

Effect of Different Zn Concentrations on Crop Growth and Root nodulation in Yard-long bean (*Vigna unguiculata* L.)

ABSTRACT

An experiment was conducted to study the effect of Zinc (Zn) as a micronutrient on growth and nodulation in *Vigna unguiculata* L. The experiment was carried out as a pot experiment in the Crop Farm, Faculty of Agriculture, Palachchola, Eastern University, Sri Lanka from August to November 2023. The experiment was laid out in Complete Randomized Design with six treatments and four replicates. The treatments are T1 (Control), T2 (50 mg ZnSO₄/kg soil), T3 (100 mg ZnSO₄/kg soil), T4 (150 mg ZnSO₄/kg soil), T5 (200 mg ZnSO₄/kg soil) & T6 (250 mg ZnSO₄/kg soil). The experiment used source of Zinc in the form of Zinc Sulphate (ZnSO₄·5H₂O) which yard-long bean plants can grow. The experiment results showed that, T2 treatment, the soil treated with 50 mg ZnSO₄/kg soil significantly increase the Plant height, Chlorophyll content, Leaf area, Fresh weight of shoot & root, Dry weight of shoot & root, Number of nodules, Effective nodule percentage, as well as Soil respiration compared to the other treatments. However, beyond the level of 200 mg ZnSO₄/kg soil, reduces the growth & nodulation of *Vigna unguiculata* L. and this shows that the level 200 mg ZnSO₄/kg soil and beyond that level were the toxic level to the *Vigna unguiculata* L. Accordingly, the treatment T2 (50 mg ZnSO₄/kg soil) was at the micronutrient level of Zn concentration which involved in many key cellular functions and show the best positive effect on growth and nodulation in *Vigna unguiculata* L. The results clearly indicate that, Zn can be use as a micronutrient up to a trace level in inducing plant growth and nodulation. However, at higher levels of Zn may act as heavy metal and cause phyto-toxicity in plants.

Keywords: Legume; Micronutrient; Nodules; Toxic; ZnSO₄; Heavy metals

1. INTRODUCTION

Yard-long bean [*Vigna unguiculata ssp. sesquipedalis* (L.) Verdc], belonging to family *Fabaceae*, is a distinctive subspecies of cowpea [*Vigna unguiculata* (L.) Walp.][1]. The yard-long bean pods are high in protein (23–32% of seed weight), lysine, tryptophan and a significant number of critical vitamins and minerals including folic acid and vitamin B [2]. Fresh yard-long beans are very high in folates [2]. It contains mineral nutrients such as Fe, Mg, Mn, K, P, Na, Ca, Cu, Zn [3]. Yard-long bean, a true legume, enriches the soil by fixing atmospheric nitrogen in nodules on its roots [3]. With the help of nitrogen fixing bacteria, the plant makes its own food, maintain soil fertility, and reduce fertilizer dependence [3].

Zinc plays a crucial role as a micronutrient in biological nitrogen fixation, and it is likely essential for the synthesis of leghaemoglobin [4]. When legumes experience zinc deficiency, it can lead to a reduction in both the number and size of their root nodules [4]. Zinc is naturally present in all soils in typical background concentrations of 10–100 mg Zn kg⁻¹ [5]. The amount of zinc present in the soil depends on the parent materials of that soil. Sandy and highly leached acid soils generally have low plant available zinc. Soils originating from igneous rocks are higher in zinc [5]. Zinc is a fundamental element that acts as a catalytic cofactor for various enzymes, influencing the folding, structural stability, and functionality of many proteins [6]. Beyond its critical role in maintaining ribosomal integrity, zinc contributes to several other vital bio-physicochemical processes in plants as facilitating protein synthesis, regulating gene expression, participating in carbohydrate metabolism, and playing a role in the structure and function of biological membranes [6]. Noxious levels of Zn in soils can result in various alterations in plants. Therefore, the study was carried out to evaluate whether the Zinc applied as ZnSO₄·5H₂O to the soil cause any toxic effect on nodules that affect the plant growth, or it enhance the nodulation and growth of yard-long bean. Hence, this experiment was conducted to assess the impact of varying Zinc concentrations on the growth and nodulation of yard-long bean and to identify the specific concentration at which Zinc functions as an essential micronutrient, instead of exhibiting toxicity as a heavy metal.

