International Journal of Plant & Soil Science

Volume 38, Issue 9, Page 408-415, 2026; Article no.IJPSS.165029
ISSN: 2320-7035

Effect of Bio-fortification of Zn and Fe on Nutrient Concentration, Uptake and Quality in *Kharif* Greengram (*Vigna radiata* L. Wilczek)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i96302

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/165029

Original Research Article

Received: 05/06/2026
Accepted: 26/08/2026
Published: 04/09/2026

Abstract

Micronutrient deficiencies, particularly those of zinc (Zn) and iron (Fe), remain important nutritional concerns and contribute to hidden hunger. Greengram (*Vigna radiata* L. Wilczek) is a nutrient-rich pulse crop, and improving its Zn and Fe concentrations through agronomic biofortification may enhance its nutritional quality. Foliar and soil applications of Zn and Fe therefore offer practical approaches for increasing micronutrient accumulation in greengram grain. A field experiment was conducted during the *kharif* season of 2022 at the Research Farm of the Pulses Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, to assess Zn and Fe biofortification in greengram (*Vigna radiata* L. Wilczek). The experiment was laid out in a randomised block design with 11 treatments and three replications. Treatments included the recommended dose of fertilisers (RDF), foliar applications of 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ applied individually or in combination at the pre-flowering and pod-initiation stages, and soil application of $ZnSO_4$ at 25 kg ha^{-1}. Nutrient concentration, nutrient uptake, protein content, protein yield, and post-harvest soil nutrient status were evaluated. The combined foliar treatment of RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ at both growth stages recorded the highest Zn content in seed

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Cite as: *Komal, Devi, U., Chaudhary, K., Neelam, Yadav, M., & Yadav, P. (2026). Effect of Bio-fortification of Zn and Fe on Nutrient Concentration, Uptake and Quality in Kharif Greengram (Vigna radiata L. Wilczek). International Journal of Plant & Soil Science, 38(9), 408–415. https://doi.org/10.9734/ijpss/2026/v38i96302*

(29.3 ppm) and straw (20.6 ppm), Fe content in seed (73.3 ppm) and straw (67.2 ppm), Zn uptake in seed (54.1 g ha^{-1}) and straw (71.1 g ha^{-1}), and Fe uptake in seed (135 g ha^{-1}) and straw (233 g ha^{-1}). It also recorded the highest protein content (22.1%) and protein yield (409 kg ha^{-1}). N, P, and K concentrations were not significantly affected by treatment, while uptake responses varied among nutrients and plant parts. Soil-applied $ZnSO_4$ produced the highest post-harvest available Zn (0.90 ppm). Under the study conditions, combined foliar Zn and Fe application at both reproductive stages produced the strongest overall micronutrient enrichment response.

Keywords: Greengram; agronomic biofortification; zinc; iron; foliar application; nutrient concentration; nutrient uptake; protein yield.

1. Introduction

Pulses constitute an essential component of diets worldwide and play a significant role in improving human nutrition, maintaining soil fertility, and ensuring global food security. Greengram (*Vigna radiata* L. Wilczek), commonly known as mungbean, moong or golden gram, is an important pulse crop. Incorporating greengram into cereal-based cropping systems enhances agricultural sustainability by improving farm income, soil productivity, human nutrition, and water-use efficiency. Among pulse crops, greengram ranks third after chickpea and pigeon pea, occupying about 16% of the total pulse-growing area in India (Kaur & Aulakh, 2021). On a dry weight basis, its seeds contain 22–28% protein, 1.0–1.5% fat, 3.5–4.5% fibre, and 60–65% carbohydrates. The iron and zinc contents in greengram range from 4.0–7.6 mg and 2.4–3.0 mg per 100 g dry weight, respectively (Dahiya *et al*., 2015). Furthermore, greengram serves as an effective cover crop and green manure, helping to reduce soil erosion. As a legume, it can fix approximately 30–40 kg atmospheric nitrogen per hectare, thereby contributing to improved soil health and fertility.

Micronutrient deficiencies affect nearly one-third of the global population. Vitamin and mineral malnourishment is referred to as "hidden hunger", as it usually does not give any visible indication. This form of malnutrition poses a major public health challenge worldwide, especially in developing countries. Micronutrients such as iron (Fe), zinc (Zn), and selenium (Se) are vital for human growth, development, and the proper functioning of the immune system (Shenkin, 2006). In plants, iron and zinc are equally important, as they are involved in numerous physiological and biochemical processes that regulate growth and development.

