

Enhancing the Nutritional Quality of Cowpea Through Iron Biofortification for Food Security

Abigail Badu

Kwame Nkrumah University of Science and Technology (KNUST)

Robert Adenyo

adenyorobert@yahoo.com

Kwame Nkrumah University of Science and Technology (KNUST)

Joseph Sarkodie-Addo

Kwame Nkrumah University of Science and Technology (KNUST)

Kennedy Agyeman

CSIR-Crops Research Institute

Godwin Amuzu

CSIR-College of Science and Technology

Research Article

Keywords: cowpea biofortification, iron fertilizer, biological nitrogen fixation, grain yield, Iron Deficiency Anaemia

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10657248/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

ENHANCING THE NUTRITIONAL QUALITY OF COWPEA THROUGH IRON BIOFORTIFICATION FOR FOOD SECURITY

Abigail Badu ¹, Robert Adenyo ^{1*}, Joseph Sarkodie-Addo ¹, Kennedy Agyeman ², Godwin Amuzu ³

¹Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

²CSIR-Crops Research Institute, Kumasi, Ghana

³CSIR-College of Science and Technology, Kumasi, Ghana

Corresponding Authors' emails: adenyorobert@yahoo.com,

ORCID; <https://orcid.org/0009-0003-2223-7455>

Abstract

This study evaluated how iron (Fe) application impacts the growth, yield, nodulation, biological nitrogen fixation (BNF), and nutritional quality of cowpea (*Vigna unguiculata* [L.] Walp) in Ghana. Conducted at the CSIR-CRI Fumesua research fields, the study utilized three cowpea varieties (Zamzam, Nketewade, and Agyenkwa) and five Fe application rates (0, 1, 2, 3, and 4 kg ha⁻¹) in a randomized complete block design.

Parameters assessed included plant growth, yield components, mineral composition, and proximate nutrient content. Results revealed that Fe application significantly influenced yield-related traits, with the highest grain yield (2,300.69 kg ha⁻¹) and Fe concentration in grain recorded at 4 kg ha⁻¹. BNF peaked at 3 kg ha⁻¹ Fe, while the Agyenkwa variety exhibited the highest Fe concentration in seeds, indicating its potential for addressing Iron Deficiency Anaemia.

Although growth parameters such as plant height and chlorophyll content showed minimal response to Fe application, significant improvements were observed in pod weight, pod numbers, and the concentrations of Fe, calcium (Ca), and zinc (Zn).

This research demonstrates the viability of agronomic biofortification as a sustainable strategy for enhancing cowpea's nutritional quality and mitigating micronutrient deficiencies, particularly in resource-constrained regions. Future studies should explore the scalability of this intervention and its integration into agricultural policies to bolster food security and public health outcomes.

Keywords: *cowpea biofortification, iron fertilizer, biological nitrogen fixation, grain yield, Iron Deficiency Anaemia*

INTRODUCTION

The crop cowpea, known scientifically as *Vigna unguiculata* [L.] Walp, belongs to the family Fabaceae and is a leguminous pulse crop which stands in the centre of human diets and contributes significantly to global food and dietary safety (Márquez-Quiroz *et al.*, 2015). Its cultivation is limited to specific geographic areas in various agroecological regions, particularly in the arid tropics of Latin America, Africa, and South Asia, due to its use in a variety of traditional and cultural practices worldwide, mainly by low-income farming communities that depend on such crops for their sustenance and income (Carvalho *et al.*, 2012; Boukar *et al.*, 2019). Sub-Saharan Africa largely consumed this crop, especially in the western and central parts of the continent. Global output is roughly 8.9 million tonnes per year (FAO 2021), while Ghana contributes about 237, 000 tonnes annually (MoFA 2019). The crop is successful in most regions and when the optimum growth conditions are provided, it can produce high grain (Mfeka *et al.*, 2019; Ramos *et al.*, 2020).

