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EFFECT OF METALLIC MICRONUTRIENT, ZINC, ON GROWTH AND BIOCHEMICAL PARAMETERS IN *PHASEOLUS VULGARIS* (COMMON BEAN) SEEDLINGS

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ABSTRACT

The present study analyses growth and biochemical parameters in common bean seedling in response to $ZnCl_2$ supply over the range of 0 – 1 mM concentrations. Bean seedlings were grown in plastic pots containing acid washed sand for 10 days and watered daily with 1/2 Strength of Hoagland Solution containing 5mM NH_4NO_3 as nitrogen source and desired concentration of $ZnCl_2$. Zn supply reduced seedling vigour measured by using %G and shoot height with later being most prominent at 1 mM Zn. However, the total chlorophyll content of leaf tissue increased at 0.1 mM Zn and glutamine synthetase activity significantly increased at all the concentrations. $ZnCl_2$ supply also increased the *in vivo* NRA at 0.001 and 0.1 mM concentration but decreased at 1 mM, while in root tissue decrease resulted at all the concentration. The protein content (a biochemical growth parameter) and proline content (an osmotic stress parameter) of the Zn treated seedling were affected reciprocally by Zn supply. NO content in root as well as leaf tissue decreased more substantially at 0.001 mM and 0.1 mM Zn. The results demonstrate that Zn exhibit hormesis effect in leaf tissue, as the biochemical growth parameters, such as, protein, Chlorophyll and NRA increased upto 0.1 mM Zn and then decreased. Zn causes toxicity in root tissue even at lower concentration suggesting root to be the more sensitive site than leaf for toxicity. Zn treatment affected protein and proline content reciprocally suggesting that Zn favors growth till 0.1 mM concentration but causes toxicity above it. As NO content decreased being most prominent at 0.001 mM Zn, tolerance seems to be mediated via NO signalling particularly in leaf tissue.

Key words: $ZnCl_2$ effects, *Phaseolus vulgaris*, Nitrate reductase activity, Nitric Oxide

Abbreviations : Nitrate Reductase – NR, Nitrite reductase – NiR, Nitric Oxide – NO, Glutamine Synthetase – GS, Chlorophyll – Chl, Guiacol Peroxidase – GPX, Percent Germination - % G.

Introduction

Zinc (Zn) being an essential element for plants, required in an optimum concentration (10-100 mg kg^{-1} soil) for appropriate growth and development to accomplish the structural and functional necessity of the plant. Zn is required for numerous biochemical and physiological roles such as, photosynthesis, respiration and chlorophyll biosynthesis (Suzuki *et al.,* 2006). Zinc scarcity and toxicity has been detected in variety of plants. Zn deficiency (<0.5 mg kg^{-1}soil) is of alarming concern as it is a chief and effective micronutrient controller in crop yield. Toxicity of Zn induce anthocyanin synthesis, results in reddening of leaf (Fontes and Cox, 1995) and retards growth and yield of *Arabidopsis thaliana* (Broadley *et al.*, 2007). Disproportionate quantity of Zn dislocates Mg from the RuBP carboxylase and affects water splitting site of PSII in *Phaseolus vulgaris* (Assche and Clijsters, 1986).

Phaseolus vulgaris also known as common bean or kidney bean is an important leguminous crop well-known for its high protein (23%) content, dietary fiber and good source of essential metals. It accounts for its higher consumption as well as demand. Nitrogen is a key element that constitutes upto 7% of dry matter in plants. It is acquired in the form of nitrate or ammonia from the soil by plant. Nitrogen is needed for the synthesis of several biomolecules, such as amino acids, proteins, nucleic acids, derived sugars and major photosynthetic pigments, chlorophylls. Nitrate is assimilated to nitrite and ammonia by sequential action of the cytosolic NR and plastidic NiR with NR being the key enzyme. Ammonia is assimilated mainly involving glutamine synthetase (GS) and glutamate synthase. NR has also been reported to be involved in the synthesis of NO in plants (Rockel *et al.,* 2002). NO production by NR has been confirmed in *Chlamydomonas reinhardii* (Chamizo-Ampudia *et al*., 2016).