Iron (Fe) is an essential micronutrient involved in the formation of chlorophyll, chloroplasts, and thylakoid structures, thereby supporting normal plant growth and development. In humans, iron deficiency can lead to anaemia, impaired cognitive development, and various health disorders, particularly among children (Sheftel *et al*., 2012). Zinc (Zn) deficiency is also a major nutritional concern and has been identified as a significant contributor to human morbidity and health-related problems worldwide (World Health Organization, 2002). In plants, zinc is required in small yet critical quantities for numerous physiological processes, including photosynthesis, protein synthesis, membrane integrity, auxin production, seedling establishment, and resistance to diseases as well as abiotic stresses such as drought.

The adoption of high-yielding and hybrid cultivars, intensive fertiliser application, multiple cropping systems, and the limited use of organic manures have contributed to widespread deficiencies of essential nutrients in agricultural soils. In view of these concerns, enhancing biofortification strategies has become necessary to meet the daily dietary requirements of iron (Fe) and zinc (Zn) in human populations. Biofortification promotes greater uptake of minerals by plants, facilitates their translocation to edible plant parts, and improves their bioavailability for human consumption. It involves increasing the concentration of bioavailable micronutrients in staple food crops through genetic improvement or agronomic practices. The primary objective of biofortification is not only to enrich grains with essential micronutrients but also to enhance their availability to humans and animals, thereby helping to alleviate micronutrient malnutrition. Among the various approaches, agronomic biofortification through the application of mineral fertilisers is considered a rapid and effective method for improving the nutritional quality of food crops (Prasad *et al*., 2014).

Recent evidence from summer mungbean indicates that soil and foliar Zn and Fe applications can improve yield and seed nutrient concentration, with responses varying according to treatment and timing (Barla *et al*., 2023). More broadly, agronomic and genetic biofortification are recognised as complementary strategies for increasing Zn and Fe concentrations in edible crop parts (Latha & Prakash, 2024). A recent review of cereals and legumes further identifies nutrient source, rate, application method, and timing as important determinants of agronomic biofortification outcomes (Fassinou Hotegni *et al*., 2024). Recent field evidence in summer mungbean also demonstrates that foliar Zn and Fe applied at flowering and pod formation can enhance seed Zn and Fe accumulation (Jassal *et al*., 2026).

Research gap: A direct comparative assessment of soil-applied $ZnSO_4$ and stage-specific foliar $ZnSO_4$ and $FeSO_4$ applications is required to determine their relative effects on nutrient concentration, nutrient uptake, and quality in kharif greengram under the experimental conditions of this study.

Objective: Therefore, the present study aimed to evaluate the effects of soil and foliar applications of Zn and Fe, applied individually or in combination at the pre-flowering and pod-initiation stages, on nutrient concentration, nutrient uptake, and quality parameters of kharif greengram.

2. Material and Methods

2.1 Site of Experimentation

A field experiment was conducted during *Kharif* 2022 at the research farm of the Pulses Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experimental site is situated at 29°10′ N latitude and 75°46′ E longitude, with an altitude of 215 m above mean sea level, and falls under a semi-arid, subtropical climatic region. The soil of the experimental field was sandy loam in texture, with a pH of 7.8, electrical conductivity of 0.30 dS m^{-1}, and 0.28% organic carbon. The soil was low in available nitrogen (154 kg ha^{-1}), medium in available phosphorus (17.4 kg ha^{-1}), and high in available potassium (380 kg ha^{-1}).

2.2 Details of Treatments

The experiment comprised eleven treatments *viz*., T_1- RDF (Control), T_2- RDF + 0.5% $ZnSO_4$ foliar spray at the pre-flowering stage (34 DAS), T_3- RDF + 0.5% $ZnSO_4$ foliar spray at the pod-initiation stage (46 DAS), T_4- RDF + 0.5% $ZnSO_4$ foliar sprays at the pre-flowering and pod-initiation stages, T_5- RDF + 0.5% $FeSO_4$ foliar spray at the pre-flowering stage, T_6- RDF + 0.5% $FeSO_4$ foliar spray at the pod-initiation stage, T_7- RDF + 0.5% $FeSO_4$ foliar sprays at the pre-flowering and pod-initiation stages, T_8- RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at the pre-flowering stage, T_9- RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at the pod-initiation stage, T_{10}- RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at the pre-flowering and pod-initiation stages, and T_{11}- RDF + soil application of $ZnSO_4$ @25 kg/ha; the experiment was replicated three times.

