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# Composition of nutrients, phytochemicals and anti-nutrients in agronomically biofortified finger millet (*Eleusine coracana* (L.) Gaertn) grains

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**Abstract**
Micronutrient intake deficiencies is a public health concern that can be alleviated by enhancing the nutrient's content in the edible plant parts. A study was carried out to determine the effect of soil application with iron and zinc sulphate fertilizers, on the nutritional composition, phytochemicals and anti-nutrients in the harvested finger millet (*Eleusine coracana* (L.) Gaertn) grains. Field experiments using improved variety of finger millet – P224, were piloted in *Tharaka Nithi* County, Kenya, during the short rainy season of 2022-2023. Five soil-applied fertilizer treatments (T1 – nitrogen, phosphorus and potassium (NPK)+calcium ammonium nitrate (CAN), T2 – manure+ZnSO4, T3 – manure+FeSO4, T4 – NPK+CAN+FeSO4, T5 – NPK+CAN+ZnSO4 and T6 – Control) were replicated three times in a randomized complete block design. After harvesting, a sample of 200g was taken from each replicate and proximate composition, minerals, total carotenoids (TC), total phenols (TP), total flavonoids (TF), phytates, tannins and oxalates were determined using standard laboratory methods. Sample preparation and analyses were done in the Food Biochemistry Laboratory, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. Results showed that iron and zinc agronomic biofortification had no significant effect ($P \leq 0.05$) on the grains proximate composition recording mean moisture content, crude ash, crude protein, crude fat and total carbohydrates of 10.06%, 2.68%, 10.56%, 1.26% and 75.45% respectively on a dry weight basis. Notably, T3 and T4 had a significant effect ($P \leq 0.05$) on grain iron concentration recording an increase of 78% and 67% respectively as compared to the control (T6). T2 and T5 also gave 7.4% and 8.9% increase of grain zinc content respectively. Total carotenoid was highest at 4.04µg/g±0.62 with the application of T5. Total polyphenols differed significantly ($P \leq 0.05$) with the various treatments applied. Total flavonoid was highest in T4 and T3 at 250.9mg/100g±24.2 and 249.6mg/100g±7.4 respectively, and was lowest in T1 at 176.6mg/100g±17.9. There was 19.92% reduction of phytates with the application of T5 as compared with T6 (control) and this may help improve mineral bioavailability. The lowest tannin content of 94.3mg/100g±11.7 was given by T1. None of the five treatments lowered the oxalate content in this experiment. The results showed that iron and zinc agronomic biofortification of finger millet has the potential to improve the micronutrients status of this crop which is an important strategy to avert micronutrient deficiencies (MNDs) and global food insecurity.

**Keywords:** Agronomic biofortification, Finger Millet, fertilizer application, micronutrient deficiencies, nutrients, anti-nutrients, phytochemicals

## Introduction

Plants are key in supporting human nutrition, either directly or indirectly (Oliver & Gregory, 2015) [33]. Micronutrient deficiencies (MNDs) also known as 'hidden hunger' resulting from unbalanced diets based on starchy staple crops are prevalent among populations around the world, affecting more than 2 billion people globally (Food and Agriculture Organization-FAO, 2014; Bouis & Saltzman, 2017) [12, 52]. Zinc and Iron are widespread crop yield-limiting factors and are also among the most prevalent deficiencies in humans (de Valença *et al*., 2017) [12]. Iron deficiency is responsible for several health complications including anemia and impairments in the endocrine and immune systems (Nissar *et al*., 2019) [31]. Zinc has a wide range of physiological functions in biological systems where about 10% of all the proteins in the human body, corresponding to nearly 3000 proteins, are Zinc-dependent (Chasapis *et al*., 2020) [9]. Plants require both macro and micro-nutrients to sustain their metabolic processes at any stage of growth. Inadequate supply of nutrients results in nutrient deficiency which impacts negatively on plants growth, yield and quality (Marschner *et al*., 2011) [29].

Finger millet is a crop adaptable to diverse agro-ecological conditions where other cereals would perform dismally (Knox *et al*., 2012) [24]. It has beneficial nutritional properties comparable to other members of Poaceae family such as maize, rice, and wheat, and outstanding agronomic features as a subsistence food crop. It has a substantial amount of calcium and other micronutrients. Devi *et al*., (2014) [13] reported that finger millet is more nutritious compared to other types of millets as it has a high mineral and proximate content. The protein profile of finger millet is equally well-balanced. It has high amounts of lysine, threonine, and valine (Ravindran, 1991) [42]. Other important amino acids contained in finger millets are isoleucine, leucine, methionine, and phenyl alanine which are deficient in other starchy meals (Verma and Patel, 2013) [60]. Finger mill*et al*so contain B vitamins, especially B6, folic acid and niacin. It is a rich source of carbohydrates with low glycemic index, which is beneficial for prevention of certain life-style diseases such as diabetes and cardio- vascular diseases (Srivastava and Sharma, 2012) [57]. Finger millet grain is gluten free with very low fat content which aids in stability of the grains (Ekta Singh and Singh E. 2016) [14].