Amongst the metallic nutrients, Zn plays a role of essentiality and toxicity that varies depending upon plant species including crop plants. Early stages of seedling growth are crucial part of any plant's life. Thus, the present study is planned to investigate the effects of Zn on growth and biochemical parameters in young seedlings of common bean, an important crop of the region, with a view to understand mechanism of Zn effect.

Materials and Method

Plant material and treatment: Seeds of *Phaseolus vulgaris* cv. Rajmah purchased from Anant seeds, Indore, were surface sterilized with 0.1 % $HgCl_2$ for 2-3 min followed by thorough washing with distilled water. The seedlings were raised in plastic pots containing acid washed sand for 10 days in continuous light of intensity 30 Wm^{-2} supplied by fluorescent tubes at 25 ± 5°C and 85% humidity inside the plant growth chamber "Scientech" model SE110. They were watered daily with ½ strength Hoagland's solution (pH 6.0) containing 5mM NH_4NO_3 as nitrogen source and desired concentration of Zinc Chloride ($ZnCl_2$). To study time course of the effect,

% germination and shoot height were determined everyday upto 10 days. For analyses of other parameters, the root and leaf tissue of the 10 days old treated seedlings were used.

Analytical Procedures: Determination of growth - Treated seedlings was used to analyse % G and shoot height on each day and vigour was calculated as %G x Shoot height.

Biochemical parameters- Total protein of root and leaf tissue was measured by the method of Lowry *et al.* (1951). For proline content determination, method of Bates *et al*. (1973) was used. Chlorophylls were extracted from the leaf tissue of treated seedlings using 80% acetone and estimated spectrophotometrically by measuring the absorbance at 646 nm, and 663 nm. The pigment content was calculated using the following equations according to the method of Lichtenthaler and Welburn (1983).

Chl *a* ($\mu g\ ml^{-1}$) = $12.21(A_{663}) - 2.81(A_{646})$

Chl *b* ($\mu g\ ml^{-1}$) = $20.13\ (A_{646}) - 5.03\ (A_{663})$

Total Chlorophylls =Chl *a* + Chl *b*

Extraction and Assay of Peroxidase activity: The peroxidase activity was measured by the method of Putter (1974). The enzyme was extracted by homogenizing with phosphate buffer (0.1 M, pH 7.0), 5 ml each for leaf and root using mortar and pestle in cold room. The extract was then centrifuged at 10,000 X g for 20 min at 4°C in cooling centrifuge and the supernatant was used for enzyme assay. The assay mixture contained 2.5 ml of phosphate buffer (0.1 M, pH 7.0), 1 ml of freshly prepared guaicol (10 mM), 0.1 ml of enzyme extract and 0.03 ml of H_2O_2 (12.3 mM having extinction of 0.436 at 240 nm). Increase in absorbance was monitored at 436 nm at the interval of 30 sec for 3 min. The enzyme activity was expressed as ΔA $min^{-1}\ g^{-1}$fr. wt.

Extraction and Assay of GS activity: GS was extracted from the treated material and assayed by the method of Lea (1987). Leaf tissue (100mg) from treated seedlings were ground in 1.0 ml of cold extraction buffer consisting of Tris HCl buffer (50 mM, pH 8.0), EDTA (1 mM), Dithiothreitol (1 mM), $MgCl_2$ (10 mM) and glycerol (10 %, v/v) using mortar and pestle in cold room (0-4°C). The crude extract was centrifuged in cold at 20,000 x g for 20 min in a cooling centrifuge "REMI C-24 plus" and the supernatant was used for enzyme activity assay. The assay mixture consisted of 0.5 ml of imidazole HCl (50 µmoles, pH, 7.2), 0.1 ml of ATP (18 µmoles), 0.2 ml of $MgSO_4$ (45 µmoles), 0.1 ml of hydroxylamine (6 µmoles), 0.1 ml of L-glutamate (92 µmoles) and 0.1 ml of enzyme preparation. The reaction mixture was incubated for 10 min at 30° C. At the end of incubation, 0.5 ml of ferric chloride reagent (0.37 M $FeCl_3$, 0.67 M HCl and 0.2 M TCA) was added. The preparation was centrifuged at 10000 x g for 10 mins the brown coloured complex with γ – glutamyl hydroxymate formed during reaction was read for absorbance at 540 nm. GS activity was calculated by using calibration curve of γ – glutamyl hydroxymate (0.3 to 3.0 µmoles). The unit of enzyme activity was expressed as nmoles of γ – glutamyl hydroxymate formed $min^{-1}g^{-1}$fresh weight and mg^{-1}protein (specific activity). For specific activity, protein content of the preparation was measured by Lowry *et al* (1951) after precipitation with 10 % TCA

and dissolving the precipitate in 0.1 N NaOH.