2.3 Experimental Details

The greengram variety MH-1142 was sown on 7 July 2022 using the Pora method, maintaining a row spacing of 30 cm. Seeds were placed at a depth of 4–5 cm with a seed rate of 15 kg ha^{-1}. Under the recommended dose of fertiliser (T_1), a basal dose of 20 kg N through urea and 40 kg P_2O_5 through single superphosphate (SSP) per hectare was applied at sowing. Foliar sprays of 0.5% zinc and 0.5% iron were administered according to the treatment schedule using $ZnSO_4{\cdot}7H_2O$ (23% Zn) and $FeSO_4{\cdot}H_2O$ (20% Fe), respectively, at the pre-flowering and pod-initiation stages, with 500 L of water ha^{-1} used as the spray volume.

2.4 Observations Recorded

The protein content was estimated by multiplying the nitrogen content in the grain by a conversion factor of 6.25. It was expressed in per cent (%).

$$\text{Protein content (\%)} = \text{Nitrogen content (\%)} \times 6.25$$

Protein yield was calculated using the following formula:

$$\text{Protein yield (kg/ha)} = \frac{\text{Protein content (\%)} \times \text{Grain yield (kg ha}^{-1}\text{)}}{100}$$

The uptake of N, P, K, Zn and Fe in seed and straw was determined using the following formula:

$$\text{Nutrient uptake (NPK kg/ha)} = \frac{\textit{Nutrient content in seed \& straw} \text{ (\%)} \times \textit{Seed \& straw yield (kg ha}^{-1}\textit{)}}{100}$$

$$\text{Zn/Fe uptake (g/ha)} = \frac{\textit{Micronutrient content in seed \& straw (ppm)} \times \textit{Seed \& straw yield (kg ha}^{-1}\textit{)}}{1000}$$

2.5 Statistical Analysis

The experimental data were statistically analysed using Fisher's analysis of variance (ANOVA) technique (Fisher, 1950). Treatment effects were evaluated through the F-test, and whenever the F-test indicated significance at the 5% probability level, the critical difference (CD) was computed to compare treatment means.

3. Results and Discussion

3.1 Effect on Zn content and Uptake

Significant differences in zinc content and uptake in seed and straw were observed with the application of zinc and iron at various growth stages (Table 1 and Fig. 1). The highest zinc content in seed (29.3 ppm) and straw (20.6 ppm) was recorded with RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at the pre-flowering and pod-initiation stages (T_{10}), whereas the lowest Zn content in seed (25.2 ppm) and straw (14.0 ppm) was recorded with RDF (T_1). The highest Zn uptake in seed (54.1 g ha^{-1}) and straw (71.1 g ha^{-1}) was also recorded with RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at both stages (T_{10}), while the lowest Zn uptake in seed (33.1 g ha^{-1}) and straw (39.5 g ha^{-1}) was recorded with RDF (T_1). The combined Zn and Fe foliar treatment at both stages recorded higher Zn content and uptake than the individual Zn- or Fe-only foliar treatments. Zinc plays a crucial role in pollen formation and reproductive development, particularly in legumes, while iron is a key component of ferredoxin and cytochromes involved in photosynthesis. Foliar application during the reproductive stage likely alleviated nutrient deficiencies caused by the translocation of micronutrients to developing grains, thereby enhancing zinc accumulation in plant tissues compared with the control. Similar results have been reported by Pal *et al.* (2021) and Dhaliwal *et al.* (2021) in chickpea, and Dhaliwal *et al.* (2023) and Farooq *et al.* (2025) in greengram.

