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ORIGINAL ARTICLE

Study Different Levels of Zinc Sulphate ($ZnSO_4$) on Fresh and Dry Weight, Leaf Area, Relative Water Content and Total Protein in Bean (*Phaseolus vulgaris* L .) Plant

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ABSTRACT
Effect of different concentrations of zinc sulphate on some physiological parameters in bean plant(Phaseolus vulgaris L .) at hydroponic culture were surveyed .7 day old seedlings were moved in pots with content of Hoagland nutrient solutionat controlled situation (temperature of 24-25 °cat day and 18-19 °c at night and 16 hours light condition and 8 hours dark condition). Three leaf seedlings were treated with different concentrations of zinc sulphate. This investigation was carried out based on Completely Randomized Designs (CRD) with four replication and nine treatments (including 0,25,50,75,150,250,350,450,550 µm zinc sulphate). Results showed that 50 µm zinc sulphate concentration significantly caused boosting of leaf area and both fresh and dry weight of shoot and root. The most Relative Water Content were obtained at 25 µm zinc sulphate concentration and after that with increasing of treatment levels, gradual decrease of this index was obtained. The most total protein content was observed at 550 µm.
Key words: Bean, Zinc Sulphate, Physiological Parameters, Total Protein

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INTRODUCTION

Pulse crops is the second mankind food sources. beans has the first grade, regarding cultivated area and economic importance among pulses crop, So researchers have tried to identify important factors for making better products and reduction of Internal and external adverse factors, because inappropriate environmental factors like as Ionic stress that directly affect on final bean yield [1]. Zinc has been known as micro and necessary element in plant nutrient and it has important role in improvement of crop quality and quantity so that ample yield of plant crops including cereal crops, forage crops, industrial crops and pulse crops, are affected by zinc[2]. This element accelerate theprocess of plant growth and development because its role as a cofactor in tryptophan amino acid biosynthesis(auxin-substrat) and also nitrogen, starch and lipid metabolism[3]. Zinc deficiency causes yield crop reduction and it even may that plant crop cannot complete its growth [4]. There are several Symptom of zinc deficiency for example reduction of root growth, decrease of stem dry matter, reduced activity of large number of enzymes (for instance carbonic anhydrase and superoxide dismutase)[2] , increase of oxygen radioactive species (ROS) density and underdevelopment of primary leaf[5]. Although low concentration of zinc, as microelement, plays fundamental role in crop physiology but high concentration of this element as like as heavy metal elements provides reduction of growth and yield by making disorder in cell metabolism. Leaf chlorosis and necrosis are some visible symptom of plant toxicity in which subject to heavy metal elements[6]. In cells that are exposed to heavy metal elements, the expression rate of some genes are changed in heavy metals stress condition, After that mRNA(related to proteins and peptides that are involved in detoxification of metals) increase, because of one reduction method of heavy metal elements toxicity is connection of metal to amino acids, proteins and polypeptides within cell[7]. Indeed Relative water content (RWC) is an estimation of plant water status and shows ability of each genotype to absorb water from soil[8]. Relative water content of leaf lessens in stress condition and the reason of this process is the effect of heavy metal on water relations of cell plant that cause inactivity of water channel in tonoplast so no water inters into plant tissue[9]. As far that heavy metal elements have a complicated interactions with many metabolic process in plant so expression just one of common mechanism for physiology behavior of heavy metal stress in plant , is difficult. Present study was done on bean (*Phaseolus vulgaris* L.) for determination of optimum concentration of zinc as microelement for stimulation of plant growth and also specification of critical concentration of zinc as heavy metal element and inhibitor of plant growth.

