



Influence of Agronomic biofortification of zinc and iron on nutrient uptake by foxtail millet and soil available nutrient status

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Abstract

Zinc (Zn) and iron (Fe) deficiencies are common among people who depend on cereals and millets and the soils that support these crops often lack the same elements. A field experiment examined how agronomic biofortification with Zn and Fe affects nutrient uptake by foxtail millet (*Setaria italica* L.) and soil fertility after harvest. The soil was a slightly alkaline clay (pH 8.20) low in DTPA-extractable Zn (0.32 mg kg⁻¹) and Fe (4.18 mg kg⁻¹). Nine treatments combining soil application (2.5 or 5 kg ha⁻¹) and foliar spray (0.25 or 0.5 %) of ZnSO₄ and FeSO₄ were compared with a control receiving only basal nutrition. Soil application of ZnSO₄ and FeSO₄ @ 5 kg ha⁻¹ each + foliar spray of ZnSO₄ and FeSO₄ @ 0.5 % each at 40 DAS gave the highest concentrations and uptake of all five nutrients. Relative to the control, it raised total uptake of N, P, K, Zn and Fe by 57, 84, 44, 150 and 84 per cent and grain Zn and Fe by 90 and 21 per cent, to 46.15 and 838.06 mg kg⁻¹. The soil application of ZnSO₄ and FeSO₄ @ 2.5 kg ha⁻¹ each + foliar spray of ZnSO₄ and FeSO₄ @ 0.25 % each at 40 DAS was statistically comparable for most plant variables. Soil pH, electrical conductivity and organic carbon were unaffected, residual K did not differ among treatments and residual N and P were lower where crop removal was greatest. Residual DTPA-Zn and DTPA-Fe were highest under the best treatment (0.34 and 4.21 mg kg⁻¹), whereas the control lost both. Combined soil and foliar Zn and Fe supply enriches foxtail millet without harming soil properties, provided the N and P removed are replaced.

Keywords: Agronomic biofortification, foxtail millet, zinc, iron, nutrient uptake, soil fertility

Introduction

Hidden hunger caused by inadequate intake of Zn and Fe is one of the most persistent nutritional problems of our time, affecting an estimated two billion people, with the heaviest burden in South Asia and sub-Saharan Africa (White and Broadley, 2009; Stein, 2010; Muthayya *et al.*, 2013) [18, 26, 31]. Zn deficiency impairs growth, immune function and cognitive development and a large share of the global population is at risk of inadequate intake (Wessells and Brown, 2012) [30], while Fe deficiency is a leading cause of anemia. Diets built around cereals are the common thread. Cereal grains are naturally low in bioavailable Zn and Fe, they are frequently grown on soils where these elements are poorly available to plants (Alloway, 2008; Cakmak, 2008) [1, 3].

In India, Zn is the most widespread micronutrient constraint in soils and Fe deficiency is also common on alkaline and calcareous soils (Singh, 2008) [25]. At high pH, Zn is adsorbed onto clay and carbonate surfaces and Fe is rapidly converted to sparingly soluble ferric oxides, so DTPA-extractable concentrations stay low even where total contents are adequate (Lindsay, 1979; Lindsay and Schwab, 1982) [14, 16]. Crops grown on such soils produce grain that is lean in both elements.

Biofortification is the effort to raise the density of these elements in edible tissue. Genetic biofortification through breeding is effective but slow and its gains depend on the soil supplying enough of the element. Agronomic biofortification, in which Zn and Fe fertilizers are applied to the soil, the foliage or both, delivers results within a single season and complements breeding (Cakmak, 2008 [3]; Bouis and Saltzman, 2017). The two routes work differently. Soil

application builds the supply in the root zone but is vulnerable to fixation, while foliar sprays bypass the soil and can feed the grain directly during filling (Fernández and Brown, 2013; Cakmak and Kutman, 2018) [4, 6]. Reviews of cereal systems generally find that combining the two routes enriches grain more than either alone (Prasad *et al.*, 2014). Millets are well placed to benefit from this approach. Foxtail millet (*Setaria italica* L.) P. Beauv.) is a short-duration, drought-tolerant crop that is grown on marginal land with few inputs and its grain supplies protein, fiber and minerals to many rural households (Saleh *et al.*, 2013; Sharma and Niranjana, 2018) [24]. Most of the agronomic biofortification literature, however, deals with wheat, rice and maize and work on foxtail millet remains limited (Sathisha *et al.*, 2019; Krupashree *et al.*, 2022) [12].

