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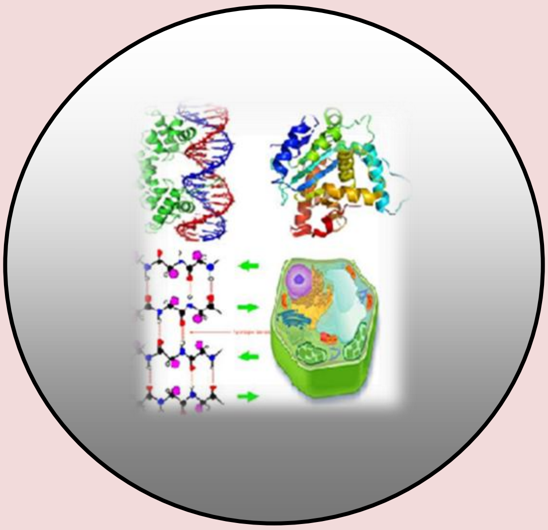

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RESEARCH PAPER

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Effect of Zinc Fertilization and Chelate (EDTA and Citric Acid) Application in Soil on Growth and Some Biochemical Constituents of *Phaseolus vulgaris* L.

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ABSTRACT

A clay pot experiment was conducted in glass house condition to study the effect of zinc management with chelate (EDTA and Citric acid) application in soil on growth (Length, dry weight) and some biochemical constituents (Pigments and Protein content) of *Phaseolus vulgaris* (french bean). Various amendment of zinc was made such as I – Control (native soil), II – 10 mg kg^{-1} $ZnSO_4$, III – 25 mg kg^{-1} $ZnSO_4$, IV – 50 mg kg^{-1} $ZnSO_4$, V – 25 mg kg^{-1} $ZnSO_4$ + EDTA (10 µM) and VI – 25 mg kg^{-1} $ZnSO_4$ + Citric acid (10 µM) were applied in soil. Experiment was made in triplicates, maximum growth (length and dry weight) was observed at the soil applied with $ZnSO_4$ at 25 mg kg^{-1} + Citric acid (10 µM). All the growth and biochemical constituents were enhanced with the application of zinc but it was more effective along with chelate (Citric acid) application. The increase in dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and protein content by +84.1%, +79.37%, +93.33%, +83.18%, +81.33% and +228.89% respectively were observed maximum in french bean at the application of 25 mg kg^{-1} $ZnSO_4$ + Citric acid (10 µM).

Keywords: Zinc sulfate, EDTA, Citric acid, *Phaseolus vulgaris* and Biochemical Constituents.

INTRODUCTION

Mostly, little attention is given to micronutrients as compared to macronutrients (particularly N, P and K). Zinc deficiency is now a days is considered as the most wide spread micronutrient deficiency disorders in several crop plants (Wissuwa et al., 2006). However, it plays a vital role in the growth and metabolism of plants, animals and human beings. Most of the soils in India are facing a large number of abiotic stress conditions like land degradation, sodicity unfavorable soil pH, toxic heavy metals, chemicals, temperature and moisture content etc. (Bronic et al., 2005; Pandey, 2010; Pandey, 2014).

Zinc is an essential element for plants, its disorders can cause adverse effect on various metabolic activities and the qualitative production of food grains (low protein, sugar and mineral contents etc) (Pandey et al., 2002; Sharma, 2006; Hafeez et al., 2013). The stresses in soils adversely affect the availability of Zn in soil (Sharma, 2006; Dutta et al., 2017). Most of the agricultural lands also facing the problems of zinc deficiency (Agarwala and Sharma, 1979), thus responsible for the development of some visual deficiency symptoms in plants such as interveinal chlorosis, inhibition of stem growth, little leaf and rosette disorder etc. (Brown et al., 1993). Deficiency of zinc can be corrected by proper fertilization, improvement of soil conditions and through growing efficient cultivars (Marschner, 1995; Cakmak et al., 2010). Micronutrient zinc is also essential for human health by supporting defense system. Zinc is also involved in defence system of plants as being constituents of enzymes and functions as anti-oxidant against reactive oxygen species (ROS) during various stress conditions (Alscher et al., 2002). The deficiency in human body leads to various diseases such as Wilson's disease, chronic liver disease, chronic kidney disease, diabetes and other chronic illness (Ahmad et al., 2012; Mukherjee et al., 2012; Bhatt et al., 2020). Human beings fulfill these mineral nutrients mostly from their food. To compensate such deficiencies in the human body various zinc supplements has been suggested. Since, there are different side effects of these supplements on human body. Therefore, there is a need of enhancement of food crops which can provide adequate amount of zinc, particularly in edible part, for the human consumption. Children, women and people in villages are mostly susceptible to zinc and other mineral deficiencies because, mostly they are depending on naturally growing food sources. If Zn is sufficient in crop they can take these nutrients naturally and such deficiency problems can be resolved (Sharma, 2006). Management of growth medium and selection of crop genotypes with the application of suitable quantity of zinc can biofortify the zinc contents in crops (Verma and Pandey, 2008; Cakmak, 2010). After the suitable management, adequate Zinc in crop may help in better growth, cellular metabolism, in qualitative and quantitative production of crops. The quality food (with sufficient protein, sugar, mineral nutrients etc.) can help human beings to stay healthy (Pandey and Verma, 2020; Pathak et al., 2012; Kannaujiya and Pandey, 2013). Therefore the study was conducted to grow *Phaseolus vulgaris* L. (french bean) at low and high doses of zinc singly or in combination with chelates (Citric acid and EDTA) to observe their effect on growth and some biochemical constituents.