MATERIALS AND METHODS

This experiment was conducted on 2011 at Tehran Research and Science Branch, Islamic Azad University. Seeds of beans (*Phaseolus vulgaris* L.) first were washed with sterile distilled water and next were sterilized in 1% (W/V) sodium hypochlorite (5 min) and finally were washed several times with sterile distilled water. these seeds were placed on filter paper in Petri dishes containing water (5ml) and kept at 25^{o}C.Each petri dishes had 8 seeds. As soon as they germinated in darkness next they were transferred in light. 7-day-old seedling were cultivated in hydroponics system and irrigated with Hoagland's solution [10]. When seedling reached at three leaf stage, they were treated at different concentration of zinc sulphate. Experimental design was Completely Randomized Designs (CRD) with 4 replications and 9 treatments (including 0,25,50,75,150,250,350,450,550 µm zinc sulphate). The plants were grown under controlled condition (day temperature of 24^{0}C/25^{0}C, night temperature of 18^{0}C/19^{0}C,16-h light) and the nutrient solution was renewed every 2 day.

Growth assay

Plants were divided into three fractions (leaves, stem and roots). Fresh weight and leaf area of each fraction were carefully determined. Dry weight of samples was measured after being dried at 70'C for 48h.

These samples were taken two times during this experiment, including at three leaf stage and twenty days later.

Calculation relative water content

To measure relative water content[11], one leaf was sampled from one plant per plot then after cutting their blade, fresh weight was determined and next the leaves were held in distilled water at petri dishes and kept in cool place for 24 hours. After that turgid weight was measured. Next the leaves were wrapped in aluminum foil and afterward dried at 70'C for 48 hours and next weighted.

relative water content was calculated by the following equation:

$$RWC(\%) = (FW - DW) / (TW - DW) \times 100$$

Where, RWC, FW, DW and TW are relative water content, fresh weight, dry weight and turgid weight respectively.

Assay of total protein:

Frozen leaf samples (0.5 g) were used for protein extraction according to the method of Bradford [12]. Samples were ground in 5 ml of 50 µm phosphate buffer (pH 7.5) using pre-chilled mortar and pestle. The phosphate buffer contained 1 µm EDTA, 1 µm PMSF and 1% PVP-40. Then the extract was centrifuged at 4°C at 15,000 × g for 30 min. The supernatant (protein extract) was used for measurement of protein content. The absorbance of the irradiated solution was read at 595 nm using a spectrophotometer[12].

Statistical analysis:

All data were analyzed statistically using SPSS package and all graphs were drown using Excel[13].

RESULTS AND DISCUSSION

Results of dry matter Measurement of shoot and root

Outcomes showed that application of 50 µm zinc sulphate had the highest and 550 µm had the lowest both shoot and root dry matter (figure 1,2). With increasing more than75 µm zinc sulphate, dry matter considerably reduced than control (table 1).

Generally low concentration of zinc causes plant growth simulation. Its clear that zinc has bold role in auxin biosynthesis and this hormone has been known as a plant growth simulation. Because with more probability, zinc plays as a cofactor in many enzyme activity so zinc has important role in tryptophan amino acid biosynthesis or conversion tryptophan amino acid to indole acetic acid. It has been reported that activity of enzyme regarding cell reproduction and enlargement can be motivated by low concentration of zinc[14].

Significantly Decrease of root and shoot dry matter was seen in high level of zinc sulphate in this study. Reduction growth was related to effect of zinc on cell wall and middle lamella because with connection of zinc to pectin cell wall, its elasticity lessens [15].

Malea et al (1995),with study of zinc effect on leaf cell death of *Halophylastipulecea*, stated that zinc in high level causes necrosis in both leaf epidermis and mesophyll cells and inhibit leaf surface growth[16]. zinc accumulation in leaf destroy mitochondria structure in plant which are exposed to high concentration of zinc and this is strong reason for reduction of leaf area[17].

Table1: Root and shoot dry weight in different concentrations of Zinc Sulphate in bean (Mean ±SE).