A second gap concerns the soil. Zn and Fe take part in protein synthesis, photosynthesis and energy metabolism, so correcting a deficiency changes how much N, P and K the crop takes up (Marschner, 2012 [17]; Kutman *et al.*, 2011). A more vigorous crop removes more of these nutrients and what happens to soil fertility after harvest is rarely reported alongside grain results. A biofortification practice that enriches grain but quietly depletes the soil would not be sustainable.

We therefore tested soil and foliar application of ZnSO₄ and FeSO₄, alone and in combination, on a Zn- and Fe-deficient clay soil. The objectives were (i) to quantify the content and uptake of N, P, K, Zn and Fe in foxtail millet grain and straw, (ii) to measure the extent of grain Zn and Fe enrichment and (iii) to assess soil pH, electrical conductivity, organic carbon and available nutrient status after harvest.

Materials and Methods

1. Experimental Site and Initial Soil Properties

The experiment was conducted at the Experimental Block of College of Agriculture, Bheemaranagudi, (UAS, Raichur) which lies in the North Eastern Dry Zone of Karnataka (Zone-II). It is located at 16°15' N latitude, 77°21' E longitude and at an elevation of 389 meters above mean sea level. The region receives on an average 630-800 mm rainfall annually. Before sowing, composite soil samples were collected from the (0-15 cm) layer, air-dried, ground and passed through a 2 mm sieve. The physical and chemical properties were determined by the methods listed in Table 1. The soil was a clay (51.12 % clay) with slightly alkaline reaction (pH 8.20), normal electrical conductivity, medium organic carbon and available N, low available P, high available K and low DTPA-extractable Zn and Fe (Table 1).

2. Crop Management

The crop foxtail millet, variety chosen was HN-46, sown at a spacing of 30 × 10 cm in plots measuring 4.8 × 3.6 m (gross plot) and 3.6 × 3.2 m (net plot). The variety is recommended for Zones I to III of Karnataka, matures in 85 to 90 days and has high yield potential with good nutritional quality.

Recommended farmyard manure at 6 t ha⁻¹ and NPK at 30:15:15 kg ha⁻¹ were applied and ZnSO₄ and FeSO₄ were applied to the soil and foliar at different doses as per the respective treatments. The recommended quantities of ZnSO₄ and FeSO₄ for soil were applied at the time of sowing and foliar application were diluted in water and sprayed using a manually operated knapsack sprayer at a spray volume of 500 l ha⁻¹. Prior to foliar application, FeSO₄ solution was neutralized appropriately to minimize the risk of leaf scorching and ensure safe and effective application to the crop.

All standard agronomic practices were followed, including land preparation, sowing on 5th November 2025, weed and pest management and harvesting at physiological maturity on 28th January 2026. The crop received timely irrigations and manual weeding and plant protection measures were adopted as per recommendations.

3. Experimental Design and Treatments

The experiment design followed was randomized complete block design with nine treatment combinations and three replications. The treatments combined soil application and foliar spray of zinc sulfate (ZnSO₄) and ferrous sulfate (FeSO₄) at two levels, alone and in combination, with a control that received only the RDF and FYM.

Table 1: Initial physical and chemical properties of the soil at the experimental site

Particulars	Value	Rating	Method followed
A. Physical properties (mechanical analysis, oven-dry basis)			
Sand (%)	26.53		International pipette method (Piper, 1966) ^[19]
Silt (%)	22.35		
Clay (%)	51.12		
Textural class	Clay		
B. Chemical properties			
Soil pH (1:2.5 soil: water)	8.20	Slightly alkaline	Potentiometry (Jackson, 1973) ^[10]
Electrical conductivity (dS m ⁻¹)	0.18	Normal	Conductometry (Jackson, 1973) ^[10]
Organic carbon (%)	0.48	Medium	Wet oxidation (Walkley and Black, 1934) ^[29]
Available N (kg ha ⁻¹)	363.80	Medium	Alkaline permanganate (Subbiah and Asija, 1956) ^[27]
Available P ₂ O ₅ (kg ha ⁻¹)	19.00	Low	Olsen extraction (Jackson, 1973) ^[10]
Available K ₂ O (kg ha ⁻¹)	369.90	High	Flame photometry (Jackson, 1973) ^[10]
DTPA-extractable Zn (mg kg ⁻¹)	0.32	Low	AAS (Lindsay and Norvell, 1978) ^[15]
DTPA-extractable Fe (mg kg ⁻¹)	4.18	Low	AAS (Lindsay and Norvell, 1978) ^[15]

AAS, atomic absorption spectrophotometry. Ratings are as assigned for the site. Concentrations reported as ppm in the original analytical records are expressed here as mg kg⁻¹ (equivalent).

