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ECOPHYSIOLOGICAL STUDIES OF A CEREAL CROP (*ORYZA SATIVA* L.) WITH ALUMINIUM OXIDE STRESS

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Abstract: *Rice is grown over an extremely wide range of climatic conditions. It is best suited to the regions that have high temperature, high humidity, prolonged sunshine and assured supply of water. Aluminium is not regarded as an essential nutrient, but low concentration can sometimes increase plant growth or induce other desirable effects. Aluminium is an important growth- limiting factor for plants in acid solution below pH 5.0 but can occur at pH levels as high as 5.5 in mine soils. The major aluminium toxicity symptom observed in plants is inhibition of root growth the root exhibit greater signs of cellular damage than other parts of the plant. Aluminum toxicity could be observed in root tips and in lateral roots. Aluminium oxide (Al_2O_3) was used as test chemical in this experiment. The laboratory experiment is carried out the actual intensity of the chemical on various levels of seedlings for seven days. First stock solution was prepared by dissolving 1gm of test chemical in 1L of distilled water. Different concentrations of solution-Control (0.0), 25, 50, 75,100 mg/L of Al_2O_3 was prepared by proportional dilution with distilled water which is used for various treatment Sterilized cotton & blotting papers are spread on the petriplates where test chemicals were taken & surface sterilized 10 healthy seeds were taken for study of pigment. The maximum germination, emergence of leaf of seeds was observed in control. There was an inverse relationship between the germination of seeds and concentration of Al2O3. The root and shoot length was decreased with the increased level of aluminum oxide. The ratio of root and shoot length in control or untreated seeds is 1.87 cm and it decreased with increase in concentration of test chemical and finally at 100mg/L concentration it was 1.52 cm. There was increase in chl-a, chl-b,total chlrophyll content with increase in the concentration of Al_2O_3. Conclusively, our results show that Al_2O_3 at higher concentration decreases seed germination, leaf emergence, root and shoot length and an increase in chl-a, chl-b and total chlorophyll content. Our results suggest that the presence of Al_2O_3 at higher concentration resulted in growth inhibition, structural damage and decline in physiological and biochemical activities.*

Key words: *Oryza sativa L.Pooja, Aluminium oxide (Al_2O_3), toxicity, Pigment analysis, Root length, Shoot length, Root/shoot ratio, Chlorophyll a,b and total chlorophyll.*

INTRODUCTION:

Rice is extensively grown food crop in India, occupying an area of over 43.77 million hectares (nearly 37% of the total area under cultivation). India is the second largest producer of rice in the world with an annual production of 95.68 million tones of paddy in 2007-08. In India, Rice is grown practically in all the states, but it is mainly cultivated in the coastal areas of Maharashtra, Andhra Pradesh, Assam, Kerala, Tamil nadu and Odisha. It is best suited to the regions that have high temperature, high humidity, prolonged sunshine and assured supply of water. An annual rainfall of 60-120 cm is for lowland varieties. Clay to clay-loam soils is more suitable for Rice cultivation. It can be grows in soils with a wide pH range of 5-9. Stress is defined as 'any force applied to an object which results in changes of objects dimensions'. With relation to plants, stress is usually defined as 'an environmental factor that exerts an adverse influence on the plant'. Stress is any change in environmental conditions that might reduce or adversely change plants growth and development. Aluminium is not regarded as an essential nutrient, but low concentration can sometimes increase plant growth or induce other desirable effects. Aluminium is an important growth- limiting factor for plants in acid solution below pH 5.0 but can occur at pH levels as high as 5.5 in mine soils. Generally, aluminium interferes with cell divisions in root tips and lateral roots, increases cell wall rigidity by cross linking pectins, reduces DNA double helix, fixes phosphorous in less available forms in soils and on surface, decreases root respiration, interferes with enzymes activity governing sugar phosphorylation and the deposition of cell wall polysaccharides and the uptake, transport and also use of several essentially nutrients (Ca, Mg, K, P & Fe). Excess aluminium even induces iron deficiency symptoms in rice, but aluminium toxicity manifested only in acid conditions, in which the phytotoxic from Al_2O_3 predominates. The salient features of aluminium toxicity and metabolism of different groups of flora are elucidated and their possible implication in the plant ecosystem are highlighted.

MATERIALS AND METHODS:

Experimental Plant: *Oryza sativa* L. is a common cereal crop in Odisha and is widely cultivated. *Oryza sativa* L.Pooja is a widely cultivated cereal crop by the local farmers in Odisha which are likely to expose to heavy metal stress and there is a need to study the effect of different concentration of heavy metal on the Pooja dhan. *Oryza sativa* L.Pooja is a short duration variety that takes

about 105-110 days duration in plane areas. Plant height is about 87.5 cm. It is a kharif (monsoon) crop sown in the month of June and harvested in the month of October-November. The seedlings are soft, brittle and fibrous. Seeds of this variety showed high percentage of germination both in field and laboratory conditions.

