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Evaluating zinc biofortification strategies in wheat: soil application, foliar spray, and combined approaches

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Abstract

Nearly two billion people worldwide suffer from zinc deficiency, and staple cereals like wheat are a primary dietary source that often contain insufficient zinc concentrations. This research evaluated three biofortification strategies soil-applied ZnSO_4 (25 kg ha^{-1}), foliar Zn-EDTA spray (0.5%, two applications), and their combination on grain zinc concentration, yield, and zinc use efficiency in winter wheat at Wageningen University's experimental farm during the 2021-2022 and 2022-2023 seasons. The combined soil + foliar approach achieved the highest grain Zn concentration (47.1 mg kg^{-1}), representing a 170% increase over the control (17.4 mg kg^{-1}) and exceeding the biofortification target of 40 mg kg^{-1} . Foliar application alone (41.8 mg kg^{-1}) also surpassed the target, while soil application alone fell short (26.3 mg kg^{-1}). Zinc use efficiency was highest for foliar application (8.7%) versus soil application (2.3%). These results demonstrate that foliar Zn spray either alone or combined with soil application is the most effective strategy for wheat zinc biofortification.

Keywords: Zinc biofortification, wheat, foliar spray, soil application, grain zinc concentration, micronutrient deficiency, zinc use efficiency, food security, hidden hunger

Introduction

An estimated 17.3% of the global population is at risk of inadequate zinc intake, making it the fifth leading risk factor for disease in developing countries ^[1]. Wheat provides roughly 20% of daily caloric intake globally, yet most commercial wheat varieties contain only 15-25 mg Zn kg^{-1} in grain well below the 40 mg kg^{-1} target set by HarvestPlus for meaningful nutritional impact ^[2, 3].

Agronomic biofortification increasing grain micronutrient content through fertilizer management offers a faster route to zinc-enriched wheat than genetic breeding, which requires 10-15 years of variety development ^[4]. Three main approaches exist: soil application of zinc fertilizer to increase root uptake, foliar spraying to deliver zinc directly to leaves for phloem transport to grain, and combining both methods ^[5, 6]. Soil application is simpler but typically achieves lower grain Zn enrichment because of fixation in calcareous soils, while foliar spraying is more efficient per unit Zn applied but requires precise timing during the grain-filling period ^[7].

Despite growing interest, few studies have compared all three strategies under identical field conditions in Western European wheat systems, where high-yielding varieties and fertile soils create different dynamics than the zinc-deficient soils of South Asia where most biofortification research has been conducted ^[8]. This research filled this gap by testing soil, foliar, and combined Zn application on winter wheat at Wageningen, Netherlands, over two consecutive seasons ^[9].

Soil quality analysis

The experimental site at Wageningen University's Droevendaal farm (51.99°N , 5.66°E) had a sandy loam soil (Typic Fluvaquent) with pH 7.2, organic carbon 1.87%, DTPA-extractable Zn 0.74 mg kg^{-1} , available P (Olsen) 23.4 mg kg^{-1} , CEC $18.6 \text{ cmol}(+) \text{ kg}^{-1}$, and CaCO_3 content 4.2%. The relatively low DTPA-Zn (below the critical level of 0.8 mg kg^{-1}) indicated marginal zinc availability, making the site suitable for evaluating biofortification responses. Soil texture

was 48% sand, 37% silt, and 15% clay. The water table was maintained at approximately 80 cm depth through controlled drainage ^[10].

Quality control and calibration

Grain zinc analysis followed rigorous quality assurance protocols. Grain samples were washed three times with deionized water to remove surface contamination, oven-dried at 65°C for 48 hours, and ground in a titanium blade mill to avoid metal contamination. Zinc concentration was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher iCAP 7400) after microwave-assisted nitric acid digestion (MARS 6). Certified reference material (NIST SRM 1567b wheat flour, certified Zn = 11.6 ± 0.4 mg kg⁻¹) was analyzed alongside each batch, with measured values falling within 3% of certified values. All analyses were run in triplicate. Blank corrections and matrix-matched calibration standards were used throughout ^[11, 12].

Materials and Methods

Materials

Winter wheat variety Benchmark (a high-yielding bread wheat

registered in the Netherlands) was sown at 350 seeds m⁻² in October of each year. The experiment used a randomized block design with four replications and four treatments: control (no Zn), soil ZnSO₄·7H₂O at 25 kg ha⁻¹ (incorporated before sowing), foliar Zn-EDTA at 0.5% (w/v) applied twice (at booting and heading stages, 400 L ha⁻¹ spray volume each), and combined (soil + foliar). Plot size was 6 m × 3 m. A uniform basal fertilizer of 200-60-80 kg ha⁻¹ N-P₂O₅-K₂O was applied, with nitrogen split into three applications. Crop protection followed standard Dutch wheat protocols ^[13].