Agronomic biofortification, implies the application of essential mineral micronutrient fertilizers to soils and/or plant leaves and is reported to increase micronutrient content in edible parts of crops during plant growth (de Valença *et al*., 2017) [12]. The cereal crops dominate diets worldwide, sustaining daily calorie intake, especially among the low and middle-income population. Genetic biofortification has been approved to be the most sustainable and cost-effective approach useful in improving nutrient concentration in grains. The breeding approach is, however, a long-term process which requires considerable resources. Agronomic biofortification on the other hand is a short term approach that offers a rapid solution to the problem, and represents useful complementary approach to other breeding programs. It therefore provide a feasible means of reaching malnourished populations who inadequately access dietary diversification, micronutrient supplementation, modification of food choices, and fortified foods (Saltzman *et al*., 2013) [48].

Agronomic biofortification of major cereals such as maize, rice and wheat with varying nutrients has resulted in significant increase in nutritional quality of the harvested grains (Joy *et al*., 2015) [23]. However, there is limited information on Zn and Fe agronomic biofortification of finger millet and its effects on grains nutritional composition, phytochemical and anti-nutrients composition. This study therefore aimed at determining quality characteristics of finger millet grains agronomically biofortified with Zinc and Iron fertilizers.

## Materials and Methods

### 1. Study site and experimental design

Agronomic biofortification of Finger Millet experiments with Zinc and Iron fertilizers was separately carried out in farmers' field in *Tharaka Nithi* County, Kenya, during 2022-2023. An improved finger millet variety -P224 was used for this experiment and 5 soil-applied fertilizer treatments were applied (T1 – nitrogen, phosphorus and potassium (NPK)+calcium ammonium nitrate (CAN), T2 – manure+ZnSO4, T3 – manure+FeSO4, T4 – NPK+CAN+FeSO4, T5 – NPK+CAN+ZnSO4, T6 – Control). The experiment was laid out in randomized complete block design (RCBD) with three replications (The agronomic work has been reported elsewhere).

### 2. Sample collection and preparation

Finger millet crop was manually harvested at physiological maturity stage, sun-dried, threshed and winnowed to obtain clean grains free from foreign matter. Approximately 200g of each replicate sample were packed in sample bags, labeled and transported to Food Biochemistry Laboratory, Jomo Kenyatta University of Agriculture and Technology, (JKUAT) for analysis. The whole-grain samples were milled using heavy duty Professional Blender (omniblend1 Model: TM-767), Tokyo, Japan and the milled flour was sieved using 1.00mm aperture test sieve, packed in airtight sample bags and stored appropriately in cool, dry place for subsequent analyses.

## Analysis of samples

### 1. Proximate composition analysis

Moisture, protein, crude fat, total ash, and total carbohydrates were determined using standard procedures of analysis (AOAC, 2004) [46].

**Moisture:** Moisture content was determined by the oven-drying method. Approximately 5g sample was weighed in dry aluminum moisture dishes, dried at 105°C for three hours and removed, cooled and weighed. Percent moisture was determined using the formula below;

$$Moisture\ \% = \frac{Sample\ weight\ before\ drying - Sample\ wieight\ after\ drying}{Sample\ weight\ before\ drying}\ x\ 100$$

**Crude protein:** Protein was determined using semi micro Kjeldahl method. Approximately 1g sample was weighed into a digestion flask together with a combined catalyst of 0.5g of copper sulphate and 5g potassium sulphate and 15ml of concentrated Sulphuric acid added. The mixture was heated in a fume chamber till the digest colour turned blue, an indication of completed digestion process. The digest was cooled, transferred to 100ml volumetric flask and topped to the mark with distilled water. A blank digestion with the catalyst was also made. About 10ml of the diluted digest was transferred into the distillation flask and washed with distilled water. 15ml of 40% (w/w) Sodium hydroxide (NaOH) was added and washed with distilled water. Distillation was done to a volume of 60ml distillate. To 10ml. of the distillate was added mixed indicator and titrated with 0.02N HCl until the colour changed to orange signifying titration end-point. Crude protein (%) was determined by multiplying % nitrogen (N) by a conversion factor of 6.25.

$$\text{Nitrogen}\ \% = (V - B)\ x\ 0.02\ x\ N\ x\ f\ x\ 0.014\ x\ \frac{100}{v}\ x\ \frac{100}{S}$$

Where V= Sample titer volume of 0.02N HCl; B= Blank titer volume of 0.02N HCl; N=Normality of standard HCL solution (0.02N); f= Factor of standard HCL solution, v= Volume of diluted digest taken for distillation (10ml); S= Weight of the sample taken (g); 0.014= Milli-equivalent weight for Nitrogen

$$\text{Protein}\ \% = Nitrogen\ \%\ x\ 6.25$$

**Crude fat:** Crude fat was determined by the Soxhlet continuous extraction method. Approximately 5g sample was weighed in cellulose extraction thimbles, plugged with defatted cotton wool and inserted in to the Soxhlet extraction apparatus. The pre-weighed receiver flask was half-filled with analytical grade petroleum ether (B.P. 40 – 60°C) and connected to the Soxhlet extraction apparatus. Extraction was done continuously for 8hours and then solvent evaporated using a rotavapour. Crude fat (%) was determined using the following formula;

$$\text{Fat}\ \% = \frac{weight\ of\ fat\ extracted}{weight\ of\ sample}\ x\ 100$$

