RESEARCH ARTICLE
Agricultural Science Digest, Volume Issue : ()

Effectiveness of Foliar and Soil Application of Zinc Fertilizer on Growth Characteristics of *Vigna unguiculata*

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10.18805/ag.D-5500

ABSTRACT

Background: The Zinc deficiency is a worldwide nutritional constraint for crop production in many types of soil. Appropriate application methods of Zn fertilizer are still unclear. The main objective of this study was to investigate the effects of Zn fertilizer and application methods on the growth characteristics of *Vigna unguiculata*.

Methods: The present study was conducted to evaluate the effects of Zinc fertilizer and application method on photosynthetic characteristics and yield of *Vigna unguiculata* from June to September of 2019, in Jaipur, Rajasthan, India. A pot experiment was conducted as a factorial design with 3 replications.

Result: The application of 15 kg $ZnSO_4.7H_2O$/ha increased chlorophyll content in leaves, improved photosynthesis and increased protein content of *Vigna unguiculata,* when applied as foliar application as opposed to being applied in basal application. These results have important implications for guiding the rational application of Zinc fertilizer and improving the yield of *Vigna unguiculata*.

Key words: Chlorophyll, Foliar, Protein, *Vigna unguiculata*, Zinc.

INTRODUCTION

Zinc (Zn) is an essential element for higher plants and its significance in agriculture is critical (Genc *et al.* 2006). Zinc deficiency is the fifth major factor affecting human health in developing countries and is one of the most prevalent disorders among various crops (Naik and Das, 2007). Zn deficiency results in decline of associated photosynthetic parameters *e.g.* rate of photosynthesis, chlorophyll content, carbonic anhydrase activity (Fu *et al.* 2016). Tryptophan, a precursor of the auxin indole-3-acetic acid-is also synthesized from Zinc (Oosterhuis *et al.* 1991). Zinc deficiency symptoms are small leaves, shortened internodes and stunted appearance. These all leads to poor fodder qualities. Effective methods of application of fertilizer are still not clear. Basal application of Zn fertilizer leads to residual effect (Liu *et al.* 2004), but in some crop plants, Zn is not assimilated as it is fixed by soil (Rengel, 2015). Availability of Zn in soils and its absorption and translocation in plants is influenced by all other plant nutrients. The cowpea (*Vigna unguiculata*) is an annual herbaceous legume which occurs in tropical and subtropical regions. *Vigna unguiculata* is a source of protein, dietary fiber, carbohydrates, vitamins, essential nutrients, phytochemicals (Lim, 2012); Awika *et al.* 2017) and animal protein. The main objective of this study was to investigate the effects of Zn fertilizer and application methods on the growth characteristics of *Vigna unguiculata*.

MATERIALS AND METHODS

Experiment location and description of materials

The present study was conducted to evaluate the effects of Zinc fertilizer and application methods on growth characteristics of *Vigna unguiculata* from June to September of 2019, in Jaipur, Rajasthan, India (26° 55' 19.4520'' N and 75° 46' 43.9860'' E) at Department of Biotechnology, IIS (Deemed to be) University. A pot experiment was conducted as a factorial design in completely randomized design with 3 replications. Soil pH (soil:water = 1:1) was estimated by a pH meter and soil organic carbon was analyzed by dichromate oxidation (Walkley and Black, 1934). EC was masured by by EC probe. Total N was estimated by semi-micro-Kjeldahl method (Bremmer and Mulvaney, 1982). Total K was analysed by the photometry method (Bao, 2002). The ammonium acetate extraction-flame photometry method was applied to detect the available K in the soil (Bao, 2002). Soil Zn concentration was determined by DTPA soil test (Lindsay and Norwell, 1978) (Table 1). Certified seeds of *Vigna unguiculata* var. Minaxi Plus were procured from Durgapura Agricultural Research station, Jaipur, Rajasthan, India. The fertilizer used in the experiment was $ZnSO_4.7H_2O$.

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How to cite this article: Batra, N. and Sharma, A. (2022). Effectiveness of Foliar and Soil Application of Zinc Fertilizer on Growth Characteristics of *Vigna unguiculata.* Agricultural Science Digest. DOI: 10.18805/ag.D-5500.

