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

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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, Palachcholai, 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 $ZnSO_4$/kg soil), T3 (100 mg $ZnSO_4$/kg soil), T4 (150 mg $ZnSO_4$/kg soil), T5 (200 mg $ZnSO_4$/kg soil) & T6 (250 mg $ZnSO_4$/kg soil). The experiment used source of Zinc in the form of Zinc Sulphate ($ZnSO_4$,$5H_2O$) which yard-long bean plants can grow. The experiment results showed that, T2 treatment, the soil treated with 50 mg $ZnSO_4$/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 $ZnSO_4$/kg soil, reduces the growth & nodulation of *Vigna unguiculata* L. and this shows that the level 200 mg $ZnSO_4$/kg soil and beyond that level were the toxic level to the *Vigna unguiculata* L. Accordingly, the treatment T2 (50 mg $ZnSO_4$/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.

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Keywords: Legume; Micronutrient; Nodules; Toxic; $ZnSO_4$; 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. Fresh yard-long beans are very high in folates [2]. It contains mineral nutrients such as Fe, Mg, Mn, K, P, Na, Ca, etc. Yard-long bean, a true legume, enriches the soil by fixing atmospheric nitrogen in nodules on its roots. With the help of nitrogen fixing bacteria, the plant makes its own food, maintain soil fertility, and reduce fertilizer dependence.

Zinc plays a crucial role as a micronutrient in biological nitrogen fixation, and it is likely essential for the synthesis of leghaemoglobin. When legumes experience zinc deficiency, it can lead to a reduction in both the number and size of their root nodules [3]. Zinc is naturally present in all soils in typical background concentrations 10–100 mg Zn kg–1. 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. Zinc is a fundamental element that acts as a catalytic cofactor for various enzymes, influencing the folding, structural stability, and functionality of many proteins. 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 [4].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 $ZnSO_4,5H_2O$ 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, ~~as opposed to~~ instead of exhibiting toxicity as a heavy metal.

2. MATERIAL AND METHODS

The pot experiment was conducted in the Crop Farm, Faculty of Agriculture, Palachcholai, 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. 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.

List 1: The treatments are as follows:

Treatments	Description
T1	Inorganic Fertilizer (Urea, TSP, MOP)
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 $ZnSO_4$concentrations were used as treatments except treatment 1. The yard-long bean cultivar “Bushita- BS 01” variety was used for this study, and the seeds were

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Commented [U14]: Always precise the quantity collected from the Agriculture Faculty Crop Farm, Palachcholai. 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, $ZnSO_4$was added to the pots filled with soil. After treated with $ZnSO_4$, 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. 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. 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.

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Field description and plant material
Experimental design
Plant growth measurement
Root nodules determination
Data analysis

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm)

The plant height of *Vigna unguiculata* L. from 2 WAP up to 6 WAP were showed in the table 1. 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 $ZnSO_4$/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. Zinc is plant micronutrient which is involved in many physiological functions and its inadequate supply will reduce crop yields [5].

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Table 1: Effect of different concentrations of Zn on plant height (cm) at 2 WAP, 4 WAP & 6 WAP.

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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

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3.2 Chlorophyll content of leaves

Chlorophyll content of *Vigna unguiculata* L. in 6WAP was showed in the table 2. 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 SPAD values in the treatments, without $ZnSO_4$ (T1), 50 mg $ZnSO_4$/kg soil (T2) and 100 mg $ZnSO_4$/kg soil (T3) were significantly different from the other treatments 150 mg $ZnSO_4$/kg soil (T4), 200 mg $ZnSO_4$/kg soil (T5) & 250 mg $ZnSO_4$/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. T1 is the NPK treatment, so it also able to produce the nutrients that useful in plant uptake. Inorganic fertilizer is a mixture of nutrients that provided early nutrient to the growing crops during the early vegetative growth stage [6]. A comparable tendency in hydroponically grown beans and low levels of chlorophyll caused leaves with low zinc concentrations to appear bright green [7]. A zinc shortage interfered with the production of chlorophyll [8]. 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 [9].

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Table 2: Effect of different concentrations of Zn on chlorophyll content at 6 WAP.

Treatment	6WAP
Control	50.90 ± 0.603[a]
50 mg $ZnSO_4$/kg soil	54.70 ± 4.37[a]
100 mg $ZnSO_4$/kg soil	47.07 ± 1.73[a]
150 mg $ZnSO_4$/kg soil	33.90 ± 0.78[b]
200 mg $ZnSO_4$/kg soil	33.27 ± 2.71[b]
250 mg $ZnSO_4$/kg soil	26.97 ± 2.32[b]

Each value represents mean ± standard error of four replicates

3.3. Leaf Area (cm^2)

Total leaf area of *Vigna unguiculata* L. at 6 WAP was shown in table 3. The results showed that changes in the different soil Zn concentration was significantly ($p<0.05$) affected the total leaf area. The highest number of total leaf area was recorded in the treatment 50 mg $ZnSO_4$/kg soil (T2) and it was significantly different from all the other treatments without $ZnSO_4$ (T1), 100 mg $ZnSO_4$/kg soil (T3), 150 mg $ZnSO_4$/kg soil (T4), 200 mg $ZnSO_4$/kg soil (T5) & 250 mg $ZnSO_4$/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 *Brachiariabrizantha*, the increase in Zn availability resulted in significant increase in leaf area. Zinc oxide increased leaf area and leaves play an important role in photosynthesis [10]. The results

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Table 3: Effect of different concentrations of Zn on total leaf area (cm^2) at 6 WAP.

