the ratio of (100:109:137 ppm) was prepared using KH_2Po_4 and NH_4No_3 and was given to the plants on 10^{th} and 20^{th} day of growth. The plants were grown under natural photoperiod. During the growth period plants were regularly monitored for any morphological changes and phytotoxicity symptoms. Each treatment including the control was replicated six times.

Sample collection

The plant samples were collected at every 15 day intervals, i.e., 15 days and 30 days. The plants were first removed from the soil, the entire plants with roots and shoots were put under constant flow of water to remove the soil particles and exogenous contaminants adhered to the plants. The water droplets were blotted dry with help of blotting paper. Sampling was done in the early hours for the measurement of various morphological, growth and biochemical parameters.

Phytotoxicity symptoms

The plants were regularly observed for any morphological changes and phytotoxicity symptoms if any at all the stages of plant growth.

Morphological parameters

Root, Shoot and Leaf length

The Maize plants were separated into roots, shoot and leaves after sampling and the length of each part was measured using a scale. Shoot height was measured from the junction of the root to the tip of the tallest leaf.

Percent phytotoxicity: was calculated using the formula of Chou and Lin, (1976).

$$\text{Percent Phytotoxicity} = \frac{\text{Radical length of control - Radical length of test x 100}}{\text{Radical length of control}}$$

Fresh weight

The plants of both treated and control of Maize (*Zea mays L) were* collected at each sampling stage and gently separated into roots and shoots and their fresh weight was recorded immediately.

Dry weight

After recording the fresh weight of the roots and shoots, they were kept in small labeled paper covers and were dried in a hot air oven at 80°C for 48 hrs till constant dry weights were obtained.

Relative growth index (%) (RGI)

According to the method of Paliouris and Hutchinson, (1991). Relative growth index of each concentration was calculated as indicated below for both the roots and shoots.

$$\text{RGI} = \frac{\text{Average dry weight of a root or shoot in treatment solution x 100}}{\text{Average dry weight of a root or shoot in control solution}}$$

Chlorophyll Estimation

The total Chlorophyll content was estimated according to the method of Arnon1949. 0.2 grams of leaf material was cut into small pieces and homogenized with 10 ml of 80% acetone in a clean mortar. The green slurry was centrifuged at 3000rpm for 12 minutes. The supernatant was transferred into a clean test tube and the residual pigment in the pellet is re-extracted with 10 ml acetone. The process is repeated till a complete white pellet is obtained. The total volume is made up to 25 ml with 80% acetone. The optical density was determined at 663&645 using 80% acetone solvent as blank in a spectrophotometer.

Total Chlorophylls = (0.D 645 × 20.2) + (0.D 663 × 8.02) V/1000× W

Chlorophyll a = (0.D 663 × 12.7) -- (0.D 645 × 2.69) V/1000× W

Chlorophyll b = (0.D 645 × 22.9) -- (0.D 663 × 4.68) V/1000× W

RESULTS AND DISCUSSIONS

Root and Shoot Growth

Aluminum does not affect the seed germination but helps in new root development and seedling establishment [Nosko P., Brassard P., Kramer J.R., Kershaw K.A., white spruce (*Picea glauca*), 1988]. Root growth inhibition was detected 2–4 days after the initiation of seed germination (Bennet R.J., Breen C.M., Fey M.V., in *Zea mays* 1987).The major Al toxicity symptom observed in plants is inhibition of root growth (Delhaize E., Ryan .R) . The roots exhibit greater signs of cellular damage than other parts of the plant (wheat (*Triticum aestivum*) cultivars 1992). Aluminum causes extensive root injury, leading to poor ion and water uptake (Barcelo & Poschenrieder, 2002). One of hypothesis is that the sequence of toxicity starts with perception of aluminum by the root cap cells, followed by signal transduction and a physiological response within the root meristem. However, recent work has ruled out a role of the root cap and emphasizes that the root meristem is the sensitive site. Root tips have been found to be the primary site of aluminum injury, and the distal part of the transition zone has been identified as the target site in maize (*Zea mays*) (Sivaguru &Horst, 1998)

Maize plant growth was adversely affected by Al toxicity stress. At 7.5mM and 9.5mM concentrations of Al, the root, shoot, leaf growth, fresh weight and dry weight were significantly reduced in both 15 day and 30 day old Maize plants. As Fig No. 1a, 1b, 1c, 1d shows that root growth was more susceptible than shoot growth with Al toxicity, particularly at 9.5mM concentration. Thick and stubby roots were clearly seen.