Submitted: 27-09-2021 **Accepted:** 17-01-2022 **Online:** 09-03-2022

Experimental design

The seeds were washed in distilled water, surface-sterilized with 0.1% $HgCl_2$ for 1 min, followed by repeated washing

Table 1: Physical and chemical properties of tested soils.

Location	Soil type	Organic matter %	Available N kg/ha	Available K kg/ha	Available Zn mg kg^{-1}	pH	ECms/cm
Mansarovar, Jaipur, Rajasthan, India	Red Sandy Loam	0.36	160	119	17.31	7.95	0.195

with distilled water and then germinated on moist filter paper in Petri dishes at 28°C in the dark for 3 days. After the germination morphologically similar seedlings having well-developed roots were selected and dibbed in pots as soil culture in a plant growth chamber at day/night temperature 25 ± 2°C /17 ± 2°C, a photosynthetic photon flux density (PPFD) of 250 to 300 µmol m^{-2} s^{-1}, day/night photoperiod of 14/10 h and relative air humidity of 65-70%. There were three treatments in experiment, including ZnO, Zn fertilizer applied to soil (15 kg $ZnSO_4.7H_2O$ ha^{-1}, respectively) as well as two foliar applications each of 7.5 kg $ZnSO_4.7H_2O$ ha^{-1}). The treatments were replicated thrice in a randomized block design (factorial). Leaf sample was taken for analysis on at 45 days after sowing (DAS).

Measurement of plant height

Plant height of seedlings at 45 days after sowing (DAS) (flowering stage) was measured manually. Furthermore, three plant per tray were randomly collected at each pot.

Shoot length and root length

Six plants were randomly selected for recording the shoot length and root length from eachpot at on 45 days after sowing (DAS). They were measured by using centimetre scale.

Relative water content

RWC express the WC (%) by ascertaining fresh weight, dry weight and turgor weight analysis (Castillo (1996) and calculated in the leaves. Samples (0.5 g) were saturated in 100 ml distilled water fo 48 h at 4°C in the dark and their turgid weights were recorded. Leaves were oven-dried at 65°C for 48 h and their dry weights were recorded. RWC was calculated as follows:

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Where,

FW, DW and TW are fresh weight, dry weight and turgid weight, respectively.

Determination of photosynthetic pigments.

Photosynthetic pigments such as Chl *a*, Chl *b* and total Chl were extracted and estimated by spectrophotometric analysis with 80% Acetone as extraction method (Arnon (1949). The optical density of the extracted Chl was measured at 645, 663 and 740 nm, using a double beam UV-VIS spectrophotometer (UV5704SS).

Estimation of lipid peroxidation

Lipid peroxidation was estimated by measuring the formation of malondialdehyde (MDA) with TBA (2-thiobarbituric acid) by spectrophotometric analysis (de Vos *et al.* 1989).

Estimation of protein

Total soluble protein content was determined by Lowry Assay: Protein by Folin Reaction (Lowry *et al.* 1951).

Proline content

Free proline content was estimated using the acid ninhydrin method (Bates *et al.* 1973).

Statistical analysis

All data were statistically analyzed with a two-way ANOVA using the SPSS 19.0 software (Chicago, USA). All the analyses were performed in triplicate and the results are expressed as mean (n=3) ± standard deviation (SD).