Test Chemical: Aluminium oxide (Al_2O_3) was used as test chemical. First stock solution was prepared by dissolving 1g of test chemical in 1L of distilled water. Different concentrations of solution-Control (0.0),25,50,75,100 mg/L of Al_2O_3 was prepared by proportional dilution with distilled water which is used for various treatment.

Seed Germination: The seeds were first kept in distilled water for 12 hrs and then soaked in wet cloth for 12 hrs which led to the germination of seeds. Appearance of plumule or radicle was considered as an index of germination.

Germination Studies: For germination studies sterilized plastic cups were used for the study by inserting holes at the bottom. Then soil was added to 3/4th volume of cups after washing it with tap water and drying and final rinsing with distilled water and drying under the sun. 10 numbers of seeds were kept in each cup at uniform distance in all the sets. Respective concentration of this test chemical (Control (0.0), 25, 50,75, 100 mg/L of Al_2O_3) was sprayed on the plastic cups before adding the seeds to the soil .The cups were kept under the sun. Seeds were allowed to germinate. Better sprouted and healthy seedling of 10 days old were used as experimental material. Care was taken to avoid drying and over flooding of test chemical in the cups.

Morphological Studies: The growth of plant was evaluated by measuring the shoot and root length of plant on 11th day.15cm scale was used for the measurement of the shoot and root length.

Estimation of Chlorophyll: The fresh samples of shoot materials of the 10 days old seedlings were collected. Care was taken for separation of sample treated with different concentrations. A known quantity of about 100 mg of samples of weighted shoot material was taken in a mortar and pestle and macerated to a paste by adding 80% acetone. Then it was stridden thoroughly and centrifuged (10 min).The pellet was discarded and the supernatant was kept for chlorophyll estimation. The optical density of each extract was determined in a spectrophotometer at a wavelength of 645 nm and 663 nm.

The total chlorophyll, chl-a and chl-b content was measured by recording the optical density of the extract at 645 nm and 663 nm wavelength and the values were calculated by using the formula given by Arnon (1949).

Chl a = (12.7*OD at 663 nm)-(2.69*OD at 645 nm)

Chl b = (22.9*OD at 663 nm)-(4.68*OD at 645 nm)

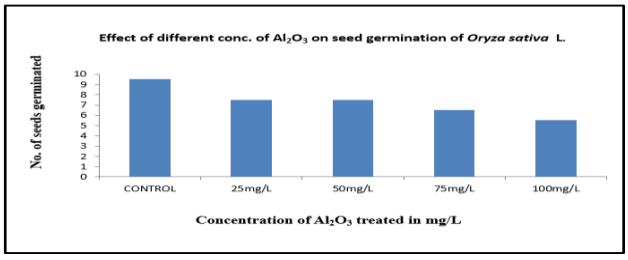
Total Chlorophyll = (20.2*OD at 663 nm) + (8.02*OD at 645 nm)

RESULT:

The radicle emerged after 72 hours incubation in a dark room at room temperature. The emergence of radicle or plumule was considered as the index of germination. The seeds were then exposed to light after germination. The following day, after the exposure of seeds to light, there was rapid elongation of radicle and plumule. By the end of 92 hours, most of the seeds showed radicle emergence. Further, there was rapid elongation of the primary root and the shoots split from coleoptiles and emerged as primary leaf. By the end of 10 days, the seedlings showed the development of primary root with nodal roots (adventitious roots), a number of rootlets and fully expanded first and second leaf in control.

Table 1: Effect of different concentration of Al_2O_3 on the number of seeds germinated of *Oryza sativa* L.

Day	Control			25 mg/L			50 mg/L			75 mg/L			100 mg/L		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	2	1	1	0	0.5	0	0	0	0	0	0	0	0	0
3	4	5	4.5	4	6	5	3	4	3.5	3	3	3	1	3	2
4	10	10	9.5	7	8	7.5	7	8	7.5	6	7	6.5	5	6	5.5



Observation: On day 4 the no. of seeds germinated in sample1 and sample2 are 9.5 & 10 under controlled medium, 7 & 8 under both 25mg/L & 50mg/L conc., 6 & 7 under 75mg/L conc. and 5 & 6 under 100mg/L conc. respectively. We had calculated the mean of the no. of seeds germinated in the taken concentrations Of Al_2O_3 up to day 4 and plotted a graph representing the no. of seeds germinated under different concentrations of Al_2O_3 in *Oryza sativa* L.