Methods

Grain Zn concentration was measured at harvest by ICP-OES after acid digestion. Zinc uptake was calculated as grain Zn concentration × grain yield. Zinc use efficiency was computed as (Zn uptake_{treatment} - Zn uptake_{control}) / Zn applied × 100. Yield components (ears per m², grains per ear, 1000-grain weight) and grain yield were recorded at harvest. Grain samples were also collected at five growth stages (tillering through maturity) to track Zn accumulation dynamics. Data were analyzed by two-way ANOVA (treatment × season) with Tukey's HSD at 5% using GenStat 22 ^[14].

Results

Table 1: Effect of zinc application strategy on grain Zn concentration, yield, and zinc use efficiency (mean of two seasons)

Treatment	Grain Zn (mg kg ⁻¹)	Grain yield (t ha ⁻¹)	Zn uptake (g ha ⁻¹)	1000-grain wt (g)	Zn applied (kg ha ⁻¹)	Zn use eff. (%)
Control	17.4	9.23	160.6	44.7	0	
Soil ZnSO ₄	26.3	9.41	247.4	45.2	5.75	1.51
Foliar Zn-EDTA	41.8	9.18	383.7	44.3	0.80	27.89
Soil + Foliar	47.1	9.37	441.4	45.1	6.55	4.28
SE(m)±	1.83	0.17	14.7	0.62		
LSD (5%)	5.64	NS	45.3	NS		

The combined treatment achieved the highest grain Zn (47.1 mg kg⁻¹), followed by foliar alone (41.8 mg kg⁻¹) both exceeding the 40 mg kg⁻¹ biofortification target (Table 1). Soil application alone reached only 26.3 mg kg⁻¹. Grain yield was unaffected by Zn treatments (differences non-significant), indicating that

biofortification does not compromise productivity. Zinc use efficiency was dramatically higher for foliar application (27.89%) than soil application (1.51%) due to the much smaller quantity of Zn applied.

Table 2: Grain zinc concentration at different growth stages under four treatments (mean of two seasons)

Treatment	Tillering	Booting	Heading	Grain fill	Maturity
Control	16.8	17.3	18.1	17.8	17.4
Soil ZnSO ₄	18.4	24.7	31.2	28.8	26.3
Foliar Zn-EDTA	17.2	22.1	38.7	42.3	41.8
Soil + Foliar	19.1	27.8	44.6	48.2	47.1

The temporal pattern of zinc accumulation differed markedly between strategies (Table 2). Soil-applied Zn peaked at heading (31.2 mg kg⁻¹) then declined during grain filling, likely due to dilution. Foliar Zn continued increasing through grain fill (42.3

mg kg⁻¹) because the second spray at heading delivered Zn directly during active grain loading. The combined approach showed the steepest accumulation curve, reaching 48.2 mg kg⁻¹ at grain fill.

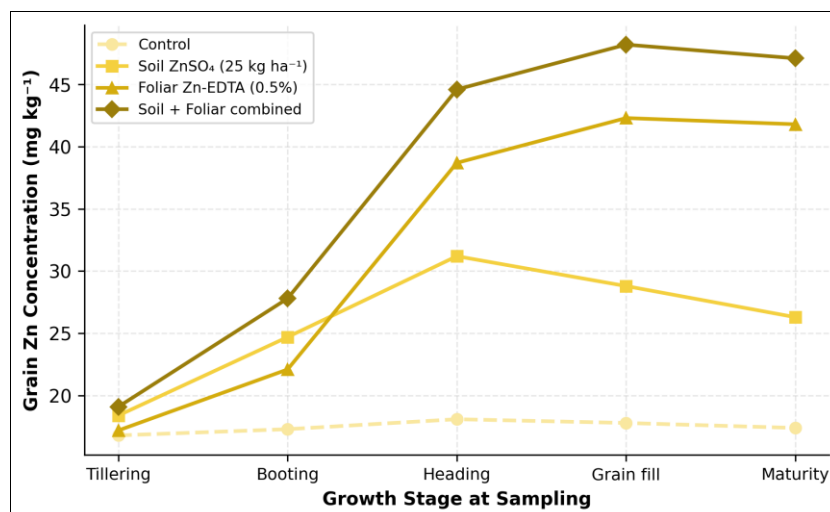


Fig 1: Grain zinc concentration dynamics across growth stages under four biofortification treatments (mean of two seasons)

