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EFFECT OF SEQUENTIAL CONCENTRATIONS OF ZINC AND ITS COMBINATION WITH CALCIUM OR GLUTATHIONE ON THE GROWTH, WATER RELATIONS AND ANATOMY OF ROOTS, STEMS AND LEAVES OF *PHASEOLUS VULGARIS* CV. CONTENDER

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Abstract

Zinc (Zn) is one of the eight essential micronutrients. It is needed by plants in small amounts, but yet crucial to plant development. A solution culture experiment was conducted to study the variation in growth, water relations and anatomy of roots, stems and leaves of *Phaseolus vulgaris* cv. contender treated with 1, 200, 400, 600, 800, 1000and 1200 mM$ZnSO_4$. Maximum significant deplete in parameters of growth (Length of root and shoot; fresh and dry weights, relative growth rate; N^o of leaves and leaf area), stomatal index and rate of transpiration was observed with, 200, 400, 600, 800, 1000and 1200 mM $ZnSO_4$. These effects were improved by the addition of Ca^{2+}than the addition of glutathione at 10 mM. Moreover, there were a significant increase at low concentration (1mM) treatment. Width of root, width of cortex and width of vascular bundles were increase with increasing Zn concentrations either alone or in combination with glutathione >Ca $(NO_3)_2$. For stem, the N^o of vascular bundles were decreased with increase in Zn concentration alone and with Ca $(NO_3)_2$, meanwhile increase with glutathione. Width of cortex and N^o of its rows were decrease with increase Zn concentrations. For leaves, the thickness of leaf blade, mid rib and vascular bundle were increase with increase the Zn concentrations alone, On other hands, they records a significant decrease in combination of Zn with $Ca(NO_3)_2$ or glutathione . In general, an increase in total uptake of zinc with increasing the concentration of Zn in all treatments. However, Ca $(NO_3)_2$ decrease these amounts than glutathione.

Keywords: Zinc; Calcium Nitrate; Glutathione; Growth; Anatomy; Phaseolus Vulgaris.

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1. Introduction

The term "heavy metal" commonly refers to metals with a specific weight in excess of 5 g / cm or anatomical number in excess of 20. Such properties are our significance for biological effects, yet the heavy metals contain essential nutrients, beneficial elements and elements that are not known to be active in humans at the present stage of science. At relatively low levels, all of them become harmful. Yet toxicity is not an exclusive aspect of heavy metals listed elements. The heavy metals are therefore a rather heterogeneous group of elements which differ greatly in their chemical properties and biological functions. The word "heavy metal" is therefore debunked **(Nieboer and Richardson 1980)**. But as Tiller (**Tiller 1989)** pointed out “heavy metal may be a useful umbrella term for metals classed as environmental pollutants”. Among the myriad of heavy metals zinc occupies the prominent position, since it plays a vital role in the growth and development of plants. Zinc, one of the essential micronutrients and an important constituent of a number of enzymes and proteins, is required only in small quantities by plants. However, plant development is crucial, as it plays a significant role in a wide range of processes. The normal range for zinc in plant tissue is between 15-60 ppm and between 0.10-2.0 ppm in the growing medium. Zinc deficiency or toxicity is not common; however, both negatively impact crop growth and performance. Any deficiency or toxicity must be addressed prior to irreversible crop damage.

Zinc release to the atmosphere may be correlated with biotic and natural atmospheric processes, with a ratio of Zn emissions from human activities to those from natural causes exceeding 20. **(Friedland 1990**). Human activities that release Zn to the atmosphere include fossil fuel combustion and the use of sewage sludge, manure and lime. Many crops may suffer from Zn toxicity in polluted and acidic soils, and species with high Zn uptake potential, such as spinach and beet, may be more prone to its abundance. (**Chaney 1993; Broadleyet al., 2007**). Bioaccumulation of trace metals in plant tissues can pose a risk to the health of wildlife and human beings (**Singh and Agrawal 2007**).

Calcium is considered to have a positive influence on plant growth and to enhance heavy metal toxicity. **(Marschner, 1995; Hagemeyer, 1999**). In addition, Ca was found to decrease the content of Cd, Cu, Mn and Zn in plant roots and/or shoots **(Kawasaki and Moritsugu, 1987; Salehet al., 1999**). In order to handle different types of metals, plants have protection techniques linked to cellular free metal content (e.g., metal exclusion, cell wall binding, chelation and sequestration) on one side. **(Hall, 2002**) However, on the other hand, control of cellular responses (e.g. repair of stress-damaged proteins, antioxidant protection). **(Hall, 2002).** The synthesis of specific chelators and subsequent sequestration of metal complexes is of major importance to limit free metal concentrations. Glutathione (GSH) is a key component of such metal scavenging due to the high metal affinity with its thiol (-SH) group and as a phytochelatin precursor (PC). In addition to metal homeostasis, plants have a well-equipped antioxidant defense system to deal with the metal-imposed oxidative challenge **(Jozefczaket.al. 2012).**

Phaseolus vulgaris, also referred to as the common bean, **Gentry, Howard Scott (1969)** green bean and French bean, among other names, is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit (both commonly called beans). The main categories of common beans, on the basis of use, are dry beans (seeds harvested at complete maturity), The common bean grows well on large variable soils with pH ranging from 4 to 9. It grows better on well-drained,

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sandy loam, silt loam or clay loam soils, rich in organic matter content. Dry beans production (theoretically only *Phaseolus* species) was about 23 million ton in 2012, cultivated on 29 million ha **(FAO, 2013)**In recent years, consumption of legumes particularly dry beans (*Phaseolus vulgaris* L.) has increased in some West European countries and the United States. This is due to an increased realization of consumers about the nutritional characteristics in foods.

The goal of this study was to investigate the effects of high nutrient solution concentrations of Zn on growth, water relationships, Zn content and anatomy characteristics composition of different parts of the plant bean model (Phaseolus vulgaris L.). The objective was also to determine the role of calcium and glutathione in improving zinc toxicity in plants of Phaseolus vulgaris.