MATERIALS AND METHODS

Clay pot experiment

A bulk composite soil samples was collected from Lucknow district (Badshabagh area, Lucknow) collected soil sample was prepared and analyzed for some physical and chemical properties like soil texture, density, porosity, organic matter content, soil pH, calcareousness, electrical conductivity, water holding capacity, available zinc in experimental soil. The above collected soil sample was used for clay pot experiment. Experiment was conducted in triplicates under glass house condition in the Department of Botany, University of Lucknow. Soil was filled in clay pots of 10 kg size and *Phaseolus vulgaris* L. (french bean) plant was selected as a test plant and amended with various levels of zinc sulfate ($ZnSO_4$) along with applications of chelates (EDTA and citric acid) to observe their effects on growth parameters (length and dry weight) and some important biochemical constituents such as pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids contents) and protein content in french bean. Visible symptoms exhibited in response to different treatments were observed regularly. The graded levels of $ZnSO_4$ was amended as follows: (i) Control (Alluvial soil) (ii) 10 mg kg^{-1} $ZnSO_4$, (iii) 25 mg kg^{-1} $ZnSO_4$, (iv) 50 mg kg^{-1} $ZnSO_4$, (v) 25 mg kg^{-1} $ZnSO_4$ + EDTA (10 µM) and (vi) 25 mg kg^{-1} $ZnSO_4$ + Citric acid (10 µM).

Growth Observations

Growth parameters (length and dry weight) and visible symptoms appeared on plants at each treatment were observed, periodically. The length and dry weight were observed at the time of harvesting of the French bean plant. Soil was determined deficient in Zn (Agrawala and Sharma, 1979).

Biochemical Responses

The biochemical parameters were determined by the prescribed standard methods: Pigments (Lichtenthaler and Welbern, 1983) and protein content (Lowry et al., 1951). Soil analysis was carried out for pH, texture, density, water holding capacity, electrical conductivity and organic matter content by the method described by Piper (1962). Available DTPA extractable zinc content in soil were determined by Lindsay and Norwell (1978) (Table 1a and 1b).

Statistical analysis

Data were statically tested with standard error (n=3) for their significance by the student 't' test method.

RESULT AND DISCUSSION

A bulk of soil samples was collected for clay pot experiment and soil samples were analyzed for some physical and chemical properties as given in table 1 a and 1 b, thereafter the above soil samples were used in clay pot experiments and results were recorded as it was observed that growth (length and dry weight), biochemical constituents and grain yield were enhanced with the application of $ZnSO_4$ but it was more effective along with chelate (Citric acid) application at 25 mg $ZnSO_4$ kg^{-1} soil with + Citric acid 10 µM. Optimum growth as dry weight by +84.1%, chlorophyll 'a' by +79.37%, chlorophyll 'b' by +93.33%, carotenoids by +81.33% and total chlorophyll by +83.18% were observed in *Phaseolus vulgaris* L (french bean) at 25 mg $ZnSO_4$ + Citric acid application in soil. Protein content also increased with the increase in zinc levels in the soil. It showed maximum value at 25 mg $ZnSO_4$ + Citric acid (10 µM) application in the soil (+228.89%) (Table 2).