When it comes to soils with low fertility, cowpea can survive and yield well because of its atmospheric nitrogen fixation ability. Additionally, it enhances soil fertility when residues are left after harvest or incorporated before planting. Therefore, it is a good choice for resource-poor farmers, as these traits help reduce production costs (Bloem *et al.*, 2009; Frankenberger and Abdelmagid, 1985). Furthermore, the crop's role in supplying dietary protein to low-income communities, especially in developing countries, has earned it the nickname “poor man’s meat” (Márquez-Quiroz *et al.*, 2015). From a nutritional perspective, cowpea grains are a valuable source of largely protein, carbohydrate and appreciable amounts of minerals, vitamins and bioactive phytochemicals (Animasaun *et al.*, 2015; Devi *et al.*, 2015).

Generally, the key micronutrient component of the crop and most staple food crops is low, coupled with meals that are predominated by cereals, which has contributed to the global health burden of micronutrient deficiency, this deficiency is popularly known as “hidden

hunger” (Welch and Graham, 2004; de Valença *et al.*, 2017). Both plants and humans require iron as a micronutrient. It forms part of several key macromolecules that are essential for metabolism, respiration, photosynthesis and DNA synthesis (Briat, 2011). Iron is a micronutrient and its deficiency is a global phenomenon affecting approximately 60% of people worldwide (FAO, 2015; Amarakoon *et al.*, 2012). Micronutrient deficiencies, especially iron (Fe) deficiency, are widespread in developing nations. Micronutrient deficiencies are caused by an over-reliance on staple foods, as well as bad eating habits and a vegetarian lifestyle in some wealthy nations (Poelman *et al.*, 2018; Garcia-Oliveira *et al.*, 2018).

In order to address the hidden hunger crisis, several initiatives have been implemented; these can be divided into direct and indirect strategies. Food diversification, micronutrient supplementation programs, and fortification are direct, nutrition-specific interventions that tackle consumption behaviour. Sadly, these attempts have made little progress as the afflicted population has limited access to these solutions (Hoekenga *et al.*, 2011; Ruel and Alderman, 2013). However, indirect solutions (nutrition-sensitive) such as biofortification provide sustainable food-based strategies to effectively combat hidden hunger (Nissar *et al.*, 2019). Biofortification can be explained as the use of agronomic approaches, conventional breeding and genetic engineering to aggregate micronutrients in economic important parts of the plant (Bouis *et al.*, 2011). Although both breeding and genetic engineering have been found to result in new varieties of crops, agronomic biofortification is increasingly recognized as a rapid and practical strategy for improving micronutrient content in food crops, particularly for resource-poor rural communities (Pal *et al.*, 2019; Nissar *et al.*, 2019).

The application of mineral fertilizers to leaves, soil, or in combination to boost the nutritional quality of crops is referred to as agronomic biofortification (Phattarakul *et al.*, 2012; Cakmak *et al.*, 2010). It works best when crops are biofortified with iron, zinc, and selenium. Cammak (2014). Iron is one of the most common elements in soil, but it is hard for plants to absorb because it easily forms insoluble combinations in aerobic environments with neutral or alkaline pH values (Gómez-Galera *et al.*, 2010). Edible parts of many staple crops contain relatively low iron (Fe) concentrations, rendering them inadequate for meeting daily dietary requirements, largely because crops such as cowpea have limited access to bioavailable soil iron. Foliar treatment has been suggested as the most effective method of supplying mineral iron to crops since it is less detrimental to the environment and results in fewer losses (Dixon, 2003).

Legumes are the world's second most significant source of nourishment after cereals, as they constitute the primary source of protein and calories for humans. It is currently a viable substitute for animal protein. Because of the nutritional worth and economic relevance of crops, innovative strategies for improving crop output and nutrient content are required (Salih, 2013). Furthermore, because cowpeas can fix nitrogen, they are the most important legume crop that can be produced in semiarid regions. Therefore, it is essential for both sustainable farming systems and human nutrition (Hemmati, 2022). Focusing on food crops and kinds that are proven to flourish in their specific environment is crucial for the success of agronomic biofortification (de Valença *et al.*, 2017).

Since the crop is largely consumed and found in the key dishes of many African delicacies, including Ghana, this demonstrates the bean's appropriateness for biofortification. Biofortification success largely relies on widely consumed staples, thus cowpea is a suitable candidate. It has the potential to alleviate iron deficiency in the senior high school populations of Ghana, as it is a major staple in their diets. In Ghana, exploring foliar application with iron fertilizer for cowpea biofortification has received limited attention. Thus, the purpose of this study was to assess how iron supplementation affected cowpea growth, production, and nutritional quality.

