SHORT COMMUNICATION

Responses of *Phaseolus vulgaris* towards Zinc and Iron Management in Soil with respect to Growth, Pigments and Protein Contents

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Abstract

An experiment was conducted to study the effect of various zinc amendment levels as well as interactions with iron doses in soil on the growth of plant (length and dry weight), some biochemical parameters such as plant pigments, protein contents, and activity of some enzymes such as catalase and peroxidase in *Phaseolus vulgaris* (French bean). The amendment of zinc and iron was made as Native soil, 5mg ZnSO4, 25 mg ZnSO4, 50 mg ZnSO4 and 100 mg kg-1 ZnSO4 in soil and their interactive doses with $FeSO_4$ was made as Native soil, 25 mg FeSO4, 25 mg FeSO4 + 25 mg ZnSO4, 50 mg FeSO4 + 5 mg ZnSO4 and 25 mg FeSO4 + 50 mg kg-1 ZnSO4 in soil. The experiment was conducted in triplicates. The maximum value of the dry weight, pigments, and protein content was found at the application of 50 mg kg^{-1} $ZnSO_4$ + 25 mg kg^{-1} $FeSO_4$ in soil. The increase in dry weight, total chlorophyll, and protein contents by 48, 44.4, and 37.3 were observed maximum in French bean at the application of 50 mg kg-1 ZnSO4 soil and 25 mg kg-1 FeSO4 in the soil. Maximum tissue accumulation of Fe was found at the high dose of Fe with low Zn-dose, the tissue accumulation showed antagonistic effects of zinc and iron.

Keywords: Soil, Micronutrients, Zinc sulfate, Iron sulfate, Alluvial soil, *Phaseolus vulgaris*.

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Introduction

Essential micronutrients zinc (Zn) and iron (Fe) are vital for the growth and metabolism of plants and human health (Kavian *et al*., 2022). In India, the area deficient in zinc and iron is spread over 50% of agricultural land (Assche *et al*., 2003). Both zinc and iron play a vital role in cellular metabolism and physiological processes related to the production and protection of plants (Marschner, 1995; Marschner, 2011; Pandey, 2020) which play an important role in crop production. Zinc plays a role in the structural integrity of cellular membranes, gene expression, and the regulation of various biochemical reactions (Cakmak, 2000; Andreini *et al*., 2006; Sadeghzadeh, 2013). Zn is actively involved in specific reactions of biosynthetic pathways of biomolecules (Pandey, 2018). Photosynthesis of C_4 plants has an enzyme system called carbonic anhydrase to fix the CO_2 at the first step, Zn is the main activator of this carbonic anhydrase enzyme (Sharma, 2006) have a significant role in the photosynthesis of C_4 plants. Also, the role of Zn in tryptophan biosynthesis which in turn is the precursor of indole-3-acetic acid (IAA) a plant growth hormone (Habiba *et al*., 2020). Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants affects various physiological and biochemical processes (Marschner, 2011). These micronutrients are very poor at adverse soil conditions, such as alkaline pH, sandy soil texture, low organic matter content and low Zn and Fe status availability (Marschner, 2011). The deficiency of Zn and Fe causes adverse effects on plant reproductive development via various metabolic activities, ultimately affecting the qualitative production of food grains such as low zinc, iron and protein content in food grains (Krouma, 2023). It has been observed that, there is frequent deficiency of Zn and Fe in alluvial soil (Pandey and Gautam, 2009). Management of soil and appropriate application of zinc and iron fertilizers can enhance crops' zinc and iron contents. The interactions of essential elements to each other greatly influence their availability and uptakes in the plant (Pandey, 2020). Sometimes, the interactive effects of these elements may be synergistic and antagonistic. In this way the concentration condition of Zn and Fe is more important. Therefore, the study was conducted to grow French beans (*Phaseolus vulgaris* L.) at low and high doses of zinc and iron applications, singly or in combination in soil to observe their effect on growth, some biochemical constituents and their accumulation in plants (Balrej, 2020). But little information is available on the doses combination of Zn and Fe in soil concerning their tissue accumulation.