2. Materials and Methods

Time Course Experiment

A homogenously-sized lot of *Phaseolus vulgaris* cv. contender) seeds was kindly supplied by the agriculture research center, Ministry of agriculture, Giza, Egypt. The seeds were selected, and surface sterilized by soaking in 0.01% $HgCl_2$ solution for about 3 min, then washed thoroughly with continuously flowing tap water for about 1 h. After this, 25 seeds were allowed to germinate in plastic dishes (length: 30 cm; width: 20 cm; height: 12 cm), covered with Whatman filter paper No. 1 and watered with equal amounts of Hoagland's nutrient solution (**Arnon and Hoagland 1940)**. The nutrient solution used was ¼ strength of **Pfeffer (1900**) nutrient mixture of macro elements. Micronutrients were supplied to the nutrient solution at concentrations used by **Arnon and Hoagland (1940).** All chemicals used were of the purest grade available from Sigma-Aldrich. The pH value of this nutrient solution was 5.7 ± 0.3. The dishes were incubated in the dark at 25 ±1°C to allow seeds to germinate. After 48 h six uniform seedlings (the length of the radical was about 2 cm; leaves had not yet differentiated were placed in black-painted beakers (600 ml) containing ¼ strength Hoagland's nutrient solution either alone or supplemented with the addition of $Ca(NO_3)_2$ or glutathione at 10 mM .The beakers were placed in a growth chamber adjusted at optimum growth conditions: temperature: 28 ± 2°C; light intensity: 3000- 5000 lux; relative humidity: 60-70%; continuous aeration from an air pump at a rate of 2 L/h/beaker according to **Steing Rover (1983).**

Throughout the experimental period, various growth parameters, stomatal index, stomatal area, rate of transpiration, content of zinc in root and shoot were determined. In addition, the changes in the internal structure of root, shoot and leaves were determined.

Data from the different groups of seedlings were statistically analyzed and comparison among means was carried out using Statgraphic Ver. 4.2, Display (one-tailed ANOVA), as described by **Snedecor and Cochran 1980).**

Growth Parameters

The plant heights from the root system intersection to the stem's growing tip were measured and at the end of the experiment (14-day old) root length was determined. The fresh weights and dry weights of the shoots and roots were obtained using an electronic balance. As well as number of leaves and leaf area were determined.

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Relative Growth Rate (RGR)

Relative growth rate (RGR) was calculated according to (Hofmann **and Poorter 2002)** formula:

$$(RGR) = (Loge W_2 - Log_e W_1) / (T_2 - T_1)$$

Where W_1 and W_2 are the dry masses at 7 and 14 - day harvest T_1 and T_2 respectively on the basis that growth was exponential during this growth period.

Rate of Transpiration

The rate of transpiration was estimated gravimetrically from the decrease in the weight of the whole plant and culture solution on the basis of root fresh mass as
mg g^{-1} fresh mass h^{-1}. (Youniset **al., 1992).**

$$\text{Rate of transpiration} = \frac{W_1 - W_2}{\text{F. wt of root x time of experiment in hours (48 hr)}} = mg/g^{-1}\text{F. Wt root}$$

W_1 = weight of plant at the beginning of experiment
W_2 = weight of plant at the end of experiment

Determination of the Stomatal Index

The stomatal number (stomatal density) is called the total number of stomata per square millimeter of epidermis. According to the stomatal index, the percentage proportional to the ultimate divisions of the epidermis of a leaf that has been converted into stomata (**Weyers and Meidner, 1990):**

$$\text{Stomatal Index} = \frac{\text{Stomatal density x100}}{\text{Stomatal density +density of epidermal cells}}$$

$$SI = \frac{S}{S + I} \times 100$$

Where SI = Stomatal index, S = number of stomata per unit area and E = number of ordinary epidermal cells in the same unit area.

Procedure

Pieces of the leaf between the margin or midrib were cleaned and mounted, and the lower surface was examined using a 4 mm objective microscope and an eyepiece with a 5 mm square micrometer disk. The numbers of the epidermal cells and the stomata within a square grid were counted, a cell being counted if at least half of its area is within the grid. The index of stomas was calculated for both surfaces of the leaf.

Chemical Analysis

The plants were harvested at the age of 14, shoots and roots, and the roots were washed with deionized water and the samples were dried for chemical analysis at 80oC in an oven for 48 h. Then dry shoots and roots were weighed and grounded. Plant samples (0.5 g) were digested with

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concentrated HNO_3 and H_2O_2 (Jackson, 1958; Han et al., 2004). The digested solution was filtered and then analyzed for Zn atomic absorption spectrophotometry (ICP-AES-Liberty series II) (**Han and Banin 1997**). Calculated as mM/100gm Dry weight.

Anatomical Preparation

For anatomical investigation, samples from plants were taken after ending the experiment (about 14-days-old). Stem sample were taken from the first internode after the 1st foliage leaf. Leaf samples were taken from 1st trifoliate leaf. Root samples were taken 5 cm away from the point of attachment of root and shoot. Plant material was fixed in FAA (Formalin , acetic acid and alcohol : 1:1:1) dehydrated , paraffin embedded, ultramicrotomed and subjected to safranin (0.1%) – fast green (0.2%) staining for further observation **(Sass 1958)**.In these sections , thickness of section , number of cortical cells (raws) , width of cortical cells , number of vascular bundels in root and stem were determined . In leaf sections, the **blade thickness**, Midrib **thickness** and **Width of vascular bundle** were determined using linear micrometr. (Shukry **1986**).

3. Results and Dissection

Zinc (Zn) is an essential component of thousands of proteins in plants, although it is toxic in excess. Zinc toxicity in crops is far less widespread than Zn deficiency. However, Zn toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn, especially in low-pH soils (**Chaney 1993**). Toxicity symptoms usually become visible at $[Zn]_{leaf}$> 300 mg Zn kg^{-1} leaf DW, although some crops show toxicity symptoms at $[Zn]_{leaf}$ < 100 mg Zn kg^{-1} DW **(Chaney, 1993; Marschner, 1995).**

Effect of Zn Treatments on Plant Growth and Water Relations of Phaseolus Vulgaris

Different growth parameters of intact *Phaseolus* plants (shoot and root length ; fresh and dry weights;) as shown in figure 1 and (number of leaves , leaf area); number of stomata , stomatal area , relative water contents and rate of transpiration as shown in table 1 showed a significant increase in growth at low concentration (1mM). This result may explained that Zn serves as a promoter of development micronutrients at low concentrations **Sridhar *et al***. (2007). Since Zn is required for the synthesis of tryptophan (**Brown *et al*., 1993; Alloway, 2004)**, which is a precursor of IAA, this metal also has an active role in the production of auxin, an essential growth hormone **(Brennan, 2005; Tsonev and Lidon 2012).**