µM(Sulphate Treatment Zinc)	g(Root Dry Weight)	g(Shoot Dry Weight)
0	0. 043 ± 0. 003	0. 144 ± 0. 002
25	0. 051± 0. 002	0. 147 ± 0. 006
50	0. 062 ± 0. 001	0. 151 ± 0. 01
75	0. 054 ± 0. 002	0. 129 ± 0. 003
150	0. 042 ± 0. 006	0. 102 ± 0. 005
250	0. 028 ± 0. 00	0. 101 ± 0. 004
350	0. 019 ± 0. 002	0. 086 ± 0. 001
450	0. 015 ± 0. 00	0. 084 ± 0. 002
550	0. 015 ± 0. 00	0. 081 ± 0. 001
F	34. 61	32. 14
P	< 0. 05	< 0. 05

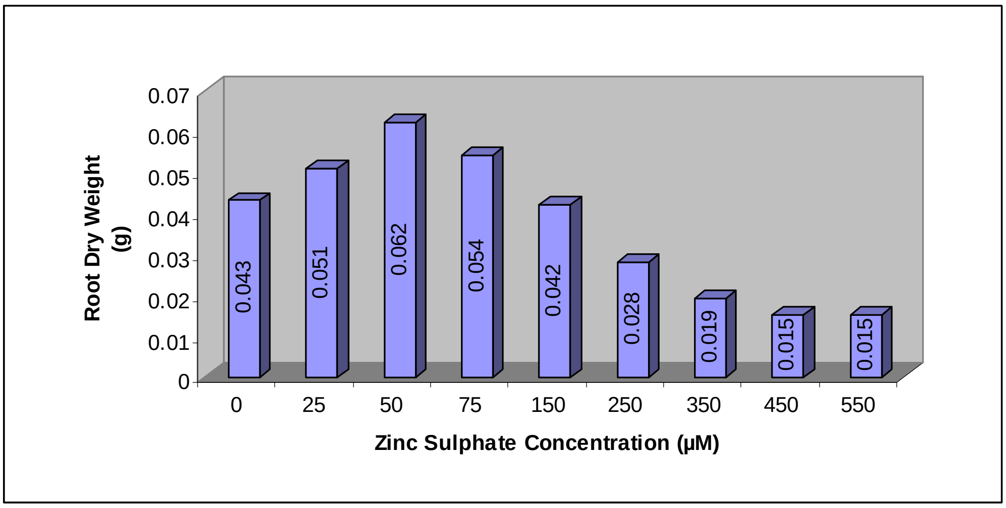

Figure1: Root dry weight in different concentrations of Zinc Sulphate in bean(Mean ±SE).

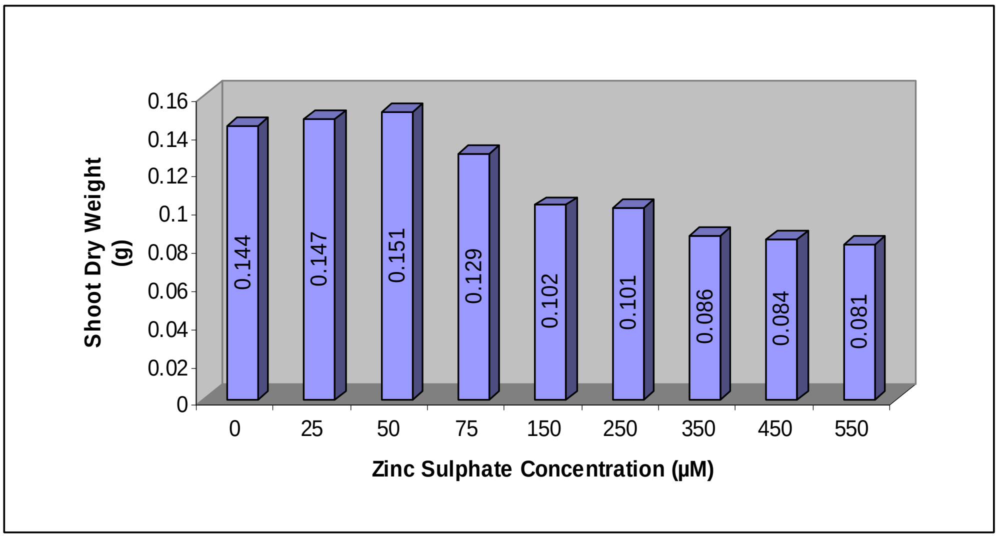

Figure2 : shoot dry weight in different concentrations of Zinc Sulphate in bean(Mean ±SE).