Table 1. Effect of zinc and iron applications on Zn and Fe content (ppm) and uptake (g ha^{-1}) in seed and straw of greengram

Symbol	Treatment	Zinc content (ppm)		Zinc uptake (g ha^{-1})		Iron content (ppm)		Iron uptake (g ha^{-1})	
		Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw
T_1	RDF (Control)	25.2	14.0	33.1	39.5	64.0	60.7	84	172
T_2	RDF + 0.5% $ZnSO_4$ foliar spray at pre flowering stage (34 DAS)	27.1	16.2	43.5	51.9	66.2	62.4	106	200
T_3	RDF + 0.5% $ZnSO_4$ foliar spray at pod initiation stage (46 DAS)	26.4	15.6	40.9	48.9	65.9	61.9	102	194
T_4	RDF + 0.5% $ZnSO_4$ foliar sprays at pre flowering and at pod initiation stages	28.3	16.7	47.1	54.2	66.5	63.0	111	204
T_5	RDF + 0.5% $FeSO_4$ foliar spray at pre flowering stage	25.8	14.6	38.1	44.3	69.6	64.7	103	196
T_6	RDF + 0.5% $FeSO_4$ foliar spray at pod initiation stage	25.7	14.3	36.6	42.3	69.0	64.2	98	190
T_7	RDF + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	26.2	14.8	39.7	45.7	70.0	65.1	106	200
T_8	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pre flowering stage	28.8	19.1	50.9	64.4	72.1	66.6	128	225
T_9	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pod initiation stage	28.4	18.6	48.4	61.4	71.4	66.1	122	219
T_{10}	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	29.3	20.6	54.1	71.1	73.3	67.2	135	233
T_{11}	RDF + soil application of $ZnSO_4$ @ 25 kg/ha	29.0	19.8	51.8	67.5	67.7	63.6	121	217
	SE (m)±	0.85	0.33	1.52	1.33	0.77	0.67	2.77	4.29
	C.D. (P=0.05)	2.50	0.96	4.49	3.91	2.28	1.98	8.16	12.66

3.2 Effect on Fe content and Uptake

Significant differences in iron content and uptake in seed and straw were observed with the application of zinc and iron at different growth stages (Table 1 and Fig. 1). The highest Fe content in seed (73.3 ppm) and straw (67.2 ppm) was recorded with RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at the pre-flowering and pod-initiation stages (T_{10}), whereas the lowest Fe content in seed (64.0 ppm) and straw (60.7 ppm) was recorded with RDF (T_1). The highest Fe uptake in seed (135 g ha^{-1}) and straw (233 g ha^{-1}) was recorded with the same combined foliar treatment (T_{10}), while the lowest Fe uptake in seed (84 g ha^{-1}) and straw (172 g ha^{-1}) was recorded with RDF (T_1). Iron plays a vital role in chlorophyll synthesis, chloroplast development, and thylakoid formation, thereby enhancing photosynthetic activity and facilitating the translocation of nutrients to sink organs. The increased iron content may be attributed to the greater availability of iron and the positive physiological response of plants, which promoted nutrient movement towards reproductive structures such as pods and seeds. In the present study, the combined application of Zn and Fe recorded greater Fe accumulation than the Fe-only foliar treatments. Foliar application of Zn and Fe during the reproductive stage also improved micronutrient concentration in plant tissues compared with the control, possibly by compensating for nutrient deficiencies arising from the translocation of these elements to developing grains. Similar findings were reported by Ramzan *et al.* (2020) in wheat, and Pal *et al.* (2021) and Dhaliwal *et al.* (2021) in chickpea, and Dhaliwal *et al.* (2023) and Farooq *et al.* (2025) in greengram.

3.3 Effect on NPK Content and Uptake

The N, P, and K contents and their uptake in seed and straw are presented in Tables 2 and 3. The concentrations of N, P, and K in both seed and straw were not significantly affected by the zinc and iron treatments (Table 2). Numerically, T_{10} recorded the highest seed N content (3.54%), seed P content (0.58%), and straw K content (2.31%). The highest straw N content (0.48%) was shared by T_4, T_7, and T_{10}; the highest straw P content (0.20%) was recorded with T_2 and T_9; and the highest seed K content (1.29%) was recorded with T_4. For nutrient uptake (Table 3), N uptake differed significantly among treatments in seed but not in straw, while P uptake differed significantly in seed but not in straw. Potassium uptake differed significantly in both seed and straw. Treatment T_{10} recorded the highest seed N uptake (65.5 kg ha^{-1}), seed P uptake (10.8 kg ha^{-1}), seed K uptake (23.6 kg ha^{-1}), and straw K uptake (80.1 kg ha^{-1}). Numerically, T_{10} also recorded the highest straw N uptake (16.7 kg ha^{-1}), whereas the highest straw P uptake (6.5 kg ha^{-1}) was recorded with T_9. Similar findings were reported by Goswami and Singh (2016) and Ranpariya *et al.* (2017) in greengram.

