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Evaluating the effects of elevated zinc concentrations on chlorophyll, reducing sugar, protein, prolein content and growth of wheat (*Triticum aestivum*)

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Abstract

Zinc is one of the essential micronutrients require to plants for growth and metabolism. Zinc is frequently used in fertilizer to promote growth and development of plants. The aim of the study is to analyze the impact of 100 ppm, 400 ppm and 800 ppm concentration of zinc on a physiological and biochemical aspects of the wheat. Spectrophotometric methods were used to determine the biochemical parameters while growth was measured manually through centimetre ruler. The results suggest that zinc affects growth parameters positively at lower concentration. Shoot growth increase at lower concentrations and reduced at highest concentration. While Root growth, enhanced with increase in concentration. Chlorophyll content increases with lowest concentration. Protein content shows16.6% increase at 100ppm then constant decline 16.7% at 400ppm and 50% decrease in protein content recorded at 800 ppm. Elevated zinc concentration reduces sugar content, minimum lower value of.03mg/g at 8000ppm and highest 1.8mg/g recorded in control. Prolein shows highest of 83.3% increase at 800ppm, 49.5% at 400ppm and 17.3% at 100ppm zinc concentration. The control shows the lowest value for prolein. It was observed that 100ppm concentration enhances wheat growth and biochemical parameters.

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Introduction

Metals are a major pollutant of soil and water now days. They are one of the major pollutants of the environment and ecosystem. Some metals like calcium, magnesium, iron, potassium, sodium and zinc are used as a micronutrient and play important role in stabilizing molecule through electrostatic interaction as a component of various enzymes and for osmotic pressure regulation (Ambler *et al.,* 1970; Alan, 1981) Excess of both essential and non essential metals disturb cell (Aery, and Sarkar, 1991; Alan, 1981; Ambler *et al.*, 1970). Zinc is an essential micro nutrient belongs to group II of the periodic table (Shier, 1994; Alan, 1981). Zinc releases in soil through mining, and smelting activities, sewage sludge, use of fertilizer and pesticides and anthropogenic activity (Chaney, 1993). Zinc is constituent of several metal enzymes and Co factor such as hydrogen, oxides, and peroxides and play important role in the regulation of cell multiplication, auxin synthesis and photosynthesis in plants (Hewitt, 1983). Zinc act as micro nutrients for crops in a permissible limit. Excess quantity of zinc may produce toxic effects. Zinc phytotoxicity cause, Fe deficiency induced chlorosis through reduction in chlorophyll synthesis and chloroplast degradation and interruption in magnesium and manganese uptake (Chaney, 1993; Foy, 1988). Wheat is one of the most important staple foods in India. Its cultivation is traditionally been *dominated* by northern reason in India. Zinc is the essential micronutrients used in fertilizer to promote growth and development of the plants. Less research was conducted on the effect of higher concentration of zinc on plants, therefore our present aim to study the impact of different concentration of zinc on the growth and biochemical Property.

Materials and methods

Estimation of root and shoot length

Seeds were surface sterilized with 0.1% mercuric chloride for 2 min. Plants were raised for twenty days in three replicates incorporated with 100ppm ,400ppm and 800ppm of zinc concentration, distilled water used for control plant .Roots length were measured on fourth day of growth while shoot length were measured on twentieth day of growth. Root and shoot length were measured by centimetre ruler.

Determination of chlorophyll

Chlorophyll was estimated by using method describe by (Arnon, 1949). 1gram fresh leaf was ground in a mortar and pestle with 10ml 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved for chlorophyll estimation. Absorbance was read at 645nm and 663nm in spectrophotometer.

Determination of protein

Protein was determined by (Bradford, 1976) using BSA as standard. Add 5 ml Bradford reagent (dissolve 50mg coomassie brilliant blue in 250ml of methanol and add 100 ml 85 % (w/v) phosphoric acid after that filters it using whatman's filter paper) in 1ml of protein samples. Absorbance was measured at 595nm.

Estimation of reducing sugar

Sugar was estimated by (Ross, 1959). Absorbance was measured at 600nm. Plant sample was dried at 60°c. The sample was ground to make a fine powder and then suspended in distilled water and filtered through whatman' s filter paper 1ml of filtrate was added in 4ml of dinitrophenol solution (0.038m) the mixture was incubated at 70 °c for 10 min and then cooled under running water absorbance was measured at 600nm. Glucose solution used as a standard. Absorbance was taken at 490nm.