Increase in growth (length and dry weight) could be attributed due to the sufficient availability of Zn in the shoot portion of *P. vulgaris* L supported growth linked cellular metabolism of the plant such as synthesis of auxin (Pandey, 2014). Optimum growth at the application of Citric acid could be due to the facilitation of zinc uptake by the Citric acid which contributed more biochemical content in test plants (Pandey, 2020). Total pigments (chlorophyll a, chlorophyll b, total chlorophyll), carotenoids content and protein content enhanced maximum at the $ZnSO_4$ + Citric acid application in soil might be due to its facilitation in uptake of zinc which supported biosynthesis of these biomolecules in test plant (Pandey and Verma, 2020) and zinc is also constituents of many enzyme its application was promotory due to normally regulated enzymatic activity in plant cells (Agarwala and Sharma, 1979) (Figure 1 A-D).

Table 1a. Physical properties of Soil.

Parameters	Values		
	Minimum	Maximum	Average
Bulk Density (g/cc)	1.2	1.4	1.35±0.05
Particle Density (g/cc)	2.64	2.68	2.66±0.05
Water holding capacity (mm/cm depth of soil)	1.45	1.60	1.52±1.0
Soil temperature (^{o}C)	27	28	27.5
Texture (%)	Sandy Loam	Sandy Loam	Sandy Loam
Color	Light gray	Light gray	Light gray

Table 1 b. Chemical Properties of Soil.

Parameters	Values (Range)
Soil pH (1:2.5 soil–water extract at 25 ^{o}C)	7.3 – 7.5 ±0.1
Electrical Conductance (m mhos / cm)	0.32 – 0.33 ±0.1
Organic Matter Content (%)	0.24 – 0.28 ±0.05
Available Zinc (ppm)	0.42 – 0.51 ±0.05

Table 2. Effect of $ZnSO_4$ application with chelate (EDTA and Citric acid) on growth and biochemical constituents (Pigments and Protein contents) of *P. vulgaris* L.

$ZnSO_4$ and Chelate (EDTA and Citric acid) Applications in Soil (mg Kg^{-1} soil)						
Parameters	I	II	III	IV	V	VI
Length (cm)	22±1.5 (0.0)	23.5±2.0 (+6.81)	25.5±2.0 (+15.9)	26.5±2.5* (+20.4)	28±1.5 (+27.2)	28.5±1.5* (+29.54)
Dry weight (g)	1.58±0.10 (0.0)	1.95±0.15 (+23.4)	2.10±0.15 (+32.9)	2.53±0.10 (+60.1)	2.73±0.5 (+72.8)	2.91±0.5** (+84.1)
Chlorophyll a (mg g^{-1} fr. wt.)	1.60±0.3 (0.0)	1.72±0.3 (+7.5)	1.97±0.3 (+23.1)	2.21±0.3 (+38.1)	2.61±0.3** (+63.1)	2.87±0.3* (+79.37)
Chlorophyll b (mg g^{-1} fr. wt.)	0.60±0.5 (0.0)	0.62±0.3 (+3.33)	0.77±0.3 (+28.3)	0.85±0.3* (+41.6)	1.10±0.3 (+83.3)	1.16±0.3 (+93.33)
Total Chlorophyll (mg g^{-1} fr. wt.)	2.20±0.1 (0.0)	2.35±0.1 (+6.81)	2.55±0.1* (+15.9)	2.98±0.4* (+35.4)	3.46±0.2 (+57.3)	4.03±0.1 (+83.18)
Carotenoids (mg g^{-1} fr. wt.)	0.75±0.1 (0.0)	0.88±0.1 (+17.3)	1.06±0.1 (+41.3)	1.13±0.1 (+50.6)	1.20±0.1 (+60.0)	1.36±0.1* (+81.33)
Protein (µg g^{-1} fr. wt.)	22.53±0.1 (0.0)	27.38±0.1 (+21.5)	36.64±0.1 (+62.6)	54.44±0.1* (+141.6)	68.73±0.1 (+205.0)	74.10±0.1* (+228.89)

Treatments: I – Control (Native soil), II – 10 mg $ZnSO_4$ kg^{-1} soil, III – 25 mg $ZnSO_4$ kg^{-1} soil, IV – 50 mg $ZnSO_4$ kg^{-1} soil, V - 25 mg $ZnSO_4$ kg^{-1} soil + EDTA (10 µM) and VI - 25 mg $ZnSO_4$ kg $soil^{-1}$ + Citric acid (10 µM).

Parenthesis indicates percentage decrease (-) or increase (+) over control, ± S.E. (n = 3).

* - Value significant at $P < 0.05$ level and ** - value significant at $P < 0.01$ level.