Assay of *in vivo* NR activity: In vivo NR activity of the treated material was assayed according to the method of Srivastava (1975). Leaf and root tissue (250 mg each) of the seedling was cut into small segments 0.5 X 0.5 cm and suspended in 10.0 ml of incubation mixture (8.0 ml of 0.1 M phosphate buffer, pH 7.4; 1.0 ml of 0.2 M KNO_3 and 1.0 ml of 25 % isopropanol) inside a tightly stoppered dark vial. After 30 min of incubation in dark at 30^0C, 1.0 ml of the incubation medium was added to 1.0 ml of acidic sulphanilamide (1 % in 1 N HCl) and 1.0 ml napthylethylenediamine (NED) (0.01 % in distilled water). After 10 min, developed pink color was read for absorbance at 540 nm. The enzyme activity was expressed as nmoles of nitrite formed $h^{-1}g^{-1}$ fr.wt using the calibration curve of Na NO_2 (10-100 nM).

Estimation of NO content: NO content was measured by the method of Kumar *et al.*, (2010). Leaf and root sample each of 100 mg from treated seedlings were ground in a mortar and pestle using 1.0 ml of cold acetate buffer (0.05 M containing 4 % zinc acetate, pH 3.6) in cold room. The homogenate was centrifuged at 10,000 X g for 20 min at 4°C. The supernatant was collected and the pellet was washed with 0.5 ml of extraction buffer and both the supernatants were pooled. Activated charcoal (0.05 g) was added to the supernatant. The suspension was vortexed and filtered through whatmann filter paper and again centrifuged. To 1 ml of supernatant, 2 ml of Greiss reagent (1.0 ml acidic sulphanilamide and 1.0 ml NED) was added. Absorbance of pink color was measured at 540 nm after incubation for 30 min at room temperature. NO content was expressed as nmoles NO_2 formed g^{-1} fr. wt. Nitrite content was calculated using calibration curve of $NaNO_2$.

Statistical Analysis: Data presented in the paper are average of at least four independent experiments with ± S.E. Significance of difference obtained for various treatments was tested by the Student's t-test. The level of significance for p values is *< 0.05, **< 0.01 and ***< 0.001.

Results

Time course of the effect of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on % Germination, Shoot height and vigour of the seedlings.

Supply of 0-1 mM $ZnCl_2$ to bean seedlings for 10 days had almost no effect till 3 days for %G, 5 days for shoot height and 6 days for seedling vigour, but thereafter all the parameters decreased with increasing conc. of Zn showing strong correlation with R^2 values of 0.884, 0.751 and 0.802 respectively (Fig 1a, b, c).

Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on Leaf weight, Total Chlorophyll and Glutamine Synthetase activity in leaf tissue of the seedlings

Supply of 0-1 mM $ZnCl_2$ to bean seedlings for 10 days increased Total chlorophyll content in leaf tissue being more prominent at 0.1 mM. Leaf weight decreased non significantly with all concentrations of Zn (Table 1). Glutamine Synthatase activity expressed as g^{-1} fr. wt. and mg^{-1} protein basis increased significantly with increasing the concentration showing perfect correlation

with R^2 values 0.978 and 0.967, respectively.

Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on Protein, Proline content and Peroxidase activity in leaf and root tissue of the seedlings.

$ZnCl_2$ supply to bean seedlings increased leaf protein content at 0.001 mM by 59% and then decreased marginally showing no correlation with R^2 value 0.202, whereas root protein content significantly increased at 0.001 mM and 1 mM, but decreased by 12% at 0.1 mM showing no correlation with R^2 value 0.156 (Table 2). Proline content in leaf as well as root decreased significantly upto 0.1 mM but increased substantially at 1.0 mM $ZnCl_2$ showing no correlation with R^2 values 0.123 and 0.314, respectively (Table 2). Guaiacol Peroxidase activity in leaf tissue increased showing strong correlation with R^2 value 0.737 but in root significantly decreased with perfect correlation having R^2 value 0.916 (Table 2).

Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on *in vivo* NR activity and NO content in leaf and root tissue of the seedlings.

Treatment of the seedlings with Zn increased in vivo NRA in the leaf tissue at 0.001 and 0.1 mM, while reduced at 1 mM concentration, though the effect was not significant (Table 3). Decrease resulted in root tissue at all the concentrations maintaining correlation ($R^2 = 0.570$). The NO content decreased substantially in both the tissue by Zn supply with the effect being more prominent at 0.001 and 0.1 mM Zn (Table 3).

Discussion

Effect of Zn on growth parameters

Several studies have demonstrated the essentially as well as toxicity of Zn, such as, Sharma *et.al* (2009) reported that lower Zn concentrations (10 and 25 µM) enhanced the seed germination, while an inhibitory effect was observed at higher levels (100 µM) in *Cicer arietinum,* whereas treatment range from 1-10 mM Zn retards the growth of ryegrass with complete inhibition at 50 mM Zn (Bonnet *et al.,* 2000). Further, in greenhouse experiment it has been found that addition of 400-1600 µM Zn inhibits the growth of *Eucalyptus maculata* and *Eucalyptus urophylla* after 5 weeks of treatment (Soares *et al*., 2001) whereas Repkina *et al*. (2023) reported growth of mustard was inhibited by the effect of Zn concentrations 100 and 150 mg kg^{-1} soil. Thus the effect appears to depend upon treatment and plant species. In the present study, Zn supply had almost no effect till 3 days for % G, 5 days for seedling height and 6 days for seedling vigour, but thereafter decreased all the parameters showing strong correlation (Fig 1a, b, c). Amongst these shoot height was more affected than % G, hence the seedling vigour seems to be affected more due to effect on height rather than % G. Further, a lag period of about 5-6 days is required to commence Zn effect.

Amongst the parameters related to leaf tissue, a study revealed that *Solanum lycopersicum* showed a substantial reduction in leaf numbers grown at 0.5, 0.75 and 1.0 mM Zn concentrations (Khateeb and Qwasemeh, 2014). Further, Zn (50 and 100 µM) levels lessen the leaf number and area, cause alterations and wrinkling of leaf margins, inward rolling of

leaves and chlorosis (Sagardoy *et.al*, 2009). Zinc at 50 ppm reduced leaf growth in *Phaseolus vulgaris* (Michael and Krishnaswamy, 2011). In current study leaf fresh weight was reduced marginally in the concentration range (0.001-1 mM) used (Table 1) indicating that leaf growth is affected by Zn to some extent.

Effect of Zn on biochemical parameters

Symptom of chlorosis in response to Zn has been reported in *Beta vulgaris* using a hydroponic culture (Sagardoy *et.al,* 2009). Treatment of Zn at 0.67 to 1000 µM reduce chlorophyll content in pea leaf (Doncheva *et al.,* 2001), in wheat leaf at 0.5, 1 and 3 mM Zn concentration (Li *et al.*,2013) and also in Duckweed leaf at 0.15 and 0.3 mM (Radic *et al*.,2010). Total chlorophyll content of Cluster bean increased at 25 mg L^{-1} Zn while decreased at 50 to 200 mg L^{-1}(Manivasagaperumal *et al.,*2011). Cui and Zhao, (2011) reported increase in total chlorophyll content with 250 mg/kg while decrease at 1000 mg/kg Zn in maize leaf. Present study reveals that supply of 0-1 mM $ZnCl_2$ to bean seedlings for 10 days increased Total chlorophyll content in leaf tissue being more prominent at 0.1 mM (Table 2). Hence it is likely that for this biochemical parameter Zn exerts hormetic effect.

