

Growth and Physiology of Wheat (*Triticum vulgare*) Seedlings under Aluminum Stress

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Abstract: Aluminum (Al) toxicity is the most wide spread form of metal toxicity to plants and its occurrence is revealed only by salinity. Al toxicity is a major agricultural problem and is intensively studied in plant system. It is an important growth limiting factor on upland soils with a pH of less than 5 worldwide. The present study was aimed to see the effect of Al on the growth and physiological aspects of Wheat plant (*Triticum vulgare*). Root and shoot growth were inhibited under high concentrations of Al. Photosynthetic pigments (Chl a and Chl b) in the leaves were gradually decreased with high concentration of Al in the present study. Al treatment elevated activities of antioxidative enzymes such as Superoxide dismutase (SOD EC 1.15.1.1), Glutathione Reductase (E.C 1.6.4.2), where as activity of Catalase (CAT E.C 1.11.1.6) was deteriorated. The results of present study supports indicate that the Al treatment cause Oxidative stress and further influence lipid peroxidation.

Keywords: Salinity, Upland Soils, *Triticum vulgare*, Antioxidative enzymes, Superoxide dismutase, Glutathione Reductase, Catalase, oxidative stress, Lipid peroxidation

1. Introduction

Heavy metals are generally considered toxic to plant cells. There are about 50 metals that are of with the toxicological impact on plants growth, human and animal health. Generally, pesticides and fertilizers are used to increase the production of the crops. They contain heavy metals, extensive use of these products can contaminate agricultural lands (Vlamiš *et al.*, 1985). Toxic metals first accumulate in soil to reach the plant through roots/are taken up by the leaves from atmosphere. Heavy metals can cause various toxic effects on plants such as inhibition of seed germination, plant growth and crop yield (Shaukat *et al.*, 1999) and also cause alteration of normal metabolic pathways including respiration and photosynthesis by disrupting the cellular enzymes (Krupa *et al.*, 1993). Al is the most common metal in the earth's crust (8.2% by weight), the main Al containing primary minerals being feldspars and micas. For years, farmers have struggled with acid soils in which overabundance of soluble Al has spelled death to crops. And when a particular type of Maize or Sorghum or Wheat seemed able to tolerate the excess Al, the farmers gratefully accepted that crop as a gift of nature and didn't ask a lot of seemingly unanswerable questions (Kochian). It has been known for nearly a century that Al suppress root growth (Miyake, 1916). And that Al restricts the uptake of nutrients by plants. Al is known to damage cell membranes by binding to sulphhydryl groups of membrane proteins and by inducing lipid peroxidation (Peixoto *et al.*, 1999). Al-mediated reactive oxygen species (ROS) generation has been demonstrated in various plant systems (Panda *et al.*, 2003; Tamas *et al.*, 2004). It is present in water, soil and earth but most of its incorporated into Aluminosilicate soil minerals and very small quantities appear in soluble forms capable of influencing biological systems. Different forms of Al occur in soil solution $Al(OH)^{+2}$ & $Al(OH)^{-2}$ at pH 4-5. Al^{+3} at pH 5.5 & $Al(OH)^{-4}$ at pH 7-8. According to Kochian's (1995) opinion toxicity has been convincingly demonstrated for Al_3 & Al^{+3} . Al toxicity commonly occurs in oxisols & ultisols as well as in heavily leached soils such

as laterite soils of the humid tropics. It is an important growth limiting factor for plants in strongly acid soils with a pH of less than 5. i.e. it is estimated that 40% of arable soils of world are acidic and therefore present Al toxicity hazards (Vonvexkull & Mutert 1995). Al toxicity is considered the most important growth limiting factor or plants in acid soils (Foy *et al.*, 1978, Foy 1984; Carver & Own by 1995; Jayasundara *et al.*, 1998). The primary response to Al stress occurs in the roots (Foy *et al.*, 1978; Foy 1984, Taylor 1988, Jayasundara *et al.*, 1998) In the present study effect of Al on root, shoot growth, lipid peroxidation and activity of antioxidative enzymes of wheat seedlings have been studied.