Results of fresh weight Measurement of shoot and root

As it was interpreted from figure 3 and 4, the highest and the lowest shoot and root fresh weight were about 50 µm and 550 µm zinc sulphate respectively.

Gradual diminish trend of fresh weight of shoot and root were obtained from 75 µm to 550 µm zinc sulphate (Table 2).

It has been reported that aquaporin channel is not limited to animal cells so these channels are found in plant cells. Heavy elements by blocking of aquaporin channel with interaction of sulfhydryl group causes to close of aquaporin channel and no water can permeate into plant tissue[18].

Results of this study was corresponded with Luderid et al *results* [19].

Table2: Root and shoot fresh weight in different concentrations of Zinc Sulphate in bean(Mean ±SE).

µM (Treatment Zinc Sulphate)	g (Root Fresh Weight)	g (Shoot Fresh Weight)
0	0. 960 ± 0. 044	1. 35 ± 0. 027
25	0. 987 ± 0. 090	1. 37 ± 0. 080
50	1. 20 ± 0. 006	1. 41 ± 0. 086
75	0. 946 ± 0. 012	1. 23 ± 0. 041
150	0. 772 ± 0. 157	1. 05 ± 0. 024
250	0. 407 ± 0. 020	0. 97 ± 0. 044
350	0. 337 ± 0. 047	0. 91 ± 0. 029
450	0. 260 ± 0. 017	0. 89 ± 0. 033
550	0. 244 ± 0. 025	0. 76 ± 0. 065
F	31. 36	20. 31
P	< 0. 05	0. 05<

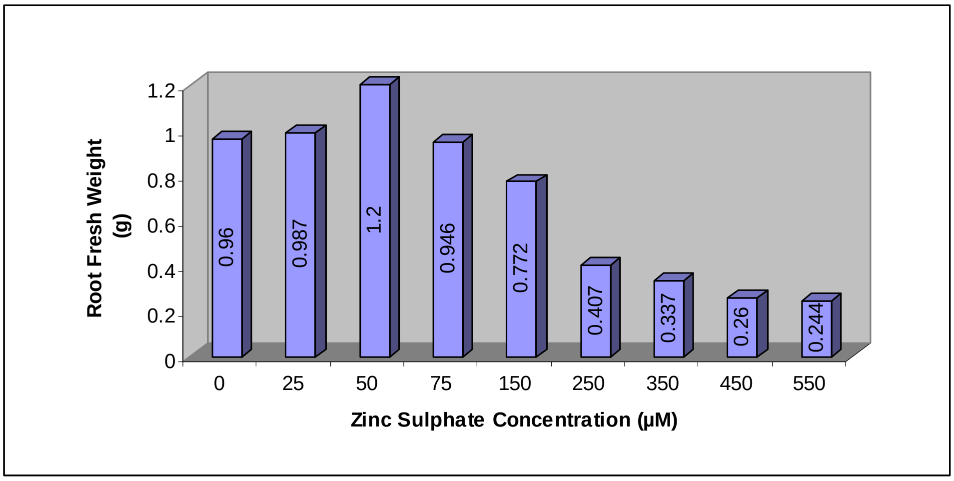

Figure3: Root fresh weight in different concentrations of Zinc Sulphate in bean(Mean ±SE).