Table 2: Treatment details

Code	Treatment
T ₁	Control (RDF and FYM only; no Zn or Fe)
T ₂	Soil application of ZnSO ₄ @ 5 kg ha ⁻¹
T ₃	Soil application of FeSO ₄ @ 5 kg ha ⁻¹
T ₄	Soil application of ZnSO ₄ and FeSO ₄ @ 5 kg ha ⁻¹ each
T ₅	Foliar spray of ZnSO ₄ @ 0.5 % at 40 DAS
T ₆	Foliar spray of FeSO ₄ @ 0.5 % at 40 DAS
T ₇	Foliar spray of ZnSO ₄ and FeSO ₄ @ 0.5 % each at 40 DAS
T ₈	Soil application of ZnSO ₄ and FeSO ₄ @ 2.5 kg ha ⁻¹ each + foliar spray of ZnSO ₄ and FeSO ₄ @ 0.25 % each at 40 DAS
T ₉	Soil application of ZnSO ₄ and FeSO ₄ @ 5 kg ha ⁻¹ each + foliar spray of ZnSO ₄ and FeSO ₄ @ 0.5 % each at 40 DAS

RDF, recommended dose of fertilizer (30:15:15 kg N: P₂O₅:K₂O ha⁻¹); FYM, farmyard manure @ 6 t ha⁻¹, common to all treatments; DAS, days after sowing.

4. Plant Sampling and Analysis

At harvest, grain and straw samples were collected from each plot, rinsed with distilled water, dried in a hot-air oven at (60-65 °C) to constant weight and ground in a stainless-steel mill to avoid contamination with Zn and Fe. Nitrogen was determined by the Kjeldahl method, P by the

vanadomolybdophosphoric yellow color method and K by flame photometry after digestion in a diacid mixture (Jackson, 1973)^[10]. A known quantity of the digested samples used for determination of zinc and iron content in plant by using Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978)^[15]. Nutrient uptake (kg ha⁻¹)

was calculated as nutrient concentration multiplied by the dry matter yield of grain or straw, with total uptake as the sum of the two. For the micronutrients, uptake was computed from the concentration in mg kg⁻¹ and expressed in kg ha⁻¹.

5. Post-Harvest Soil Sampling and Analysis

After harvest, a composite soil sample was drawn from each plot at the same depth as the initial sampling and processed as described above. Soil pH, EC, organic carbon, available N, P₂O₅, K₂O and DTPA-extractable Zn and Fe were determined by the methods given in Table 1.

6. Statistical Analysis

Data were subjected to analysis of variance appropriate to the experimental design (Gomez and Gomez, 1984) [8]. Treatment means were compared using the critical difference (CD) at the 5 per cent level of probability ($P = 0.05$) and standard errors of the mean (S.E.m. $\pm$) are reported. Differences that did not reach this level are denoted NS (non-significant).

Results and Discussion

1. Nitrogen, phosphorus and potassium content and uptake

Zn and Fe supply raised the concentrations of N, P and K in both grain and

straw and the pattern were the same for all three nutrients (Table 3). Soil application of ZnSO₄ and FeSO₄ @ 5 kg ha⁻¹ each + foliar spray of ZnSO₄ and FeSO₄ @ 0.5 per cent each at 40 DAS (T₉) gave the higher concentrations in grain and straw: N 1.86 and 0.55 per cent, P 0.35 and 0.14 per cent and K 0.45 and 1.32 per cent, respectively. These values were statistically on par with those of soil application of ZnSO₄ and FeSO₄ @ 2.5 kg ha⁻¹ each + foliar spray of ZnSO₄ and FeSO₄ @ 0.25 per cent each at 40 DAS (T₈; N 1.75 and 0.53 %, P 0.33 and 0.13 %, K 0.43 and 1.29 %) and with the foliar spray of ZnSO₄ and FeSO₄ @ 0.5 % each at 40 DAS (T₇; N 1.69 and 0.49 %, P 0.32 and 0.10 %, K 0.41 and 1.24 %). For K, soil application of ZnSO₄ and FeSO₄ @ 5 kg ha⁻¹ each (T₄; 0.40 and 1.22 %) was also on par with T₉. The control had the lowest concentrations throughout (N 1.45 and 0.40 %, P 0.25 and 0.08 %, K 0.35 and 1.10 %).

Total uptake followed the same order. T₉ removed 66.04 kg N, 14.04 kg P and 76.78 kg K ha⁻¹, against 41.96, 7.64 and 53.22 kg ha⁻¹ in the control, which corresponds to increases of 57, 84 and 44 per cent, respectively. T₈ was on par with T₉ for all three nutrients (59.98, 12.53 and 70.57 kg ha⁻¹). The nutrients were partitioned differently. In T₉, grain held 57 per cent of total N uptake (37.83 of 66.04 kg ha⁻¹) and 51 per cent of total P uptake (7.13 of 14.04 kg ha⁻¹), but only 12 per cent of total K uptake, because K accumulated mostly in straw (67.62 of 76.78 kg ha⁻¹).