Wheat is the most important cereal grain in the world. It is a kind of grass whose fruit is a head of Wheat with edible seeds belongs to *Poaceae* (*Gramineae*) family. Wheat grows best in alluvial clayey soil, which can retain water, moisture and it prefers a nearly neutral soil about (6.4pH). It has a higher protein content than other major cereals such as Maize or Rice. Research has already proven that Wheat is extremely beneficial for healthy living. Wheat has lot of health benefits to humans. It considered to lower risks of heart diseases because of its low fat content. It also regulates glucose levels in diabetic patients.

2. Materials and Methods

The seeds of Wheat (*Triticum vulgare*) were collected randomly from the research field of Agricultural University, Polasa, Jagityal, Karimnagar, Telangana, India. The selected healthy seeds were soaked for 16hrs in double distilled water and surface sterilized with 0.01M Mercuric chloride for 3 minutes and thoroughly washed with double distilled water several times. The seeds of uniform size were spread in large size Petri plates lined with four layers of *Wattman* No1 filter paper containing 12 ml of varying concentration of Aluminum. 10 seeds of Wheat were placed in each Petri plate. The different concentrations of Al selected for the present experiment was 1.5 mM, 3.5mM,

5.5mM,7,5mM,9,5mM given in the form of Aluminum Sulphate. For control the seeds were kept in Deionized Distilled water for germination. For each treatment five replicates were maintained. The experiment was carried out under normal laboratory conditions with a photo period of 8 hours per day and a temperature of approximately $30 \pm 2^{\circ}\text{C}$ during the day and $22 \pm 2^{\circ}\text{C}$ during the dark period. The seeds were allowed to germinate for 12 days. They were then removed separated into individual parts further analysis. Different parameters like Morphological changes, root and shoot length, percent phytotoxicity, Dry weight, relative growth index (RGI), chlorophyll a, chlorophyll b, total chlorophyll, lipid peroxidation, super oxide dismutase (SOD), Glutathione Reductase and catalase enzymes were assayed.

Growth Parameters

Morphological Changes: - Seedlings were observed for morphological changes. The visual symptoms of toxicity if any were noted on the 12th day.

Root and Shoot length: - The seedlings were separated into roots and shoots and length of each part was measured using a graph paper.

Percent phytotoxicity: - It was calculated as follows:

$$\text{Percent phytotoxicity} = \frac{\text{root length of control} - \text{root length of test}}{\text{root length of control}} \times 10$$

Dry weight: - The seedlings were separated into roots and shoots, gently blotted and their fresh weight was recorded, the same were dried in a hot air oven at 90°C for 48 hours to obtain constant dry weights.

Chlorophyll Estimation:

Through the Arnon method (1949) the total chlorophyll content was estimated. 0.2 grams of leaf material was cut into small pieces and blended with 10 ml of 80% acetone in a clean mortar. The green slurry was centrifuged at 3000 rpm for 12 minutes. The supernatant was transferred into a clean test tube 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 spectro photometer.

$$\text{Total Chlorophylls} = (0.D\ 645 \times 20.2) + (0.D\ 663 \times 8.02) / 1000 \times W$$

$$\text{Chlorophyll a} = (0.D\ 663 \times 12.7) - (0.D\ 645 \times 2.69) / 1000 \times W$$

$$\text{Chlorophyll b} = (0.D\ 645 \times 22.9) - (0.D\ 663 \times 4.68) / 1000 \times W$$

Assay of Lipid Peroxidation :

Lipid peroxidation in roots of 12 days old Wheat seedlings were determined by estimating the malondialdehyde content according to the method of Stewart & Bewley 1980.