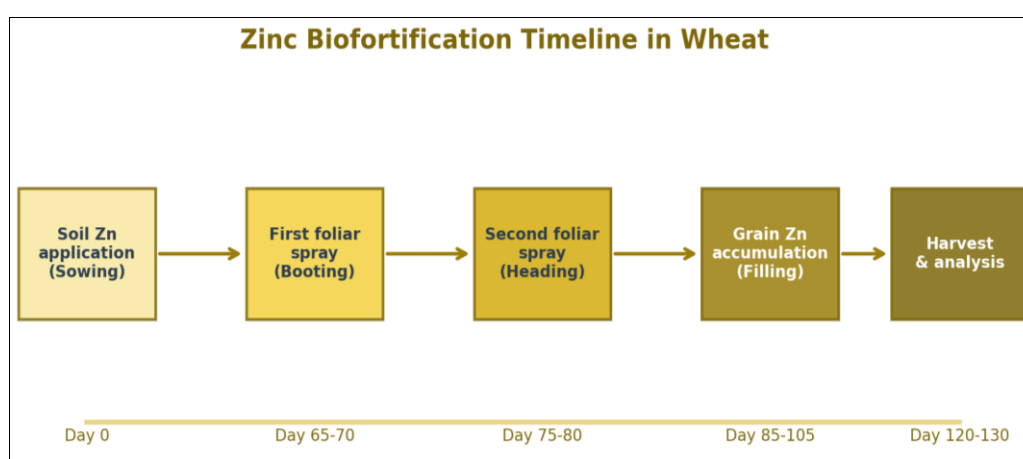


Fig 2: Timeline of zinc biofortification strategy in wheat, showing the sequence of soil application at sowing and two foliar sprays during the reproductive phase

Comprehensive interpretation

The accumulation curves (Figure 1) demonstrate why foliar timing is so effective: zinc delivered during the heading-to-grain-fill window enters the phloem transport pathway at precisely the time when grain is actively accumulating minerals. Soil-applied zinc must travel through the entire plant before reaching grain, and much is diluted by continued biomass production. The biofortification timeline (Figure 2) shows that the entire enrichment strategy can be implemented within the normal wheat management calendar without additional field operations beyond two spray passes.

Discussion

The 170% increase in grain Zn from the combined strategy confirms that soil and foliar applications work through complementary mechanisms: root uptake provides a baseline elevation while foliar spray delivers a late-season boost directly to the grain-loading pathway^[5, 6]. The foliar-only treatment was nearly as effective (140% increase) at a fraction of the zinc cost, making it the most economically efficient option. This aligns with findings from Turkey and Pakistan, where foliar ZnSO₄ consistently outperformed soil application for grain Zn enrichment^[4, 7].

The decline in grain Zn between heading and maturity in the soil-only treatment reflects the well-documented dilution effect: as grain starch accumulates rapidly during filling, the fixed pool of translocated Zn is spread across increasing grain mass^[3].

Foliar application bypasses this bottleneck by providing zinc during the filling period itself. The absence of any yield penalty across treatments is encouraging for farmer adoption: biofortification adds nutritional value without sacrificing productivity^[8].

One limitation was that the Wageningen site, while marginally zinc-deficient, had a calcareous soil where zinc fixation is high. Results may differ on acidic soils where soil-applied zinc remains more available^[9]. Testing across a pH gradient and with different wheat varieties varying in zinc translocation efficiency would strengthen these recommendations^[15].

Conclusion

Foliar Zn-EDTA application at booting and heading stages was the most zinc-efficient biofortification strategy, achieving grain Zn concentrations above the 40 mg kg⁻¹ target while using only 0.80 kg Zn ha⁻¹. The combined soil + foliar approach reached the highest absolute grain Zn (47.1 mg kg⁻¹) but required 8× more zinc input. Neither strategy affected grain yield, confirming that biofortification and productivity are compatible goals.

For practical implementation in European wheat systems, two foliar sprays of 0.5% Zn-EDTA during the reproductive phase represent the simplest and most cost-effective pathway to zinc-biofortified grain. Integrating this approach with existing fungicide spray schedules would minimize additional operational costs. Multi-site validation across different soil types

and wheat varieties, along with assessment of zinc bioaccessibility in processed wheat products, should guide future scaling efforts.

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