The leaf comes out after 4 days of sowing seeds. The number of leaf emergence data is given in the next table.

Table 2: Effect of different concentration of Al_2O_3 on the number of leaf emerged of *Oryza sativa*.L.

Day	Control			25 mg/L			50 mg/L			75 mg/L			100 mg/L		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
1	1	2	1.5	2	0	1	2	1	1.5	2	2	2	0	0	0
2	3	4	3.5	4	3	3.5	4	3	3.5	3	4	3.5	1	0	0.5
3	5	4	4.5	4	5	4.5	4	5	4.5	4	5	4.5	1	2	1.5
4	7	6	6.5	5	6	5.5	6	5	5.5	5	6	5.5	1	2	1.5
5	9	8	8.5	6	7	6.5	6	6	6	5	6	5.5	3	4	3.5
6	10	10	10	8	9	8.5	7	6	6.5	5	6	5.5	4	5	4.5
7	10	10	10	8	9	8.5	7	6	6.5	5	6	5.5	4	5	4.5

Observation: From the Table-2, we observed that after 4 days of seed sowing, the no. of leaves emerged from the germinated seeds. The number of leaves emerged were listed in the table regarding the last 7 days after seeds sowing. The means of the 10th day of seed sowing were taken as the final values for determination of graph by putting their mean values.

This graph was plotted representing the number of leaves emerged in the different concentrations of Al_2O_3 and also in controlled condition of *Oryza sativa* L.

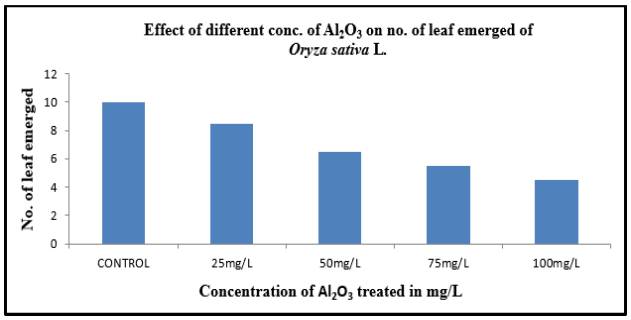
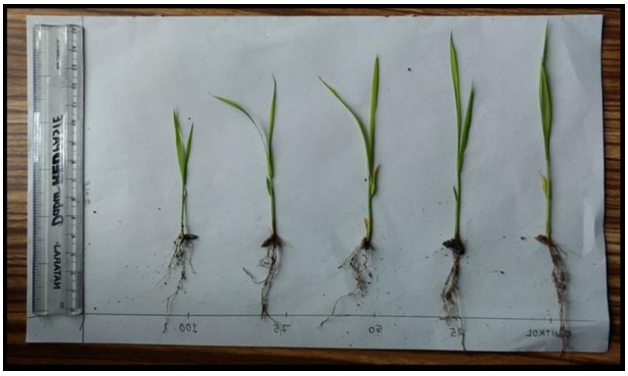


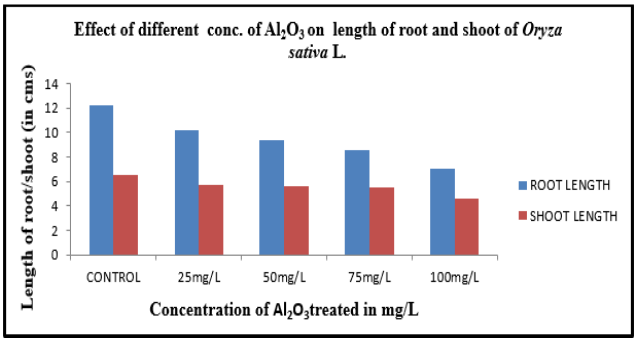
Table 3: Effect of different concentration of Al_2O_3 on the root and shoot length and Root and shoot ratio with respect to control of the 10 days old seedlings of *Oryza sativa.*

Conc.	Root Length	Shoot Length	Root/Shoot Ratio
Control	12.2	6.5	1.87
25 mg/L	10.2	5.7	1.78
50 mg/L	9.4	5.6	1.7
75 mg/L	8.6	5.5	1.56
100 mg/L	7	4.6	1.52

Photograph:



Observation: From the above table we observed the root length and shoot length and the root/shoot ratio of the ***Oryza sariva*. L.** plant under different conc. with Al_2O_3. Under controlled condition the root/shoot ratio is 1.87, 25mg/L is 1.78, 50mg/L is 1.70,75mg/L is 1.56. and under the conc. Of 100mg/L the root/shoot ratio is 1.52. Basing up on the above information we had plotted a chart showing the length of the root and shoot of ***Oryza sativa* L.** plants under the different concentrations of Al_2O_3