**Crude ash:** Total ash was determined by dry ashing method. About 5g of the sample was charred by heating on a hot plate in fume chamber till smoking stopped. The charred samples were then incinerated in a muffle furnace at 550C until the ash turned grayish. The samples were then cooled in a desiccator before weighing again.

$$\text{Total ash \%} = \frac{ash\ weight}{sample\ weight}\ x\ 100$$

**Total carbohydrate content:** This was determined by the difference.

$$Total\ available\ carbohydrates\ \% = 100 - (moisture + fat + protein + total\ ash)\%$$

**2. Determination of mineral content**

Minerals (Fe, Zn, Ca, P, K and Mg) were determined by the dry ashing method and atomic absorption spectrophotometry (AAS), AOAC, (2005). About 5g of samples were incinerated at 550˚C for 6 hours. The ash was quantitatively transferred to a 100 ml beaker using 20 ml of 0.5N HNO3, then heated at 80-90˚C on a hot plate for 5 minutes. The contents were transferred to a 100 mL volumetric flask and filled to the mark using 0.5N HNO3. The insoluble matter was filtered and the filtrate used to read absorbance by the AAS (Shimadzu AA-7000) to determine Fe, Zn, Ca and Mg. Phosphorus (P) content was measured spectrophotometrically at 420nm using UV-Vis-1800 Shimadzu, Tokyo Japan. Potassium (K) was determined using Wagtech Flame Photometer Model AE-SFP401. Various mineral standards were prepared to make the calibration curve used for calculation of the actual mineral concentration.

**3. Determination of Phytochemicals**

The phytochemicals analyzed in the biofortified grains were total flavonoids, total polyphenols and total carotenoids.

**Sample extraction for determination of total phenolic (TP) and total flavonoids (TF) contents**

Sample extraction for the analysis of TP and TF was done as described by Waterman and Mole, (1994) and Harborne (1998) [19], respectively with modifications suggested by Barros *et al*., (2007) [4]. Five grams of the samples were weighed into a conical flask and 100 mL methanol added. The flasks were closed using parafilm and covered with aluminum foil to keep off the light. The samples were put in a shaker, shaken for 3 hours and then kept in the dark to extract for 72 hours. The samples were then filtered through Whatman filter paper No.4, and the filtrate topped up to 100ml using methanol, centrifugation followed at 4500rpm for 10minutes at 25˚C.

**Determination of total phenol (TP) content.**

Total phenolic of the sample extracts were estimated by Folin-Ciocalteu (FC) method. Briefly, 1mL extract was micro-filtered into a test tube, and 2mL of 10% Folin-Ciocalteu reagent added and mixed well. To the mixture, 4mL of 0.7M Sodium Carbonate was added and vortexed. The mixture was allowed to stand at room temperature for 2 hours to develop the colour and absorbance measured at 765nm using a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The absorbance values were compared with those of known gallic acid concentrations and the total polyphenol content expressed as mg equivalent gallic acid per 100g of sample taken.

**Determination of Flavonoids**

***Qualitative analysis***

A preliminary qualitative analysis was done to test for the presence of flavonoids. Five mL of dilute ammonia solution was added to a small quantity of aqueous filtrate of extract, followed by addition of concentrated sulphuric acid ($H_2SO_4$). A yellow coloration was observed which indicated the presence of flavonoids. The yellow coloration disappeared on standing.

**Quantitative analysis**

Quantitative determination of flavonoids was done by the aluminum chloride colorimetric method (Jagadish *et al*., 2009) [22]. To a 10ml volumetric flask, 1ml of the sample extract and 4ml distilled water was added. After 3 minutes, 0.3 mL of 5 % sodium nitrite solution was added, followed by addition of 0.3 mL of 10 % aluminum chloride after another 3 minutes. After 5 minutes, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with distilled water. Absorbance was measured at 415nm using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Total flavonoids were calculated from the calibration curve of the standard solution prepared from quercetin.

**Determination of total carotenoids (TC)**

Total carotenoids was determined by UV-Vis Spectrophotometric method (Rodriguez-Amaya & Kimura, 2004) [46]. Approximately 2g sample was mixed with 10 ml cold acetone and ground with pestle and mortar. The extract was filtered and the extraction repeated till colorless with about 50ml acetone. The extracts were pooled and mixed with 50 ml petroleum ether in a separating funnel. Acetone was removed by washing with cold water about 2-3 times. The petroleum ether layer with carotenoids was separated and dried with anhydrous sodium sulphate and made up to 100ml with petroleum ether. The absorbance was measured at 450nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) and the total carotenoids content was calculated based on the molar extinction co-efficient of β-carotene.

$$\text{Total carotenoids μg/g} = Abs\ x\ Volume\ of\ extract\ x\ \frac{10^4}{A^{1\%}}\ X\ \frac{1}{sample\ weight}$$

where, Abs= absorbance; volume = total volume of extract, $A^{1\%}$ = molar absorptivity of total carotenoids in petroleum spirit = 2592

**4. Anti-nutrients composition**

The anti-nutrients tested were phytates, tannins and oxalates.