However, at the high concentrations (200, 400, 600 ,800 ,1000 &1200mM ZnSO4), showed a general decrease in all growth parameters with increasing Zn concentration. These results are in accordance with those obtained by **Bonnet *et al* (2000)** in ryegrass plants and **Shute and Macfie (2006**) in soybean . The same trend was observed in RGR. Meanwhile, S/R ratio was increased with increasing Zn concentration, this indicate that, the root is more sensitive than the shoot. The reason for the different responses of root and shoot growth to heavy metals is not clear, but may be partly due to faster accumulation of heavy metals in the root than in shoots or a faster detoxification rate in the shoot than the root (**Al-Yemeni andAl-Helal.2002**Who reported that ZnCl2 significantly inhibited the root and shoot elongation of rice seedlings and increased the degree of inhibition as the concentration increased. Radical elongation has been more detrimental than shooting elongation. **Zengin (2006)** It stated that, Phaseolus vulgaris cv treatments. For Zn^{2+},

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the abscisic acid content in the root and in the leaves increased significantly. The rise in leaf abscisic acid was correlated with the root material.

Shaukat, *et.al* (1999) suggested that shooting heavy metal concentrations lead to high phenolic compounds that could be responsible for germination and growth inhibition. Phenolic acids have been shown to exert dramatic effect on membrane permeability and membrane electrical potentials. Zn concentrations of 100–400 µg g-1(soil d.m.) cause significant decrease in root and shoot growth parameters at different developmental stages of Artemisia annua plants and the biomass decline and inhibition of cell elongation and division (**Khudsar *et al.*, 2004**)

Zinc In Zn- (Table 2), accumulation in plant shoots and roots increased significantly ($P < 0.05$) either alone or in incompatibility with Ca^{+2} or glutathione treated groups with increased concentration of applied metal solution. There were a negative correlations between Zn accumulation in shoots and roots to RWC, plant fresh and dry weight and plant height (Fig. 1). Meanwhile , there were a positive correlations with S/R ratio , this may indicate that , the root is more sensitive to Zn than shoot. **Sresty and MadhavaRao (1999)** based on transmission electron microscopy concluded that radicle elongation was more adversely affected than the plumule extension. The major change was seen in the nucleus of the root tip cells due to zinc toxicity. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless, disintegration of cell organelles and the development of vacuoles were also observed. **Rout and Das (2003).** Also, it was found that, Phenolic contents were substantially elevated in both shoots and roots following treatment with heavy metals particularly at high concentrations (200 and 400 ppm). **Shaukat *et.al.(*1999).**

A significant decrease in dry weight may be due to decrease in protein content, this in accordance with those obtained by **Zengin,. (2006)**. The contents of total protein decreased with the concentration of zinc. Number of leaves and leaf area was significantly decrease with increase Zn concentration, this is in agreement with those obtained by**(Khudsar *et al* (2004))**, where they found that, the Responses of *Artemisia annua* to different concentrations of zinc [50, 100, 200, 300 and 400 µg g^{-1}(soil dry mass)]. Total leaf area, dry mass of leaves, length and dry mass of shoots and roots declined significantly under the influence of Zn treatment .Similar results were also reported by **Chamon et al., (2008**) who ,showed that the application of Zn to soils had a slight negative influence on nitrogen content in stems of spinach, may be the reason for negative influence in case of red amaranth **(Malik *et al*. 2011)**. **Sedberry *et al*., (1988)** found that Zn application resulted in a reduction in P concentration in rice plant tissue at first joint, may also be another reason for yield reduction. As clear from table 1, the rate of transpiration increased significantly with increasing Zn concentration, this also correlated with increasing the stomatal area although the no of stomata decreased with decrease the leaf area. In this respect, Hoe ***et.al*. (2012)** stated that, Transpiration of plants has an important role in heavy metal absorption. When the transpiration is flourishing, plants accumulate more heavy metals, and its enrichment capability is also stronger.

Meanwhile the stomatal index was decreased with increasing Zn concentrations at all treatments, this results was confirmed with **Kasim (2007)**, who found that, The Zn-induced 6-fold increase in

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stomatal deformation, reduction in frequency of normal stomata in of *Phaseolus vulgaris* L. cv. Limburgsvroege were sown in peat moss supplemented with $ZnSO_4$ (600 mg kg).

Glutathione combination can mitigate the plant's toxic effect of Zn. In particular, when taken in excessive amounts, all metals can contribute to toxicity and oxidative stress, which poses a serious threat to the environment. Plants have defensive strategies in which glutathione (GSH; π-glu-cys-gly) plays a central role as a chelating agent, antioxidant and signaling element in order to cope with different types of metals. This analysis therefore emphasizes GSH's role in: (1) metal homeostasis; (2) antioxidant defense; and (3) metal stress signal transduction. GSH's various functions come from the cysteine sulfhydryl group, allowing GSH to chelate metals .and participates in redox cycling. **Jozefczak *et.al.* (2012)**.

The combination of Ca+2 can alleviate the toxic effect of Zn on plants more than glutathione. For $Ca^{2+,}$ three mechanism of alleviation have been identified **(Kinraide, 1998).**

Mechanism I is the electrostatic displacement of cationic toxicants from the plasma membrane (PM)surface. Addition of Ca2+ salts to the rooting medium causes a reduction in the negative potential at the outer surface of the PM because of ionic screening and binding, thereby reducing the electrostatic attraction of cationic toxicants. Because of their equal charge and strength of binding to the PM, Ca2+ and Mg^{2+}haveequal effectiveness as Mechanism I ameliorants. Al3+and H+ have even higher Mechanism I effectiveness (**Grauer and Horst, 1990; Kinraide, 2003**) even though both ions are also intoxicating. Na+ and K+ also alleviate toxicity by Mechanism I, but much more weakly than Ca2+ and Mg2+.*Mechanism II* is the restoration of Ca2+ at the PM surface. Extracellular $Ca^{2+}$is essential for root elongation even in the absence of toxicants. If a toxic anthas sufficiently displaced Ca2+ from the PM surface (by toxicant-induced reduction of surface negativity, or by other means), then the addition of Ca^{2+}willengage Mechanism II. Mg2+, of course, has no Mechanism II effectiveness; in fact, it may induce Ca^{2+}insufficiency. Induced Ca^{2+}insufficiency is a component, though not usually the major component, of toxicity induced by low pH or high salinity (Kinraide,1998, 1999). *Mechanism III* is the residual alleviation beyond *Mechanisms I and II*. It is a heterogeneous suite of mechanisms that may entail interactions between $Ca^{2+}$and the toxicant at the PM surface