Table 3: Nitrogen, phosphorus and potassium content in grain and straw and total uptake by foxtail millet as influenced by agronomic biofortification with zinc and iron.

Treatment	Nitrogen		Phosphorus		Potassium		Total uptake (kg ha ⁻¹)		
	Grain (%)	Straw (%)	Grain (%)	Straw (%)	Grain (%)	Straw (%)	N	P	K
T ₁	1.45	0.40	0.25	0.08	0.35	1.10	41.96	7.64	53.22
T ₂	1.55	0.46	0.28	0.09	0.38	1.18	47.31	8.81	57.52
T ₃	1.52	0.44	0.27	0.09	0.37	1.16	45.83	8.65	56.58
T ₄	1.65	0.48	0.30	0.12	0.40	1.22	52.51	11.02	63.03
T ₅	1.58	0.46	0.28	0.09	0.39	1.19	50.26	9.29	62.57
T ₆	1.55	0.46	0.28	0.10	0.37	1.18	48.79	9.67	60.00
T ₇	1.69	0.49	0.32	0.10	0.41	1.24	54.38	10.43	65.53
T ₈	1.75	0.53	0.33	0.13	0.43	1.29	59.98	12.53	70.57
T ₉	1.86	0.55	0.35	0.14	0.45	1.32	66.04	14.04	76.78
S. Em $\pm$	0.06	0.02	0.01	0.00	0.02	0.04	2.05	0.49	2.38
CD ($P=0.05$)	0.17	0.05	0.03	0.01	0.05	0.13	6.15	1.47	7.14

Treatment details are given in Table 2. S. Em $\pm$, standard error of the mean; CD, critical difference.

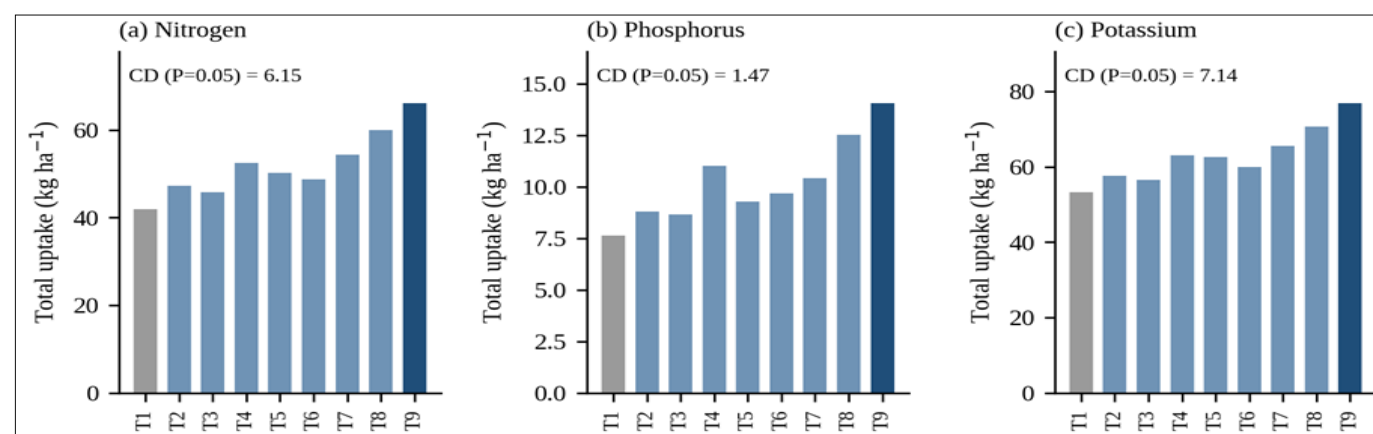


Fig 1: Total uptake of (a) nitrogen, (b) phosphorus and (c) potassium by foxtail millet (grain plus straw) under different methods of Zn and Fe application. The grey bar is the control and the dark bar is T₉.

The enhanced macronutrient uptake with Zn and Fe application may be attributed to their complementary roles in root growth, enzyme activity, nutrient transport and photosynthesis. Zn supports carbohydrate and protein metabolism, ribosome and RNA polymerase function, and root membrane integrity, while Fe is essential for ferredoxin, cytochromes, nitrate and nitrite reductases and chlorophyll synthesis (Marschner, 2012; Hänsch and Mendel, 2009) [9, 17]. Improved N status also promotes Zn and Fe uptake and grain loading (Kutman *et al.*, 2011) [13]. Since the soil was deficient in Zn and Fe and had low available P (19.00 kg ha⁻¹), their application likely improved overall N, P and K utilization without the Zn-induced suppression of P uptake associated with high P ratios. Similar increases in macronutrient uptake following Zn and Fe application were reported by White and Broadley (2009), Qureshi *et al.* (2021) [21, 31] and Krupashree *et al.* (2022) [12]. The non-significant difference between T8 and T9 indicates that nutrient uptake was approaching a plateau, with the higher dose providing little additional benefit.