2. MATERIAL AND METHODS

2.1 Experimental Site

The pot experiment was conducted in the Crop Farm, Faculty of Agriculture, Palachchola, Eastern University, Sri Lanka from August to November 2023. It is located in the latitude of 7° 43a N and the longitude of 81° 42a E which belongs to the Agro Ecological Zone of Low Country Dry Zone in Sri Lanka (DL2). The mean annual rainfall of this area ranges from 1400 mm to 1680 mm. The annual mean temperature varies from 30°C to 36°C and the average annual percentage of humidity is 76%. The major soil type of the experimental area is classified as Sandy Regosol.

2.2 Experimental Design

The experiment was arranged in a Complete Randomized Design (CRD) includes six treatments and four replicates. Twenty-four pots were maintained in this experiment under field condition.

Table 1: Description of Treatments

Treatments	Description
T1	Inorganic Fertilizer [Urea(35kg/ha), TSP(130kg/ha), MOP(35kg/ha)]
T2	50 mg ZnSO ₄ /kg soil + Inorganic Fertilizer
T3	100 mg ZnSO ₄ /kg soil + Inorganic Fertilizer
T4	150 mg ZnSO ₄ /kg soil + Inorganic Fertilizer
T5	200 mg ZnSO ₄ /kg soil + Inorganic Fertilizer
T6	250 mg ZnSO ₄ /kg soil + Inorganic Fertilizer

Different ZnSO₄ concentrations were used as treatments except treatment 1.

2.3 Agronomic Practices

The yard-long bean cultivar “Bushita- BS 01” variety was used for this study, and the seeds were collected from the Agriculture Faculty Crop Farm, Palachchola. Plastic pots which are with 22cm diameter and 17cm height were used for the experiment. Each pot was filled with air dried sandy regosol soil and according to the respective treatments, ZnSO₄ was added to the pots filled with soil. After treated with ZnSO₄, soil was pre-incubated for one week and soil in each pot was brought to 50% of water holding capacity. Seeds of Bushita BS- 01 variety were soaked for 24 hours and then, they were kept on a filter paper to drain water. After drain water, four seeds were planted in each 24 pots. Two days before planting urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP) were incorporated in to the potting mixture as basal fertilizer. Top dressing was done in one month after planting. Twice a day irrigation was done depend upon requirement of crop to maintain optimal moisture for growth. The increase of moisture content may cause collar rot. After two weeks of planting, two plants were removed by thinning out and keep two vigorous plants in each pot. At the time of active growth of seedlings, the seedlings were protected from different pests by applying relevant organic or synthetic pesticides.

2.4 Growth and Nodule Parameters

At the peak level of the nodulation, plants were uprooted to take the parameters related to the growth and the nodulation. The plants reach the peak level of the nodulation at the flowering stage at 6th week after planting. Plant height, chlorophyll content (SPAD-502plus, United States of America), Leaf area (cm²) (LI-3100C, United States of America), dry shoot biomass (g), dry root biomass (g), number of nodules, effective nodule percentage and soil microbial respiration were measured.

2.5 Analysis of Data

Data measured were statistically analyzed using ANOVA at 5% significant level based on Complete Randomized Design. Data evaluations were analyzed through Minitab 17 statistical software. Mean separation was done by *Tukey's Test*.