3.4 Effect on Quality Parameters

Protein content was not significantly affected by the zinc and iron treatments, whereas protein yield differed significantly among treatments (Table 4).

Numerically, protein content ranged from 18.0% with RDF (T_1) to 22.1% with RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at the pre-flowering and pod-initiation stages (T_{10}), but the differences in protein content were non-significant. Protein yield differed significantly, with the highest value of 409 kg ha^{-1} under T_{10} and the lowest value of 236 kg ha^{-1} under T_1. Based on the critical difference (54.42 kg ha^{-1}), the protein yield under T_{10} was statistically at par with T_{11} (387 kg ha^{-1}) and T_8 (375 kg ha^{-1}). Protein content in seeds is closely associated with their nitrogen content; therefore, no significant variation in protein content was observed among the treatments. However, protein yield increased significantly, primarily due to the higher grain yield recorded under the micronutrient treatments. Iron and zinc play important roles in the structure and functioning of enzymes involved in amino acid metabolism and protein synthesis, which may contribute to improved protein accumulation. Zinc, in particular, serves as an essential component of several enzymes such as dehydrogenases, proteinases, and peptidases, and is involved in the synthesis of various amino acids. The enhanced activity of these enzymes following zinc application may have contributed to the observed improvement in protein yield. These results are in close agreement with the findings of Pal *et al.* (2021) in chickpea, and Boradkar *et al.* (2023) and Singh *et al.* (2025) in greengram.

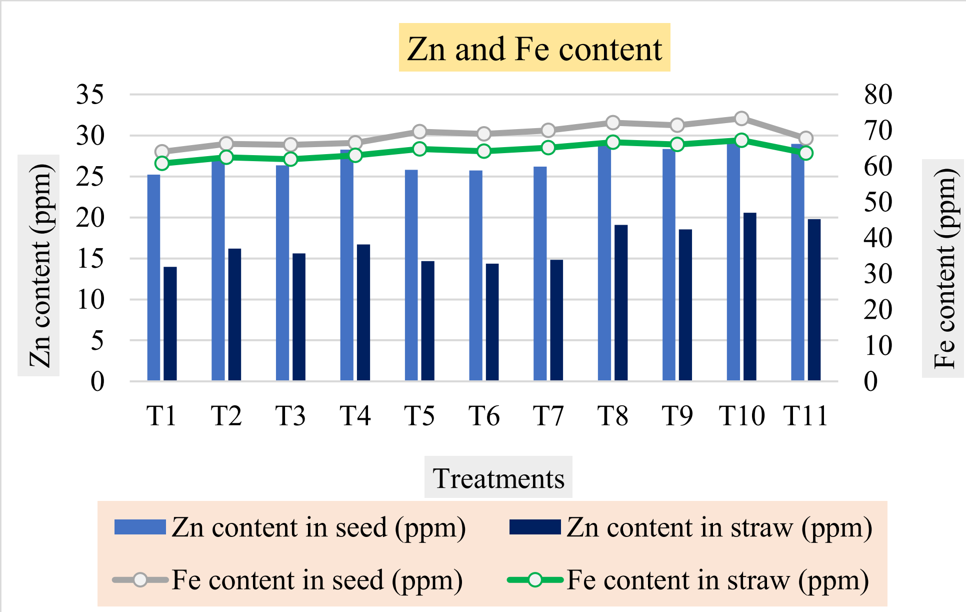

Fig. 1. Effect of zinc and iron applications on Zn and Fe content (ppm) in seed and straw of greengram

Table 2. Effect of zinc and iron applications on NPK content (%) in seed and straw of greengram

