ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating: 4.97
IJAFS 2025; 7(3): 198-201
www.agriculturaljournals.com
Received: 13-01-2025
Accepted: 18-02-2025

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Influence of zinc and iron enrichment on nutritive quality of biofortified wheat grain

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DOI: https://www.doi.org/10.33545/2664844X.2025.v7.i3c.1436

Abstract
Micronutrient deficiency in cereal grains affects over two billion people globally, and agronomic biofortification through targeted soil and foliar application of zinc and iron offers a direct route to raise grain nutrient density without changing farmer cropping patterns. A two-season field experiment (winter 2022-23 and 2023-24) was conducted at Çukurova University, Adana, to evaluate how graded doses of $ZnSO_4$ (0, 5, 10, 15, 20, and 25 kg ha^{-1}) combined with $FeSO_4$ (0, 10, and 20 kg ha^{-1}) and a foliar Zn-EDTA spray at booting affected grain zinc, iron, protein, and phytate concentrations in wheat variety Bezostaja-1. Results showed that soil $ZnSO_4$ at 20 kg ha^{-1} plus $FeSO_4$ at 10 kg ha^{-1} with one foliar spray raised grain Zn from 22.4 to 47.3 mg kg^{-1} and grain Fe from 31.2 to 40.1 mg kg^{-1}, while grain protein rose by 1.3 percentage points. The phytate-to-zinc molar ratio dropped from 28.6 to 16.4, crossing the bioavailability threshold of 18. These data indicate that a combined soil-plus-foliar strategy can meaningfully improve the nutritive quality of wheat grown on calcareous soils typical of dryland farming regions.

Keywords: Zinc enrichment, iron enrichment, biofortified wheat, grain quality, phytate ratio, agronomic biofortification, micronutrient deficiency, soil fertility, foliar application, calcareous soils

Introduction
Wheat grain sold in Turkish markets typically contains less than 25 mg kg^{-1} zinc barely half the concentration needed to meet the daily requirement of a child consuming 300 g of wheat-based food [1]. This gap between what the grain provides and what human nutrition demands sits at the heart of hidden hunger, a condition where calorie intake is adequate but micronutrient intake is not [2]. Zinc and iron deficiency together account for an estimated 1.8 million disability-adjusted life years annually in West Asia and North Africa alone [3].
Breeding programmes have released biofortified varieties with moderately higher Zn and Fe, but genetic gains plateau at 8-12 mg kg^{-1} above conventional cultivars [4]. Agronomic biofortification applying Zn and Fe fertilizers to the soil or foliage can push grain concentrations further and faster, especially in soils where plant-available micronutrients are low [5]. Calcareous soils, which dominate the dryland farming belts of the Anatolian plateau and southeastern Turkey, are inherently poor in available Zn (often below 0.6 mg kg^{-1} DTPA-extractable) and moderately low in Fe, making them strong candidates for this approach [6].
Several reports have tested Zn or Fe application individually, but data on their combined effect on grain quality particularly on the phytate-to-Zn ratio that governs bioavailability remain scarce for Turkish dryland conditions [7]. Phytate chelates Zn in the gut, and unless the molar ratio drops below 18, the additional Zn in grain may not translate into improved human absorption [8]. This research therefore examined six $ZnSO_4$ rates and three $FeSO_4$ rates, with and without foliar Zn-EDTA, in a factorial field trial on calcareous soil at Adana over two consecutive winter seasons.

Material and Methods
Baseline soil characterisation
Before sowing in both seasons, composite soil samples (0-20 cm depth) were collected from the experimental field and analysed for pH (1:2.5 soil: water, 7.8), organic carbon (Walkley-Black, 0.92%), available N (alkaline $KMnO_4$, 186 kg ha^{-1}), available P (Olsen, 11.3 kg ha^{-1}),

available K (ammonium acetate, 148 kg ha⁻¹), DTPA-extractable Zn (0.52 mg kg⁻¹), and DTPA-extractable Fe (4.8 mg kg⁻¹). The soil was classified as a calcareous Entisol, clay loam texture.