Another biochemical parameter, Protein is a versatile macromolecule responding to metal stress by triggering the expression of certain stress related and heat shock protein that helps to provide defence against metallic stress. Further it is an important factor in plant defense system under stress conditions and protein level has been observed to vary in *Vicia faba* (Corduk *et al.,* 2016). Protein was found to be affected variedly by Zn supply. Thus Chaoui *et al*. (1997) reported that concentration of protein is significantly reduced in roots of *Phaseolus vulgaris* by 100 µM Zn and in duckweed plants at 0.15 mM Zn concentration (Radic *et al.* 2010). Further it has been observed that leaves of *Artemisia annua* showed significant reduction in protein levels by Zn treatment at 50 to 400 µg g^{-1} soil dry mass (Khudsar *et al.,* 2004). Increase in protein content at 1 and 2 µM Zn was seen in hydroponically grown mungbeans plant (Samreen *et al.,* 2013) and also in roots of cotton plant at 2 and 4 µM Zn treatment (Santos *et .al.*, 2021). Thus Zn effect on protein depends upon Zn concentration, plant species investigated and also organ. In this study, $ZnCl_2$ increased leaf protein content at 0.001 mM by 59% and then decreased marginally showing no correlation, whereas root protein content increased tremendously at 0.001 mM and 1 mM, but decreased by 12% at 0.1 mM showing no correlation (Table 3). Thus increased protein content at 0.001 mM Zn in leaf tissue and at 0.001 and 1 mM in root may be consequence of development of tolerance and toxicity.

Proline accumulation (stress marker) in metal treated plants is an indicator of primary defense mechanism implemented by plants to maintain the osmotic pressure. Proline notified a remarkable protective role for the detoxification of ROS (Tripathi and Gaur, 2004). Li *et al,* (2013) reported Zn stress promotes accumulation of proline content at 1 mM Zn concentration in roots of *Triticum aestivum* and also in 15 days old seedlings of *Vigna unguiculata* at 250 µM and 500 µM Zn concentrations (Singh *et al*.

2014). Comparative studies between *Solanum nigrum* and *Solanum lycopersicum* suggested that with increase in Zn concentrations (0.25, 0.50, 0.75 and 1.00 mM) significant increase in accumulation of proline was observed in *S. nigrum* is higher as compared to *S. lycopersicum* (Khateeb and Qwasemeh.,2014). In current study, proline content in leaf as well as root decreased significantly upto 0.1 mM but increased substantially at 1.0 mM $ZnCl_2$ showing no correlation (Table 3). This indicates that Zn supports osmotic stress tolerance upto 0.1 mM Zn and toxicity results at 1 mM.

Guaicol peroxidase, peroxidative stress marker, is a heme containing protein that is widely accepted as a "stress enzyme" (Sharma *et al.*, 2012). It is associated with many important biosynthetic processes and defence mechanism, against metal toxicity. In *Phaseolus vulgaris*, Zn concentration at 100 µM enhanced the activity of GPX by 90% in stem (Chaoui *et al*, 1997) and at 50 µM Zn in both root and leaf (Cuypers *et al.,* 2002) whereas decrease in GPX activity at 100 mM Zn in wheat leaves were reported by Panda *et al.* (2003). Svadkoohi *et al.* (2017) reported increase in GPX activity in roots at 300 µM Zn decrease at 500 µM in *Plantago major L.* In current study, Guiacol Peroxidase activity in leaf increased showing strong correlation but in root decreased with perfect correlation (Table 2). Thus Zn effect seems to reduce peroxidative stress in roots, but in leaf it causes peroxidative stress.

In plants, nitrogen metabolism is considered as a crucial parameter for their response to metal stress. NR is the key enzyme for nitrate assimilation in plant cells. Panda and Choudhary (2005) reported decrease in NR activity by 11%, 19% and 21% at 1, 10 and 100 mM Zn concentration in *Polytrichum commune* whereas Luna *et.al* (2000) reported increase in NR activity at 0.05 mM Zn but decreased at 0.1 to 5 mM in leaf of *Triticum aestivum*. In *Artemisia annua* plants Zn supply under varied concentration (50, 100, 200, 300 and 400 µg g^{-1} Soil dry mass), found to decrease nitrate reductase activity upto 62.8 % at 400 µg g^{-1}(soil dry mass) as compared to control (Khudsar *et al*, 2004). Exposure of 3 and 9 mg L^{-1} Zn caused a significant inhibition of nitrate reductase activity in *Ceratophyllum demersum* (Umebese and Motajo, 2008). Thus effect seems to depend upon treatment and plant species. In present study, treatment with Zn increased *in vivo* NRA in the leaf tissue at 0.001 and 0.1 mM, while reduced at 1 mM concentration (Table 3). However, decrease resulted in root tissue at all the concentrations maintaining correlation (Table 3). Thus Zn favours nitrate assimilation in leaf tissue upto 0.1 mM while in roots inhibits at all the concentrations tested. Another enzyme of nitrogen metabolism, Glutamine Synthatase increased significantly with Zn treatment showing perfect correlation (Table 2). Thus Zn supply increases ammonia assimilation in leaf tissue. Sun *et al* (2020) also reported improved activity of GS at 5 µM Zn supply in wheat (*Triticum aestivum L*). Nitrate reductase not only mediates the reduction of nitrate to nitrite but also reduces nitrite to Nitric Oxide, a relevant pathway for NO production in higher plants that are