Effect of Zinc Treatment on Zn Concentration in P. Vulgaris

The effect of zinc treatment on its come into roots and shoots of *P. vulgaris* are presented in table 2. The results were highly significant at all levels of Zinc in the solution culture influenced it's concentration in *P. vulgaris*. It was observed that when the zinc levels in solution increased its concentrations in roots were also increased. Zinc was accumulated in roots and shoots. The average Zn concentration ranged from 0.416 to 35.91mM in roots in treatment with Zn alone, and from 0.60 to 33.00mM in combination of Zn with Ca $(NO_3)_2$ and from 0.69 to 61.63 in combination with glutathione. A gradual increase of zinc concentration was observed with the increasing Zn levels. In shoots Zn concentration was also found to increase with increasing Zn levels. The Zn concentration in *P.vulgaris* shoots was found lowest than in the roots. Roots accumulate more Zn than shoots. In general, total uptake of Zn decreased in supplementation of $ZnSO_4$ with $Ca(NO_3)_2$ than with glutathione .

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Effect of Zn Accumulation on Plant Internal Structure

Following the results in Table (3) and images (2), there was an increase in the root thickness. This coincided with an increase in the density of the vascular area and vascular bundles in the case of zinc alone or glutathione. This may be due to the increased surface exposure of the elements. **Gadallah and Ramadan (1997)** show that high concentrations of zinc enhanced xylem formation in the roots of *Carthamustinctorius*L. While **Rosolem *et al.* (2005)** stated that Plants grown without Zn showed an increase in root and in root stele diameter.

For the stem , as shown in table (4) and plate (3) It was found that there was a decrease in width of the cortex and concurrent with the decrease in number of rows of cortical cells and the number of vascular bundles with increased concentration of zinc alone or in combination with calcium nitrate or glutathione, noting that there is an improvement in the case of the addition of glutathione.This may be due to the fact that high concentrations of zinc may affect the rate of formation of auxins, which affects the rate of growth. On the other hand, **Alpaslan *et al.* (1999)** added that the addition of zinc to tomato plant with sodium chloride lead to increase the number of vascular bundles in the stem.

In leaf anatomy as shown in table (5) and plate (4), it was shown that, the thickness of the leaf in the zinc-treated plants alone was increased with increasing zinc concentration. This was coinciding with the increase in thickness of the midrib area and the expansion of the vascular bundle area. With the addition of calcium nitrate, there was a decrease in these measurements with increasing zinc concentration. However, with the addition of glutathione, the thickness of leaf and a decrease in the mid rib area, while the thickness of the vascular bundle area did not show a change. **Sidhar *et al*. (2007)** suggests that, the, microscopic structural changes, such as a decrease in intercellular spaces, breakdown of vascular bundles, and shrinkage of palisade and epidermal cells, occurred in leaves, stems and roots of plants treated with high concentrations of Zn.

Shoots and roots of *P. vulgaris* seedlings seemed to show differential sensitivity to Zn stress. Reduction in shoot growth criteria seemed to result from a decrease in parenchyma cell size and diameters of metaxylem vessels in the leaf midrib. Scanning Electron Microscope (SEM) revealed the presence of compacted grana with reduced thylakoids in chloroplasts, which might have contributed to the recorded loss of chl-a, chl-b and carotenoids **Kasim (2007)**

4. Conclusion

Zinc added at the rates of, 200, 400, 600, 800, 1000 and 1200 mM ZnSO4. Maximum significant decrease in the growth affected the height of *Phaseolus vulgaris* plants significantly. At 1mM Zn, plant height was found to be highest (29.21 cm/plant) and then decreased with increasing Zn treatments. Fresh and dry matter production of *Phaseolus vulgaris* decreased with increasing Zn levels and found highest at 1 mM. Zn concentration in plants increased with increasing Zn treatment and was highest at 1200 mM in all treatments either in application of Zn alone or in combination with Ca^{+2} or with glutathione in both for root and shoot. There was a differential variation in anatomical structure of roots, stems and leaves owing to all treatments with zinc.

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Table 1: Effect of different ZnSO4 concentrations in the culture medium either alone or in combination with 10mM Ca(NO3)2 on shoot/root ratio , relative growth rate (RGR);number leaves; leaf area; stomatal Index; stomatal area and rate of transpiration

Treatment	Concentrations	S/ R	RGR	Nº of leaves	leaf area (cm^2)	Stomatal Index	Stomatal area µ2	Rate of transpiration mg/g-1 F.Wt root
ZnSO4	Control	.79 ±.058	.0176 ±.001	4.0 ±.277	90.81 ±.006	24.46 ±.058	13.520 ±.006	0.304 ±.000
	1mM	.74* ±.006	.0197* ±.001	4.6* ±.058	95.77 ±.006	19.07 ±.006	15.020 ±.006	0.342 ±.000
	200mM	.72* ±.011	.0153* ±.000	3.1* ±.058	71.69 ±.058	17.25 ±.058	15.770 ±.058	2.025 ±.006
	400mM	1.22 ±.064	.0073 ±.000	2.7* ±.058	6.09 ±.577	16.81 ±.006	18.023 ±.006	3.644 ±.000
	600mM	1.47 ±.058	.0026 ±.000	2.3 ±.058	5.58 ±.058	13.47 ±.058	19.520 ±.006	3.883 ±.000
	800mM	1.48 ±.115	.0013 ±.000	2.3 ±.058	4.18 ±.006	9.38 ±.173	19.550 ±.058	4.333 ±.000
	1000mM	1.50 ±.058	.000 ±.000	2.0 ±.177	3.29 ±.115	8.48 ±.058	20.27 ±.058	6.510 ±.006
	1200mM	2.00 ±.077	.000 ±.000	2.0 ±.155	1.90 ±.177	6.46 ±.058	21.026 ±.006	7.030 ±.006
	L.S.D	**0.636**	**0.008**	**1.505**	**0.879**	**0.232**	**0.109**	**0.009**
ZnSO4+ $Ca(NO3)_2$	Control	.77 ±.006	.0431 ±.000	5.7 ±.058	103.70 ±.058	30.246 ±.058	13.520 ±.006	.125 ±.006
	1mM	.76* ±.006	.0461* ±.000	6.0* ±.577	116.63 ±.006	25.982 ±.000	13.520* ±.011	.127* ±.006
	200mM	.71* ±.058	.0312* ±.000	3.2 ±.058	79.51 ±.006	25.791 ±.000	15.020 ±.006	0.218 ±.000
	400mM	1.27 ±.058	.0270 ±.000	3.0 ±.277	26.32 ±.006	23.801 ±.000	18.027 ±.000	0.493 ±.000
	600mM	1.61 ±.058	.0240 ±.006	3.0 ±.077	15.86 ±.006	23.163 ±.000	19.529 ±.058	0.581 ±.000
	800mM	2.21 ±.006	.0211 ±.000	3.0 ±.155	12.18 ±.006	22.581 ±.000	19.529 ±.006	0.847 ±.006
	1000mM	2.23 ±.006	.0207 ±.000	3.0 ±.077	9.39 ±.058	21.302 ±.000	20.278 ±.058	1.234 ±.000
	1200mM	2.28 ±.058	.0179 ±.001	3.0 ±.077	3.80 ±.058	18.174 ±.000	21.030 ±.006	1.300 ±.058
	L.S.D	**0.109**	**0.012**	**2.124**	**0.134**	**0.095**	**0.095**	**0.055**
ZnSO4+ glutathione	Control	0.75 ±.006	.0329 ±.000	4.8 ±.058	92.84 ±.006	26.076 ±.000	18.027 ±.006	0.134 ±.000
	1mM	0.68* ±.058	.0279* ±.002	5.0* ±.577	96.19 ±.058	20.741 ±.000	19.520 ±.006	0.151 ±.006
	200mM	0.70* ±.058	.0218 ±.000	3.3 ±.058	25.06 ±.006	19.271 ±.000	19.520 ±.058	0.533 ±.000