0.2 gram of root samples were homogenized in 5 ml of double distilled water. An equal volume of 0.5% Thiobarbituric acid (TBA) in 20% Trichloroacetic acid solution was added and the sample incubated at 95°C for 32 minutes. The reaction was stopped by putting the reaction

tubes in the ice bath. The samples were then centrifuged at 18,000 rpm for 32 minutes. The supernatant removed, absorption was read at 532 nm, and the amount of nonspecific absorption was read at 600 nm and subtracted from this value. The amount of MDA present calculated from the extinction coefficient of 155 mM-1cm-1. Enzyme activity and MDA content of samples were recorded in triplication, and expressed as nM/gr.fr.wt.

$$\text{MDA (nM gr-1 fr.wt.)} = [(A_{532} - A_{600}) \times V \times 1000 / \epsilon] \times W$$

Where ϵ is the specific extinction coefficient (155 mM Cm⁻¹), V is the volume of crushing medium, W is the fresh weight of root, A₆₀₀ is the absorbance at 600 nm wave length and A₅₃₂ is the absorbance at 532 nm wave length.

Enzymatic Antioxidants :

The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), Glutathione Reductase (GR) were analysed.

Enzyme Extraction :

0.2 grams leaf tissue was blended in pre chilled mortar and pestle with 10 ml of 100 mM potassium phosphate buffer (pH 7.0) under ice-cold conditions. The homogenized material was centrifuged at 4°C for 20 minutes at 18,000 rpm and the supernatants were used for determining the activities of SOD, CAT, and GR.

Assay of Superoxide dismutase (SOD) EC 1.15.1.1 :

According to the method of Sen Gupta *et al.*, (1993). Superoxide dismutase (SOD), a metal containing enzyme plays a vital role in scavenging superoxide (O_2^-) radical.



Assay

SOD activity was estimated by recording the decrease in absorbance of superoxide nitroblue tetrazolium complex by the enzyme. About 3 ml of reaction mixture, containing 0.1ml of 200mM methionine, 0.01 ml of 2.25 mM nitro-blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1ml deionised water and 0.05 ml of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. Two reaction tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml of 60 μM riboflavin and placing the reaction tubes below a light source of 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and keeping the tubes in the dark. Reaction tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture which did not develop colour served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

Assay of catalase (CAT) EC 1.11.1.6 :

Catalase activity was assayed by the method of Barber, (1980).

Assay

To 0.5 ml enzyme extract 2.0 ml of hydrogen peroxide and 3.5 ml of phosphate buffer (pH 7.0) was added and was incubated for 2 minutes. The reaction was stopped by adding 10.0 ml of 2% (v/v) concentrated sulphuric acid, and the leftover hydrogen peroxide was titrated against 0.01M KMnO₄ until a faint purple colour that persisted for at least 15 sec. The activity of the enzyme was expressed as mg of H₂O₂ decomposed min⁻¹ gr⁻¹ fr. wt. of tissue.

Assay of Glutathione Reductase (GR) EC 1.6.4.2 :

Glutathione reductase was assayed by the procedure adopted by David and Richard, (1983). Glutathione reductase catalyse the conversion of oxidized glutathione to reduced glutathione employing NADPH as substrate. The amount of NADPH utilized is a measure of enzyme activity.

Procedure :

The assay system contained 1ml (0.12 M, pH 7.2) of phosphate buffer, 0.1 ml (15mM) of EDTA, 0.1 ml (10 mM) of sodium azide, 0.1 ml (6.3 mM) of oxidized glutathione and 0.1 ml of enzyme source and the volume was made up to 2 ml with distilled water. The tubes were incubated for 3 minutes and 0.1 ml (9.6 mM) of NADPH was added. The absorbance was read at 340 nm in a spectrophotometer at every 15 seconds interval for 2 to 3 minutes. For each series of measurement, controls were set up that contained water instead of oxidized glutathione. The enzyme activity was expressed as μM of NADPH oxidized/minute/gr.fr.wt.

3.Results and Discussions

Wheat seedlings were observed after 6 days of germination. At higher concentration of Al i.e at 9.5 mM the root and shoot growth was greatly reduced. Fageria(1985) noted differential response of Rice to different levels of Al⁺³ while Delhaize *et al.*, (1991) , Petterson and Strid (1989) reported significant inhibitory effect on root growth in Al⁺³ sensitive Wheat. Similar results were observed by Purcell *et al.*, (2002) that the inhibitory effect on water uptake , growth and grain yield on soyabean.