2. Zinc and Iron Content and Uptake

Zn and Fe concentrations responded strongly to the treatments (Table 4). The highest grain and straw Zn (46.15 and 39.20 mg kg⁻¹) occurred in T₉ and was statistically on par with T₈ (43.62 and 36.31 mg kg⁻¹) and T₇ (41.38 and 35.13 mg kg⁻¹). The control had the lowest values (24.25 and 17.99 mg kg⁻¹). Total Zn uptake rose from 0.12 kg ha⁻¹ in the control to 0.30 kg ha⁻¹ in T₉, a 2.5-fold increase, with T₈ again on par (0.26 kg ha⁻¹). Applying ZnSO₄ alone to the soil (T₂) raised grain and straw Zn significantly (33.61 and 29.39 mg kg⁻¹), whereas soil or foliar FeSO₄ alone (T₃ and T₆) left Zn concentrations within the critical difference of

the control. In other words, Fe supply neither suppressed nor promoted Zn accumulation.

Fe followed a similar order, with a notable difference between tissues. T₉ recorded the highest grain and straw Fe (838.06 and 542.11 mg kg⁻¹), on par with T₈ (830.25 and 534.90 mg kg⁻¹), T₇ (824.03 and 523.41 mg kg⁻¹) and foliar FeSO₄ alone (T₆; 800.97 and 516.71 mg kg⁻¹). Soil application of both salts (T₄; 817.19 and 522.77 mg kg⁻¹) was similarly high and all of these treatments exceeded the control by more than the critical difference. The control had 695.11 mg kg⁻¹ in grain and 291.38 mg kg⁻¹ in straw. Total Fe uptake increased from 2.44 to 4.48 kg ha⁻¹ (84 %) in T₉ and T₈ reached 4.21 kg ha⁻¹. Soil application of ZnSO₄ alone (T₂) did not change grain or straw Fe beyond the critical difference, which again argues against any strong Zn-Fe antagonism at these doses.

The proportional response was far greater in straw than in grain (86 % versus 21 % for Fe in T₉). This is typical of Fe, whose movement into grain is tightly regulated and depends on remobilization from leaves and on phloem transport as Fe-chelate complexes (Kobayashi and Nishizawa, 2012) [11]. The grain was also not starting from a low base, since untreated grain already carried about 695 mg Fe kg⁻¹. Zn followed the same logic in a less extreme form. Grain held only about 30 per cent of the total Zn taken up by T₉ and 38 per cent of the total Fe, so most of the added micronutrient stayed in vegetative tissue. The treatments that performed best are those that gave the plant a second, later opportunity to load grain, namely the foliar sprays at 40 DAS (Cakmak and Kutman, 2018) [4].

The physiology behind the Zn and Fe responses is consistent with the literature. Zn regulates enzymatic reactions, auxin synthesis and membrane permeability, which improves root growth and nutrient acquisition.

Table 4: Zinc and iron content in grain and straw, total uptake and percentage increase in grain concentration over the control, as influenced by agronomic biofortification.

Treatment	Zinc			Iron			Grain gain over control (%)	
	Grain (mg kg ⁻¹)	Straw (mg kg ⁻¹)	Total uptake (kg ha ⁻¹)	Grain (mg kg ⁻¹)	Straw (mg kg ⁻¹)	Total uptake (kg ha ⁻¹)	Zn	Fe
T ₁	24.25	17.99	0.12	695.11	291.38	2.44	–	–
T ₂	33.61	29.39	0.19	727.47	328.24	2.72	38.6	4.7
T ₃	25.28	18.99	0.13	736.35	509.41	3.49	4.2	5.9
T ₄	38.95	34.56	0.23	817.19	522.77	3.90	60.6	17.6
T ₅	37.35	30.01	0.21	781.75	356.06	3.07	54.0	12.5
T ₆	27.49	19.99	0.14	800.97	516.71	3.78	13.4	15.2
T ₇	41.38	35.13	0.24	824.03	523.41	3.98	70.6	18.5
T ₈	43.62	36.31	0.26	830.25	534.90	4.21	79.9	19.4
T ₉	46.15	39.20	0.30	838.06	542.11	4.48	90.3	20.6
S. Em±	1.73	1.39	0.01	28.95	17.99	0.13	-	-
CD (P =0.05)	5.20	4.18	0.02	86.79	53.95	0.39	-	-

Treatment details are given in Table 2. Percentage gain was calculated from the treatment means.