**Determination of tannin content**

Condensed tannins was assayed by the vanillin-hydrochloric acid method as described by Price *et al*., (1978); Liang, (2006) [27, 38]. Quarter-gram of ground sample was extracted with 10 ml of 4% HCl in Methanol by shaking for 20 minutes and then separated using a centrifuge at 4,500 rpm for 10 minutes. The supernatant was put into a 25ml volumetric flask and extraction from the residue repeated with 5ml of 1% HCl in methanol. The second supernatant was combined with the first one and diluted to 25ml. Standards were prepared using catechin hydrate at 0, 10, 20, 40, 60, 80, and 100 μg/ml. Duplicate aliquots of 1 ml of sample extracts was put into test tubes where one served as a sample blank. The samples and standard solutions were reacted with five ml vanillin-HCl reagent (equal volumes of 8% HCl in methanol and 1% Vanillin in methanol) and allowed to stand for 20 min. To the samples, and to the blanks was added 5 ml of 4% HCl in methanol. Absorbance for all prepared solutions were read in a UV-Vis

spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) at 500nm and tannin content calculated as percent Catechin Equivalent (CE) using the standard calibration curve. The percent catechin equivalents (% CE) was calculated using the formula below;

$$CE\,\% = \frac{CC\ X\ VM}{VE\ X\ SW}\ X\ 100$$

where CC = catechin concentration (mg/ml); VM = volume made up (25ml); VE = volume of extract (1ml); and SW = weight of sample

**Determination of phytates**

Phytates was analyzed by the HPLC analysis method of phytic acid (Camire & Clydesdale, 2006) [8]. Approximately 0.5g of the sample was weighed into extraction flask (125ml Erlenmeyer flask) and 10ml of 3% $H_2SO_4$ added for extraction. The flasks were placed on a shaker at a moderate speed for 30minutes for extraction and the contents filtered. The filtrate was transferred to a boiling water bath (BWB) for 5minutes and 3ml of $FeCl_3$ solution (6mg ferric iron per ml in 3% $H_2SO_4$) added. A second boiling water bath (BWB) heating was done for 45minutes to complete the precipitation of the ferric phytate complex. The contents were centrifuged at 2500 rpm for 10 minutes and the supernatant discarded. The precipitate was washed with 30ml distilled water, centrifuged and the supernatant discarded. Three ml of 1.5N NaOH was added to the residues and the volume brought to 30ml with distilled water. Contents were heated in a boiling water bath for 30minutes to precipitate the ferric hydroxide, cooled, centrifuged, and the supernatant transferred into a 50ml volumetric flask. The precipitate was rinsed with 10ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. The samples were micro-filtered for HPLC analysis. HPLC analysis was done using Shimadzu Refractive Index Detector (RID-10A). The mobile phase was 0.005 N sodium acetate in distilled water, at a flow rate of 0.5µL/min. A stock solution of the standard containing 10 mg/ml of sodium phytate (Inositol hexaphosphoric acid $C_6H_6$ $(OPO_3Na_2)_6$+$H_2O$) in distilled water was prepared and serial dilutions made for the preparation of a standard curve. The equation of the standard curve line was obtained and used for calculating the phytate content.

$$\text{Phytate content mg/g} = \frac{y}{b}\ x\ \frac{\text{dilution factor}}{\textit{sample weight}}$$

Where, y = y intercept obtained from the standard curve of phytates, b = the peak area of the injected sample.

**Determination of Oxalates**

Oxalates was analyzed by the HPLC method according to de Guevara *et al*., (1990) [11] with modifications suggested by Yu *et al*., (2002). Two-and-a-half-gram samples was homogenized with 10mL of 0.5N HCL. The homogenate was heated to $80^0$ C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added to a volume of 25 ml, and centrifuged at 10,000 rpm for 10 minutes. About 1 ml of supernatant was passed through a micro filter (0.45µ) before HPLC analysis. Standards were prepared using Sodium Oxalate at varying concentrations for quantification (10-100ppm). HPLC analysis was done using Shimadzu UV-VIS detector, Hypsil C18_column (5µ M, 4.6 mm *250 mm), equipped waters 550 was used as the static phase and the mobile phase was a solution of 0.01 N $H_2SO_4$. Flow rate was 0.6 mL min-1, pressure of 62kgf and detection wavelength of 221 nm. Oxalate concentration was calculated from the corresponding peak areas and a standard curve generated.

**5. Data Analysis**

Samples analysis was carried out in triplicates and statistically analyzed using analysis of variance (ANOVA) at 5% level of significance under the Genstat analytical tool, $14^{th}$ Ed to determine whether there was significant difference in nutritional composition, phytochemicals and anti-nutrients among the various treatments applied.