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm)

The plant height of *Vigna unguiculata* L. from 2 **Weeks After Planting** (WAP) up to 6 **Weeks After Planting** (WAP) were showed in the table 2. The results showed that changes in the different soil Zn concentrations were significantly ($p < 0.05$) affected the plant height. The plant height in the treatment 50 mg ZnSO₄/kg soil (T2) was more

pronounced than other treatments from 2 WAP to 6 WAP. Accordingly, the results at 6 WAP, the plant height on T2 was 29.33 cm while the least height recorded on T6 was 16 cm. The reason for this significant difference in growth rate between plants in T2 which was the lower Zn concentration and T6 which was the highest Zn concentration could be due to the action of Zn as a micronutrient in lower concentration treatments and it is extremely important in many ways for plant growth as well as productivity and also higher concentration of Zn was act as toxic for plants. This observation also supported by an investigation which presents that, Zinc is plant micronutrient which is involved in many physiological functions and its inadequate supply will reduce crop yields [7].

Table 2: Plant height (cm) measures in different concentrations of Zn at 2 WAP, 4 WAP & 6 WAP.

Treatment	2 WAP	4 WAP	6 WAP
Control	9.00 ± 0.57 ^{ab}	17.33 ± 2.33 ^a	26.33 ± 0.33 ^a
50 mg ZnSO ₄ /kg soil	10.33 ± 0.33 ^a	24.33 ± 1.20 ^a	29.33 ± 0.82 ^a
100 mg ZnSO ₄ /kg soil	9.33 ± 0.33 ^{ab}	17.00 ± 2.31 ^{ab}	27.00 ± 0.57 ^a
150 mg ZnSO ₄ /kg soil	9.33 ± 0.33 ^{ab}	16.67 ± 0.82 ^b	24.00 ± 0.57 ^{ab}
200 mg ZnSO ₄ /kg soil	8.00 ± 0.00 ^b	15.67 ± 1.33 ^b	22.67 ± 4.67 ^{ab}
250 mg ZnSO ₄ /kg soil	7.33 ± 0.82 ^b	14.33 ± 0.82 ^b	16.00 ± 2.08 ^b

Each value represents mean ± standard error of four replicates

3.2 Chlorophyll content of leaves

Chlorophyll content of *Vigna unguiculata* L. in 6WAP was showed in the table 3. The results showed that changes in the different soil Zn concentration was significantly ($p < 0.05$) affected the chlorophyll content of the leaves or SPAD value of leaves. The chlorophyll content in the treatments, without ZnSO₄ (T1), 50 mg ZnSO₄/kg soil (T2) and 100 mg ZnSO₄/kg soil (T3) were significantly different from the other treatments 150 mg ZnSO₄/kg soil (T4), 200 mg ZnSO₄/kg soil (T5) & 250 mg ZnSO₄/kg soil (T6). This is because, in the treatments T2 and T3, the Zn concentrations were act as a micro nutrient for the plant and the Zn concentrations in T4, T5 & T6 are at the toxic level to the plants. An experiment presents that, a comparable tendency in hydroponically grown beans and low levels of chlorophyll caused leaves with low zinc concentrations to appear bright green with high chlorophyll content [8]. An investigation shows that, zinc shortage interfered with the production of chlorophyll when low Zn concentration low chlorophyll content in plant leaves [9]. According to an experiment Zinc is responsible for the increased levels of chlorophyll since it is a structural and catalytic element of proteins and enzymes and a co-factor for the proper growth of pigment biosynthesis [10].

Table 3: Chlorophyll content measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	50.90 ± 0.603 ^a

50 mg ZnSO₄/kg soil	54.70 ± 4.37 ^a
100 mg ZnSO₄/kg soil	47.07 ± 1.73 ^a
150 mg ZnSO₄/kg soil	33.90 ± 0.78 ^b
200 mg ZnSO₄/kg soil	33.27 ± 2.71 ^b
250 mg ZnSO₄/kg soil	26.97 ± 2.32 ^b