responsible to activate stress tolerance mechanism to detoxify the detrimental effects. Production of stress-induced endogenous NO is generally time-dependent and plays differential roles in response to stress (Sun *et al.,* 2013). In our study, the NO content decreased substantially in both the tissue with Zn supply and the effect being more prominent at 0.001 and 0.1 mM Zn but increased at 1 mM (Table 3). This suggests that tolerance seems to be mediated via NO signalling and toxicity causes NO stress.

Conclusion

Zn supply reduced seedling vigour measured by using % G and shoot height with later being most prominent at 1 mM Zn. For biochemical parameters Zn exhibit hormesis effect in leaf tissue as, protein, Chlorophyll and NRA increased upto 0.1 mM Zn and then decreased. Zn causes toxicity in root tissue even at lower concentration suggesting root to be the more sensitive site than leaf for toxicity. Although peroxidative stress was more prominent in leaf tissue. Zn treatment affected protein and proline content reciprocally suggesting that Zn favors growth till 0.1 mM concentration but causes toxicity above it. As NO content decreased being most prominent at 0.001 mM Zn, tolerance seems to be mediated via NO signalling particularly in leaf tissue.

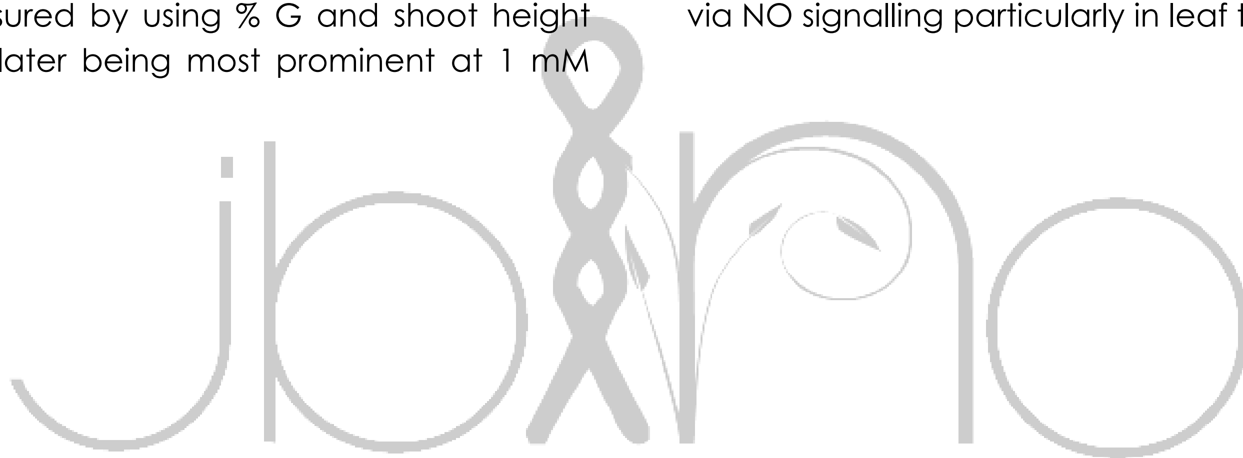


Table 1. Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on Leaf weight, Total Chlorophyll and Glutamine Synthetase activity in leaf tissue.

Common bean seedling were treated with varying concentration (0-1 mM) of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 were grown for 10 days in plastic pots containing acid washed sand at continuous light intensity of 30 Wm^{-2} and temperature 25 ºC ± 5ºC and humidity 85%.