MATERIALS AND METHODS

Sample collection and preparation: The experiment was carried out at the research fields of the Council for Scientific and Industrial Research's Crops Research Institute, Fumesua, Ashanti Region (01°36' W, 06°43' N), during the main cropping season from April to June 2023. Fumesua is situated within the semi-deciduous forest zone and receives an average annual rainfall of approximately 1,727.2 mm, exhibiting a bimodal distribution with a major rainy season from March to July and a minor season from August to November. Mean relative humidity ranges from 95 % in the morning to 61 % at midday, with temperatures varying between 21 °C and 31 °C. The experimental site is characterized by Ferric Acrisol soils of the Asuansi series, featuring a 16–20 cm sandy-loam topsoil on gentle slopes of 1–5 % (Adu, 1992).

The varieties used as test crops for the experiment were Zamzam, Nketewade, and Agyenkwa were sourced from CSIR-CRI. The varieties used were all released in 2015, and they exhibit similar growth patterns that are semi-erect, early maturing, and all high-yielding.

The source of Fe was a laboratory-grade FeSO₄, 19.7% Fe from Park Scientific Limited. The experiment followed a 3 × 5 factorial layout, arranged in a randomized complete block design with three replicates. The study examined two main variables: three cowpea varieties Zamzam, Nketewade, and Agyenkwa and five iron-application levels (0, 1, 2, 3, and 4 kg Fe ha⁻¹). To prevent burning in a 0.25% solution, the iron was administered using the split application approach. The Fe was applied every five days, giving 1 kg Fe/ha per application, starting from 20 DAS when there was enough foliage to intercept the sprayed chemical.

In each plot, five plants were marked in the two middle rows. Plant height was measured with a meter rule from the soil surface to the tip of the last leaf at 15, 25, 35 and 45 days after sowing (DAS). At the same growth stages, the number of trifoliate leaves on each tagged plant was counted and the average leaf count per plant was recorded. (The leaf counts were taken at 15, 25, 35 and 45 DAS). Three leaves were randomly selected from each of the five tagged plants and the greenness was measured using a SPAD chlorophyll meter. Each tagged plant was represented by the mean value of the three leaves at 15, 25, 35 and 45 DAS. Samples used for dry matter accumulation were taken at 20, 30, 40, and 50 DAS.

From each plot, three plants were selected at random, cut at ground level, and their fresh shoots were weighed with an electronic balance in the Legumes Division of the Crops Research Institute. The fresh material was then oven-dried at 84 °C for 48 hours, after which the dry-matter weight was recorded for each plot. Crop growth rate (C) was calculated using Radford's formula (1967).

$$CGR = \frac{W_2 - W_1}{T_2 - T_1}$$

Where;

C stands for crop growth rate.

W₁ = dry weight per unit area at the first harvest,

W₂ = dry weight per unit area at the subsequent harvests

The time units between the two harvests are T₂-T₁.

Using the g/m²/day units

From each plot, five plants that had reached physiological maturity were harvested. After the pods were removed, the remaining plant material and the pods were oven-dried at 80 °C for 48 hours. The total nitrogen fixed in the seeds and the residual biomass was estimated

using the total nitrogen difference (TND) method (Peoples et al., 1989). The calculation of total N fixed was then adjusted with the modified formula of Viera-Vargas *et al.* (1995).

Total N in plants is calculated as follows:

Total N in legumes minus Total N in reference crop = Dry matter (kg/ha) x % N in plants / 100
N fixed (kg/ha).

In each plot, the pods from the five marked plants in the two central rows were collected and the average pod count per plant was calculated. After threshing the pods, the seeds were counted, and the number of seeds per pod was obtained by dividing the total seed count by the total number of pods for that plot. Harvesting from a net plot inside each plot's two central rows allowed for the determination of grain yield. After the pods were threshed, the seeds were oven-dried for 48 hours at 80 degrees Celsius before being weighed. After that, the weight in grams per harvested area was extrapolated to kilograms per hectare (Okogun *et al.*, 2005).