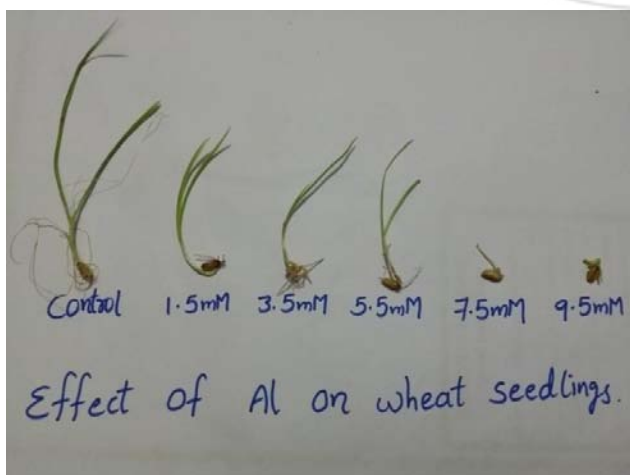


Figure 1: Effect of Al on Wheat seedlings.

Table 1: Effect of Aluminum on shoots and root length of Wheat

Metal conc (in mM)	Shoot Length (in cm)	Root Length (in cm)
Control	3.4	11.9
1.5 mM	2.3	1.3
3.5 mM	2.2	1.9
5.5 mM	2.1	1.2
7.5 mM	1.8	0.7
9.5 mM	1.2	0.2

Percent Phytotoxicity:

It was calculated on the basis of root length and is shown in Table no 2. A undeviating interaction was observed between the percentage phytotoxicity and the concentration of Al treated in Wheat seedlings at 9.5mM percent phytotoxicity of Al was 98.31% i.e maximum similar results were observed in Rice (Fageria 1982)

Table 2: Effect of Aluminum on % phytotoxicity

Metal conc (in mM)	% phytotoxicity
Control	0
1.5 mM	89.07
3.5 mM	84.03
5.5 mM	89.91
7.5 mM	94.11
9.5 mM	98.31

Dry weight:

Root dry weight was decreased at all concentration of Al maximum decrease was observed at 9.5mM Narayanan and Symala (1989) noted of Cajanus Cajan with the rise of Al⁺³ concentrations. Similar results were observed in Cowpea and Cucumber (Jemo *et al.*, 2006; Pereira *et al.*, 2006)

RGI:

The RGI was calculated from the dry weight of root and shoot of Wheat. RGI of root and shoot of Wheat decreased with increasing concentration of Al. Similar results were obtained for Wheat and Rice (Jan and Patterson 1989, Foy 1997). At 9.5 mM concentration of Al , RGI was 10.49% in Wheat seedlings and at 1.5mM Al concentration . RGI was gradually decreased.

Table 3: Effect of Aluminum on Fresh weight, Dry weight and RGI (relative growth index) of Wheat

Metal conc (in mM)	Fresh weight (in mg)	Dry weight (in mg)	RGI (in %)
Control	4.92	3.24	100
1.5 mM	3.59	2.32	71.60
3.5 mM	3.62	2.41	74.38
5.5 mM	3.21	2.19	67.59
7.5 mM	1.82	0.97	29.93
9.5 mM	0.94	0.34	10.49