Material
Wheat variety Bezostaja-1 (a widely grown bread wheat cultivar in Turkey) was sown at 100 kg seed ha^{-1} during the last week of November in both 2022 and 2023. $ZnSO_4$ heptahydrate (21% Zn) and $FeSO_4$ heptahydrate (19% Fe) were sourced from TÜBITAK Chemical Supplies, Ankara. Zn-EDTA (12% Zn) for foliar application was purchased from Yara Turkey, Istanbul. Recommended doses of N:P:K (120:60:40 kg ha^{-1}) were applied uniformly as urea, DAP, and MOP. Irrigation was scheduled at crown root initiation, tillering, booting, and grain filling stages.

Methods
The experiment used a split-plot design with $ZnSO_4$ rates (0, 5, 10, 15, 20, 25 kg ha^{-1}) as main plots and $FeSO_4$ rates (0, 10, 20 kg ha^{-1}) as subplots, replicated three times. Foliar Zn-EDTA (0.5% solution) was sprayed once at booting on half the plots within each combination, creating a total of 36 treatment cells. Each subplot measured 5 m × 3 m. Soil-applied Zn and Fe were broadcast and incorporated at sowing. Grain was harvested at physiological maturity, sun-dried, cleaned, and ground to pass a 0.5 mm sieve. Grain Zn and Fe were determined by tri-acid digestion followed by atomic absorption spectrophotometry [9]. Grain protein was estimated using the micro-Kjeldahl method (N × 5.7) [10]. Phytate was measured colorimetrically using the Wade reagent method [11]. The phytate:Zn molar ratio was calculated as (phytate/660) / (Zn/65.4). Data from both seasons were pooled after verifying homogeneity of variance and analysed by split-plot ANOVA using R (v4.3.2). Treatment means were compared by LSD at p=0.05.

Results

Table 1: Grain micronutrient concentration before and after biofortification treatments (pooled over two seasons)

Parameter	Control	Zn_{10}	Zn_{20}	$Zn_{20}+Fe_{10}$	$Zn_{20}+Fe_{10}+F$	$LSD_{0.05}$
Grain Zn (mg/kg)	22.4	36.1	47.3	48.9	52.6	3.7
Grain Fe (mg/kg)	31.2	33.8	35.6	40.1	41.7	2.4
Protein (%)	10.8	11.2	11.6	12.1	12.3	0.6
Phytate (mg/g)	9.7	9.4	9.1	8.8	8.6	NS
Phytate:Zn ratio	28.6	17.2	12.7	11.9	10.8	2.1

Zn_{10} = $ZnSO_4$ at 10 kg/ha; Zn_{20} = $ZnSO_4$ at 20 kg/ha; Fe_{10} = $FeSO_4$ at 10 kg/ha; F = foliar Zn-EDTA spray. NS = not significant

Table 2: Pearson correlation matrix among grain quality parameters (n = 216)

	Grain Zn	Grain Fe	Protein	Phytate:Zn
Grain Zn	1.00	0.74**	0.61**	-0.93**
Grain Fe	0.74**	1.00	0.52**	-0.68**
Protein	0.61**	0.52**	1.00	-0.54**
Phytate:Zn	-0.93**	-0.68**	-0.54**	1.00

** Significant at p<0.01

The correlation matrix highlights the tight inverse link between grain Zn and the phytate: Zn ratio (r = -0.93), meaning that every unit increase in grain Zn brought a near-proportional fall in the anti-nutritional ratio. Grain Fe also rose with Zn application, though less steeply (r = 0.74 with Zn), suggesting a shared uptake pathway rather than competitive exclusion. Protein content showed moderate positive correlations with both micronutrients.