Estimation of prolein

Prolein was determined according to (Bates *et al.,)*.Absorbance was taken at 520 nm. 1gram of plant tissue was homogenized in 10 ml 3 per cent aqueous sulphosalicylic acid. The homogenate was filtered through Whatmann No. 42 filter paper. Two ml of acid ninhydrin (1.25 g) ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid in a test tube was heated for an hour at 100 C. The reaction mixture was extracted with 4 ml toluene and mixed vigorously by using a vortex mixture for 15-20 Sec. The observance of the toluene layer was measured in a spectrophotometer at 520 nm.

Statistical analysis

Statistical analysis was carried out by IBM SPSS 16.0 using ANOVA for comparing mean. Statistical significance was selected as the probable value of p<0.05.

Results and discussion

Growth parameter

Results show increase in plant height at 100 ppm and 400 ppm as compare to control while decrease at 800ppm concentration of zinc (Table 1), increase in plant height at lower zinc concentration reported by (Bameri, 2012).

A higher concentration of zinc retards the growth and development of plant and disturbs the important metabolic process. (Alia and Saradhi, 1991; Ebbs and Kochaian, 1998; Cherif, 2001) supports the present observation of decline in plant height at higher concentration. Similar findings were also observed in rice seedling treated with a high concentration of zinc show growth inhibition in rice (Song, 2011).

Table 1. Effect of zinc on wheat shoots and root length. (Data mean of three replicates ± SD, statistically significant at p<0.05).

Treatment	Shoot length (cm) (mean±S.E)	Root length (cm) (mean± S.E)
Control	19.29 ±0.329	4.85 ± 0.350
Zn100ppm	20.86 ±0.145	5.433 ±0.233
Zn400ppm	21.75 ±0.180	5.36 ± 0.290
Zn800ppm	19.86 ±0.233	5.73 ±0.290

Chlorophyll content

Various abiotic stresses decrease the chlorophyll content in plant that lead to disruption of photosynthetic machinery (Ahmad *et al.*, 2007). In the present study zinc affects the chlorophyll content positively at 100ppm, it shows highest of 0.718 mg/g chlorophyll a and 800ppm shows lowest 0.505mg/g chlorophyll a. Similar trend shown in total chlorophyll perhaps chlorophyll b show little variations (Fig 1).

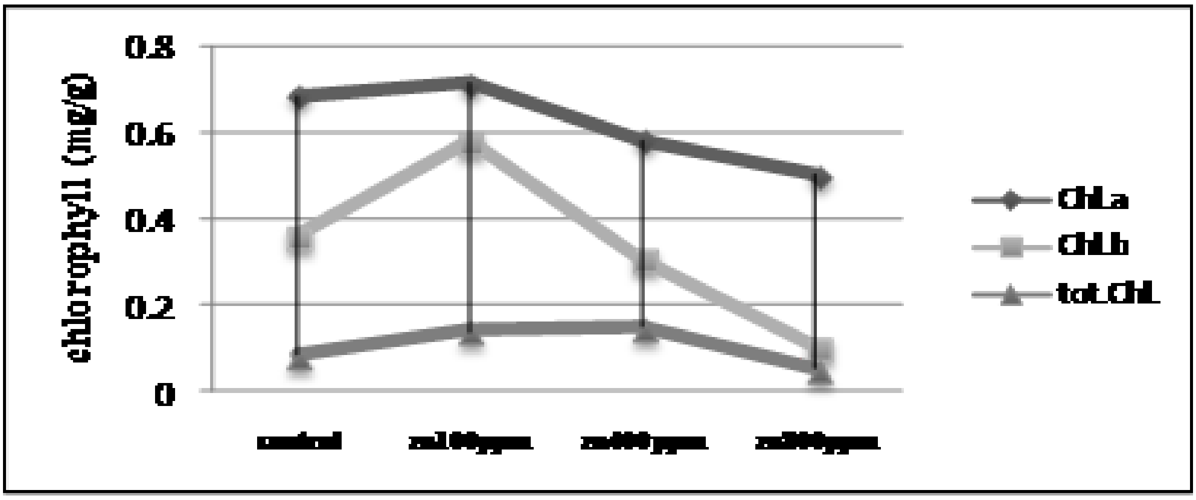

Fig. 1. Effect of zinc on chlorophyll content of wheat. (Data mean of three replicates, statistically significant at p<0.05).