RESULTS AND DISCUSSION

Effect of Zinc fertilization on the plant growth and development

Plant height is reliable index of growth of the plant, which represents the infrastructure build-up over a period of time. The scrutiny of the data presented in Table 2, indicated that plant height of the cowpea increased significantly with foliar application of $ZnSO_4.7H_2O$ over control and basal application. The increase in plant height may be due to effect of Zinc fertilizer on cell division and enlargement, which is ultimately reflected in terms of increased plant height. Teixeira *et al.* 2004 reported that in foliar application plants can have higher nutrient uptake and efficiency than soil application which in turn is reflected in plant height. Similar results were also reported by (Meena *et al.* 2014). Leaf surface characteristics influence in retention of nutrient ions. Surface area of leaf increased 8.3 per cent as compared to control in basal application and in foliar treatment it increased 30 per cent as compared to control (Table 2). Many studies have showed that a high density of stomata significantly increase the rate of foliar uptake mainly under the condition of stomata opening during the light phase. These results were similar to previous research (Ojeda-Barrios *et al.* 2014), who observed that applications of foliar Zn fertiliser led to increases in leaf surface area. There was a significant difference in shoot length (Table 2) under foliar application treatment (1.44 times) as compared to basal treatment. RWC increased in basal and foliar treatments as compared to control (Table 2). The RWC is an appropriate indicator of turgidity in plants and Zn may play an important role in this regard because it affects IAA synthesis through tryptophan, consequently increasing the root system that is more effective in water absorption from soil. Moreover, increasing RWC due to Zn-fertilizer could be ascribed to its role in enhancing stomatal conductance that reflected in maintaining RWC (Hejaji *et al.* 2011; Alamer *et al.* 2020).

Effects of Zn fertilizer and application methods on chlorophyll concentrations

As compared to control Zn application (basal or foliar) resulted in (70.89 and 69.15 per cent) a significant increase ($p \leq 0.05$) concentrations of Chlorophyll a (Table 3) respectively. In the present research $ZnSO_4.7H_2O$ application lead to an increase in the rate of photosynthesis pigments. Zinc fertilizer enhances chlorophyll content, net photosynthetic rat of higher plants and results in increases of leaf photoassimilates as well as grain yield of maize (Du and Zhang, 2008). The effectiveness of Zn fertilizer for crops growth and yield depends on crop species and application methods (Mao *et al.* 2014). Zarrouk *et al.* (2005) reported a positive correlation of Zn concentrations with leaf chlorophyll content in plants. In general, Zn applications increased the carotenoid content of *Vigna unguiculata*. either as soil (83.5%) and or foliar (90.84%) over control. The observed improvement in vegetative growth and the parameters (root length, carotenoids and crude protein) as affected by Zinc nutrition can be explained on the basis of that Zinc promotes growth hormone biosynthesis, the formation of starch and maturation (Van der Meer, 2000).

Effect of Zinc fertilizer on proline content

The result revealed a significant effect of $ZnSO_4.7H_2O$ on free proline content (Table 4).In foliar treatment, proline decreased by 50 per cent as compared to basal treatment (41%) The major reason for decrease in the proline concentration by application of Zn may be due to lesser incorporation of continuously synthesised proline amino acid during synthesis of proline. It is also known to act as an osmo regulator under stress conditions (Ashraf and Foolad, 2007). Similar results were obtained by Shahriaripour *et al.* (2010).

Effect of Zinc fertilizer on protein content

Protein content increased more in foliar treatment as compared to basal treatment. Protein content in ($ZnSO_4.7H_2O$) foliar treatment showed a significant increase of 1.53 times and in soil treatment increased about 1.36 times (Table 4). inc is a structural component of the ribosomes and plays a pivotal role in synthesis of proteins, RNA and DNA (Kobraee *et al.* 2011). Zinc-deficient plants have low protein contents and high amino acid contents,

Table 2: Effect of $ZnSO_4.7H_2O$ (Soil and foliar application) fertilizer on growth parameters of 45days old *Vigna unguiculata*.

Treatments	Height (cm)	S.A leaf (cm^2)	Length (cm) Root	Length (cm) Shoot	RWC (%)
Control	28.00 ± 1.00	6.00 ± 0.87	6.50 ± 2.78	16.67 ± 1.52	96.213 ± 1.454
Soil-$ZnSO_4.7H_2O$	31.33 ± 2.52	6.50 ± 1.50	6.67 ± 0.57	16.67 ± 1.15	96.790 ± 0.518
Control	27.00 ± 1.00	5.50 ± 0.87	4.67 ± 1.15	13.17 ± 0.76	84.340 ± 2.327
Foliar-$ZnSO_4.7H_2O$	32.67 ± 1.53*	7.17 ± 1.04	6.33 ± 0.57	19.00 ± 3.00*	84.820 ± 5.544

*significant at $p<0.05$.

Values are means of three replicates ± standard deviation.