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Materials And Methods

Clay Pot Experiment

Clay pots (10 kg size) were filled with soil collected from the Badshah bagh area of district Lucknow. Soil was characterized for some important parameters related to plant growth (Texture, pH, organic matter content and available Zn and Fe status: Available DTPA extractable Zn and Fe were determined by the method of Lindsay and Norvel (1978). Two sets of experiments were made in this study. In the first set of experiments, various levels of zinc were applied. Various graded levels of zinc were applied, as zinc sulphate in the soil as: I- Native soil, II- $ZnSO_4$ (5 mg kg^{-1}), III- $ZnSO_4$ (25 mg kg^{-1}), IV- $ZnSO_4$ (50 mg kg^{-1}) and V- $ZnSO_4$ (100 mg kg^{-1}). Also, iron and zinc fertilizers were applied in the second set of experiments. The treatments were made as: I- Alluvial soil (Control), II- $FeSO_4$ (25 mg kg^{-1}), III- $FeSO_4$ (25 mg kg^{-1}) + $ZnSO_4$ (25 mg kg^{-1}), IV- $FeSO_4$ (50 mg kg^{-1}) + $ZnSO_4$ (5 mg kg^{-1}) and V- $FeSO_4$ (25 mg kg^{-1}) +$ZnSO_4$ (50 mg kg^{-1}soil) to study their interactive effects at their various doses. Experiments were conducted in triplicates.

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 and Fe (Agrawala and Sharma, 1979).

Biochemical Responses

The biochemical parameters were determined by the prescribed standard methods: Pigments (Lichtenthaler and Welbern, 1983), catalase (Euler and Josephson, 1927), Peroxidase (Luck, 1963) and Protein (Lowry *et al.*, 1951). Soil analysis was carried out for pH, texture, and organic matter content by the method described by Piper (1962). Available DTPA extractable zinc and iron content in soil were determined by Lindsay and Norwell (1979).

Statistical Analysis

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

Result And Discussion

It is observed that growth, biochemical constituents, and grain yield were enhanced with the application of high dose of $ZnSO_4$. The soil (Table 1) applied with graded Zn levels showed promotery effects, which was dose dependent Pathak and Pandey (2010). Also, maximum dry weight was observed at 100 mg kg^{-1} $ZnSO_4$ application in soil (+91.7%). The application of $ZnSO_4$ was found to be promotery maximum at 50 mg kg^{-1} $ZnSO_4$ application in soil. Promotery effects up to higher doses of zinc sulphate (50 & 100 mg) could be due to their sufficient availability to the plants (Marschner, 2011) and their effect on cellular functions supported growth (Pandey, 2020). The normal dose of Fe fertilizer applied with low dose increased the dry weight (+151%). Whereas a high dose of zinc (50 mg $ZnSO_4$ kg^{-1} soil) with low Fe reduced dry weight (+48%) as compared with single application of $ZnSO_4$ (100 mg $ZnSO_4$ kg^{-1} soil) (Table 2). Thus, result showed antagonistic effects of Zn and Fe by the uptake of French beans as reported earlier in other crops (Sharma, 2006). At low/nil Zn application in soil, catalase activity was low as compared to its high dose (100 mg kg^{-1} soil) (Table 3). Low Zn in plant tissues produces reactive oxygen species

Table 1: physicochemical parameters of soil such as texture, pH, O.M. (%), and DTPA extractable (ppm) Zn and Fe

Soil Texture	*pH*	*O.M. (%)*	*DTPA extractable (ppm)*	
			Zn	Fe
Sandy loam	7.8	0.31	0.52	3.8

Table 2: Effect of graded levels of zinc in soil on growth, tissue zinc and some biochemical constituents in French bean.