**Results and Discussion**

**1. Proximate composition of biofortified finger millet grains**

Table 3.1 below indicate that there was no significant difference (P≤0.05) in proximate composition among the various treatments. The significant differences in total carbohydrates as shown was due to difference method applied in its determination and not due to the biofortification. The highest moisture content of 10.33±0.69% was due to T2(manure+ZnSO4) and the lowest of 9.76±0.66% was as a result of T3(Manure+$FeSO_4$) while mean moisture content was 10.06%. Mean total ash was 2.68%, while crude protein ranged from 9.95±1.18% to 11.05±0.83% with a mean of 10.56%. T5 (NPK+CAN+ZnSO4) gave the highest protein content of 11.05±0.83%. T1(NPK+CAN) resulted in the highest fat content of 1.45±0.25% while T3 had the lowest fat content of 1.09±0.16%.

**Table 1:** Proximate composition (% DWB) of finger millet grains agronomically biofortified with Iron and Zinc

| Treatments | Moisture | Ash | Proteins | Fats | Carbohydrates |
|---|---|---|---|---|---|
| T1 | 10.03±0.13$^a$ | 2.79±0.16$^a$ | 10.44±1.32$^a$ | 1.45±0.25$^a$ | 75.44±1.18$^{abc}$ |
| T2 | 10.33±0.69$^a$ | 2.57±0.23$^a$ | 10.97±0.99$^a$ | 1.14±0.20$^a$ | 74.98±1.75$^{bc}$ |
| T3 | 9.76±0.66$^a$ | 2.68±0.15$^a$ | 10.28±1.39$^a$ | 1.09±0.16$^a$ | 76.19±1.04$^{ab}$ |
| T4 | 9.84±0.29$^a$ | 2.49±0.03$^a$ | 9.95±1.18$^a$ | 1.16±0.22$^a$ | 76.56±0.86$^a$ |
| T5 | 10.29±0.15$^a$ | 2.75±0.25$^a$ | 11.05±0.83$^a$ | 1.37±0.18$^a$ | 74.32±0.67$^c$ |
| T6 | 10.14±0.13$^a$ | 2.79±0.11$^a$ | 10.69±1.76$^a$ | 1.34±0.26$^a$ | 75.02±1.73$^{bc}$ |
| Mean | 10.06 | 2.68 | 10.56 | 1.26 | 75.45 |
| F pr Value | 0.492 | 0.285 | 0.733 | 0.374 | 0.067 |
| LSD (5%) | 0.7539 | 0.3283 | 1.780 | 0.4195 | 1.425 |

Values are presented as Mean±SD, n = 3. Means with common superscripts within a column were not significantly (P≤ 0.05) different. LSD = Least Significant Difference at 5% level of significance. T1= (NPK+CAN); T2= (manure+ZnSO4); T3= (manure+FeSO4); T4= (NPK+CAN+$FeSO_4$); T5= (NPK+CAN+ZnSO4); T6= (control).

Proximate composition include biological materials that are required in major components by human body (Pragya Singh, 2012) [36], and proximate content is essential for the determination of the nutritional value of food (George *et al*., 2023) [16]. Agronomic biofortification had no significant ($P \leq 0.05$) effect on grain moisture content. The mean moisture values obtained are in accordance with the Codex standard specifications which dictates finger millet grain moisture to a maximum of 14% (World Health Organization, 2007) [63]. The suitable mean moisture of 10.06% is attributable to grains harvesting at proper physiological maturity and adequate drying before milling. Moisture is a specific measure of food quality that has a direct impact on the stability during storage. Storing grains with a higher than ideal moisture content may lead to deterioration of grain quality as a result of starch breakdown caused by microbial enzymes (Victor *et al*., 2013). Ramashia *et al*., (2019) and Shibairo *et al*., (2014) [40, 52, 61] reported similar findings on finger millets grain moisture content.

The ash content which is the residue after complete incineration of organic matter represents the amount of mineral matter in the finger millet grains. Crude ash results ranged from 2.49±0.03%– 2.79±0.16% which is significant for mineral analysis. George *et al*., (2023) [16] reported similar results for different varieties of finger millet.

Crude protein results ranged from 9.95±1.18% to 11.05±0.83% in this study. Protein is the second major component in finger millet and various factors are reported to contribute to high protein levels in finger millet grains as compared to other common cereals (Thapliyal & Singh, 2015) [59]. Finger millet is known to have high nitrogen use efficiency (NUE) and therefore can thrive on little to no nitrogen inputs yet accrues high quality proteins enhanced with essential amino acids in the grains (Goron *et al*., 2015; Kumar *et al*., 2009) [17, 25]. Additionally, finger millet grain endosperms are the main sinks for nitrogen and carbon compounds which boosts protein content (Wafula *et al*., 2018). Pragya *et al*., (2015), Sarita and Singh (2016) [14, 37, 62] also reported similar findings on the finger millet protein content. Singh and Srivastava (2006) [53] analyzed 16 finger millet varieties and found out that protein ranged from 4.88% to 15.58% with a mean value of 9.728%. Sher *et al*., (2022) [51] reported that Zn soil fertilization significantly improved quality traits of cereal crops including crude protein content differing with our findings.

The crude fat (%) in this study ranged between 1.09±0.16% and 1.45±0.25%, which is good for the stability of the finger millets. Fat content along with moisture is a decisive factor in appreciating the shelf stability of flours, thus high fat content aids oxidation of unsaturated acids producing off-flavours and rancid odours. The crude fat results were similar to those reported by Pragya Singh, (2012). Antony *et al*., (1996) [3, 36] reported a slightly higher percentage of 2.0%.