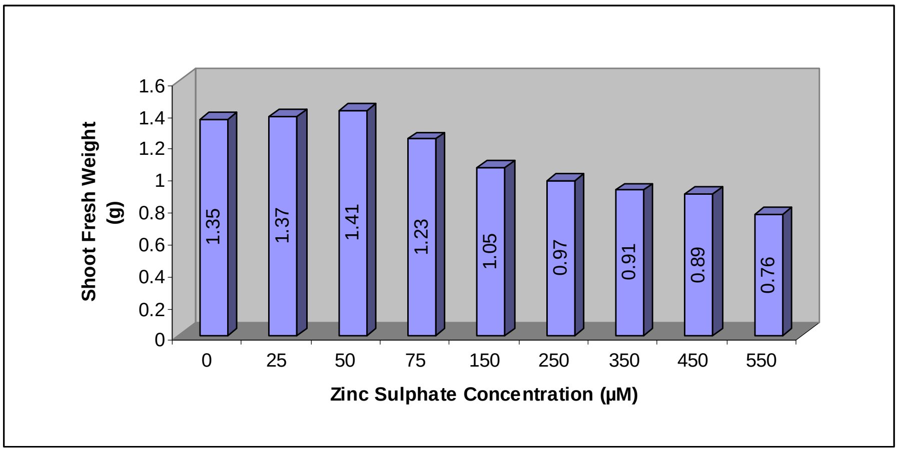

Figure4: shoot fresh weight in different concentrations of Zinc Sulphate in bean(Mean ±SE).

Result of leaf area measurement

There were significant difference between treatments regarding leaf area(table 3, figure 5). Increasing trend of leaf area was occurred with boosting concentration of zinc sulphate till 50 µm but upper than 75 µm , significantly reduction of leaf area was seen(table 3). These outcomes could be due to accumulation of heavy metals in cell wall so this action made disorder at cell natural metabolism and decrease of cell division and cell growth and finally reduction of leaf area were done [20].

Table3: leaf area in different concentrations of Zinc Sulphate in bean(Mean ±SE).

(Treatment Zinc Sulphate µM)	Leaf Area (cm^2)
0	80. 28 ± 8. 92
25	81. 67 ± 10. 30
50	85. 46 ± 1. 09
75	67. 16 ± 9. 20
150	38. 33 ± 5. 16
250	28. 23 ± 2. 54
350	21. 64 ±3. 18
450	21. 05 ± 1. 08
550	9. 9 ± 0. 45
F	25. 96
P	0. 05<

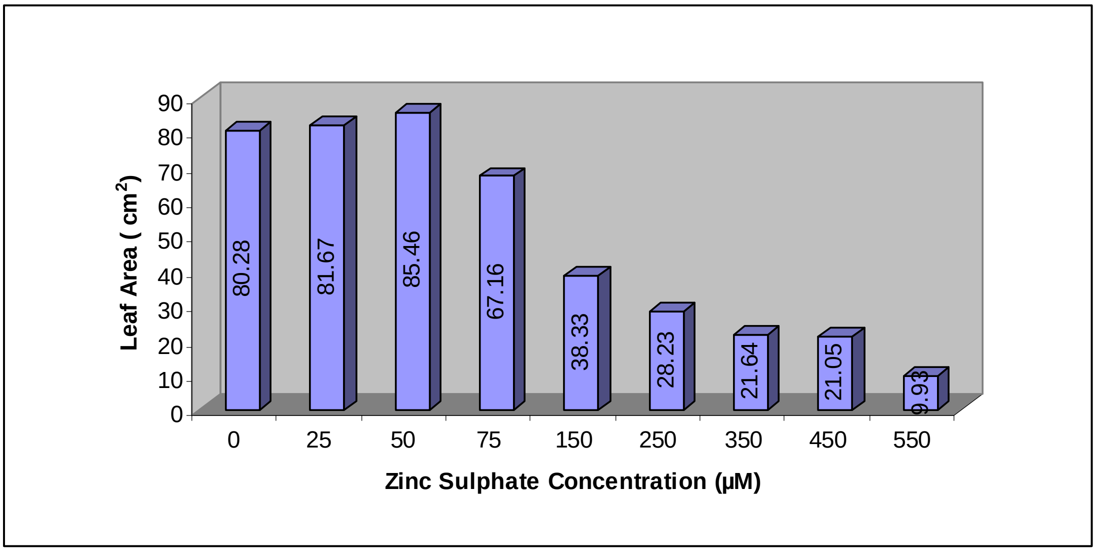

Figure5: leaf area in different concentrations of Zinc Sulphate in bean(Mean ±SE).