Fe underpins chlorophyll synthesis, photosynthetic electron transport and respiration, thereby sustaining biomass production and nutrient translocation. Zn also promotes root growth, enzyme activation and membrane stability, which can facilitate Fe uptake and use and foliar feeding during the reproductive period further enhances translocation to the developing grain. Our results agree with the findings of Sathisha *et al.* (2019) [23] and Krupashree *et al.* (2022) [12] in foxtail millet, Charishma *et al.* (2022) and Sudeep *et al.*

(2025) [5, 28] in sorghum and White and Broadley (2009) and Qureshi *et al.* (2021) [21, 31] for cereals in general.

3. Grain zinc and iron enrichment over the control

Calculated from the tabulated means, grain Zn in T₉ was 90.3 per cent higher than in the control, followed by T₈ (79.9 %) and T₇ (70.6 %) (Fig. 2a). Grain Fe increased by 20.6, 19.4 and 18.5 per cent in the same three treatments (Fig. 2b). The half-rate combination (T₈) is worth noting: it delivered about 88 per cent of the Zn enrichment and 94 per

cent of the Fe enrichment of T₉ while using half the quantity of both salts in the soil and half the spray concentration. The comparison between application routes is informative. For Zn, foliar spray alone (T₅; +54.0 %) was numerically higher than a soil application of the same salt (T₂; +38.6 %), although the difference was within the critical difference. This is in line with the observation that foliar Zn is often the more efficient route for grain in soils that immobilize applied Zn

(Cakmak, 2008; Alloway, 2008) [1, 3], which is certainly relevant in a clay soil of pH 8.2. For Fe the contrast was clearer. Soil FeSO₄ (T₃) raised grain Fe by only 5.9 per cent, within the critical difference of the control, while foliar FeSO₄ (T₆) raised it by 15.2 per cent, which was significant. Applied Fe²⁺ is oxidized quickly in an aerated alkaline soil and precipitates as ferric hydroxides, so little of it reaches the plant (Lindsay and Schwab, 1982) [16]. A leaf spray avoids that step.

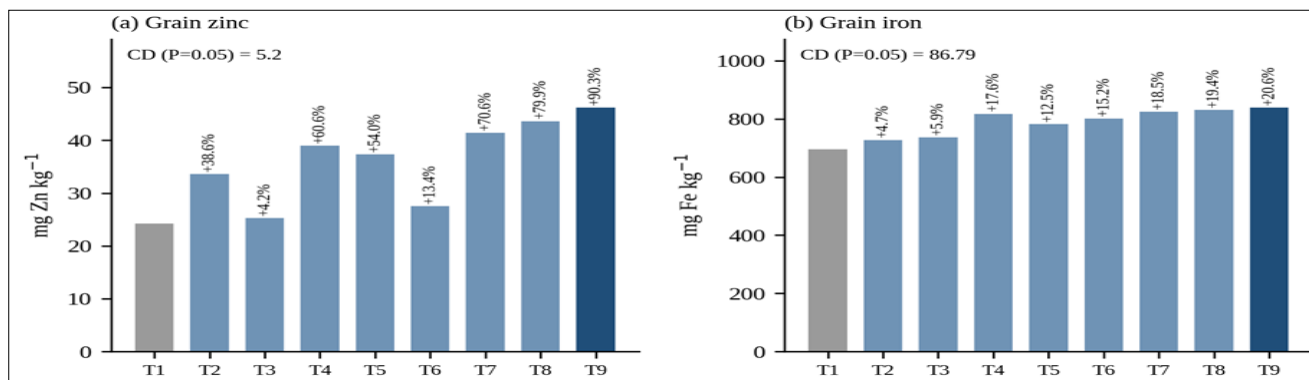


Fig 2: Grain (a) zinc and (b) iron concentrations in foxtail millet under different methods of Zn and Fe application. Values above the bars are percentage increases over the control (grey bar); the dark bar is T₉.

Zn and Fe can compete for uptake and transport sites at excessive concentrations. At the doses used here, however, combined soil and foliar supply produced balanced micronutrient nutrition. The complementary roles of Zn in enzyme activation and root development and of Fe in chlorophyll synthesis and energy metabolism evidently outweighed any competition and both elements accumulated together. This agrees with the mechanisms described by White and Broadley (2009) [31] and the improved growth and uptake reported by Qureshi *et al.* (2021) [21].