## 2. Effect of Iron and Zinc based fertilizers on mineral content of finger millet grains.

Finger millet grains shows a significance response to iron fertilizers (Table 3.2). T3 (Manure+$FeSO_4$) gave the highest concentration of 3.84±0.12mg/100g which was 78% increase compared to T6 (control). This was followed by T4 (NPK+CAN+$FeSO_4$) which had iron content of 3.60±0.10mg/100g, representing 67% increase. T5(NPK+CAN+ZnSO4) and T2(Manure+ZnSO4) gave an increase of 8.9% and 7.4% respectively of grain zinc content. These effects on grain zinc content were however statistically ($P \leq 0.05$) non-significant in the current experiment. The treatments showed no significant effects on Ca, P and Mg compared to the control. Higher means of Ca, Mg, and P of 433.6±19.5mg/100g, 198.9±3.2mg/100g and 319.3±11.8mg/100g respectively were all recorded with the application of NPK+CAN+$FeSO_4$(T4). Potassium (K) content of finger millet grains however varied significantly with the application of the various treatments. The highest concentration of 907.9±59.3mg/100g was due to T3(Manure+$FeSO_4$).

**Table 2:** Mineral composition (mg/100g DWB) of finger millet grains agronomically biofortified with Iron and Zinc

| Treatments | Iron | Zinc | Calcium | Magnesium | Phosphorus | Potassium |
|---|---|---|---|---|---|---|
| T1 | 2.11±0.05[b] | 2.06±0.06[a] | 373.0±24.9[a] | 155.3±2.7[a] | 292.4±17.3[a] | 819.0±74.1[a b] |
| T2 | 2.66±0.28[b] | 2.17±0.10[a] | 402.9±28.1[a] | 177.6±3.1[a] | 277.0±21.7[a] | 824.3±59.4[a b] |
| T3 | 3.84±0.12[a] | 2.10±0.15[a] | 405.1±31.1[a] | 183.2±2.6[a] | 302.8±8.6[a] | 907.9±59.3[a] |
| T4 | 3.60±0.10[a] | 2.10±0.05[a] | 433.6±19.5[a] | 198.9±3.2[a] | 319.3±11.8[a] | 711.7±132.6[c] |
| T5 | 2.40±0.17[b] | 2.20±0.06[a] | 382.9±20.9[a] | 161.3±1.7[a] | 290.3±14.5[a] | 826.2±68.8[a b] |
| T6 | 2.15±0.19[b] | 2.02±0.09[a] | 410.0±23.1[a] | 190.6±5.8[a] | 289.6±8.7[a] | 770.0±48.6[b c] |
| Mean | 2.79 | 2.11 | 401.2 | 177.8 | 295.2 | 810.0 |
| F pr-Value | <.001 | 0.764 | 0.406 | 0.312 | 0.447 | 0.007 |
| LSD | 0.6056 | 0.2971 | 63.33 | 45.25 | 44.50 | 83.2 |

Values are presented as Mean±SD, n = 3. Means with common superscripts within a column were not significantly ($P \leq 0.05$) different. LSD = Least Significant Difference at 5% level of significance. T1= (NPK+CAN); T2= (manure+ZnSO4); T3= (manure+FeSO4); T4= (NPK+CAN+$FeSO_4$); T5= (NPK+CAN+ZnSO4); T6= (control).

Presence of minerals in human diet is crucial for maintenance of various physiological processe s which are essential to life even though they are required in small quantities (Soetan *et al*., 2010) [55]. The elevated iron and zinc contents could be attributed to the increased organic matter in the soils as a result of the application of T3 (manure+FeSO4) and T2 (manure+ZnSO4). Manure supplies both macro- and micronutrients to the soil, increases soil organic carbon (SOC), and raises soil pH (Zingore *et al*., 2007) [67]. The rise in SOC increases cation exchange capacity and improves the soil's physical properties resulting to improved assimilation and transfer of micronutrients to the edible parts of the crop. Rengel, (2015) [45] demonstrated that increase in organic matter in soils promotes the process of micronutrients and macronutrients diffusion to plant roots hence re-mobilization and re-localization to plants edible parts. Teklu *et al*., (2023b) [58] also reported a 17.8% increase in finger millet grain iron in response to iron-fertilization. Iron fertilization in wheat reported a 19.4% increase in grain Fe concentration due to

soil application of $FeSO_4$ in other studies (Aciksoz *et al*., 2011) [1]. Use of NPK as basal fertilizer may have improved soil phosphorus (P) nutritional status and P is key in activating several metabolic processes and building plant tissues (Wafula *et al*., 2018) [62]. It also impacts root development that results in higher uptake of iron (Y *et al*., 2022). However, soil-iron fertilization has been reported to be ineffective in other studies. For example, iron biofortification of wheat and barley showed no significant effect on grain Fe concentration. Iron is rapidly converted into unavailable forms when applied to calcareous soils, and the poor mobility of Fe in phloem makes Fe fertilization unsuccessful (Zhang *et al*.,2008). Sakellariou & Mylona, (2020) [47, 66] reported that micronutrient fertilization is most effective when combined with NPK and organic fertilizers. NPK boosts nitrogen (N) supply to plants and this is stated to enhance the grain Fe and Zn concentration. This is because N is an integral element for effective biofortification of food crops with Zn and Fe due to numerous physiological and molecular mechanisms which are dependent on N nutritional status of the plant (Kumar *et al*., 2016) [26]. Nitrogen fertilization causes an increase in the growth of plants, adjusts the pH of the root zone and improves root uptake and shoot translocation which has a positive effect on the grain Zn concentration (Singh *et al*., 2014) [54].