Each value represents mean ± standard error of four replicates

3.3. Leaf Area (cm²)

Total leaf area of *Vigna unguiculata* L. at 6 WAP was significantly ($p < 0.05$) affected (Table 4). The highest number of total leaf area was recorded in the treatment 50 mg ZnSO₄/kg soil (T2) and it was significantly different from all the other treatments without ZnSO₄ (T1), 100 mg ZnSO₄/kg soil (T3), 150 mg ZnSO₄/kg soil (T4), 200 mg ZnSO₄/kg soil (T5) & 250 mg ZnSO₄/kg soil (T6). Accordingly, the results, at the micronutrient level of zinc plays an important role in photosynthesis as in T2 than in other treatments. The study of Photosynthesis and Leaf Area of *Brachiaria brizantha*, the decrease in Zn availability resulted in significant increase in leaf area. Zinc oxide increased leaf area and leaves play an important role in photosynthesis [11]. The results were shown that, the lowest leaf area was recorded in the T6 followed by T5, T4, T1, T3 & T2. No significant difference was recorded in T1 & T3 and also in T4 & T5. Zn is an important micronutrient however at the toxic level it reduces the photosynthesis and the plant growth. The study have shown that element toxicity caused a delay in plant maturity due to a delay in leaf appearance and an increase in the vegetative growth period [12].

Table 4: Total leaf area (cm²) measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	565.70 ± 17.90 ^b
50 mg ZnSO₄/kg soil	727.50 ± 22.80 ^a
100 mg ZnSO₄/kg soil	625.50 ± 11.40 ^b
150 mg ZnSO₄/kg soil	449.50 ± 13.70 ^c
200 mg ZnSO₄/kg soil	410.98 ± 5.66 ^c
250 mg ZnSO₄/kg soil	282.50 ± 15.10 ^d

Each value represents mean ± standard error of four replicates

3.4 Dry Shoot Biomass (g)

The different soil Zn concentration was significantly ($p < 0.05$) affected the dry shoot biomass of *Vigna unguiculata* L. at 6 WAP (Table 5). The results showed that the higher dry shoot weight was in 50 mg ZnSO₄/kg soil (T2) however, there was no statistically significant difference with the treatments without ZnSO₄(T1), 100 mg ZnSO₄/kg soil (T3)

and 150 mg ZnSO₄/kg soil (T4). Applications of inorganic N (NPK) greatly enhanced the biomass of plants' shoots in both early growth stages and mature plants in faba beans [13]. The results revealed by the current study showed that, T3, T4, T5 & T6 were not statistically significant and they showed statistically significant different with T2 and lower dry shoot biomass than T2. This was due to the effect of Zn as micronutrient and toxic levels. Accordingly Zn as a micronutrient T2 shows the highest dry shoot biomass. An investigation presents, lower concentrations of zinc promote plant growth, while excessive concentrations can lead to toxicity symptoms [14].

Table 5: Dry shoot biomass (g) measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	3.877 ± 0.416 ^{ab}
50 mg ZnSO ₄ /kg soil	4.567 ± 0.869 ^a
100 mg ZnSO ₄ /kg soil	3.107 ± 0.491 ^{ab}
150 mg ZnSO ₄ /kg soil	2.050 ± 0.885 ^{ab}
200 mg ZnSO ₄ /kg soil	1.423 ± 0.429 ^b
250 mg ZnSO ₄ /kg soil	1.060 ± 0.503 ^b

Each value represents mean ± standard error of four replicates

3.5 Dry Root Biomass (g)

Dry root biomass of *Vigna unguiculata* L. at 6 WAP was showed in table 6. The results showed that changes in the different soil Zn concentration was significantly ($p < 0.05$) affected the dry root biomass. The results indicated by the study showed, the treatment 50 mg ZnSO₄/kg soil (T2) has the higher dry root weight and it did not show a statistically significant difference with the treatments without ZnSO₄ (T1) & 100 mg ZnSO₄/kg soil (T3) treatments. The Zinc increases the dry weight up to a certain level and concentration beyond 100 ppm causes significant decrease in dry weight of root due to the Zn toxicity [15]. Furthermore, the results were showed that T1, T3 and the treatments 150 mg ZnSO₄/kg soil (T4), 200 mg ZnSO₄/kg soil (T5), 250 mg ZnSO₄/kg soil (T6) were not statistically significant. The amount of Zn in the roots of the control bean plants reached 143 mg/kg. This value is in within the range of typical concentrations of Zn in the plants, as the concentration of Zn increased to 200 µM [16].