4. Soil Reaction, Salinity and Organic Carbon after Harvest

Soil reaction did not change significantly (Table 5). Post-harvest pH ranged from 8.18 in T₄ to 8.26 in the control, around the initial value of 8.20. Zn and Fe sulfates are mildly acidifying, particularly as Fe²⁺ oxidizes and hydrolyzes, but the amounts added were small relative to the

buffering capacity of a clay soil. Electrical conductivity ranged from 0.19 to 0.22 dS m⁻¹ and did not differ among treatments. The addition of soluble sulfate salts may have raised the ionic strength of the soil solution temporarily, but the rates were low, much of the added Zn and Fe was taken up or immobilized and EC stayed within the normal range, with no risk to crop growth. Similar absence of salinity effects has been noted in biofortification studies by White and Broadley (2009), Sathisha *et al.* (2019) [23, 31] and Krupashree *et al.* (2022) [12] in foxtail millet.

Organic carbon was also unaffected statistically. It ranged from 0.47 per cent in the control to 0.52 per cent in T₇, T₈ and T₉, against 0.48 per cent at the start. The numerical advantage of the treatments that produced the most biomass is consistent with greater root and stubble residues, but a single season is rarely long enough to detect a real shift in a slow pool such as soil carbon and no inference is drawn here.

Table 5: Soil pH, EC, organic carbon and available nutrients after harvest of foxtail millet as influenced by agronomic biofortification with zinc and iron.

Treatment	pH	EC (dS m ⁻¹)	OC (%)	Available N (kg ha ⁻¹)	Available P ₂ O ₅ (kg ha ⁻¹)	Available K ₂ O (kg ha ⁻¹)	DTPA-Zn (mg kg ⁻¹)	DTPA-Fe (mg kg ⁻¹)
Initial	8.20	0.18	0.48	363.80	19.00	369.90	0.32	4.18
T ₁	8.26	0.19	0.47	339.07	23.78	328.52	0.27	3.07
T ₂	8.21	0.21	0.48	331.23	22.51	322.66	0.33	3.07
T ₃	8.20	0.22	0.49	333.52	23.04	324.67	0.28	4.11
T ₄	8.18	0.21	0.51	314.82	21.69	316.67	0.32	4.17
T ₅	8.22	0.21	0.50	321.09	22.67	319.34	0.30	3.07
T ₆	8.21	0.19	0.51	320.54	22.80	318.27	0.28	3.41
T ₇	8.22	0.19	0.52	305.84	21.36	312.51	0.31	3.32
T ₈	8.20	0.20	0.52	289.24	20.00	307.10	0.31	3.71
T ₉	8.19	0.21	0.52	285.45	18.57	300.91	0.34	4.21
S. Em±	0.29	0.01	0.02	11.48	0.86	18.50	0.01	0.03
CD (P = 0.05)	NS	NS	NS	34.42	2.57	NS	0.02	0.09

Initial values are those at the start of the experiment (Table 1). NS, non-significant. Treatment details are given in Table 2.

5. Residual Available Nitrogen, Phosphorus and Potassium

Available N, P and K after harvest told a different story from the plant data (Table 5). Residual N was highest in the control (339.07 kg ha⁻¹) and was on par with all treatments except T₈ (289.24 kg ha⁻¹) and T₉ (285.45 kg ha⁻¹), which were significantly lower. Every treatment, including the control, ended below the initial 363.80 kg ha⁻¹, with the decline reaching 21.5 per cent in T₉ and 6.8 per cent in the control. The reason is arithmetic. Crop N removal (41.96 to 66.04 kg ha⁻¹) exceeded the 30 kg N ha⁻¹ applied as fertilizer in every treatment, so the crop was drawing on native soil N and on mineralization from FYM. The better-growing treatments, with greater root development and N uptake, left the least behind. The progressive fall in residual N with more intensive Zn and Fe supply signals greater extraction and, for the crop, better nutrient use efficiency.

Residual P showed the same trend. The control had the highest value (23.78 kg P₂O₅ ha⁻¹), on par with all treatments except T₈ (20.00) and T₉ (18.57). Interestingly, every treatment but T₉ finished above the initial 19.00 kg ha⁻¹, which indicates that the basal 15 kg P₂O₅ ha⁻¹ and the FYM supplied more P than the less responsive treatments removed. Where Zn and Fe supply stimulated root growth and metabolic activity, P demand rose and crop removal consumed the surplus, returning T₉ to roughly its starting level.