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	400mM	0.94 ±.006	.0217 ±.000	3.1 ±.058	11.25±.006	18.472 ±.058	19.526 ±.006	0.582 ±.000
	600mM	1.45 ±.006	.0216 ±.000	3.0 ±.377	8.11±.058	18.437 ±.006	20.27 ±.006	0.959 ±.006
	800mM	1.59 ±.058	.0213 ±.002	3.0 ±.377	7.48±.058	18.333 ±.058	20.27 ±.006	1.225 ±.000
	1000mM	1.65 ±.006	.0193 ±.000	2.3 ±.058	4.51±.115	16.639 ±.006	21.029 ±.006	2.063 ±.000
	1200mM	1.71 ±.006	.0173 ±.000	2.0 ±.058	3.20±.115	14.863 ±.000	22.534 ±.000	2.304 ±.000
	L.S.D	**0.109**	**0.008**	**1.23**	**0.205**	**0.095**	**0.055**	**0.008**
* Nonsignificant L.S.D. at 5%								

Table 2: Effect of different ZnSO4 concentrations in the culture medium either alone or in combination with (10mM) Ca (NO3)2 or glutathione on the zinc content in root ,in shoot and in the total uptake of 14-day-old Phaseolus vulgaris plants .Each value is the mean of 3 Sample calculated as m mole 100g-1 dry weight

Treatment	**Concentrations**	**Zn in Root**	**Zn in Shoot**	**Total uptake of Zn**
ZnSO4	Control	0.333±.008	0.073±.002	0.406±.000
	1mM	0.416*±.000	0.087*±.000	0.503*±.000
	200mM	22.51±.058	1.36±.006	23.87±.006
	400mM	28.78±.006	1.73±.006	30.51±.058
	600mM	31.73±.006	2.00±.577	33.75±.058
	800mM	34.76±.006	2.74±.006	37.50±.058
	1000mM	35.91±.006	3.78±.006	39.69±.058
	1200mM	35.91±.006	4.55±.006	40.46±.115
	L.S.D	**0.095**	**0.612**	**0.173**
ZnSO4+ Ca(NO3)2	Control	0.27±.002	0.068±.000	0.338±.006
	1mM	0.60*±.005	0.075*±.000	0.675±.006
	200mM	4.55±.006	1.23*±.058	5.78±.000
	400mM	11.97±.006	1.27*±.006	13.24±.000
	600mM	18.45±.012	1.32±.012	19.77±.000
	800mM	28.84±.006	1.77±.006	30.61±.001
	1000mM	32.67±.006	2.13±.155	34.80±.000
	1200mM	33.00±.577	2.70±.058	35.70±.000
	L.S.D	**0.627**	**1.228**	**0.008**
ZnSO4+ glutathione	Control	0.52±.006	0.108±.000	0.628±.000
	1mM	0.69±.058	.160*±.006	0.85±.012
	200mM	17.32±.006	1.303±.000	18.623±.012
	400mM	24.43±.006	1.460±.006	25.89±.058
	600mM	29.84±.006	1.598±.000	31.438±.058
	800mM	35.24±.006	1.656±.001	36.896±.000
	1000mM	36.93±.006	2.679±.077	39.609±.058
	1200mM	61.63±.006	5.039±.006	66.669±.058
	L.S.D	**0.055**	**0.612**	**0.122**

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Table 3: Effect of ZnSO4 concentrations either alone or incombination with (10 Mm) Ca(NO3)2 or glutathione on the root anatomy of 14-day-old aseolus vulgaris Each value is the mean of 3 samples.

Treatment	Concentration	Width of T.S.µ	Width of cortex µ	Width of vascular bundle µ
ZnSO4	Control	60.9±.058	16.8±.058	25.2±.115
	1mM	64.05±.006	21.0±.577	29.4±.058
	200mM	68.95±.577	23.1±.058	31.5±.577
	600mM	78.75±.006	25.2±.887	42.0±.155
	1200mM	100.8±.058	31.5±.289	52.5±.289
	L.S.D	0.822	4.169	1.873
ZnSO4+ Ca(NO3)2	Control	70.35±.058	17.85±.006	24.15±1.155
	1mM	73.5±.058	18.9*±.577	26.25*±.006
	200mM	75.6±.058	19.95±.006	31.5±.058
	600mM	76.65±.006	21.0±.577	33.6±.115
	1200mM	105.0±.577	35.7±.058	42±1.155
	L.S.D	0.826	1.154	2.308
ZnSO4+ glutathione	Control	69.3±.058	23.1±.058	31.5±.577
	1mM	73.50±.577	26.25±.006	34.65±.006
	200mM	75.60±.115	31.50±.577	36.75±.006
	600mM	97.65±.006	36.75±.006	45.15±.006
	1200mM	105.0±1.155	42.0±.155	46.2±.115
	L.S.D	1.828	1.821	0.829
* Non significant L.S.D. at 5%				