Fig No 1: Maize plants (in pots) under different concentrations of Aluminum (15 days old plants)

15 Days old Maize plants

Fig No 1a: Maize plants under different concentrations of Aluminum (15 days old plants)

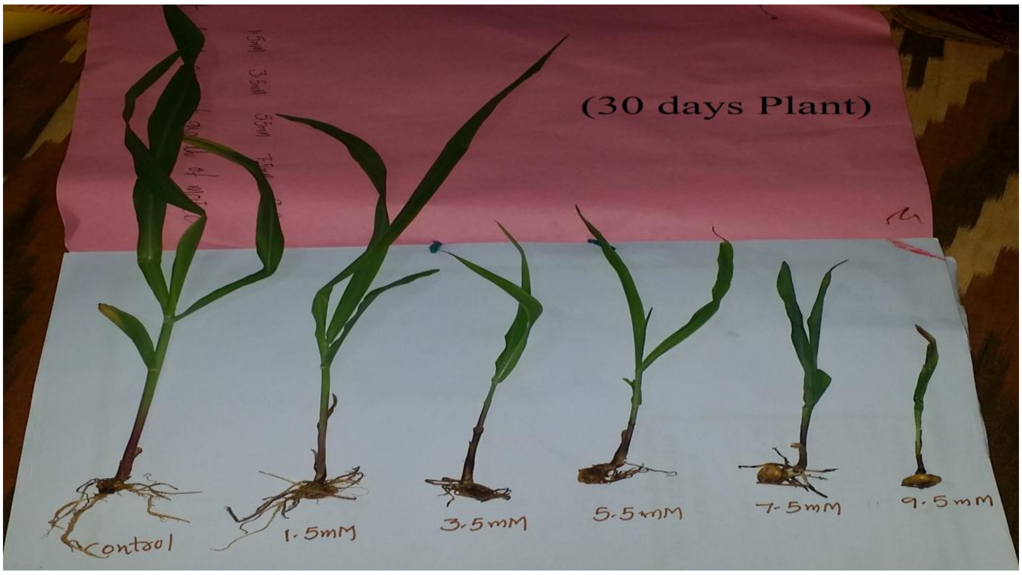

Fig No 1b: Maize plants under different concentrations of Aluminum (30 days old plants)

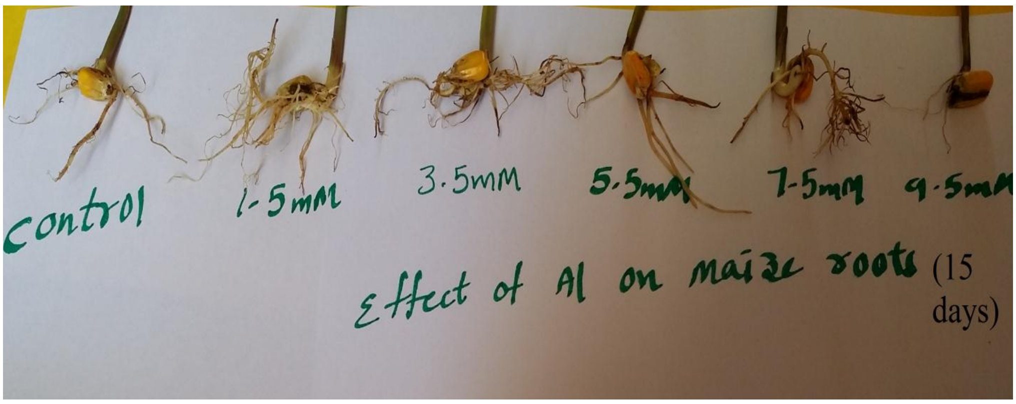

Fig No 1c: Effect of Aluminum on root morphology (15 days old Maize plants)