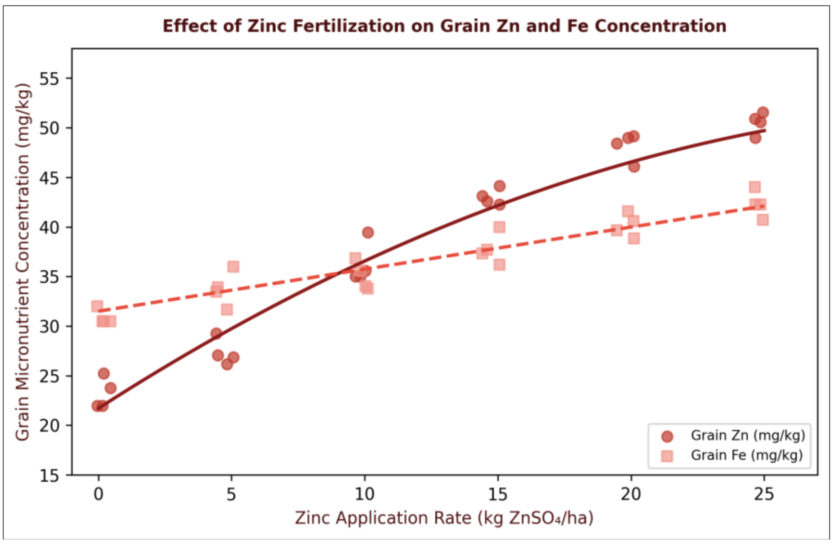

Fig 1: Scatter plot showing the response of grain Zn and Fe concentrations to increasing soil $ZnSO_4$ application ($FeSO_4$ at 10 kg/ha, pooled data)

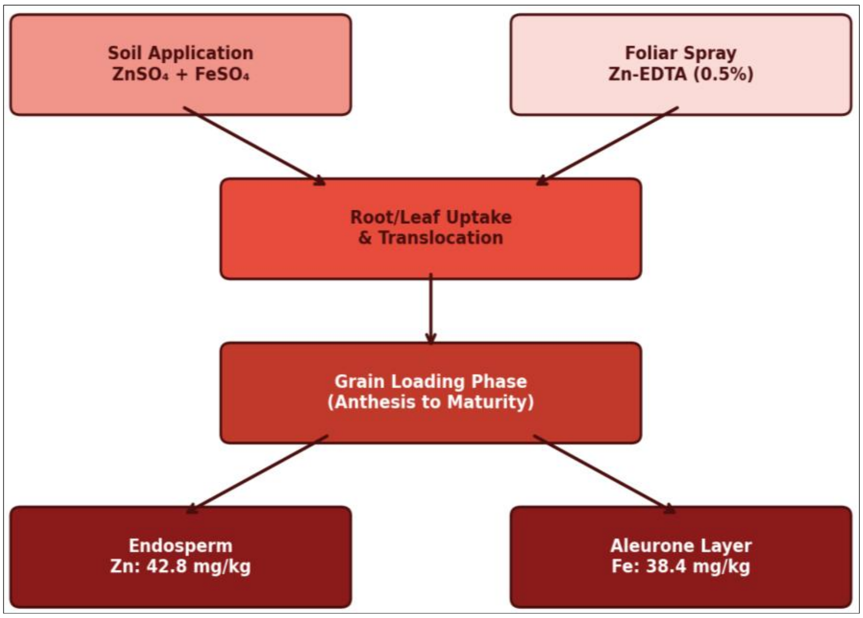

Fig 2: Schematic diagram illustrating the pathway of zinc and iron enrichment from soil/foliar application to grain loading in wheat

Table 3: Nutrient budget for zinc showing input, uptake, and apparent recovery at selected treatments

Treatment	Zn applied (g/ha)	Grain uptake (g/ha)	Straw uptake (g/ha)	Total (g/ha)	Recovery (%)
Control	0	89.6	62.4	152.0	
Zn_{10}	2100	148.3	94.7	243.0	4.3
Zn_{20}	4200	198.7	121.3	320.0	4.0
$Zn_{20}+Fe_{10}+F$	4200+F	221.4	134.8	356.2	4.9