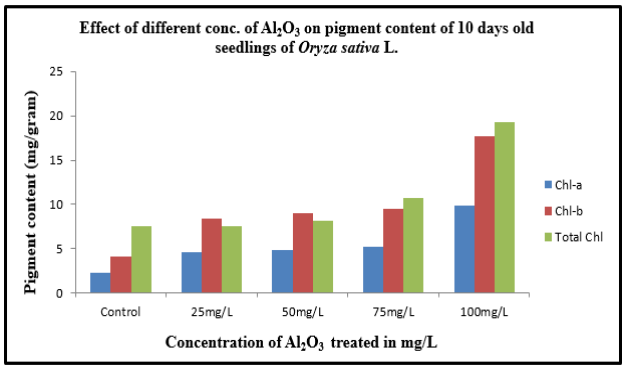
Pigment contents: Chlorophyll: Effect of test chemical on the chlorophyll contents against control and percent of pigments was presented. A significant change occur in the chlorophyll pigment was observed with increase in Al_2O_3 concentration. The percentage of chl-a, chl-b and total chlorophyll of seedlings exposed to 100mg/L showed a fall.

Table 4: Effect of Al_2O_3 on the pigment content (mg/l) of *Oryza sativa* L. on 10 days old seedlings.

Conc.	Chlorophyll- a			Chlorophyll- b			Total chlorophyll		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Control	2.45	2.07	2.26	4.43	3.74	4.08	10.74	4.28	7.51
25 mg/L	4.84	4.48	4.66	8.74	8.09	8.41	9.4	5.69	7.54
50mg/L	4.72	4.97	4.84	8.64	9.27	8.95	8.53	7.87	8.2
75 mg/L	5.18	5.33	5.22	9.37	9.63	9.5	10.09	11.41	10.75
100 mg/L	10.21	9.4	9.8	18.5	17	17.7	20.7	18	19.2

Observation: The amount of chlorophyll pigments such as chl-a, chl-b and total chlorophyll contents of *Oryza sativa* L. placed in controlled condition and in the different Al_2O_3 concentration were listed in the above table and their mean values were calculated. Thus the mean values of chl-a, chl-b and the total chlorophyll content of the plantlets in the controlled condition were 2.26, 4.08, and 7.51 respectively, that of the plantlets in 25mg/L concentration of Al_2O_3 were 4.66, 8.41 and 7.54, in 50mg/L concentration of Al_2O_3 were 4.84, 8.95 and 8.20, in 75mg/L concentration of Al_2O_3 were 5.22, 9.50 & 10.75, in 100mg/L concentration of Al_2O_3 were 9.81, 17.74 & 19.23 respectively.

Then the graph was plotted regarding these chlorophyll pigmentation values at different concentrations of *Oryza sativa* L.



DISCUSSION:

In the present study on exposure of rice seedlings to high concentration of Al_2O_3 increases the chl-a, chl-b, total chlorophyll. In general, decrease in germination has been one of the important manifestations of metal toxicity. The phytotoxicity of Al_2O_3 indicated by decrease in growth and development, metabolism and an induction of oxidative damage in various plant species. Al_2O_3 toxicity in plants limited the growth of both root and shoot. Al_2O_3 though an essential element for growth, showed toxicity symptoms at higher concentration and inhibited root growth. Al_2O_3 toxicity was marked in root system particularly in root blunt of *Oryza sativa* L.thickening and caused restraint on both cell division and cell elongation.

SUMMARY AND CONCLUSION:

In the present study of ecophysiological effects of aluminium oxide was evaluated by taking local cultivated cereal *Oryza sativa* L. Plants exposed to aluminium stress at high concentration inhibit seed germination, seedling growth and development. The germination data showed that there is negative impact of concentration of Al_2O_3 on the germination of seed. There is decrease in seed germination at higher concentration in comparison to control. Root and shoot growth of seedling was negatively affected when exposed to high concentration of Al_2O_3. Shoots were more affected and much reduced than roots. Effects of different concentrations of Al_2O_3 were visible in different pigment concentration of leaves. Aluminium toxicity increases chlorophyll formation which stimulated the formation of green pigment in shoots exposed to different concentrations of Al_2O_3. With increase in concentration of the toxicant exposed seedlings showed enhancement in chl-a, chl-b and total chlorophyll had direct impact on photosynthesis. Conclusively, our results show that Al_2O_3 at higher concentration decreases seed germination, leaf emergence, root and shoot length and an increase in chl-a, chl-b and total chlorophyll content. Our results suggest that the presence of Al_2O_3 at higher concentration resulted in growth inhibition, structural damage and decline in physiological activities.

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