$ZnSO_4$ (mg kg^{-1} soil)					
Parameters	Nil	5	25	50	100
Dry weight(g)	1.33± 0.01 (0.0)	1.48 ± 0.05 (+11.2)	1.92 ± 0.5 (+ 44.3)	1.96 ± 0.5* (+47.3)	2.55 ± 0.1* (+91.7)
Length (cm)	22 ± 1.5 (0.0)	26.5 ± 1.0 (+20.4)	26 ± 2.0 (+18.1)	28.5 ± 1.5* (+29.5)	26 ± 1.5 (+18.1)
Chlorophyll a (mg g^{-1}fr. wt.)	1.9 ± 0.1 (0.0)	2.5 ± 0.1 (+31.5)	2.6 ± 0.2 (+36.8)	2.7 ± 0.2 (+42.1)	1.5 ± 0.1 (-21.0)
Chlorophyll b (mg g^{-1} fr. wt.)	0.6 ± 0.1 (0.0)	0.9 ± 0.1 (+50)	1.0 ± 0.1 (+66.6)	1.2 ± 0.1 (+100)	0.5 ± 0.1 (-16.6)
Total chlorophyll (mg g^{-1}fr. wt.)	2.6 ± 0.1 (0.0)	3.4 ± 0.2 (+30.7)	3.7 ± 0.5 (+42.3)	3.9 ± 0.5* (+50)	2.0 ± 0.1* (+23.0)
Carotenoids (mg g^{-1}fr. wt.)	0.9 ± 0.1 (0.0)	1.1 ± 0.1 (+22.2)	1.2 ± 0.2 (+33.3)	1.3 ± 0.2* (+44.4)	0.7 ± 0.1 (-22.2)
Peroxidase	12.78 ± 1.5 (0.0)	22.75 ± 2.5 (+78.0)	20.22 ± 2.5** (+58.2)	16.12 ± 5.0 (+26.1)	18.37 ± 5.0* (+43.7)
Catalase (mMol H_2O_2 decomposed fr.wt./100mg)	200 ± 10 (0.0)	300 ± 25 (+50)	100 ± 20 (-50)	400 ± 25 (+100)	700 ± 20 (+250)
Protein (µg g^{-1}fr. wt.)	42.83 ± 2.0(0.0)	52.16 ± 5.0(+21.7)	54.25 ± 5.5* (+26.6)	79.55 ± 5.0**c (+85.7)	53.80 ± 4.8* (+25.6)
Zn tissue	9.57 ± 0.1 (0.0)	11.0 ± 0.1 (+14.9)	21.5 ± 0.5 (+124.6)	27.8 ± 0.5* (+190.4)	48.6 ± 0.5*c (+407.8)

±- S.E. value (n=3), *- value significant at P < 0.05 level and **- value significant at P < 0.01 levels.

Table 3: Interactive effects of zinc and iron applications in soil, (at their various doses) on the growth of French bean

Various levels of $ZnSO_4$ and $FeSO_4$ application in soil (mg/kg)					
Parameters	*I*	*II*	*III*	*IV*	*V*
Dry weight(g)	1.3 ± 0.01 (0.0)	2.53 ± 0.05* (+87.4)	1.63 ± 0.01 (+ 20.7)	3.39 ± 0.05* (+151.1)	2.00 ± 0.05 (+48.1)
Length (cm)	22 ± 1.0 (0.0)	24 ± 0.5 (+9.0)	23 ± 1.5 (+4.5)	28 ± 1.5** (+27.2)	25 ± 2.0 (+13.6)
Chlorophyll a (mg g^{-1}fr. wt.)	2.0 ± 0.1 (0.0)	1.8 ± 0.2 (-10)	1.5 ± 0.5 (-25)	2.3 ± 0.5** (+15)	2.6 ± 0.1* (+30)
Chlorophyll b (mg g^{-1} fr. wt.)	0.7 ± 0.1 (0.0)	0.6 ± 0.1 (-14.2)	0.5 ± 0.1 (-28.5)	0.6 ± 0.1 (-14.2)	1.2 ± 0.1 (+71.4)
Total chlorophyll (mg g^{-1}fr. wt.)	2.7 ± 0.5 (0.0)	2.5 ± 0.2 (-7.4)	2.0 ± 0.2* (-25.9)	2.6 ± 0.5* (-3.7)	3.9 ± 0.5 (+44.4)
Carotenoids (mg g^{-1}fr. wt.)	1.0 ± 0.1 (0.0)	0.8 ± 0.1 (-20)	0.7 ± 0.1 (-30)	0.8 ± 0.2 (-20)	1.4 ± 0.1 (+40)
Peroxidase	14.22 ± 1.5 (0.0)	23.16 ± 2.5 (+62.8)	14.94 ± 2.0 (+5.0)	15.12 ± 2.0 (+6.3)	17.36 ± 1.5 (+22.0)
Catalase (mMol H_2O_2 decomposed fr.wt./100mg)	700 ± 20 (0.0)	500 ± 40 (-28.5)	100 ± 25 (-85.7)	500 ± 50 (-28.5)	600 ± 50 (-14.2)
Protein (µg g^{-1}fr. wt.)	55.9 ± 0.5 (0.0)	60.89 ± 0.5 (+8.7)	28.28 ± 1.0* (-49.4)	72.16 ± 0.5 (+28.9)	76.86 ± 1.5* (+37.3)
Tissue Zn	8.6 ± 0.5 (0.0)	7.5 ± 0.1 (-12.7)	18.6 ± 0.1* (+116.2)	9.5 ± 0.5 (+10.4)	40 ± 1.5 (-697.6)
Tissue Fe	15.5 ± 0.5 (0.0)	30.5 ± 0.5 (+96.7)	28.5 ± 0.1 (+83.8)	49.6 ± 1.5 (+220)	35.0 ± 1.0 (+125.8)