Treatment	6WAP
Control	565.70 ± 17.90[b]
50 mg $ZnSO_4$/kg soil	727.50 ± 22.80[a]
100 mg $ZnSO_4$/kg soil	625.50 ± 11.40[b]
150 mg $ZnSO_4$/kg soil	449.50 ± 13.70[c]
200 mg $ZnSO_4$/kg soil	410.98 ± 5.66[c]
250 mg $ZnSO_4$/kg soil	282.50 ± 15.10[d]

Each value represents mean ± standard error of four replicates

3.4 Dry Shoot Biomass (g)

Dry shoot biomass of *Vigna unguiculata* L. at 6 WAP was showed in table 4. The results showed that changes in the different soil Zn concentration was significantly ($p<0.05$) affected the dry shoot biomass. The results showed that the higher dry shoot weight was in 50 mg $ZnSO_4$/kg soil (T2) however, there was no statistically significant difference with the treatments without $ZnSO_4$(T1), 100 mg $ZnSO_4$/kg soil (T3) and 150 mg $ZnSO_4$/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 [12]. 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. Optimal concentrations of zinc promote plant growth, while excessive concentrations can lead to toxicity symptoms [13].

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Table 4: Effect of different concentrations of Zn on dry shoot biomass (g) 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 5. 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 $ZnSO_4$/kg soil (T2) has the higher dry root weight and it did not show a statistically significant difference with the treatments without $ZnSO_4$ (T1) & 100 mg $ZnSO_4$/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 [14]. Furthermore, the results were showed that T1, T3 and the treatments 150 mg $ZnSO_4$/kg soil (T4), 200 mg $ZnSO_4$/kg soil (T5), 250 mg $ZnSO_4$/kg soil (T6) were not statistically significant. The amount of Zn in the leaves and roots of the control bean plants reached 138 mg Zn/kg and 143 mg/kg, respectively. These values are within the range of typical concentrations of Zn in the plants, as the concentration of Zn increased to 200 μM [15].

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Table 5: Effect of different concentrations of Zn on dry root biomass (g) 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 $ZnSO_4$/kg soil	0.587 ± 0.139[b]
200 mg $ZnSO_4$/kg soil	0.477 ± 0.221[b]
250 mg $ZnSO_4$/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 showed in table 6. The results showed that changes in the different soil Zn concentrations were significantly ($p<0.05$) affected the number of nodules. The results were indicated that, the maximum number of nodules was in the treatment 50 mg $ZnSO_4$/kg soil (T2), however, T2 was not statistically significant with the treatment 100 mg $ZnSO_4$/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 [16]. Further results, treatments without $ZnSO_4$ (T1), 100 mg $ZnSO_4$/kg soil (T3), 150 mg $ZnSO_4$/kg soil (T4) & 200 mg $ZnSO_4$/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 $ZnSO_4$/kg soil (T6). The mean values were decreasing as T1, T3, T4, T5 & T6 respectively. This showed the similar effect recorded by NPK as in Zn treatment on number of nodules. The concentration of Zn increased, and the number of nodules per plant decreased dramatically. 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 [17].

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Table 6: Effect of different concentrations of Zn on Number of nodules at 6 WAP.

Treatment	6WAP
Control	25.67 ± 1.33[b]
50 mg $ZnSO_4$/kg soil	36.33 ± 3.71[a]
100 mg $ZnSO_4$/kg soil	28.33 ± 0.33[ab]
150 mg $ZnSO_4$/kg soil	22.67 ± 1.20[b]
200 mg $ZnSO_4$/kg soil	19.67 ± 0.33[b]
250 mg $ZnSO_4$/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 7. 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 $ZnSO_4$/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 $ZnSO_4$/kg soil (T3). This was due to that, at the micronutrient level of Zn it increases the effectiveness of the nodules than at higher concentrations. 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 [16].

Accordingly, the results revealed that, the 250 mg $ZnSO_4$/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 $ZnSO_4$/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 [18].

Table 7: Effect of different concentrations of Zn on Effective nodule percentage (%) 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

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3.8 Soil Microbial Respiration

Soil respiration of *Vigna unguiculata* L. at 6 WAP was shown in the table 8. 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 CO_2 in the treatment 50 mg $ZnSO_4$/kg soil (T2) when compare to the other treatments. However, T2 was not statistically significant with the treatments without $ZnSO_4$ (T1), 100 mg $ZnSO_4$/kg soil (T3), 150 mg $ZnSO_4$/kg soil (T4) & 200 mg $ZnSO_4$/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 [19]. 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 [20].
The results showed that, the lesser CO2 production was in 250 mg $ZnSO_4$/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 [21,22]. 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 [23].

Table 8: Effect of different concentrations of Zn on Soil microbial respiration 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

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4. CONCLUSION

The experiment concluded that, T2 which used 50 mg $ZnSO_4$/kg soil showed the higher performance in growth and nodulation in *Vigna unguiculata* L. than the other treatments. As Zn a micronutrient, it increases the nutrient availability of the plants which result in increasing the growth and nodulation. Accordingly, the experiment results revealed that, the micronutrient level of Zn was the 50 mg $ZnSO_4$/kg soil. 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 $ZnSO_4$/kg soil was toxic to the plants. 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.

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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 $ZnSO_4$/kg soil – milligram Zinc Sulphate per kilo gram soil