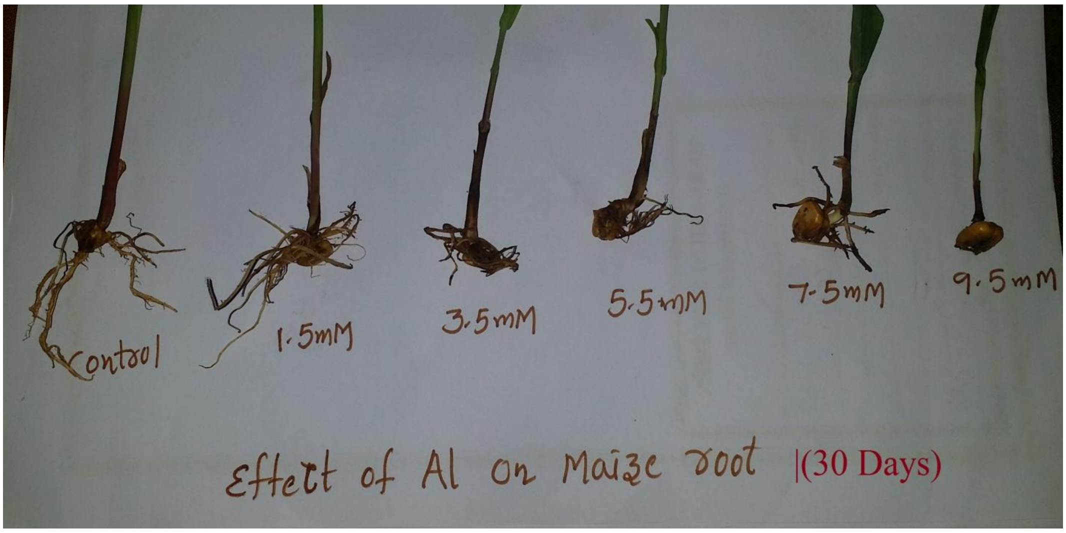

Fig No 1d: Effect of Aluminum on root morphology (30days old Maize plants)

A gradual reduction in growth parameters were observed with increased Al3+ concentration. Al3+ exhibited injurious effects followed by the death of *Maize* seedlings when added at the highest (9.5mM) level. Inhibition of growth and reduction of biomass production are general responses of some plants to metal toxicity and are often a reliable indication of plant's sensitivity to their stresses.

Table No I: Effect of Soil applied Aluminum on root, shoot and leaf length and width of 15 days old Maize plants.

Al conc. (in mM)	Root length (in cm)	Shoot length (in cm)	Leaf length (in cm)	Leaf width (in cm)
Control	6.1	8.4	8.5	2.7
1.5mM	5.0	5.2	6.7	2.6
3.5mM	4.3	4.6	5.6	2.9
5.5mM	3.5	4.2	4.2	1.5
7.5mM	3.0	3.9	4.2	2.4
9.5mM	1.4	3.2	2.7	1.0

Table No Ia: Effect of Soil applied Aluminum on root, shoot and leaf length and width of 30 days old Maize plants.

Al conc. (in mM)	Root length (in cm)	Shoot length (in cm)	Leaf length (in cm)	Leaf width (in cm)
Control	13.0	15.7	32.0	1.8
1.5mM	10.5	11.0	20.5	1.4
3.5mM	6.4	8.6	12.5	1.2
5.5mM	6.0	8.0	10.2	1.0
7.5mM	4.3	5.1	9.5	0.9
9.5mM	1.2	2.8	6.5	0.7

Leaf Growth

The development of maize leaves at all Aluminum levels was delayed compared to control plants .In the presence of Al, the fully developed leaves had a clearly smaller size than the leaves of the control plants. The length and width of leaves as shown in Fig No 1e & 1f decreased in proportion to the increasing doses of Aluminum. Similar results were obtained by Konarska in Capsicum annuum leaves. Al also resulted in the formation of smaller young leaves that are curled along the margin with yellow tips and having necrotic spots. And Al induced impairment in growth has been reported previously in a number of crops such as Buck Wheat (Shen *et al.,* 2004), Tomato (Simon *et al.,* 1994), Citrus (Chen, 2005). Aluminum also resulted in the formation of smaller young leaves that are curled along the margin with yellow tips and having necrotic spots. Older leaves show marginal chlorosis with subsequent lethality in Maize. (Pavan & Bingham 1982 & Foy 1984).