Sample analysis

Mineral and Proximate Analysis

The Department of Crops and Soil Sciences' Soil Science Laboratory was used to analyze the grains' nutritional composition.

Plant Total Nitrogen and Crude Protein Determination

After being weighed, 2.0 grams of the material were put into a 30-millilitre digestion flask. Twenty millilitres of concentrated H₂SO₄ were added to a digesting flask containing 20 g of a mixture of selenium powder, copper sulphate, and sodium sulphate. The sample was then treated with digestion chips, and the digestion process was continued until the mixture became colourless. After the digest has cooled down, a small amount of distilled water that is free of ammonia was added to dilute it before transferring it to the distillation apparatus. Small amounts of water were used to rinse the Kjeldahl flask several times. Five millilitres of boric-acid solution and a few drops of mixed indicator were placed in a 100-mL conical flask, and the condenser tip was submerged beneath the liquid surface. The test solution was mixed with 10 millilitres of sodium hydroxide-sodium thiosulphate solution. Boric acid was used to distil and collect 50 millilitres of ammonia. The endpoint was determined by rinsing the condenser tip and titrating the solution against the standard acid until a violet tinge developed.

Lastly, the sample titration volume was deducted from the volume obtained from the titration of the reagent blank using an equivalent volume of distilled water (Okalebo *et al.*, 1993).

Calculation:

The nitrogen content of the seeds and residues was calculated as:

$$N \text{ (g/Kg)} = 14 \times \frac{(\text{Vol. of acid in Titration} - \text{Vol. of acid in blank titration}) \times \text{Normality}}{10 \times \text{Weight of the sample}}$$

Exact crude protein (CP) is calculated by multiplying the total nitrogen (NT) content by the conversion factor 6.25, giving the percentage of protein in the sample.

Calcium

To start, 5 mL of the sample solution was transferred into a 100-mL Erlenmeyer flask. Ten millilitres of 10 % potassium hydroxide and 1 mL of 30 % triethanolamine (TEA) were added, followed by three drops of 10 % potassium cyanide (KCN) and a few drops of eriochrome black T (EBT) indicator. The flask was shaken vigorously to ensure a uniform mixture, then titrated with 0.02 N ethylenediaminetetraacetic acid (EDTA) until the endpoint, marked by a colour change from red to blue.

The calcium concentration (in mg) is calculated by multiplying the EDTA titre value by 0.4008.

$$\text{Calcium (\%)} = \frac{\text{mg Calcium} \times 100}{\text{Weight of sample} \times \text{volume}}$$

Potassium and Sodium

After drying for 4 h at 105 °C, 1.908 g of oven-dried analytical-grade KCl and 2.542 g of NaCl were each dissolved in 200 mL of deionized water. The two solutions were combined and made up to a final volume of 1000 mL. A reference solution containing 1000 ppm of each element was prepared as a standard. Calibration curves were constructed using K concentrations of 200, 400, 600 and 800 ppm, and Na concentrations of 20, 40, 60 and 80 ppm. Absorbance readings were taken with a flame photometer, which was also used to analyze the sample solution containing HClO₄ (perchloric acid) and HNO₃ (nitric acid). By comparing the sample's absorbance to the calibration curves, the potassium and sodium contents of the sample could be determined precisely.

Calculation:

$$K, Na \text{ content (g) in 100 g plant sample, (\% K, Na)} = \frac{c \times df \times 100}{1000 \ 000} = \frac{c \times 100 \times 100}{1000 \ 000}$$

$$= \frac{C}{100}$$

Where;

According to the standard curve, C is the concentration of K and Na ($\mu\text{g/ml}$); df is the dilution factor, which is $100 \times 1 = 100$, or 1.0g of the sample produced up to 100 ml (100 times).