Table 6: Dry root biomass (g) measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	0.857 ± 0.139 ^{ab}
50 mg ZnSO ₄ /kg soil	1.547 ± 0.229 ^a
100 mg ZnSO ₄ /kg soil	0.973 ± 0.134 ^{ab}

150 mg ZnSO₄/kg soil	0.587 ± 0.139 ^b
200 mg ZnSO₄/kg soil	0.477 ± 0.221 ^b
250 mg ZnSO₄/kg soil	0.290 ± 0.078 ^b

Each value represents mean ± standard error of four replicates

3.6 Number of Nodules

Number of nodules in *Vigna unguiculata* L. at 6 WAP was significantly ($p < 0.05$) affected the different concentrations of Zn (Table 7). The results were indicated that, the maximum number of nodules was in the treatment 50 mg ZnSO₄/kg soil (T2), however, T2 was not statistically significant with the treatment 100 mg ZnSO₄/kg soil (T3). This may due to Zn as a micronutrient it increases the nutrient availability of the plants. The number of nodules formed on the roots of beans for zinc treatments were higher and has positive significant effect by increasing the number of nodules [17]. Further results, treatment without ZnSO₄ (T1), 100 mg ZnSO₄/kg soil (T3), 150 mg ZnSO₄/kg soil (T4) & 200 mg ZnSO₄/kg soil (T5) did not show a statistically significant different among each other on number of nodules. The minimum number nodules were recorded in the treatment 250 mg ZnSO₄/kg soil (T6). The mean values were decreasing as T1, T3, T4, T5 & T6 respectively. The concentration of Zn increased, and the number of nodules per plant decreased dramatically. Similar results showed in an experiment which, heavy metals did not significantly affect the number of nodules at low concentrations; however, once the concentration reached >0.5 mmol kg⁻¹ soil, nodulation considerably decreased at high concentrations. The availability of heavy metals to impede biological and biochemical processes involved in root growth, development, and nodule formation is most likely the cause of this [18].

Table 7: Number of nodules measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	25.67 ± 1.33 ^b
50 mg ZnSO₄/kg soil	36.33 ± 3.71 ^a
100 mg ZnSO₄/kg soil	28.33 ± 0.33 ^{ab}
150 mg ZnSO₄/kg soil	22.67 ± 1.20 ^b
200 mg ZnSO₄/kg soil	19.67 ± 0.33 ^b
250 mg ZnSO₄/kg soil	6.33 ± 3.33 ^c

Each value represents mean ± standard error of four replicates

3.7 Effective Nodule Percentage (%)

Effective nodule percentage in *Vigna unguiculata* L. at 6 WAP was showed in table 8. The results showed that changes in the different soil Zn concentrations were significantly ($p < 0.05$) affected the effective nodule percentage. The results revealed that, the treatment 50 mg ZnSO₄/kg soil (T2) showed the higher effective nodule percentage (63.33%) when compare to the other treatments. However, T2 was not statistically

significant with the treatment 100 mg ZnSO₄/kg soil (T3). This was due to that, at the micronutrient level of Zn it increases the effectiveness of the nodules. This is in consequence with an investigation which presents, sufficient quantities of zinc can encourage nodule development and expansion, resulting in a more successful symbiosis due to zinc is necessary for the action of enzyme involved in nitrogen fixation, which converts atmospheric nitrogen into ammonia [19].

Accordingly, the results revealed that, the 250 mg ZnSO₄/kg soil (T6) showed the minimum effective nodule percentage (20%) than the other treatments. T6 was no statistically significant difference with the treatment 200 mg ZnSO₄/kg soil (T5). This was due to the toxicity of the Zn as a heavy metal. This was in consistent with the findings that, effectiveness of the nodules was decreases at the toxic level of heavy metals [20].