Fig No 1e: Effect of Aluminum on leaf morphology (15 days old plants)

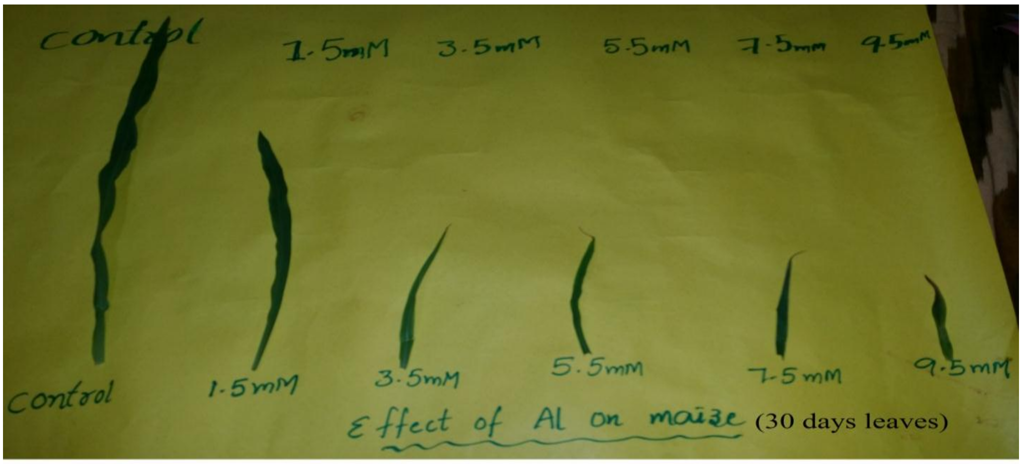

Fig No 1f : Effect of Aluminum on leaf morphology (30 days old plants)

RGI and Percent (%) Phytotoxicity

Our results indicated that the RGI in Al treated 15 days & 30 days old Maize plants was reduced compared to control plants where as the % phytotoxicity increased compared to control plants. A linear relation was observed between the concentration of Al and % of the phtotoxicity in 15 days& 30 days old Maize plants. At 9.5mM % phtotoxicity of Al was maximum i.e. 77.04% in 15 days old Maize plants, it was increased to 90.16% in 30 days old Maize plants. Similar trend in % phtotoxicity as a result of Al was observed in wheat and Flax by Saritha et al, 2016. A marked correlation was observed between % phtotoxicity and RGI in 15 days &30 days old Maize plants. Increased in % phtotoxicity resulted in the decrease of RGI (Table No II, II a and Fig No 3a, 3b).

Table No II: Effect of Soil applied Aluminum on Fresh weight, Dry weight, RGI, % Phytotoxicity of 15 days old Maize plants.

Al conc. (in mM)	Fresh weight (in mg)	Dry weight (in mg)	RGI (in %)	% Phytotoxicity
Control	6.23	4.72	100	0
1.5Mm	4.98	3.28	69.49	18.03
3.5mM	2.86	1.92	40.67	29.50
5.5mM	2.49	1.54	32.62	42.62
7.5mM	1.82	1.08	22.88	50.81
9.5mM	1.06	0.52	12.99	77.04