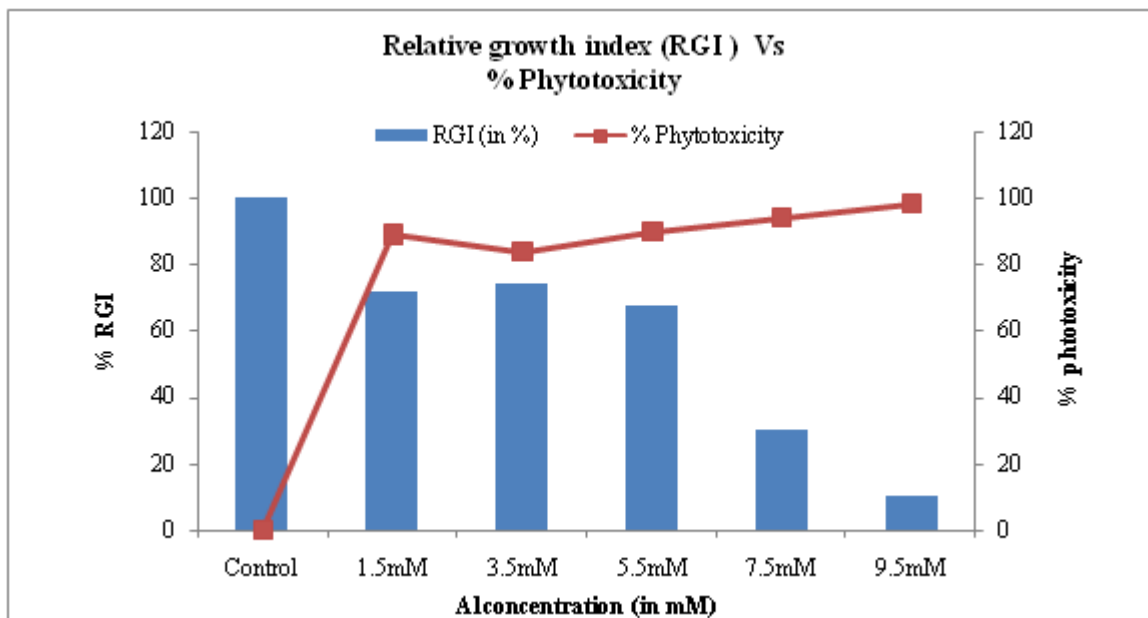


Figure 4: Relative growth index decreased with increase in % phytotoxicity in Wheat seedlings

Chlorophyll

The effect of AL on the chlorophyll pigments is represented in Table no 4 chl a , chl b and total chlorophylls decreased with increasing concentrations of Al in Wheat seedlings (Figure no 5). The total chlorophyll (a+b) in Spinach (*Spinacia aleracea L. var. viroflay*) significantly decreased under Al stress reported by Melika Karimei. Vahid Poozesh (2016). From the literature review it is evident that heavy metals including Aluminum reduce photosynthesis reduction in many plant species (Jiang *et al.*, 2008). It has been shown that Al decrease in chlorophyll synthesis by restrains the activity of aminolevulinic acid dehydratase responsible for the formation of monopyssale parphobilongen (Pereira *et al.*, 2006).

Table 4: Effect of Aluminum on chlorophyll content

Metal conc (in mM)	Chl a	Chl b	Chl a+b
Control	2.97	1.69	4.66
1.5 mM	2.52	1.48	4.00
3.5 mM	2.23	1.24	3.47
5.5 mM	1.86	1.02	2.88
7.5 mM	1.42	0.77	2.19
9.5 mM	0.89	0.48	1.37

indicator of lipid peroxidation. Al is known to damage cell membranes by binding to sulphhydryl groups of membrane proteins and by inducing lipid peroxidation (Peixoto *et al.*, 1999). Xiao *et al.*, (2003) showed that Al increased the permeability of plasma membrane the generation of superoxide anion and the concentrations of H₂O₂ and MDA an indicator of lipid peroxidation in longer leaves. (Sharma and Dubey 2007).