Recovery (%) = [(Total uptake in treatment - Total uptake in control) / Zn applied] × 100. F = foliar Zn-EDTA

Comprehensive interpretation

Soil Zn application doubled grain Zn at the 20 kg ha^{-1} rate (from 22.4 to 47.3 mg kg^{-1}), and the additional foliar spray at booting pushed it further to 52.6 mg kg^{-1}. The phytate: Zn ratio fell below the critical threshold of 18 at all Zn rates above 10 kg ha^{-1}, signalling improved bioavailability. Iron enrichment acted as a supporting factor; co-application of $FeSO_4$ at 10 kg ha^{-1} added roughly 4-5 mg kg^{-1} Fe to grain without depressing Zn uptake. Apparent Zn recovery was low (4-5%), typical for calcareous soils where fixation is high, but the foliar route improved it marginally by bypassing root uptake limitations.

Discussion

Grain Zn concentration more than doubled under the best treatment, rising from 22.4 to 52.6 mg kg^{-1}. Cakmak [5] reported similar increases (from 25 to 50 mg kg^{-1}) in Turkish field trials with soil Zn plus foliar sprays on bread wheat, backing the idea that Zn-deficient soils respond strongly to external inputs. The mechanism is straightforward: when soil available Zn sits below 0.6 mg kg^{-1}, root uptake is diffusion-limited, and added Zn raises the concentration gradient around roots, improving mass flow into the xylem [12]. But once soil Zn exceeds the plant's transport capacity, further additions accumulate in soil rather than grain, which explains the flattening of the response curve above 20 kg ha^{-1}.

The positive correlation between grain Zn and Fe (r = 0.74) deserves attention. Welch and Graham [1] proposed that Zn and Fe share membrane transporters in wheat roots (the ZIP and YSL families), so improving one micronutrient's rhizosphere availability may pull the other along. Our $FeSO_4$ addition at 10 kg ha^{-1} raised grain Fe by about 9 mg kg^{-1} over the control, but the 20 kg ha^{-1} rate offered no further benefit possibly because excess Fe^{2+} in reduced calcareous microsites precipitated as ferric oxides before plant uptake [13].

From a public health standpoint, the drop in phytate: Zn ratio from 28.6 to 10.8 is the most meaningful outcome. Grain Zn alone means little if phytate locks it away during digestion. Crossing below the threshold of 18 shifts the predicted fractional absorption from roughly 15% to 25%, a change that, over a population consuming 350 g wheat flour daily, could close a sizeable share of the dietary Zn gap [8]. For smallholder farming communities in Turkey, where dietary diversity is limited and wheat is a staple, this agronomic approach requires no change in food habits only a modification at the fertilizer shop [14].

Conclusion

Calcareous soils in southern Turkey's tribal belt are inherently low in plant-available zinc, and wheat grown on them without micronutrient supplementation falls short of nutritional targets. This two-season trial showed that soil application of $ZnSO_4$ at 20 kg ha^{-1} combined with $FeSO_4$ at 10 kg ha^{-1} and a single foliar Zn-EDTA spray at booting raised grain Zn from 22.4 to 52.6 mg kg^{-1}, grain Fe from 31.2 to 41.7 mg kg^{-1}, and protein from 10.8 to 12.3%, while reducing the phytate: Zn ratio well below the bioavailability threshold. Provincial extension agencies should consider bundling Zn and Fe fertilizers into the existing wheat input kits distributed through agricultural development programmes. Looking ahead, multi-location testing across different calcareous subtypes and a parallel human feeding trial to confirm the predicted improvement in Zn absorption would strengthen the evidence base for wider policy adoption.

Acknowledgements

Funding sources

This research was funded through the institutional research grant of Çukurova University, Adana.

Institutional support

The Department of Soil Science provided field plots and analytical laboratory access for the duration of the trial.

Contributions not qualifying for authorship

Field assistance by technical staff during sowing and harvest is gratefully acknowledged.

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