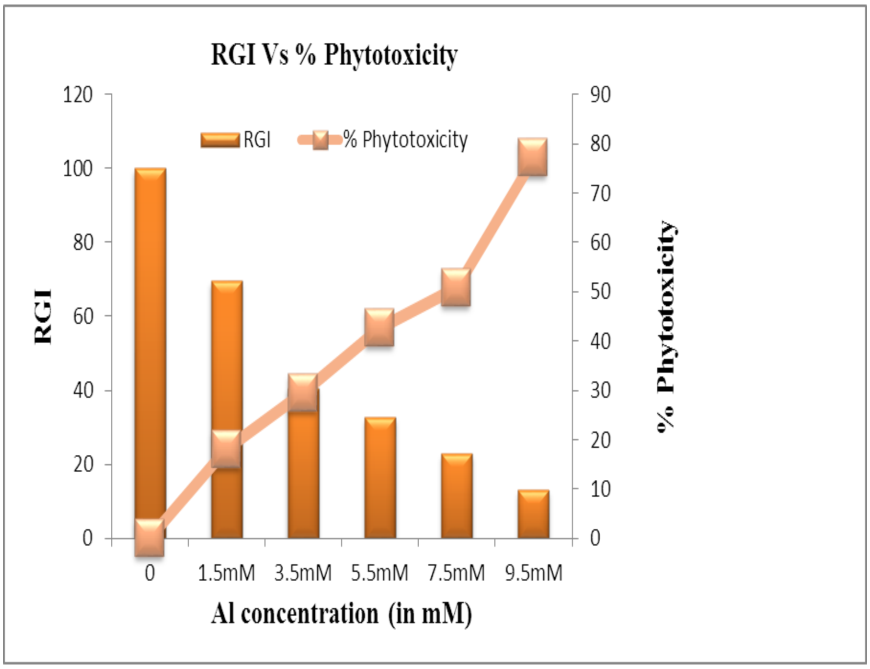

Fig 3a: Relative Growth Index (RGI) decreased with increase in %phytotoxicity in 15 days old Maize plants.

Table No IIa: Effect of Soil applied Aluminum on Fresh weight, Dry weight, RGI, % Phytotoxicity of 30 days old Maize plants.

Al conc. (in mM)	Fresh weight (in mg)	Dry weight (in mg)	RGI (in %)	% Phytotoxicity
Control	8.72	5.02	100	0
1.5Mm	5.16	4.61	91.83	19.13
3.5mM	4.82	4.01	79.88	50.34
5.5mM	4.04	3.15	62.74	53.26
7.5mM	2.32	2.02	40.23	66.79
9.5mM	1.49	0.72	14.34	90.16

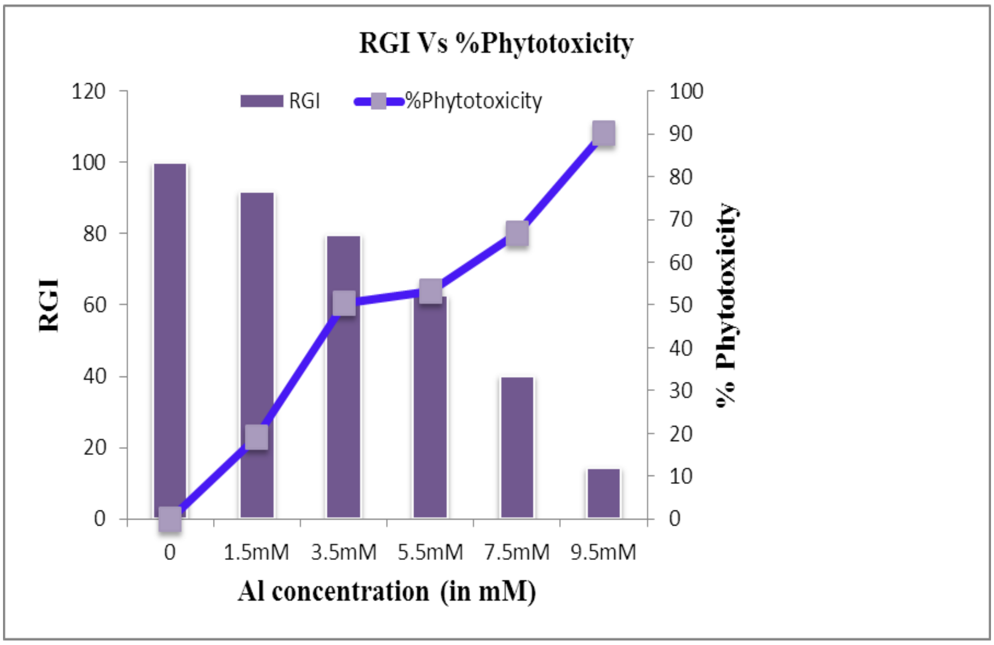

Fig 3b: Relative Growth Index (RGI) decreased with increase in % phytotoxicity in 30 days old Maize plants.