Table 3: Effect of $ZnSO_4.7H_2O$ (Soil and foliar) fertilizer application on chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll a + b content (Chl a + b), carotenoid (Car), carotenoid: total chlorophyll (Car: Chl) of 45 days *Vigna unguiculata* seedlings.

Treatment	Chl a (mg/gFW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Chla/ Chl b	Car (mg/g FW)	Car : Chl
Control	1.03 ± 0.02	0.80 ± 0.01	1.83 ± 0.03	1.30 ± 0.02	1.40 ± 0.90	0.77 ± 0.51
Soil- $ZnSO_4.7H_2O$	1.76 ±0.41*	0.90 ± 0.20	2.66 ± 0.59	1.95 ± 0.18*	2.17 ± 0.38	0.87 ± 0.35
Control	1.07 ± 0.15	0.71 ± 0.15	1.78 ± 0.12	1.59 ± 0.55	1.42 ± 0.94	0.80 ± 0.52
Foliar- $ZnSO_4.7H_2O$	1.81 ± 0.07*	0.96 ± 0.29	2.77 ± 0.30	2.00 ± 0.57	2.71 ± 0.20*	0.97 ± 0.01

*significant at $p<0.05$.

Values are means of three replicates ± standard deviation.

Table 4: Change in proline, protein and lipid peroxidation content in *Vigna unguiculata* when treated with soil and foliar application of fertilizer ($ZnSO_4.7H_2O$) at 45days of treatment.

Treatment	Proline (µg g^{-1} F.W.)	Protein (mg g^{-1} F.W.)	MDA (nMg^{-1} F.W.)
Control	8.68 ± 0.58	0.745 ± 0.042	393.33 ± 12.909
Soil-$ZnSO_4.7H_2O$	5.12 ± 0.07*	1.018 ± 0.055	322.58 ± 6.45*
Control	7.56 ± 0.44	0.760 ± 0.069	370.32 ± 19.86
Foliar-$ZnSO_4.7H_2O$	3.77 ± 0.05*	1.165 ± 0.016*	284.08 ± 5.41*

*significant at $p<0.05$.

Values are mean of three replicates ± standard deviation.

showed reduced transcription and translation and increased RNA degradation (Alloway, 2004).

Effect of Zinc fertilizer on on lipid peroxidation content

The plant showed a significant decrease in MDA (Malondialdehyde) content (a measure of lipid peroxidation) (Table 4). The foliar application (284.08 nMg^{-1} F.W.) and soil treatment (322.58 nMg^{-1} F.W) recorded significantly lower values as compared to both the controls (370.32 nMg^{-1} F.W) and (393.33 nMg^{-1} F.W), respectively. Rapid and non-specific reaction of ROS result in several cell biochemical changes during oxidative stress including lipid peroxidation and damage to protein and DNA, which may lead to cell death. Zinc acts as an integral structural component for the normal functioning of Cu Zn SOD Zinc deficiency leads to decrease in SOD activity and increase in $O_2^{\cdot -}$ production and higher contents of MDA in leaves. MDA is regarded as a marker for a evaluation of lipid peroxidation or damage to plasma lemma and organelle membrane that decrease with Zinc nutrition (Das and Mukherjee, 2011).

CONCLUSION

Zn nutrient have an effect on metabolism and biological activity and growth parameters. Zinc treated plants showed an increase in photosynthetic pigments due to higher photo harvesting pigment in leaf resulting in taller, greater number and weight of leaves, enzyme activity which encourages vegetative branches, pods /quality of plant and regulation of enzymatic process. Zn applications, either as soil or foliar application increased germination percentage, root length, shoot length, fresh weight, dry weight and relative water content. Similarly the biochemicals such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, protein content also increased with a decreased proline and lipid peroxidation of cowpea plant. These agronomic side effects of Zinc fertilization may result in better bioavailability of Zinc in the human digestive system. Soil and/or foliar applications of Zinc also bring several agronomic benefits for crop production. In addition, seedlings from seeds containing high Zinc have better ability to withstand adverse environmental conditions.

AKNOWLEDGEMENT

This study was supported by IIS (Deemed to be) University. The authors extend their sincere appreciation to IIS (Deemed to be) University for facilitating this research study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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