Treatments: I- nil (Control), II- 25 mg $FeSO_4$, III- 25 mg $FeSO_4$+ 25 mg $ZnSO_4$, IV- 50 mg $FeSO_4$ + 5 mg $ZnSO_4$ and V- 25 mg $FeSO_4$+ 50 $ZnSO_4$$kg^{-1}$ soil. ± S.E. (n = 3); *- value significant at P < 0.05 level and **- value significant at P < 0.01 level.

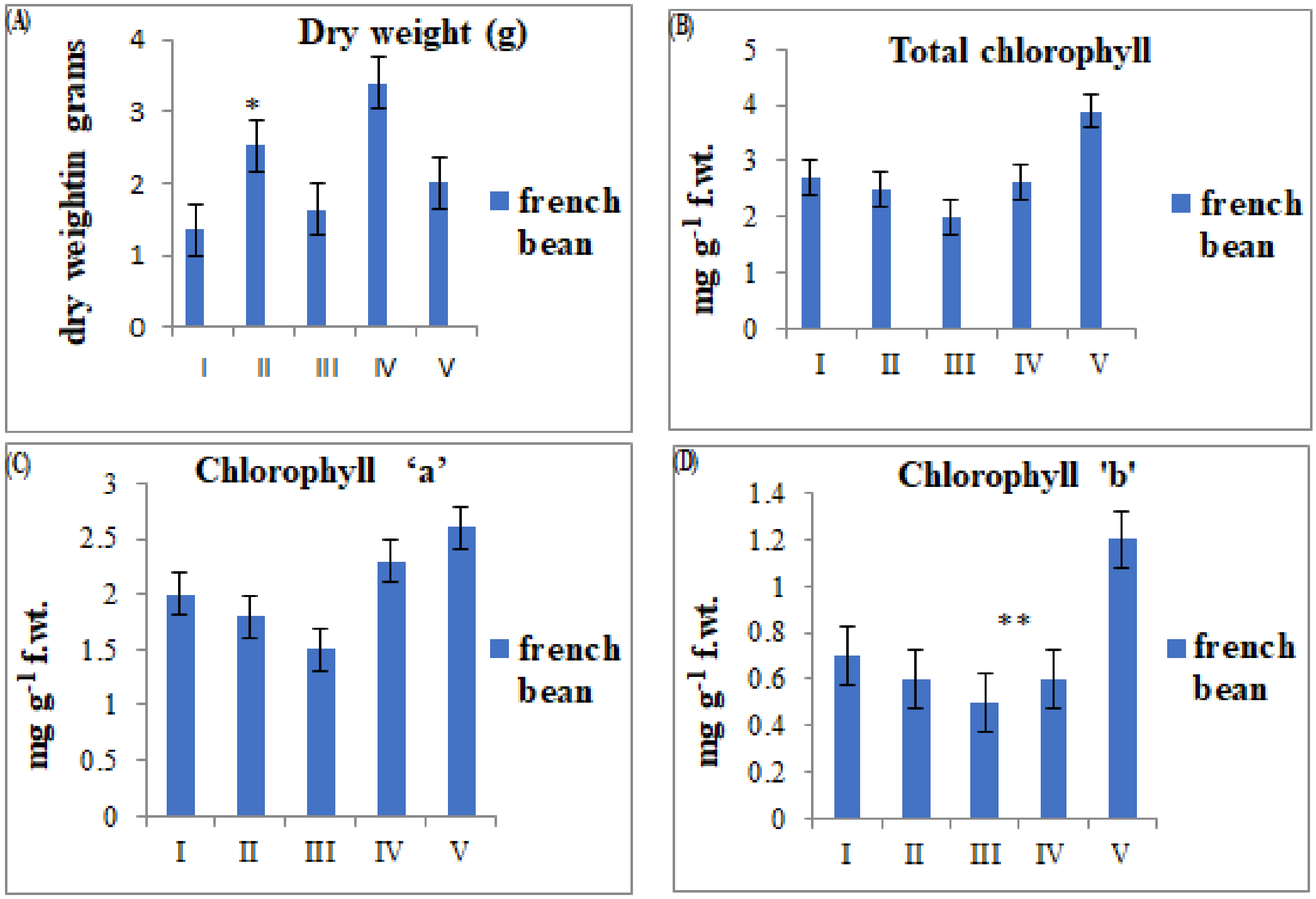

Fig 1: Interactive effects of various levels of $ZnSO_4$ and $FeSO_4$ applications in soil on dry weight (A), Total chlorophyll (B), chlorophyll a(C), and chlorophyll b contents (D) in french bean. Treatments: I- Alluvial soil (Control), II- $FeSO_4$ (25 mg kg^{-1}), III- $FeSO_4$ (25 mg kg^{-1}) +$ZnSO_4$ (25 mg kg^{-1}), IV- $FeSO_4$ (50 mg kg^{-1}) + $ZnSO_4$ (5 mg kg^{-1}) and V- $FeSO_4$ (25 mg kg^{-1})+$ZnSO_4$ (50 mg kg^{-1}). ± S.E. (n = 3); *- value significant at $P < 0.05$ and **- value significant at $P < 0.01$ levels.

(ROS), which is neutralized by antioxidative enzymes like catalase (Assche *et al.*, 2003). Insufficient Zn reduces activity of catalase has been reported (Cakmak, 2000). Elevated peroxidase activity was observed at low Zn (+78%) and its normal dose (25 mg kg^{-1} soil) (+58%). zinc with iron (Table 3). Maximum promoter effect on chlorophyll b (+100%) followed by total chlorophyll content (+50%) as compared to chlorophyll a (+42%) and carotenoid content (+44.4%). The application of Zn in a high Zn deficient soil showed promotery effects also in consonance with Pandey and Gautam (2009) and Verma and Pandey (2020). The activity of catalase was increased at 100 mg kg^{-1} of $ZnSO_4$ but decreased at 25 mg kg^{-1} $ZnSO_4$ soil level. The graded concentration of nutrient supply enhanced protein content (maximum by +85%), whereas, comparatively, protein content was decreased. The results could be due to the antagonistic effects of Zn and Fe at these levels (Habiba *et al.*, 2020). Zinc applications (1 to 100 mg kg^{-1} soil) increased protein content in French bean, but maximum protein content was enhanced at 50 mg $ZnSO_4$ kg^{-1} soil. Maximum protein content was also increased at all the level of zinc applications. It was maximum by 85.7% at 50 mg kg^{-1} $ZnSO_4$ soil application. The maximum value of the dry weight, pigments, and protein content was determined at the application of 50 mg kg^{-1} $ZnSO_4$ with 25 mg kg^{-1} $FeSO_4$ in soil.