Available data on quality characteristics of finger millet grains agronomically biofortified with soil Zn and Fe-only fertilizers is scarce. Reported available data shows an increase of 18.9% in FM grain Zn in response to application of Zn-fertilizers (Teklu *et al*., 2023b) [58]. Soil application of Zn fertilizers on maize, rice and wheat crops had incremental effect on grain zinc concentrations of up to 20%, 7% and 19% respectively (Joy *et al*., 2015) [23]. Another research by Botoman *et al*., (2022) [6] showed that maize grain zinc concentration was increased by 15% as a result of application of soil zinc. In other studies, Y *et al*., (2022) [64] reported a substantial increase in grain mineral concentration due to application of combined Fe and Zn fertilizers. The mechanisms of Fe and Zn interactions is not well studied. Niyigaba *et al*., (2019) [32] reported that combined iron and zinc fertilizers is more beneficial as it increases grain macro- and micronutrients. Soil application along with foliar application is also claimed to be more effective in increasing grain nutrient compared to soil or foliar alone (Maqbool & Beshir, 2019; Nakandalage *et al*., 2016). Blindauer & Schmid (2010) [5, 28, 30] demonstrated that from biofortification point of view, foliar application strategy can be more reliable than soil fertilization as micronutrients enters the phloem through cuticle and stomata on leaf surface directly hence it's a shorter pathway than root uptake. Phattarakul *et al*., (2012) [34] also demonstrated that foliar Zinc application or a combination of soil and foliar zinc is more effective in enhancing grain zinc content.

Application of iron and zinc fertilizers had no significant effect ($P \leq 0.05$) on the grains calcium content. Finger millet grain is known especially for its elevated calcium content having averagely 0.34% as compared with 0.01-0.06% in most of other common cereals (Gupta *et al*., 2017) [18]. The mechanisms behind calcium transport and grain filling are not well studied but it is associated with higher expression of Ca signaling transporter genes (Ceasar *et al*., 2018) [2].

## 3. Phytochemical composition of biofortified finger millet grains

There were significant ($P \leq 0.05$) differences on phytochemical contents among different fertilizer treatments (Table 3.3). Total carotenoid was highest at 4.04±0.62µg/g with the application of NPK+CAN+ZnSO4 (T5), and low due to T4 (NPK+CAN+$FeSO_4$). Total phenolic (TP) compounds and total flavonoids (TF) were highest due to T4(NPK+CAN+$FeSO_4$). T2 (Manure+$ZnSO_4$) presented low values of TP, while T1 (NPK+CAN) reported lowest TF of 176.6±17.9mg/100g.

**Table 3:** Phytochemical composition of finger millet grains agronomically biofortified with Iron and Zinc

| Treatments | Total carotenoids ((µg/g) | Total Phenolic (mg/100g) | Total flavonoids (mg/100g) |
|---|---|---|---|
| T1 | 2.81±0.31$^{b}$ | 117.8±5.5$^{b}$ | 176.6±17.9$^{c}$ |
| T2 | 2.48±0.25$^{bc}$ | 106.0±6.4$^{b}$ | 207.0±13.3$^{b}$ |
| T3 | 1.96±0.11$^{cd}$ | 118.1±8.8$^{b}$ | 249.6±7.4$^{a}$ |
| T4 | 1.56±0.20$^{d}$ | 153.6±5.6$^{a}$ | 250.9±24.2$^{a}$ |
| T5 | 4.04±0.62$^{a}$ | 147.6±13.1$^{a}$ | 187.7±12.5$^{b\ c}$ |
| T6 | 1.94±0.28$^{cd}$ | 111.1±13.0$^{b}$ | 211.8±12.1$^{b}$ |
| Mean | 2.47 | 125.7 | 213.9 |
| Fpr | <.001 | <.001 | <.001 |
| LSD (5%) | 0.6536 | 11.39 | 26.39 |

Values are presented as Mean±SD, n = 3. Means with common superscripts within a column were not significantly ($P \leq 0.05$) different. LSD = Least Significant Difference at 5% level of significance. T1= (NPK+CAN); T2= (manure+ZnSO4); T3= (Manure+FeSO4); T4= (NPK+CAN+$FeSO_4$); T5= (NPK+CAN+ZnSO4); T6= (control).