Table 8: Effective nodule percentage (%) measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	40.00 ± 5.77 ^{bc}
50 mg ZnSO ₄ /kg soil	63.33 ± 3.33 ^a
100 mg ZnSO ₄ /kg soil	53.33 ± 3.33 ^{ab}
150 mg ZnSO ₄ /kg soil	43.33 ± 3.33 ^{bc}
200 mg ZnSO ₄ /kg soil	26.67 ± 3.33 ^{cd}
250 mg ZnSO ₄ /kg soil	20.00 ± 0.00 ^d

Each value represents mean ± standard error of four replicates

3.8 Soil Microbial Respiration

Soil respiration of *Vigna unguiculata* L. at 6 WAP was shown in the table 9. The results showed that changes in the different soil Zn concentration was significantly ($p < 0.05$) affected the soil respiration. The results obtained from the study showed, there was a higher production of CO₂ in the treatment 50 mg ZnSO₄/kg soil (T2) when compare to the other treatments. However, T2 was not statistically significant with the treatments without ZnSO₄ (T1), 100 mg ZnSO₄/kg soil (T3), 150 mg ZnSO₄/kg soil (T4) & 200 mg ZnSO₄/kg soil (T5). Therefore, the microbial percentage in the T2 was higher when compare to other treatments. This was due to the nutrient availability (N, P, K, Ca, Mg & micronutrients) and it is a function of nutrient chemical properties, soil and climate properties, and plant species. This was in consistent with that, the soil amended with metal had a higher cumulative respiration than soils receiving no metals. Significant increases in soil microbial biomass after incorporation of Zn have been reported at soil concentrations [21]. Chemical fertilization can also influence the enzyme activities of soil microbes, soil pH, and soil structure. It is therefore pertinent to apply the optimum amounts of fertilization in the field [22].

The results showed that, the lesser CO₂ production was in 250 mg ZnSO₄/kg soil (T6) however, it was not statistically significant with T1, T3, T4 & T5. Soil microbiological equilibrium can be disrupted by cadmium, copper, and zinc. Different groups of microorganisms are affected differently by these heavy metals due to unique physiological, morphological, and genetic traits of the former [23,24]. Exceeding levels of cadmium, copper, and zinc in the soil can disturb the biological balance by disrupting physiological functions, denaturing proteins, and destroying the cellular membranes of soil microorganisms [25].

Table 9: Soil microbial respiration measures in different concentrations of Zn at 6 WAP.

Treatment	6WAP
Control	0.000374 ± 1.25 ^{ab}
50 mg ZnSO ₄ /kg soil	0.000613 ± 0.08 ^a
100 mg ZnSO ₄ /kg soil	0.000432 ± 1.24 ^{ab}
150 mg ZnSO ₄ /kg soil	0.000228 ± 1.95 ^{ab}
200 mg ZnSO ₄ /kg soil	0.000148 ± 0.262 ^{ab}
250 mg ZnSO ₄ /kg soil	0.000046 ± 0.109 ^b

Each value represents mean ± standard error of four replicates

4. CONCLUSION

The experiment concluded that, at 50 mg ZnSO₄/kg soil showed the higher performance in growth and nodulation in *Vigna unguiculata* L. than the other concentrations. As Zn a micronutrient, it increases the nutrient availability of the plants which result in increasing the growth and nodulation. As Zn increases the plant growth up to a certain level however at the toxic level it reduces the photosynthesis and the plant growth. It affects the plant growth and nodulation at the toxic level. The experiment shows that, the treatment T5 starts to reduce the plant growth and nodulation. According to the experiment, beyond the level of 200 mg ZnSO₄/kg soil has a deleterious effect on overall growth performance including the nodule formation in yard long bean. Consequently, as Zn enhances the growth and nodulation of *Vigna unguiculata* L., in future it can be modified the fertilizer recommendation up to the trace level of Zn.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that no AI have been used during writing the manuscript.

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ABBREVIATIONS

WAP – Weeks After Planting

mg ZnSO₄/kg soil – milligram Zinc Sulphate per kilo gram soil

UNDER PEER REVIEW