Table 4: Effect of ZnSO4 concentrations either alone or incombination with (10 Mm) Ca(NO3)2 or glutathione on the stem anatomy of 14-day-old Phaseolus vulgaris Each value is the mean of 3 samples

Treatment	Concentration	Number of rows of cortical cells	with of cortex µ	Number of vascular bundle
ZnSO4	Control	10±.887	157.5±.289	23.0±.577
	1mM	6*±.577	136.5±.577	22*±1.155
	200mM	6*±.155	115.5±2.887	21*±1.155
	600mM	5±.155	105±.577	21*±1.732
	1200mM	5±.4	105±2.887	20.0*±.577
	L.S.D	4.813	5.881	3.546
ZnSO4+ Ca(NO3)2	Control	11±.577	168.0±.577	24±.577
	1mM	7±.155	157.5±.289	21±1.155
	200mM	6±.155	126.0±.577	11.0±.577
	600mM	6±.577	115.5±.577	10±1.155
	1200mM	6±.732	115.5±.058	20±.577
	L.S.D	3.546	1.469	2.698
	Control	13±.577	199.5±.058	21±.577
	1mM	8±.155	189*±5.77	26±1.155
	200mM	8±.577	168.0±5.77	29±.577

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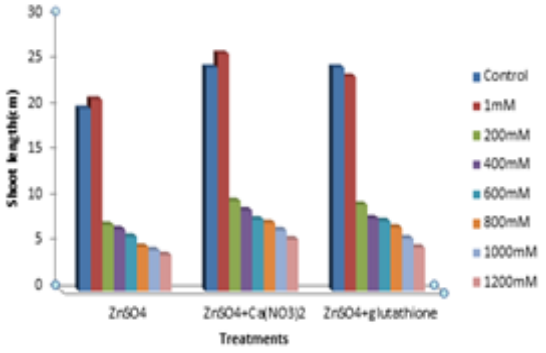
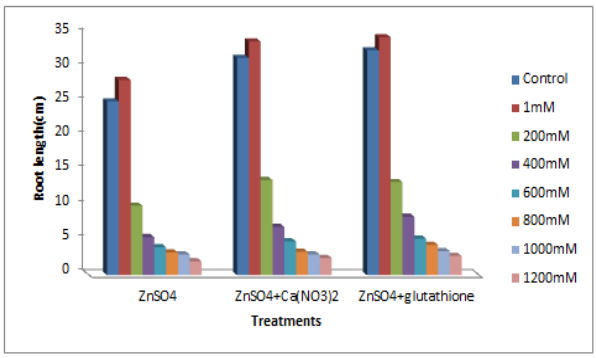
ZnSO4+ glutathione	600mM	7±.155	157.5±.289	30±1.155
	1200mM	6±.155	157.5±.058	30±.577
	L.S.D	3.04	11.513	2.698

Table 5: Effect of ZnSO4 concentrations either alone or incombination with (10 Mm) Ca (NO3)2 or glutathione on the leaf anatomy of 14-day-old Phaseolus vulgaris Each value is the mean of 3 samples.

Treatment	**Concentrations**	**blade thickness µ**	**Midrib µ thickness**	**Width of vascular bundle µ**
ZnSO4	Control	9.9750±.000	76.125±.577	18.375±.058
	1mM	9.975*±.006	78.75±.006	21.0±.577
	200mM	11.130±.577	86.10±.058	24.15±.577
	600mM	11.550±.064	90.30±.173	24.15±1.155
	1200mM	18.375±.058	96.075±.000	25.20±.115
	L.S.D	0.822	0.853	2.001
ZnSO4+ Ca(NO3)2	Control	11.02±.006	84.0±.577	21.0±.577
	1mM	11.55±.064	84*±1.155	25.2±.115
	200mM	12.92±.006	94.5±.577	26.25±.006
	600mM	6.30±.173	79.8±.058	16.8±.058
	1200mM	6.30±.058	78.7±.058	15.75±.006
	L.S.D	0.269	1.996	0.834
ZnSO4+ glutathione	Control	10.5±.577	89.25±.058	26.25±.006
	1mM	10.5*±.289	87.15±.006	26.25*±.577
	200mM	9.45*±.577	84.0±.577	26.22*±.064
	600mM	5.780±.058	78.75±.006	26.2*±.115
	1200mM	2.60±.058	75.6±.115	26.0*±.577
	L.S.D	1.226	0.834	1.165





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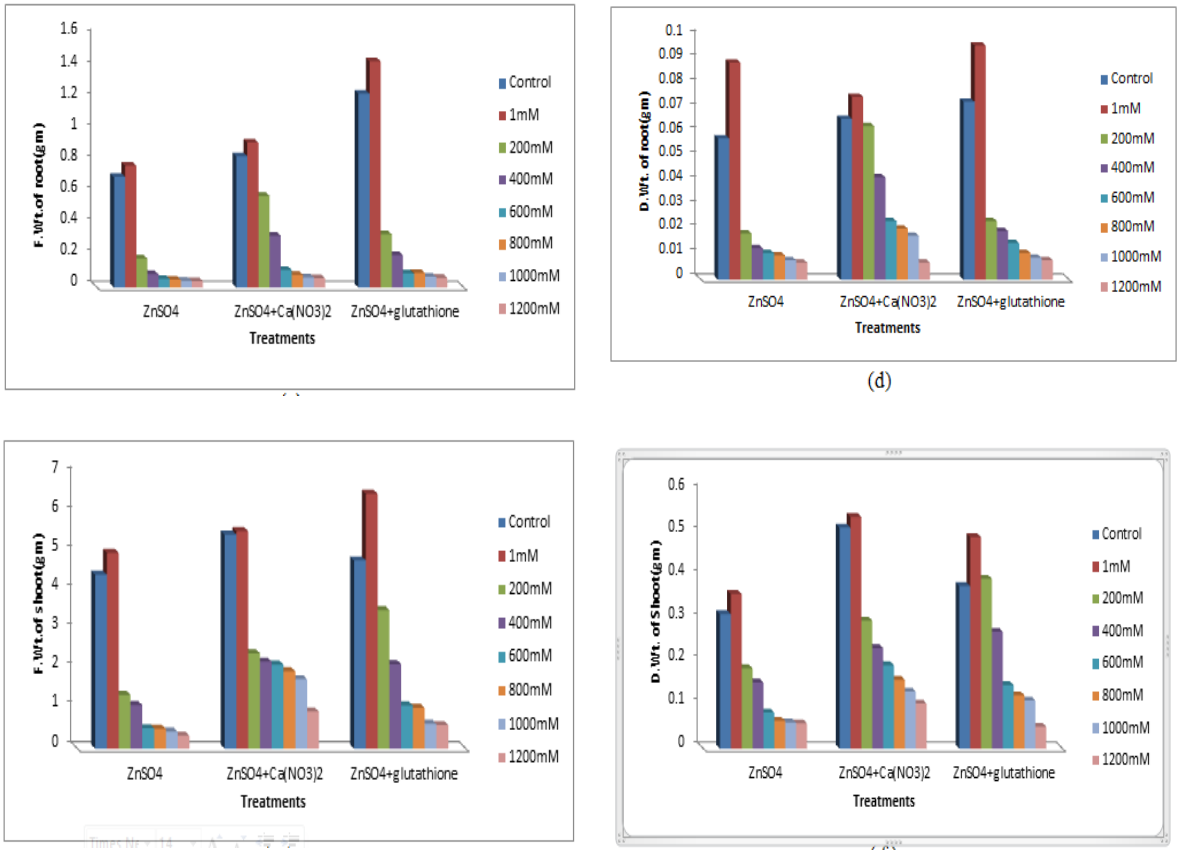