Figure 3: Root Lipid peroxidation of Wheat seedlings

Lipid Peroxidation:

Figure no 3 and figure no 5 reveals that Aluminum treatment resulted in a remarkable increase in MDA content as an

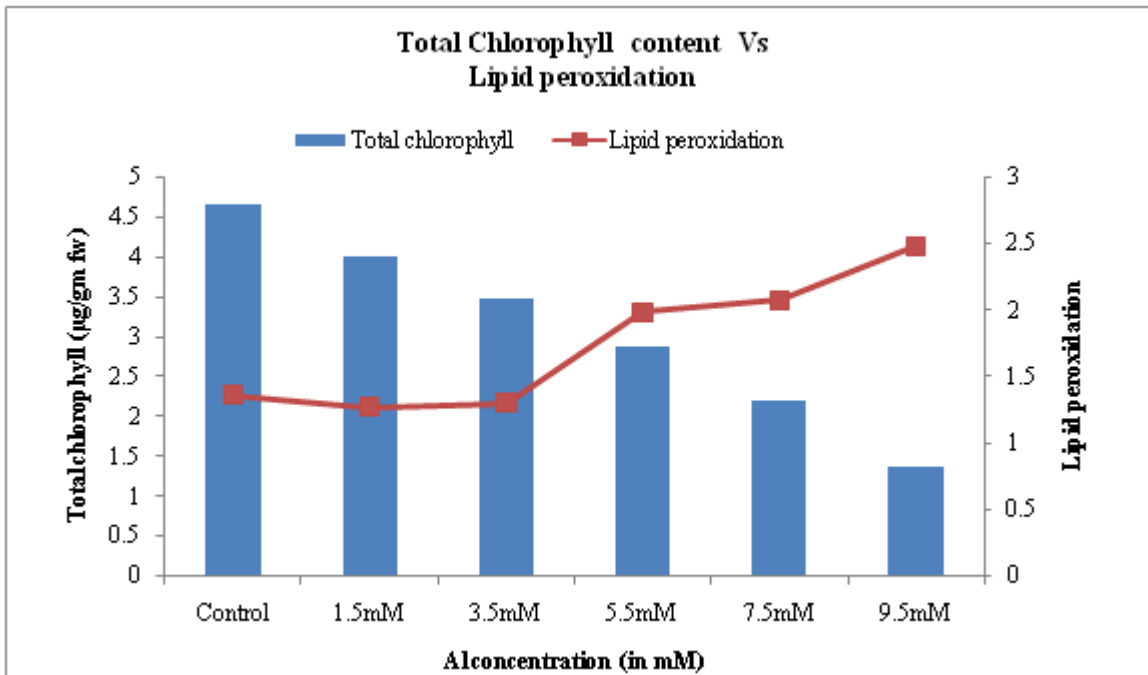


Figure 5: Total chlorophyll decreased with increase in Lipid peroxidation in wheat seedlings.

SOD , CAT , GR :

Figure no 2, 6 and 7 shows that the increased activities of SOD and GR in leaves of Wheat seedlings treated with Al. The decreased activity of Catalase was noted in Wheat seedlings. It is clear that Al treated Wheat seedlings had higher H₂O₂ content compared to control (H₂O treated seedlings) resulted in the reduction of seedling growth. Al induced oxidative stress inhibits plant growth, changes in antioxidative enzyme activities, and increase in the content of H₂O₂ and lipid peroxidation in the seedling of Wheat.