With single application of zinc in soil, tissue Zn content was increased with increase in zinc doses. Zn was determined at 100 mg $ZnSO_4$ kg^{-1} soil application. The result was similar of dose-dependent reported earlier Pandey and Verma (2020). The concentration of zinc in tissue was maximum at high dose of $ZnSO_4$ application in soil (+407.8%). The increase in dry weight, total chlorophyll, and protein contents by 48.1, 44.4, and 37.3% were observed maximum in French bean at the application of $ZnSO_4$50 mg kg^{-1} with 25 mg kg^{-1} $FeSO_4$. There was an increase in the concentration of zinc in tissue at the application of high dose of 50 mg kg^{-1} $ZnSO_4$ and at a medium dose of 25 mg kg^{-1} $FeSO_4$ (+697.9%). The concentration of iron in tissue was increased at low dose of 5 mg kg^{-1} $ZnSO_4$ and high dose of 50 mg kg^{-1} $FeSO_4$ (+220%) (Krouma, 2023). It was observed that the high dose of $ZnSO_4$ suppress $FeSO_4$ at low dose thus showing antagonistic effects of this interaction. Similarly, at the application of high dose of $FeSO_4$, the low dose of $ZnSO_4$ was suppressed, thus showing antagonistic effects of this interaction also. The increase in growth of the French bean could be attributed due to the normal uptake and transportation of zinc in plants (Pandey, 2018). Zinc might support cellular metabolism and defense system against stress conditions which was an increase in growth (Kumar *et al.*, 2016). The increase in pigment content could be due to the sufficient iron content in leaves, which was maximum at low zinc and high iron concentrations (Fig. 1). Due to the presence of sufficient essential micronutrients, zinc and iron-supported protein synthesis (Stanon, 2022). The interaction of $ZnSO_4$ with $FeSO_4$ was found antagonistic. The results could be due to the higher competition among the species. The maximum dry-weight production of French beans was found at 50 mg $FeSO_4$ in combination with 5 mg $ZnSO_4$ kg^{-1} soil. At this condition, tissue Zn was 9.5 ug g^{-1} dry weight and Fe was 49.6 ug g^{-1} d. wt. Other parameters determined, pigments, protein and antioxidative enzymes were maximum with tissue Zn 40 and Fe 35 ug g^{-1} dry weight, where the $ZnSO_4$ at 25 mg and $FeSO_4$ 50 mg kg^{-1} soil was amended. The combined applications of Zn and Fe fertilizers in the soil reduced tissue content of Zn and Fe. Therefore, study was concluded that the applications of $ZnSO_4$, singly in a Zn-deficient alluvial soil promoted growth, some biochemical constituents and antioxidative enzymes determined were maximum in french bean shoot at the soil with 50 mg $ZnSO_4$ kg^{-1} soil, where tissue Zn was 27.8 ug g^{-1} dry weight. But the combined applications of $ZnSO_4$ and $FeSO_4$ reduce tissue Zn and Fe content.

Conclusion

The study concluded that the soil was moderately deficient in zinc. This soil was applied with $ZnSO_4$ fertilizer (@ 50 mg kg^{-1}) that enhanced the growth of *P. vulgaris*. The interactive effects of Zn and Fe fertilizers amendment in soil showed antagonistic effects on their uptake in plants. The maximum increase in growth, pigments and protein contents in French bean was found at the high dose of $ZnSO_4$ (50 mg kg^{-1} soil) in combination with $FeSO_4$ (25 mg kg^{-1} soil). Adequate $ZnSO_4$ applications singly or in combination with $FeSO_4$ promoted growth, pigments and protein content in French beans. At this fertilizers level, tissue accumulation of Zn (40 ug g^{-1} dry weight) and Fe (35 ug g^{-1} dry weight) determined was higher to growth and various biochemical constituents in French bean determined.

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Author's Contribution

Authors Samridhi Malviya, Girish Chandra Pathak and Shyam Narain Pandey jointly designed and performed experimental work. Then it was compiled as manuscript to the publication in the journal.

Conflict Of Interest

There is no any conflict of interest.

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