Result of Relative Water Content (RWC)

Effect of zinc on RWC was demonstrated (figure 6 and table 4). RWC content with boosting of zinc gradually decreased. RWC reduction, in high level of zinc sulphate, was significant and outstanding. Almost any plant process directly or indirectly affects on plant water content and water can be a major factor in the regulation of plant growth. Thus, most research, related to plant responses to environmental stresses, have specific attention to water relations in entire plant [18].

In the present study, the relative water content of leaves, affected by zinc toxicity, was reduced. High concentration of zinc, in root atmosphere, prevents the absorption of potassium. On the other hand zinc toxicity is the reason of potassium leakage from cells. Reduction of relative water content could be as a result of decrease of hydraulic conductivity of root or decline of water flow from root to shoot [21,15].Similar results about the effect of heavy metals on the RWC in wheat and maize has been reported [22].Also arsenic effect as a heavy metal had similar results on bean [23].

Table4: RWC in different concentrations of Zinc Sulphate in bean(Mean ±SE).

μM (Treatment Zinc sulphate)	RWC %
0	77. 08 ± 0. 815 %
25	81. 25 ± 1. 28 %
50	76. 40 ± 1. 20 %
75	74. 19 ± 1. 03 %
150	70. 56 ± 0. 348 %
250	67. 91 ± 0. 847 %
350	60. 39 ± 0. 431 %
450	58. 44 ± 0. 297 %
550	55. 30 ± 0. 660 %
F	75. 97
P	0. 05<

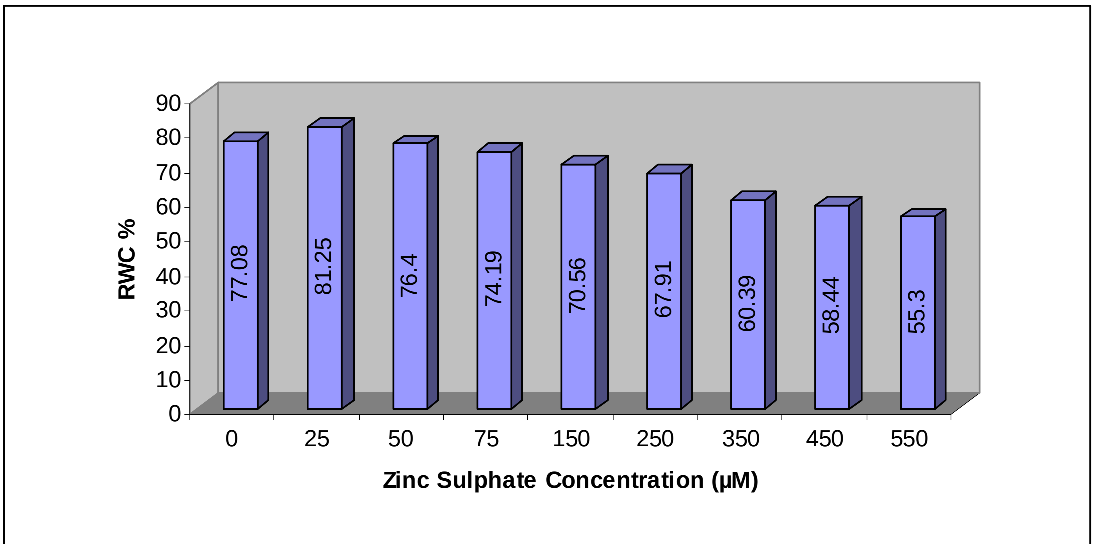

Figure6: RWC in different concentrations of Zinc Sulphate in bean(Mean ±SE).