Potassium and Sodium

Vanadomolybdate reagent preparation was done by dissolving 1.25 g of ammonium vanadate in 300 mL of boiling distilled water and, separately, 2.25 g of ammonium molybdate in 400 mL of distilled water. The two solutions were mixed and allowed to cool to room temperature. An addition of 250 mL of analytical-grade HNO was made to make 1 L with deionized water. The phosphate standard solution was prepared by dissolving 0.2195 g of KH_2PO_4 (analytical grade) in 1 L of filtered water to obtain a $50 \mu\text{g P mL}^{-1}$ stock solution. In order to create a calibration curve, transfer of 1, 2, 3, 4, 5, and 10 mL of the stock solution was poured into separate 50 mL volumetric flasks. The addition of 10 mL of vanadomolybdate reagent to each flask and dilution to 50 mL with deionized water was carried out. This yielded standards of 1, 2, 3, 4, 5, and $10 \mu\text{g P mL}^{-1}$. The absorbance was measured at 420 nm using a Spectronic 20 spectrophotometer and plotted absorbance versus concentration. A pipette was used to transfer 10 mL of the sample solution into a 100 mL volumetric flask. Vanadomolybdate reagent was added in 10 mL increments until the total volume reached 100 mL. The mixture was allowed to stand for 30 minutes for the characteristic golden colour to develop. The absorbance was measured at 420 nm and compared to the calibration curve to determine the phosphorus level in the sample.

$$(\% \text{ P}) = \frac{C \times df \times 100}{1\,000\,000} = \frac{C \times 1000 \times 100}{1\,000\,000} = \frac{C}{10}$$

The dilution factor (df) is 1000, which is the product of two separate dilutions: first, 1g of sample is dissolved in 100 ml (a 100-fold dilution) and then 5 ml of that solution is further diluted to 50 ml (a 10-fold dilution). To convert the measured μg values to grams, a factor of 1000 000 is applied.

Zinc and Iron

The instrument was set up with the usual AAS parameters: air pressure between 50 – 60 psi, acetylene pressure between 10 – 15 psi, and a power supply of 208 – 240 V. For the analyses

we chose the characteristic lines of iron (Fe 248.3 nm) and zinc (Zn 213.9 nm) using the appropriate hollow-cathode lamps. Calibration curves for each element were prepared from stock standards (Buck Scientific) and the samples were measured after suitable dilution of the digest. In the calibration equation, Y is the absorbance of the element and X is its concentration in the sample. By inserting the sample's absorbance into the equation, we obtained X in mg L⁻¹. Multiplying that concentration by the volume of the sample solution (0.1 L) gave the total amount of the element present in the 100 mL solution, which corresponds to the overall mass of the analyte in the mixture. Finally, the percentage of the analyte in the original material was calculated by dividing the analyte mass in the solution by the initial sample weight and multiplying by 100.

Cal:

$$\text{Concentration. (Iron, and Zinc) (mg/kg)} = \frac{\text{Conc.recorded from AAS} \times \text{Normal volume (100 ml)}}{\text{Sample weight (1.00g)}}$$

Moisture

A total of five grams of the sample were placed on a plate or moisture container. It was then transferred to a drying oven maintained at 105°C. The sample was allowed to dry until it has a uniform weight, indicating that all moisture had been removed.

$$\text{Moisture Content (\%)} = \frac{\text{Weight of fresh Sample} - \text{Weight of dry Sample}}{\text{Weight of Fresh Sample}} \times 100$$

(AOAC,1990; Bisiw, 2018)

Crude Fat

Extraction thimble was used to contain two (2) grams of the dried sample. The extraction thimble was then inserted into a Soxhlet apparatus. A dry solvent flask that has been pre-weighed was connected beneath the apparatus, ensuring that it is free of any moisture. The required quantity of solvent, approximately 150-200 ml of petroleum ether, was placed in the flask. The condenser was linked to the apparatus and the extraction process was initiated and allowed to run for 2 – 3 hours. After the extraction, the thimble was removed from the apparatus, and the ether was recovered by using the appropriate techniques. To ensure the complete removal of any remaining traces of ether, the flask was subjected to a boiling bath. The container was oven-dried at 105 °C for 30 minutes, cooled in a desiccator, and its final mass was measured precisely.

$$\text{Crude fat (\% of DM)} = \frac{\text{Weight of fat}}{\text{Weight of Sample}} \times \frac{100}{1}$$

(AOAC,1990; Bisiw, 2018)