Manivasagaperumal *et al.* (2011) states that excess zinc interfere in chlorophyll synthesis (Granick, 1951).

Reducing sugar content

Sugar content shows constant decreasing trend 1.2mg/g in 100ppm, 0.08mg/g at 400ppm and 0.03 mg/g at 800ppm, control show highest value 1.8mg/g (Fig. 2).

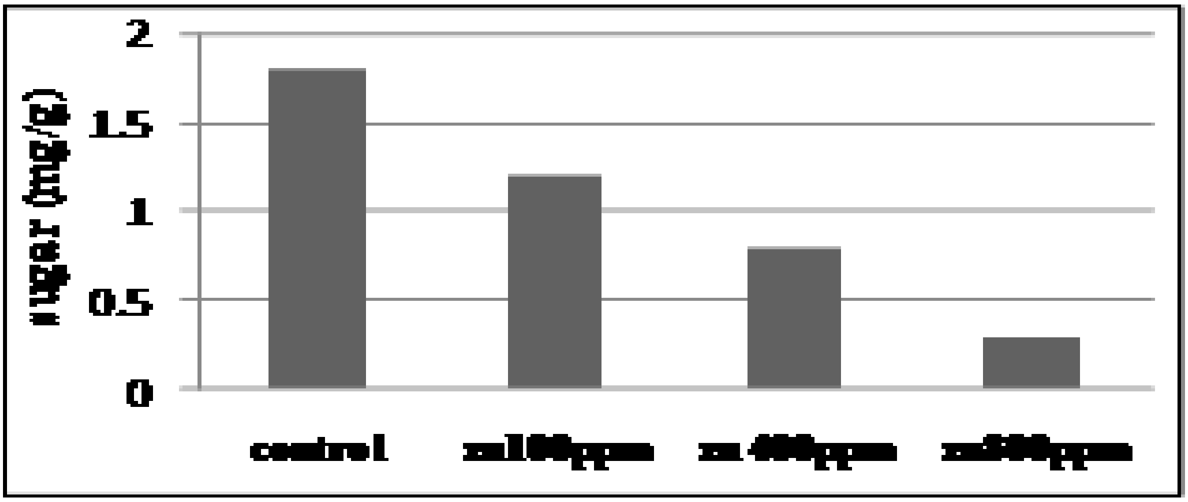

Fig. 2. Effect of zinc on wheat reducing sugar content. (Data mean of three replicates, statistically significant at p<0.05).

Similar trend also observed by(Samarakoon and Rauser, 1979; Singh, 2000) in zinc stress. In Sugar beet (Greger. and Lindberg, 1986) and(Cherif ,2001) in maize reported decrease sugar content at higher concentration.

Protein content

Protein show slight increase of 16.6% at 100ppm as compare to control then shows constant decline with an increase in concentration. Highest 800ppm zinc concentration affects protein concentration by 50%, while 400ppm moderately decreases protein concentration up to 16.6% (Fig. 3).