Symbol	Treatment	Nitrogen content (%)		Phosphorus content (%)		Potassium content (%)	
		Seed	Straw	Seed	Straw	Seed	Straw
T_1	RDF (Control)	2.88	0.45	0.43	0.13	1.05	2.00
T_2	RDF + 0.5% $ZnSO_4$ foliar spray at pre flowering stage (34 DAS)	3.15	0.47	0.49	0.20	1.28	2.13
T_3	RDF + 0.5% $ZnSO_4$ foliar spray at pod initiation stage (46 DAS)	3.10	0.47	0.47	0.18	1.26	2.11
T_4	RDF + 0.5% $ZnSO_4$ foliar sprays at pre flowering and at pod initiation stages	3.21	0.48	0.50	0.18	1.29	2.15
T_5	RDF + 0.5% $FeSO_4$ foliar spray at pre flowering stage	2.98	0.47	0.46	0.17	1.19	2.05
T_6	RDF + 0.5% $FeSO_4$ foliar spray at pod initiation stage	2.92	0.46	0.45	0.15	1.18	2.03
T_7	RDF + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	3.02	0.48	0.47	0.17	1.22	2.08
T_8	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pre flowering stage	3.40	0.46	0.54	0.15	1.25	2.27
T_9	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pod initiation stage	3.31	0.46	0.52	0.20	1.23	2.23
T_{10}	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	3.54	0.48	0.58	0.18	1.28	2.31
T_{11}	RDF + soil application of $ZnSO_4$ @ 25 kg/ha	3.46	0.47	0.56	0.18	1.26	2.29
	SE (m)±	0.15	0.02	0.04	0.02	0.05	0.08
	C.D. (P=0.05)	NS	NS	NS	NS	NS	NS

Table 3. Effect of zinc and iron applications on NPK uptake (kg ha^{-1}) in seed and straw of greengram

Symbol	Treatment	Nitrogen uptake (kg/ha)		Phosphorus uptake (kg/ha)		Potassium uptake (kg/ha)	
		Seed	Straw	Seed	Straw	Seed	Straw
T_1	RDF (Control)	37.8	12.8	5.6	3.7	13.8	56.5
T_2	RDF + 0.5% $ZnSO_4$ foliar spray at pre flowering stage (34 DAS)	50.6	15.1	7.8	6.3	20.6	68.0
T_3	RDF + 0.5% $ZnSO_4$ foliar spray at pod initiation stage (46 DAS)	48.2	14.8	7.3	5.7	19.6	65.9
T_4	RDF + 0.5% $ZnSO_4$ foliar sprays at pre flowering and at pod initiation stages	53.5	15.7	8.3	5.9	21.5	69.8
T_5	RDF + 0.5% $FeSO_4$ foliar spray at pre flowering stage	44.2	14.2	6.8	5.1	17.7	62.0
T_6	RDF + 0.5% $FeSO_4$ foliar spray at pod initiation stage	41.5	13.7	6.4	4.4	16.7	60.0
T_7	RDF + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	45.7	14.9	7.1	5.2	18.4	64.1
T_8	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pre flowering stage	60.0	15.6	9.6	5.0	22.1	76.6
T_9	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pod initiation stage	56.7	15.3	8.9	6.5	20.9	73.8
T_{10}	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	65.5	16.7	10.8	6.3	23.6	80.1
T_{11}	RDF + soil application of $ZnSO_4$ @ 25 kg/ha	61.9	16.0	10.1	6.2	22.5	78.1
	SE (m)±	2.95	0.79	0.63	0.72	0.98	2.45
	C.D. (P=0.05)	8.71	NS	1.86	NS	2.88	7.23

Table 4. Effect of zinc and iron applications on quality parameters of greengram

Symbol	Treatments	Protein content (%)	Protein yield (kg ha^{-1})
T_1	RDF (Control)	18.0	236
T_2	RDF + 0.5% $ZnSO_4$ foliar spray at pre flowering stage (34 DAS)	19.7	316
T_3	RDF + 0.5% $ZnSO_4$ foliar spray at pod initiation stage (46 DAS)	19.4	301
T_4	RDF + 0.5% $ZnSO_4$ foliar sprays at pre flowering and at pod initiation stages	20.1	335
T_5	RDF + 0.5% $FeSO_4$ foliar spray at pre flowering stage	18.6	276
T_6	RDF + 0.5% $FeSO_4$ foliar spray at pod initiation stage	18.2	260
T_7	RDF + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	18.9	286
T_8	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pre flowering stage	21.2	375
T_9	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pod initiation stage	20.7	354
T_{10}	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	22.1	409
T_{11}	RDF + soil application of $ZnSO_4$ @ 25 kg/ha	21.6	387
	SE (m)±	0.96	18.45
	C.D. (P=0.05)	NS	54.42