Crude Fibre

Two grams of the defatted, dried material were placed in a digestion flask. The flask was charged with 200 mL of boiling sulfuric acid and positioned under a condenser. The mixture was brought to a boil and held at a gentle simmer for exactly 30 minutes, with an antifoam agent added to control foaming. After heating, the liquid was passed through a linen filter and rinsed thoroughly with boiling water. The retained solid was returned to the flask, mixed with 200 mL of boiling sodium hydroxide solution, and again refluxed for 30 minutes. The resulting suspension was filtered through a porous crucible and washed with hot water followed by 15 mL of 95 % ethanol. The crucible was dried to constant weight at 105 °C, cooled in a desiccator, and weighed. The crucible contents were then ashed at 550 °C for 30 minutes, allowed to cool, and reweighed. The difference between the two weights gave the mass of the fibre.

Calculation:

$$\text{Crude fibre (\% of fat-free DM)} = \frac{\text{Weight of crucible+dried residue} - (\text{Weight of crucible+ashed residue})}{(\text{Weight of sample})} \times 100$$

(AOAC,1990; Bisiw, 2018)

Ash

Two grams of the sample were placed in a pre-weighed, clean porcelain dish. The dish was then heated in a muffle furnace at 550 °C for four hours. After the heating period, the dish was removed, allowed to cool to room temperature in a desiccator, and re-weighed together with the sample to obtain the final weight.

Calculation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

(AOAC,1990; Bisiw, 2018)

Carbohydrate

The proportion of total carbohydrate was calculated using the formula below, which includes the values obtained for NFE (non-fibre carbohydrates) and crude fibre. This calculation allows for a comprehensive assessment of the overall carbohydrate content present in the sample. By utilizing the individual measurements of NFE and crude fibre, the formula provides a reliable

means of quantifying the percentage of total carbohydrates, enabling a thorough analysis of the sample's nutritional composition.

$$\% \text{ Carbohydrate} = \% \text{ NFE} + \% \text{ Fibre}$$

(AOAC,1990; Bisiw, 2018)

DATA ANALYSIS

All data were subjected to analysis of variance (ANOVA) using R statistical software. Differences among treatment means were evaluated with the Least Significant Difference (LSD) test at the 5 % significance level. Where appropriate, data were transformed before the analysis.

RESULT AND DISCUSSION

Effects of Iron fertilizer on growth and yield

Table 1: Effect of Variety and Iron Fertilizer Application Rates on Plant height of Cowpea

Varieties	Plant Height (cm)			
	15 DAS	25 DAS	35 DAS	45 DAS
Agyenkwa	8.84	16.89	21.82	27.81
Nketewade	8.26	15.09	20.60	29.05
Zamzam	9.04	17.00	22.69	27.79
LSD (5%)	0.80	1.09	1.13	2.25
Rates (kg ha ⁻¹)				
0	8.39	16.37	21.46	27.85
1	8.97	16.47	21.90	28.83
2	8.39	15.95	21.81	28.65
3	9.41	16.79	21.80	28.67
4	8.40	16.06	21.55	27.09
LSD (5%)	1.03	1.41	1.46	2.91
CV (%)	12.22	8.91	6.99	10.66
p-value(Var*Rates)	0.73	0.24	0.09	0.41

DAS = Days After Sowing, CV = Coefficient of Variation, LSD = Least Significant Difference

The ANOVA of plant height measured at several days after sowing indicated that there were no statistically significant differences ($P > 0.05$) among the varieties at 15 days and at 45 days after sowing. However, substantial ($P < 0.05$) differences were seen at 25 and 35 DAS (Table 4.1). At 25 and 35 DAS, Zamzam had the highest plant height ($P < 0.05$), higher than that of Nketewade on both occasions. Application of iron fertilizer did not affect cowpea plant height substantially ($P > 0.05$) cowpea plant height on all sampling occasions (Table 1). Also, the variety by iron fertilizer interaction was not significant on all sampling days. The non-significant differences/effects observed in Table 1 among the cowpea varieties for plant height is not affected by variety and the varieties were similar in terms of these parameters. As such, selecting a variety to bring about differences in terms of the two parameters of the cowpea plant would prove to be futile. This result contrasts with the observations of El Naim and Jabereldar (2010), who reported significant differences in plant height and branch number among cowpea varieties. The lack of significant variation in the present study may be attributed, in part, to the growth habits of the selected varieties. Supporting this, Haruna and Usman (2013) and Singh et al. (2011) noted that spreading and semi-spreading cowpea types exhibit greater growth and developmental potential compared to non-spreading types. The non-significant differences observed among the iron (Fe) application rates and the interaction between the varieties and iron application rates for plant height are an indication that the iron rates did not influence the cowpea plant height. The lack of a significant effect may be due to the application rates being insufficient to influence cowpea growth. This result aligns with Weldu et al. (2012), who observed comparable outcomes when zinc was applied at similar rates.