Results of zinc sulphate on total protein content

Results showed that with increasing of zinc sulphate, raising of stem total protein happened (figure 7,table 5).

Because zinc as a cofactor for RNA polymerase enzyme that has a fundamental role in biosynthesis of amino acid and protein[14]. In spite of decreasing of growth, increasing total protein could be related to boosting of some kind of protein with low molecular weight that synthesis of this kind of proteins was gained in stress condition so accumulation of proteins are happened at same time with accumulation of zinc in plant. For protection of plant in heavy metal stress , these proteins connect to heavy metal and reduce toxic effects of heavy metal[17].

In addition , when plant are exposed to stress condition like as high temperature, salt stress, drought and heavy metal stress ,Heat Shock Protein are made in plant[24].

Table5: total protein content in different concentrations of Zinc Sulphate in bean(Mean ±SE).

μM (Treatment Zinc Sulphate)	Total Protein Content (mg g^{-1} f. w.)
0	1. 61 ± 0. 02
25	1. 68 ± 0. 01
50	1. 64 ± 0. 02
75	1. 95 ± 0. 05
150	2. 03 ± 0. 01
250	2. 08 ± 0. 01
350	2. 21 ± 0. 02
450	2. 41 ± 0. 02
550	2. 58 ± 0. 02
F	332. 6
P	0. 05<

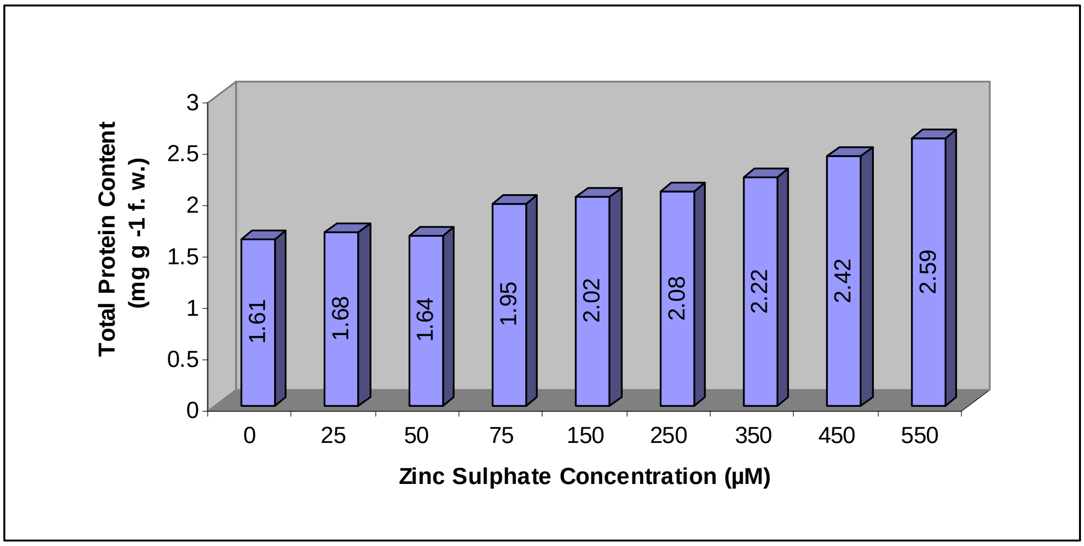

Figure7: total protein content in different concentrations of Zinc Sulphate in bean(Mean ±SE).

CONCLUSION

With importance of zinc as a micro nutrient in improvement and Complement of growth and development stage also on the other role of the zinc as a heavy metal in high concentration, for better understanding of optimal concentration of zinc on a some physiological traits of bean, this investigation was done. Results showed that low concentration of zinc sulphate (25 until 50 µm) toward control had positive effects on surveyed parameters but high concentration of zinc had stopple role.

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