Figure 1: Effect of different ZnSO4 concentrations either alone or incombination with (10Mm) Ca(NO3)2 or glutathione on growth parameters(a) root length;(b) shoot length ;(c) fresh weight of root;(d)dry weight of root;(e) fresh weight of shoot;(f) dry weight of shoot;(g) of 14-day- old Phaseolus vulgaris plant.

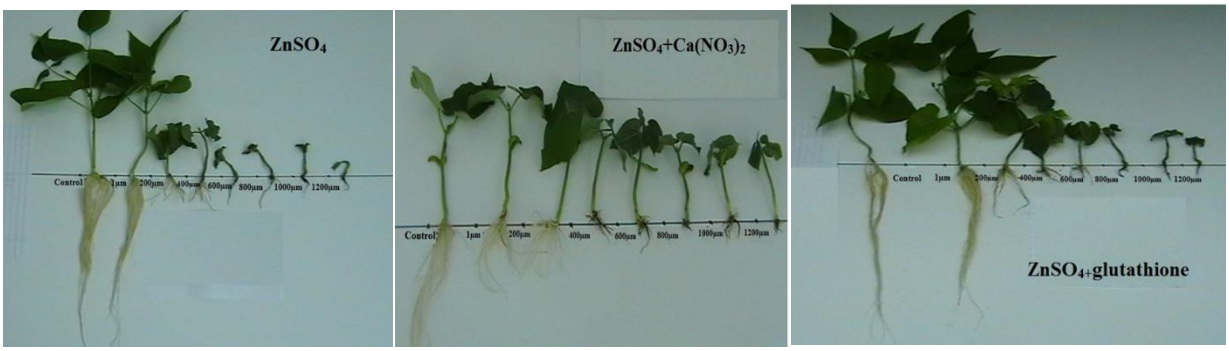

Plate 1: Effect of different Zinc concentrations alone or incombination with (10Mm) Ca(NO3)2 or glutathione on growth of *Phaseolus vulgaris* plant

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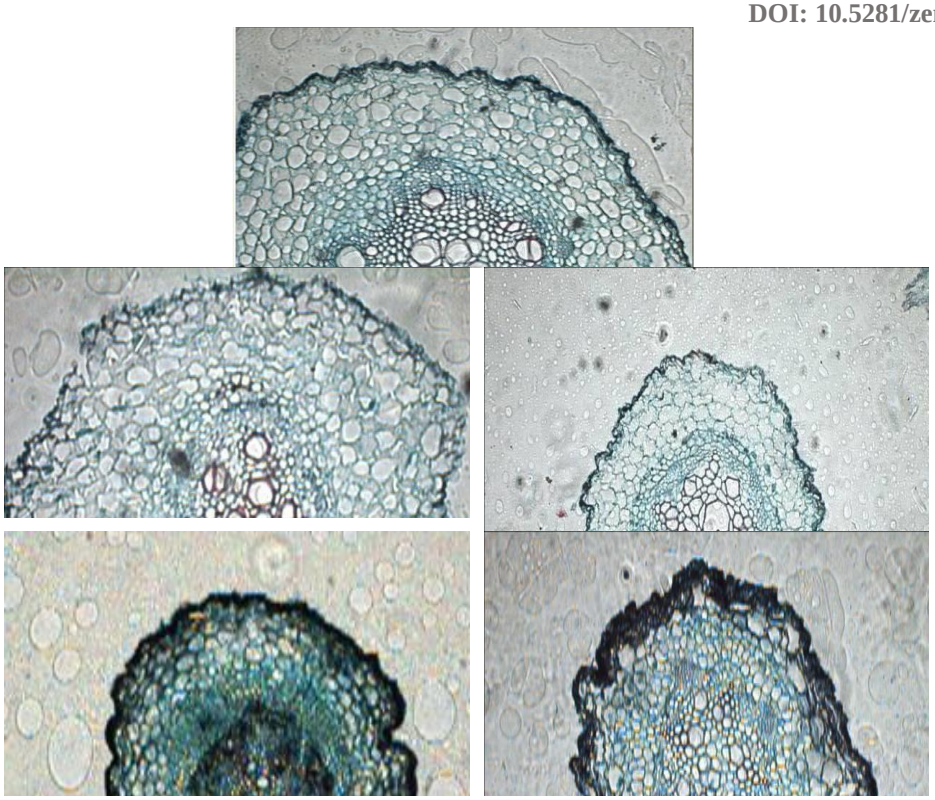

Plate 2: Photographs showing the effect of different ZnSO4 concentrations on the root anatomy of Phaseolus *vulgaris* plants. (a) control; (b) 1mM; (c) 200mM; (d) 600mM and (e) 1200 mM.