$ZnCl_2$ Conc, mM	Leaf wt. (mg)	Total Chl ($\mu g\ g^{-1}$ fr.wt)	Glutamine Synthatase (GS) (micromoles of γ- glutamyl hydroxmate min^{-1})	
			g^{-1}fr.wt	mg^{-1} protein
0.0	80±2(100)	36±3(100)	425±30(100)	29.89±5(100)
0.001	68±1(85)	37±3(103)	481±11(113)	36.85 ± 1.7(123)
0.1	74±1(93)	44±4(120)	542±20*(128)	48.35 ± 7.7(162)
1	67±1(84)	40±4(109)	570±28*(134)	64.92 ± 3.3**(217)
R^2	0.136	0.407	0.978	0.967

Values relative to control are given in parentheses.

Level of significance: p value *< 0.05, **< 0.01.

Table 2. Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on Protein, Proline and Peroxidase activity in leaf and root tissue.

Common bean seedling treated with varying concentration (0-1 mM) of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 were grown for 10 days in plastic pots containing acid washed sand at continuous light intensity of 30 Wm^{-2} and temperature 25 ºC ± 5ºC and humidity 85%.

$ZnCl_2$ Conc, mM	Protein content (mg g^{-1} fr. wt))		Proline content (µg g^{-1} fr. wt)		Peroxidase activity (ΔA min^{-1} g^{-1} fr. wt.)	
	Leaf	Root	Leaf	Root	Leaf	Root
0.0	13±0.2 (100)	3.94±0.008 (100)	246±21 (100)	248±29 (100)	1.4±0.009 (100)	3.8±0.001 (100)
0.001	19±0.23 (159)	8.39±0.05* (213)	169±6** (68)	130±17** (53)	1.6±0.005 (113)	3.1±0.03 (82)
0.1	11±0.17 (96)	3.46±0.01 (88)	196±7* (80)	175±13* (71)	1.7±0.02 (118)	2.2±0.01*** (60)
1	10±0.18 (81)	8.34±0.08 (212)	284±18 (116)	412±84 (167)	2.9±0.009** (208)	2.2±0.03** (58)
R^2	0.202	0.156	0.123	0.314	0.737	0.916

Values relative to control are given in parentheses.

Level of significance: p value *< 0.05, **< 0.01, ***< 0.001.

Table 3. Effect of supply of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on NR activity and NO content in leaf and root tissue.

Common bean seedling treated with varying concentration (0-1 mM) of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 were grown for 10 days in plastic pots containing acid washed sand at continuous light intensity of 30 Wm^{-2}, temperature 25ºC ± 5 ºC and humidity 85%.

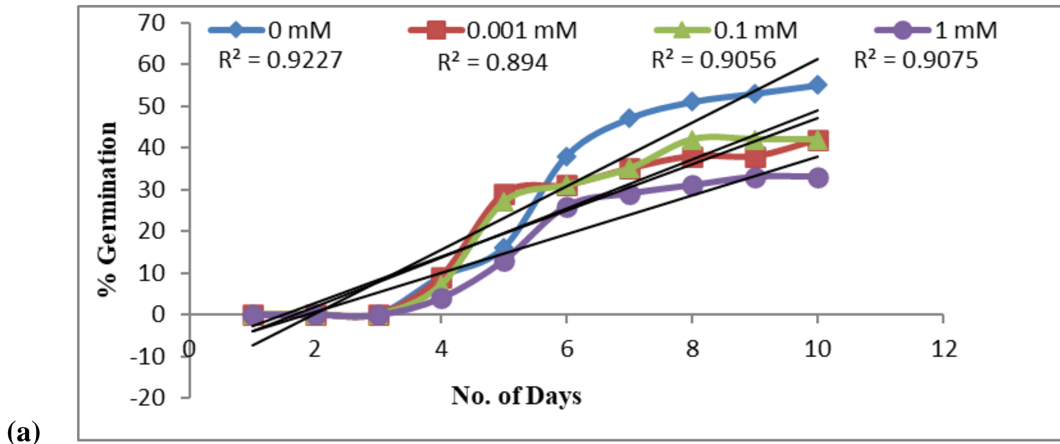
$ZnCl_2$ Conc, mM	NR Activity (in vivo) (nmoles NO_2 hr^{-1} g^{-1} fr. wt)		NO Content (nmoles NO_2 g^{-1} fr. wt)	
	Leaf	Root	Leaf	Root
0.0	90±0.001(100)	65±0.001(100)	20±3(100)	11±0.9(100)
0.001	113±0.08(126)	19±0.0007*(75)	6±0.4***(29)	3±0.7*** (27)
0.1	100±0.05(112)	36±0.0006*(41)	9±0.4***(45)	5±0.6*(46)
1	80±0.004(80)	16±0.0001**(22)	13±1.3*(64)	9±0.8 (78)
R^2	0.168	0.570	0.147	0.02

Values relative to control are given in parentheses.