Figure 2: Enzyme Extraction of Wheat leaves

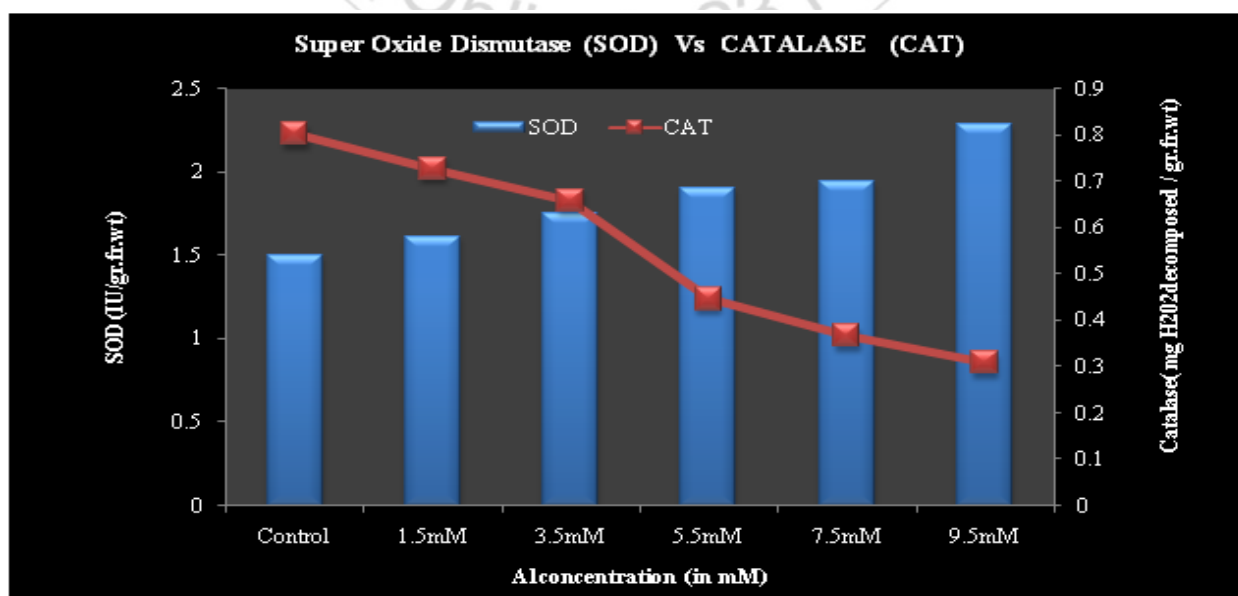


Figure 6: Super Oxide Dismutase increased with decrease in catalase activity in Wheat seedlings.

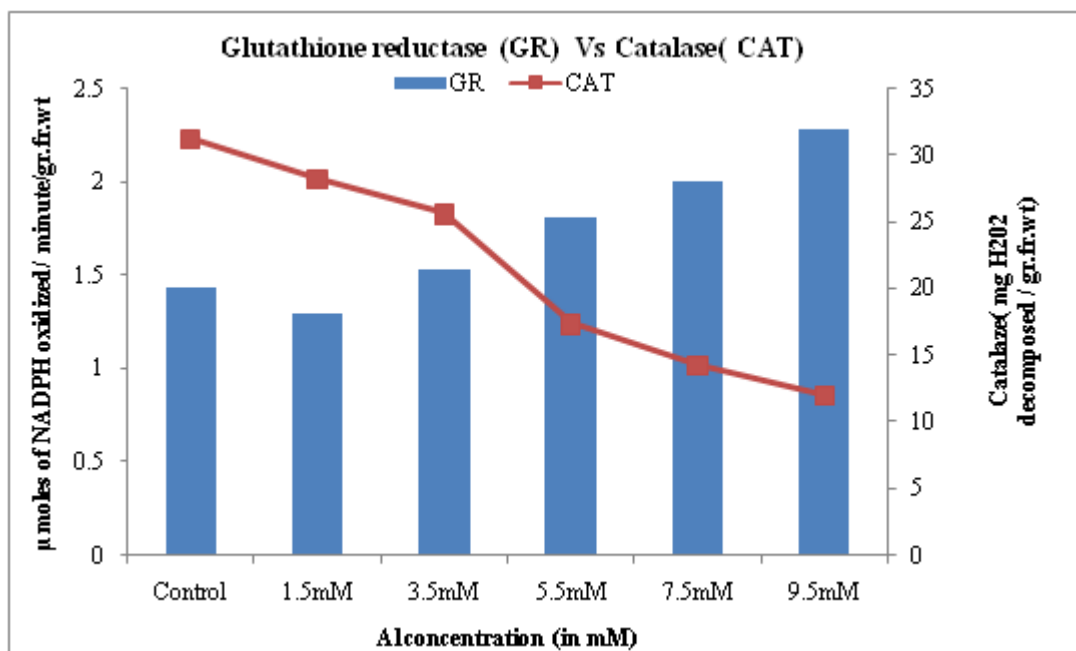


Figure 7: Glutathione reductase increased with decrease in catalase activity in wheat seedlings

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