3.5 Effect on Nutrient Status of Soil

The soil analysis results (Table 5) indicated that the available nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) contents were not significantly affected by the application of zinc and iron at different growth stages. However, the available zinc (Zn) content in the soil was significantly influenced by the treatments. The maximum available Zn (0.90 ppm) was recorded with RDF + soil application of $ZnSO_4$ @25 kg/ha (T_{11}) and the minimum available Zn (0.80 ppm) was recorded with RDF (T_1), RDF + 0.5% $FeSO_4$ foliar spray at pre-flowering stage (T_5) and RDF + 0.5% $FeSO_4$ foliar sprays at pre-flowering and pod-initiation stages (T_7). The quantities of nutrients supplied through foliar application were relatively small and, therefore, had little effect on the soil nutrient status at harvest. In contrast, soil-available zinc increased significantly due to the application of zinc sulphate ($ZnSO_4$) to the soil. Similar observations have also been reported by Goswami and Singh (2016) and Ranpariya *et al.* (2017).

Table 5. Effect of zinc and iron applications on nutrient status of soil after harvest of greengram

Symbol	Treatments	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	Zinc (ppm)	Iron (ppm)
T_1	RDF (Control)	149	15.0	368	0.80	7.89
T_2	RDF + 0.5% $ZnSO_4$ foliar spray at pre flowering stage (34 DAS)	145	14.3	355	0.84	7.92
T_3	RDF + 0.5% $ZnSO_4$ foliar spray at pod initiation stage (46 DAS)	145	13.6	359	0.84	7.91
T_4	RDF + 0.5% $ZnSO_4$ foliar sprays at pre flowering and at pod initiation stages	147	13.6	352	0.85	7.93
T_5	RDF + 0.5% $FeSO_4$ foliar spray at pre flowering stage	145	15.1	363	0.80	7.97
T_6	RDF + 0.5% $FeSO_4$ foliar spray at pod initiation stage	149	16.6	365	0.82	7.94
T_7	RDF + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	147	15.1	361	0.80	8.02
T_8	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pre flowering stage	149	14.3	342	0.81	7.99
T_9	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar spray at pod initiation stage	145	16.6	347	0.83	7.96
T_{10}	RDF + 0.5% $ZnSO_4$ + 0.5% $FeSO_4$ foliar sprays at pre flowering and at pod initiation stages	147	14.3	341	0.84	8.04
T_{11}	RDF + soil application of $ZnSO_4$ @ 25 kg/ha	145	15.0	346	0.90	7.91
	SE (m)±	3.99	1.10	15.85	0.02	0.25
	C.D. (P=0.05)	NS	NS	NS	0.05	NS
	Initial (before sowing)	154	17.4	380	0.87	8.27

4. Conclusion

The study showed that the combined foliar application of 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ with RDF at both the pre-flowering and pod-initiation stages produced the highest measured Zn and Fe concentrations and uptake in greengram seed and straw. The same treatment also recorded the highest protein content (22.1%) and protein yield (409 kg ha^{-1}). Although N, P, and K concentrations in seed and straw were not significantly affected by the treatments, nutrient uptake responses differed among nutrients and plant parts, with significant effects evident for seed N and P uptake and for K uptake in both seed and straw. Soil application of $ZnSO_4$ at 25 kg ha^{-1} with RDF resulted in the highest post-harvest available soil Zn (0.90 ppm). Overall, the findings indicate that combined foliar Zn and Fe application at both reproductive stages was the most effective treatment for improving micronutrient concentration, micronutrient uptake, and protein yield under the conditions of this experiment. Wider recommendation would require confirmation under additional environments and seasons.

5. Limitation

The experiment was conducted during one kharif season at a single location using one greengram variety, which limits the extent to which the findings can be generalised across environments, seasons, and genotypes. The study assessed nutrient concentration and uptake but did not directly measure micronutrient bioavailability or human nutritional outcomes. Multi-location and multi-season validation would strengthen the applicability of the findings.

Acknowledgements

The authors are thankful to the Department of Agronomy, CCS Haryana Agricultural University, Haryana, India, for providing necessary facilities during the research work.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://pr.sdiarticle5.com/review-history/165029