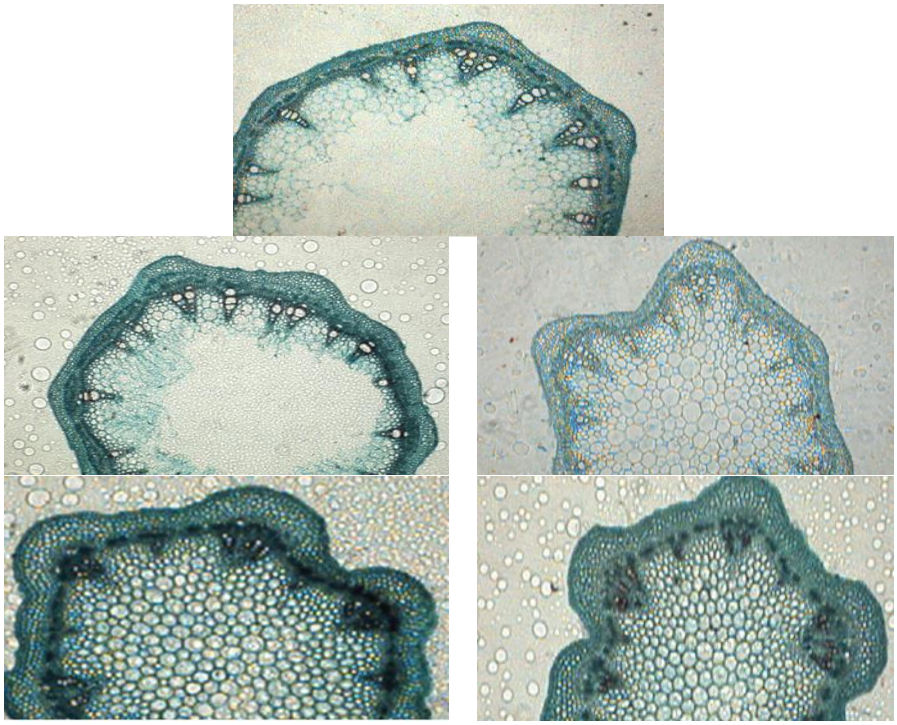

Plate 3: Photographs showing the effect of different $ZnSO_4$ concentrations on the stem anatomy of *Phaseolus vulgaris* plants. (a) control; (b) 1mM; (c) 200 mM; (d) 600 mM and (e) 1200 mM.

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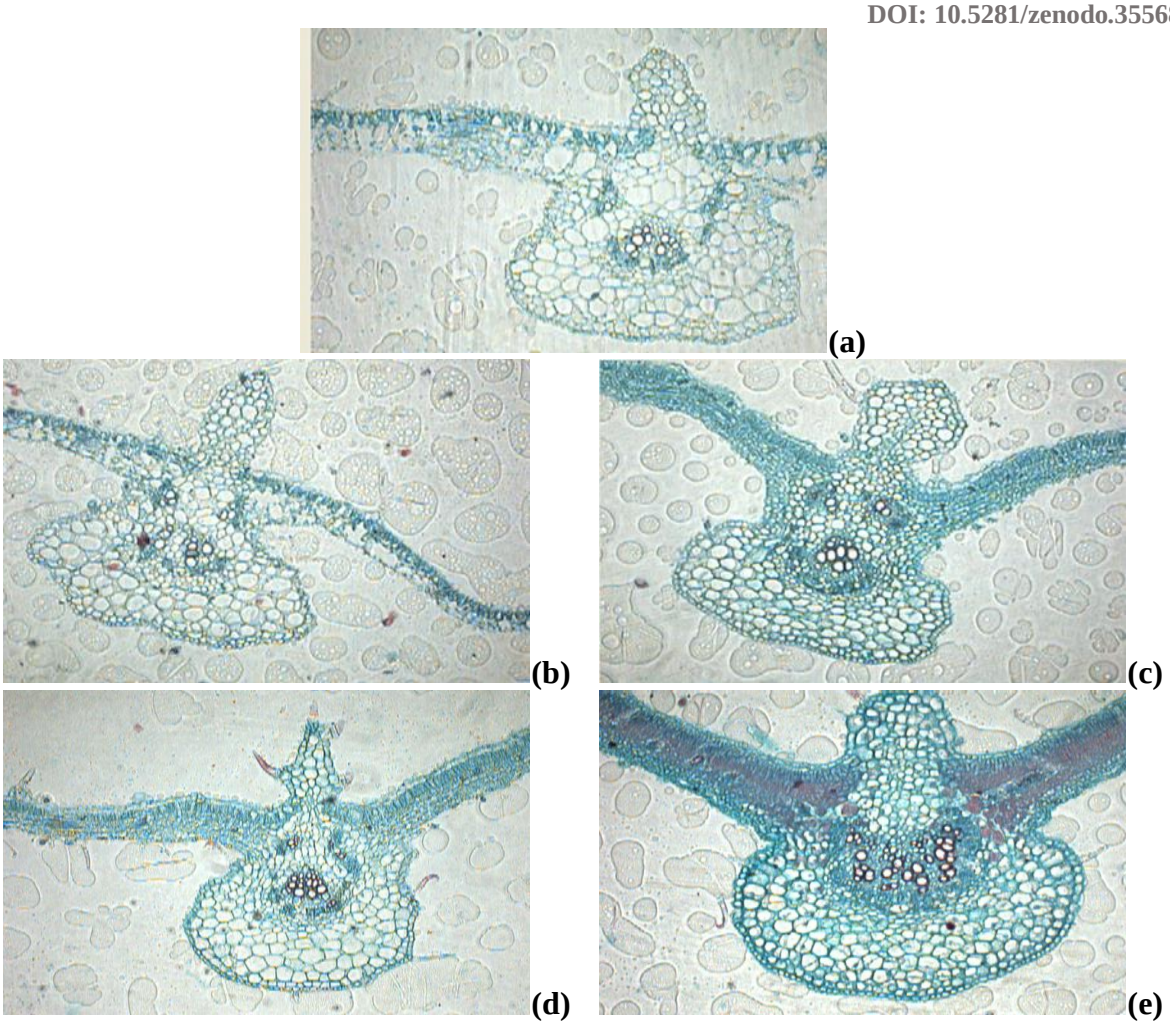

Plate 4: Photographs showing the effect of different ZnSO4 concentrations on Leaf anatomy of *Phaseolus Vulgaris* plants. (a) control; (b) 1 mM; (c) 200 mM; (d) 600 mM and (e) 1200 mM

Conflict of Interest

The authors declare that there is no conflict of interest .

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