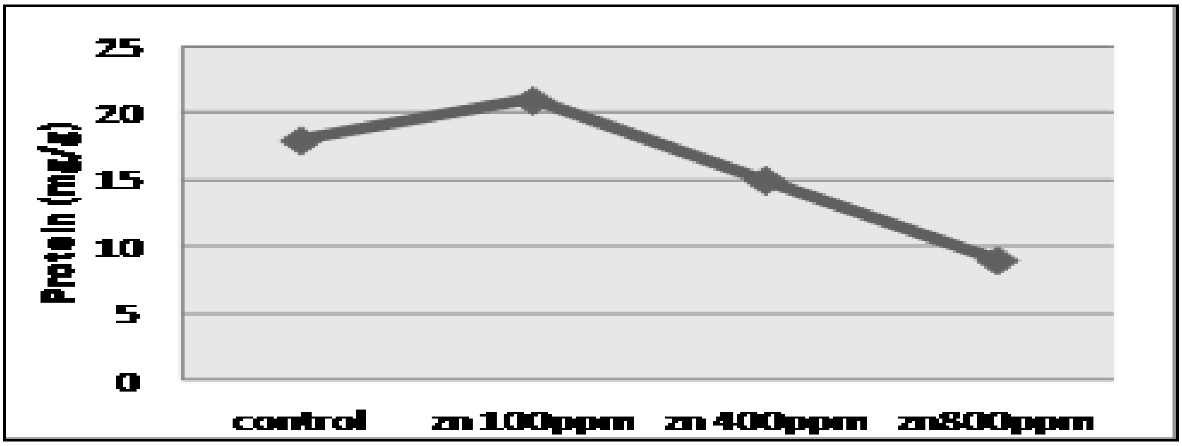

Fig. 3. Effect of zinc on wheat protein content. (Data mean of three replicates, statistically significant at p<0.05).

Nag, *et al.* (1981) marks an increase in protein content at lower zinc concentration in rice due to metals like zinc, lead, cadmium and mercury. Similar results was observed by Kastori *et al.* (1992) with zinc, copper and cadmium. Nitrogen acts as a precursor of amino acid, which are building blocks of protein, Metals decrease the nitrogen content of the plant leads to decrease the protein content (Granick, 1951; Welch, 1995; Mayz and Cartwright, 1984).

A similar result was observed in present study where protein content decreases at higher concentration.

Prolein content

Prolein show a constant increasing trend with progression in zinc concentration. 800ppm shows highest of 83.3 % increase in prolein content and lowest dose100ppm show lowest 17.3 % increase as compared to control (Fig. 4).This finding supported by (Kastori *et al.*,1992) in zinc stress.

Alia and Saradhi (1991) suggested increase prolein content under heavy metal stress is common. Proline content increase in plants stress condition is a defensive mechanism (Kuznetsov and Shevyakova, 1997). Prolein increase the stress tolerance of plant by several mechanisms such as osmoregulation, protect enzymes against denaturation and helps in protein synthesis stabilization (Kuznetsov and Shevyakova, 1997).

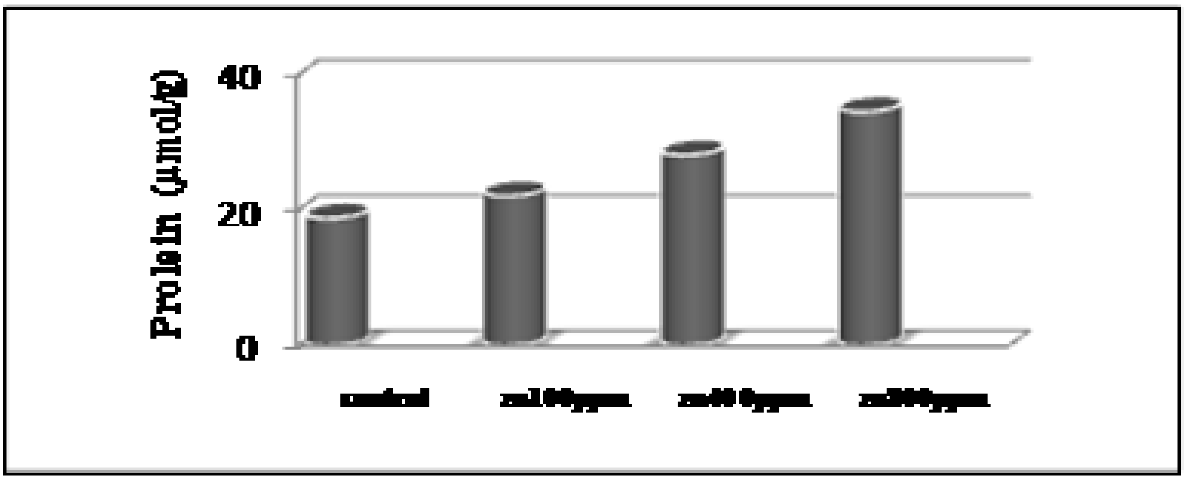

Fig. 4. Effect of zinc on wheat prolein content. (Data mean of three replicates, statistically significant at $p<0.05$).

Conclusion

Present observation reveals the fact that zinc is an essential micronutrient for plant growth and development. 100ppm concentration of zinc is beneficial for plant development whereas 400ppm accelerates the growth only and 800ppm reduces the growth. Zinc produces a positive effect on root, shoot growth and biochemical parameters at lower concentration. It is suggested that use of 100ppm zinc concentration improve plants development and health.

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