The current study reported an increase of 108% of total carotenoids on the grain sample as a result of T5(NPK+CAN+ $ZnSO_4$) as compared to T6 (Control). The increase in total carotenoids is attributed to the role of zinc sulphate in enhancing the concentrations of photosynthetic pigments (Rehman *et al*., 2023) [44] owing to the essential role of Zn in plants as a catalytic and structural component of enzymes, proteins and as a co-factor for development of pigment biosynthesis (Impa *et al*., 2013) [21].

TP and TF were high due to application of T4(NPK+CAN+$FeSO_4$) differing from a report by Rehman *et al*., (2023) [44] who reported a significant increase in TP as a result of ZnSO4 fertilization on rice cultivars, and Song *et al*., (2015) [56] who reported an increase in TF with zinc sulphate fertilization.

## 4. Level of anti-nutrients in biofortified finger millet grains

Table 3.4 below shows the effect of agronomic biofortification on finger millet grains where it indicates significant effect ($P \leq 0.05$) on phytates. Tannins and oxalates also varied with the application of various fertilizer treatments. Lowest phytate value of 261.8±9.16mg/100g was recorded with the application of T5 (NPK+CAN+ZnSO4). This was 19.92% lower as compared to control (T6). Tannin concentration ranged from

94.3±11.7mg/100g - 253.6±46.4mg/100g. The highest concentration reported was due to application of T6 (Control). Zinc and Iron biofortification did not lower the level of anti-nutrient oxalates.

**Table 4:** Anti-nutrients composition of finger millet grains agronomically biofortified with Iron and Zinc

| Treatments | Phytates (mg/100g) | Tannins (mg/100g) | Oxalates (mg/100g) |
|---|---|---|---|
| T1 | 383.5±11.9$^{a}$ | 94.3±11.7$^{c}$ | 73.6±14.2$^{b}$ |
| T2 | 277.9±29.0$^{cd}$ | 150.1±23.5$^{b}$ | 79.0±14.6$^{b}$ |
| T3 | 359.9±45.8$^{ab}$ | 178.9±23.5$^{b}$ | 109.2±10.4$^{a}$ |
| T4 | 293.0±30.3$^{cd}$ | 172.7±22.2$^{b}$ | 66.1±1.7$^{b}$ |
| T5 | 261.8±9.2$^{d}$ | 162.0±11.0$^{b}$ | 75.2±10.2$^{b}$ |
| T6 | 326.9±13.1$^{bc}$ | 253.6±46.4$^{a}$ | 63.5±9.0$^{b}$ |
| Mean | 317.2 | 168.6 | 77.8 |
| FPr Value | <.001 | <.001 | 0.007 |
| LSD (5%) | 47.39 | 47.14 | 20.55 |

Values are presented as Mean±SD, n = 3. Means with common superscripts within a column were not significantly (P≤ 0.05) different. LSD = Least Significant Difference at 5% level of significance. T1= (NPK+CAN); T2= (manure+ZnSO4); T3= (Manure+FeSO4); T4= (NPK+CAN+$FeSO_4$); T5= (NPK+CAN+ZnSO4); T6= (Control).

The significant reduction of phytates and tannins could be attributed to increased nutrient uptake as a result of biofortification, use of organic manure and inorganic fertilizers amendments which eventually lowered the anti-nutrients level. This is because phytate has antagonistic effect with metal ions (Hussain *et al*., 2012). Ramzani *et al*., (2016) [20, 41] reported similar findings in the study of Zn and Fe fertilization in fortification of maize and wheat. Reddy *et al*., (2000) [43] reported that grain phytic acid correlates negatively with micronutrient absorption. Thus as micronutrient concentration increases phytate contents were decreased. Finger millet is reported to have highest tannin concentration among millets. Interestingly, there are several other strategies of reducing tannins and other anti-nutrients to a safer level to improve nutrition (Selvaraj *et al*., 2023) [50]. Furthermore, partial retention of the anti-nutrients is on the other hand beneficial for their contributions to certain health benefits. Plants also use anti-nutrients for their own defense against pests (Popova & Mihaylova, 2019) [35]. In this study, agronomic biofortification of finger millet with zinc and iron fertilizers had no significant effect on the oxalate levels of the finger millet grains. Reports on effect of agronomic biofortification on oxalates are however limited.

## Conclusion

The findings of this study reveal that finger millet is highly nutritious crop. Agronomic biofortification through application of Fe and Zn mineral fertilizers has however resulted in increased Fe and Zn nutritional value of the finger millet grains, which may help to meet Fe and Zn requirements in human population thus reducing micronutrient deficiencies (MNDs). T3(Manure+FeSO4) and T5(NPK+CAN+ZnSO4) were most significant in increasing Fe and Zn contents respectively. T5(NPK+CAN+ZnSO4) had 19.9% reduction of phytate level as compared to the control which is important in improving nutrients bioavailability. Increasing Fe and Zn concentration in staple food crops such as finger millet reduces rural households' nutritional vulnerability emerging from climatic and economic challenges without the need for dietary change. The effect on macronutrients composition was however non-significant.

The findings of this study provide a basis for taking evidence-based policy direction in the agricultural sectors in addressing Zn and Fe deficiencies. In the short term, agronomic biofortification could provide an immediate and effective way to alleviate micronutrient deficiencies among the rural populations especially of developing countries.


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