Residual K ranged from 300.91 to 328.52 kg K₂O ha⁻¹ and did not differ significantly among treatments. Zn and Fe do not directly alter K availability in soil and the native K-supplying capacity of the soil, together with the basal K dose, kept values similar across treatments. Even so, all values were lower than the initial 369.90 kg ha⁻¹ and K uptake of 53 to 77 kg ha⁻¹ comfortably exceeded the 15 kg K₂O ha⁻¹ applied, so the crop drew on the reserve. The soil remains in the high K range, but repeated seasons of this kind of removal would eventually matter. Similar observations, in which Zn and Fe application affected plant growth and nutrient uptake rather than soil K, were reported in foxtail millet by White and Broadley (2009) and Sathisha *et al.* (2019). Gajbhiye *et al.* (2018)^[7, 23, 31] in maize and Charishma *et al.* (2022)^[5] in sorghum attributed changes in soil nutrient status under Zn fertilization to altered root activity and nutrient cycling.

6. Residual DTPA-Extractable Zinc and Iron

Residual DTPA-extractable Zn was highest in T₉ (0.34 mg kg⁻¹), on par with soil ZnSO₄ alone (T₂; 0.33 mg kg⁻¹) and soil ZnSO₄ plus FeSO₄ (T₄; 0.32 mg kg⁻¹) and lowest in the control (0.27 mg kg⁻¹), which had lost about 16 per cent of its initial 0.32 mg kg⁻¹ (Table 5). Soil-applied ZnSO₄ replenished the plant-available pool and the foliar spray in T₉ reduced the crop's reliance on soil Zn during later growth, conserving the residual pool. The sulfate form is also soluble, which favors availability in the rhizosphere. With no external Zn, the control was simply mined by the crop. Even so, residual Zn in all treatments stayed below the commonly adopted critical limit of about 0.6 mg kg⁻¹, so the soil remained Zn-deficient and the treatments maintained rather than cured the problem. Similar improvements in residual Zn after combined soil and foliar Zn have been reported by Krupashree *et al.* (2022)^[12] in foxtail millet.

DTPA-Fe was likewise highest in T₉ (4.21 mg kg⁻¹), on par with T₄ (4.17 mg kg⁻¹) and lowest in the control (3.07 mg kg⁻¹), a 26.6 per cent decline from the initial 4.18 mg kg⁻¹. Note that every treatment without soil-applied FeSO₄, including all foliar-only treatments, ended between 3.07 and 3.41 mg kg⁻¹. Foliar Fe feeds the plant but does not rebuild the soil pool. The drop in untreated soil reflects removal in harvested biomass and the conversion of soluble Fe into less extractable ferric oxides under aerobic, alkaline conditions (Lindsay and Schwab, 1982)^[16]. Soil FeSO₄ at 5 kg ha⁻¹ offset these losses and only T₉ marginally exceeded the starting value. For farmers, the practical meaning is that soil application maintains the Fe fertility of the field and foliar application targets the grain and each does a job that the other cannot.

7. Practical Implications and Limitations

Three practical points follow. First, the half-rate combination (T₈) was statistically comparable to the full-rate combination for N, P, K, Zn and Fe content and uptake and offers most of the grain enrichment for half the input, which should be considered when economics matter. Second, because the best treatments depleted soil N and P (and K numerically), biofortification should be accompanied by adequate NPK fertilization or integrated nutrient management so that the larger crop does not erode soil fertility over successive seasons. Third, soil and foliar routes are complementary rather than interchangeable. Soil application sustains the residual Zn and Fe pools, while foliar spray is the more efficient way to load grain, especially with Fe.

The conclusions are bounded by the design. The experiment was conducted on one clay soil with a pH of 8.2 and responses may differ on lighter or more acidic soils. Grain Zn and Fe concentrations were measured, but their bioavailability to humans, which depends on phytate and other inhibitors, was not.

Conclusion

Agronomic biofortification with ZnSO₄ and FeSO₄ substantially improved nutrient uptake and grain quality in foxtail millet grown on a Zn- and Fe-deficient, slightly alkaline clay soil. Soil application of both salts at 5 kg ha⁻¹ each plus foliar spray of both at 0.5 per cent at 40 DAS gave the highest content and uptake of N, P, K, Zn and Fe and raised grain Zn by 90 per cent (to 46.15 mg kg⁻¹) and grain Fe by 21 per cent (to 838.06 mg kg⁻¹) over the control. The half-rate combination was statistically comparable for most plant variables and is the more economical option.

The treatments did not disturb soil pH, electrical conductivity or organic carbon. Residual DTPA-Zn and DTPA-Fe were best maintained where Zn and Fe were applied to the soil, whereas the control lost both. Residual N and P were lowest where crop removal was greatest, which means that biofortification should be paired with adequate NPK fertilization to keep soil fertility from declining over successive seasons. Combining soil and foliar application is therefore a practical route to nutrient-dense foxtail millet and, with proper nutrient replacement, to soil-sustaining production on Zn- and Fe-deficient soils.

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Conflict of interest

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