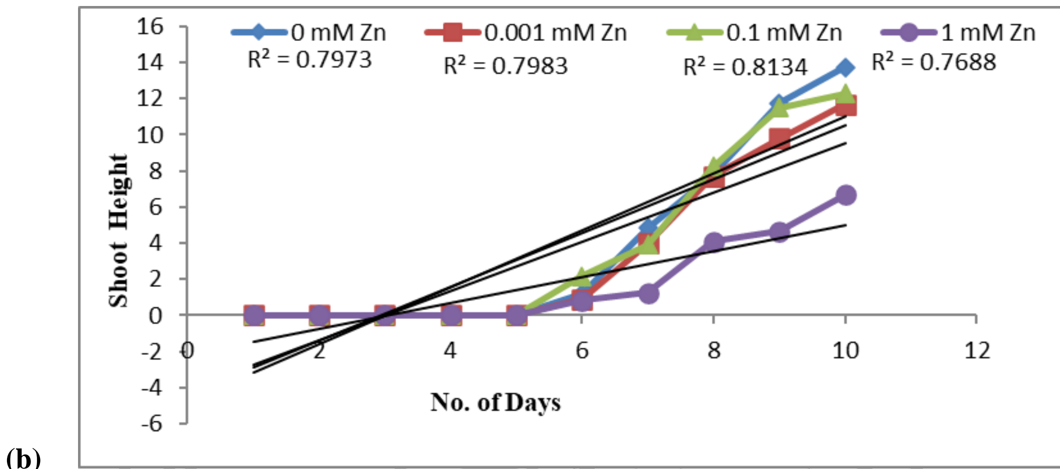
Level of significance: p value *< 0.05, **< 0.01, ***< 0.001.

Figure 1 (a, b, c). Time course of the effect of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 on % Germination, Shoot height and Seedling vigour.

Bean seedling were treated with varying concentration of $ZnCl_2$ in the presence of 5 mM NH_4NO_3 upto 10 days in plastic pots containing acid washed sand at continuous light intensity of 30 Wm^{-2} at temperature 25 ± 5 ºC and humidity 85% . Everyday % G and shoot height were measured.



(a)



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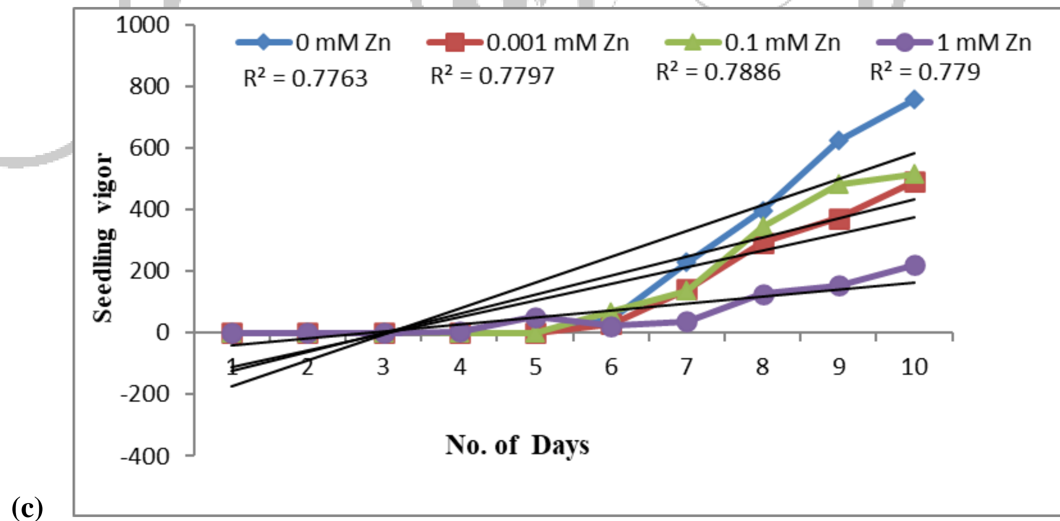

(c)

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