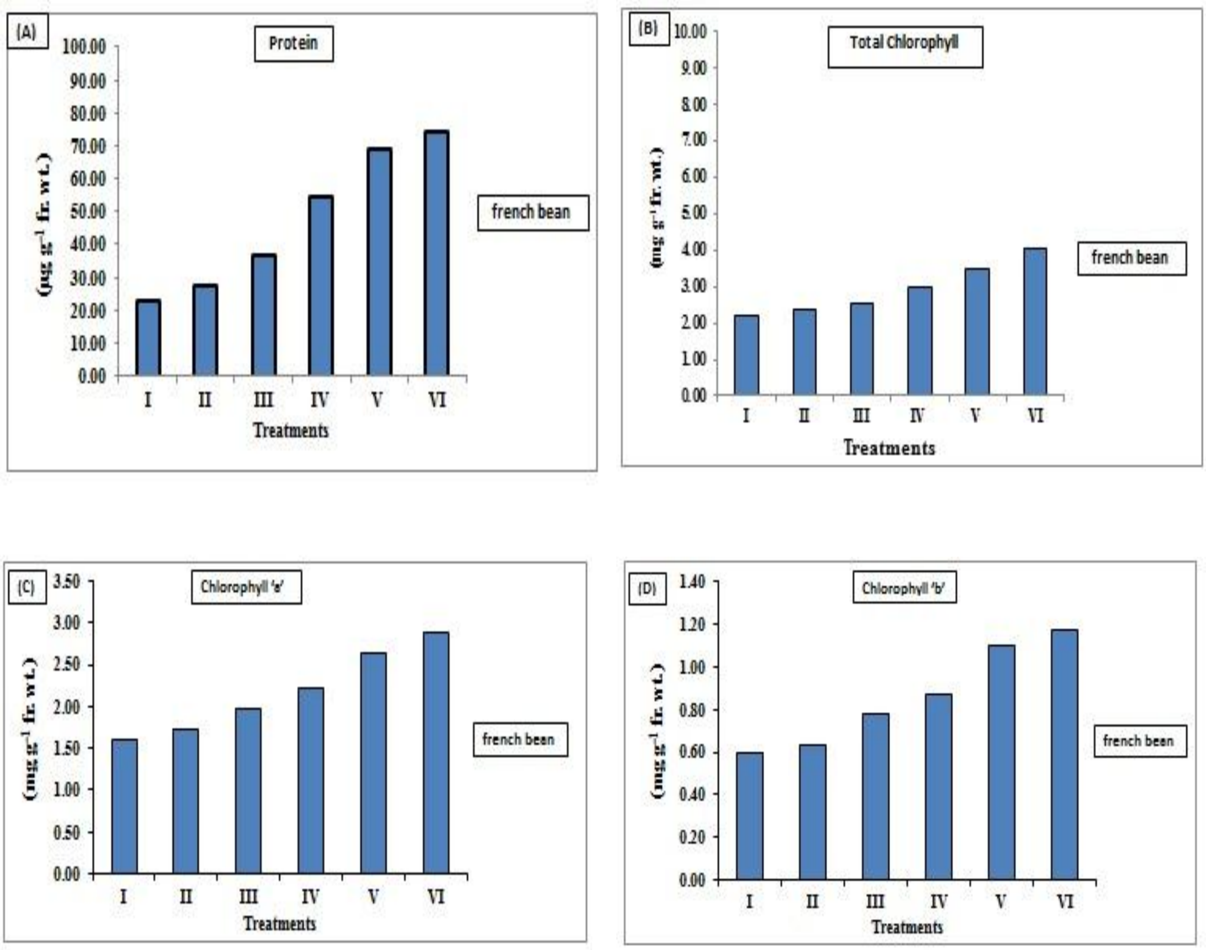

Figure 1. Effect of $ZnSO_4$ application with chelate (EDTA and Citric acid) on protein content (A), total chlorophyll (B), chlorophyll 'a' (C) and chlorophyll 'b' (D) in french bean.

Treatments: (I) Control (Alluvial Soil), (II) $ZnSO_4$ (10 mg^{-1}Kg), (III) $ZnSO_4$ (25 mg^{-1}Kg), (IV) $ZnSO_4$ (50 mg^{-1}Kg), (V) $ZnSO_4$ (25 mg^{-1}Kg) + EDTA (10 µM) and (VI) $ZnSO_4$ (25 mg^{-1}Kg) + Citric acid (10 µM).

CONCLUSION

There this study is concluded that application of Citric acid (chelate) enhances growth and biochemical constituents (Pigments and Protein content) of *P. vulgaris* L crop. The promontory effect shown by Citric acid in uptake of zinc as compared to other treatments due to chelating agents increases the absorption of zinc as the roots have more affinity for the chelated micronutrients.

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