Chlorophyll Content

The effect of Al on the chlorophyll content in 15 days and 30 days old plant of Maize is represented in Table No III, III a & and Fig No 1g & 3c. Chl a, Chl b content noted decreased with the increased Al treatment when compared to the control plant. Chl a, Chl b content decreased with increase in Al treatment as compared to the control plant. Chl(a+b), Chla/b ratio also decreased with increasing concentrations of Al. Maximum decrease was recorded at 9.5mM concentration.The similar results was observed by Zhang et al (2007) and Radic *et al.*,(2010)in soyabean (Glycine max L) and duck weed(Lemna minor L).Haider et al (2007) found that Al decreased chlorophyll content that coincided with decline Mg and Fe absorption.

Table No III: Effect of Soil applied Aluminum on Chl a, Chl b, Chl (a+b) and Chl a/b of 15 days old Maize plants.

Al conc. (in mM)	Chl a	Chl b	Chl (a+b)	Chl a/b
Control	4.055	3.072	7.127	1.319
1.5mM	3.995	2.081	6.076	1.919
3.5mM	3.805	2.086	5.573	1.839
5.5mM	3.213	2.042	5.255	1.573
7.5mM	2.488	2.031	4.519	1.225
9.5mM	1.214	1.021	2.235	1.189

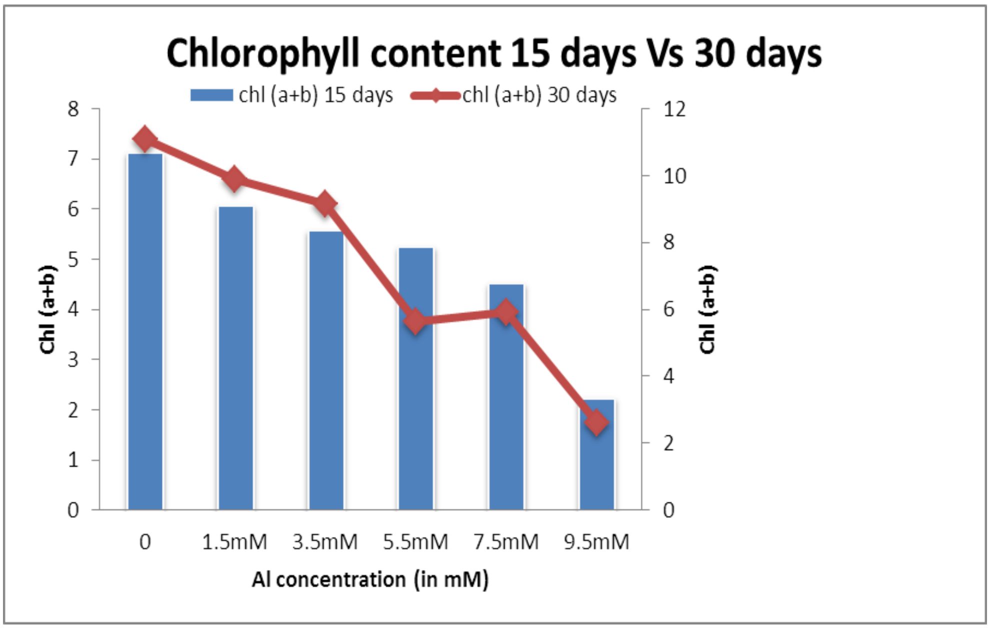

Fig No 3c: Effect of Al on Chlorophyll content in 15 days and 30 days old plants of Maize.

Table No III a: Effect of Soil applied Aluminum on Chl a, Chl b, Chl (a+b) and Chl a/b of 30 days old Maize plants.

Al conc. (in mM)	Chl a	Chl b	Chl (a+b)	Chl a/b
Control	6.412	4.681	11.093	1.369
1.5mM	5.360	4.551	9.911	1.177
3.5mM	5.580	3.591	9.171	1.553
5.5mM	3.361	2.271	5.632	1.479
7.5mM	2.661	2.252	5.913	1.625
9.5mM	1.371	1.223	2.594	1.121

Fig No 1g: Effect of Al on chlorophyll content of Maize 15 days &30 days old plants

CONCLUSION

The results demonstrated that the root growth, shoot and leaf growth inhibition were part of the overall expression of Al toxicity in Maize plants resulted that the Maize plants were very sensitive to Aluminum.

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