Effect of Variety and Iron Fertilizer Application Rates on the Number of Branches of Cowpea

Table 2: Effect of Variety and Iron Fertilizer Application Rates on Number of Branches of Cowpea

Variety	Number of Branches			
	15 DAS	25 DAS	35 DAS	45 DAS
Agyenkwa	2.91	3.28	4.25	4.33
Nketewade	2.72	3.03	3.91	4.17
Zamzam	2.91	3.05	4.19	4.33

LSD (5%)	0.21	0.36	0.48	0.45
Rates (kg ha ⁻¹)				
0	2.82	3.09	4.40	4.53
1	2.80	3.42	4.29	4.40
2	2.91	3.00	4.22	4.36
3	2.82	2.92	3.80	3.93
4	2.87	3.16	3.87	4.18
LSD (5%)	0.27	0.46	0.62	0.58
CV (%)	9.87	15.35	15.51	14.03
p-value (Var* Rates)	0.871	0.443	0.882	0.491

DAS = Days After Sowing, CV = Coefficient of Variation, LSD = Least Significant Difference

The cowpea variety had no significant effect ($P > 0.05$) on branch number. In contrast, iron fertilizer significantly influenced branching at 25 and 45 days after sowing (DAS). At 25 DAS, plants receiving 1 kg Fe ha⁻¹ produced a significantly higher number of branches ($P < 0.05$) compared with those treated with 3 kg Fe ha⁻¹ alone. At 45 DAS, the control treatment had the greatest effect, but it was more than that of 3 kg ha⁻¹ alone. All other treatment methods were comparable. A variety of fertilizer interactions had no significant effect on branch output ($P > 0.04$). (Table 2). Fe application did not affect the production of branches and leaves in cowpea; therefore, application of Fe would not result in a significant increase in them, as indicated by the non-significant differences also seen in Table 2 for the Fe application rates and the interaction between the varieties and Fe application rates for the number of leaves and branches. This could be due to the rates of application of Fe used in this study. This finding conforms to the findings from the work of Schon and Blevin (1990), who indicated similar finding using boron on soybean. This result, however, runs contrary to those of Chatterjee and Bandyopadhyay (2017) and Desta et al. (2015), who found that molybdenum, zinc, and boron had a substantial impact on both parameters. This contradiction could be due to the different rates required in using the various micro-elements.

Effect of Variety and Iron Fertilizer Application Rates on the Number of Leaves of Cowpea

Table 3: Effect of Variety and Iron Fertilizer Application Rates on Number of Leaves of Cowpea

Variety	Number of Leaves			
	15 DAS	25 DAS	35 DAS	45 DAS
Agyenkwa	2.88	14.64	21.83	26.40
Nketewade	2.67	13.53	20.60	29.71
Zamzam	2.89	14.11	20.32	25.51
LSD (5%)	0.22	1.96	2.23	3.12
Rates (kg ha ⁻¹)				
0	2.78	14.33	21.76	27.00
1	2.80	14.76	21.49	28.76
2	2.87	14.58	21.98	27.89
3	2.80	13.09	19.36	26.27
4	2.82	13.71	20.00	26.11
LSD (5%)	0.27	2.53	2.88	4.03
CV (%)	10.04	18.63	14.26	15.34
p-value(Var* Rates)	0.766	0.932	0.770	0.727

DAS = Days After Sowing, CV = Coefficient of Variation, LSD = Least Significant Difference

The cowpea leaf count was similar across all kinds at 15, 25, and 35 DAS (Table 3), except for 45 DAS. At 45 DAS, Nketewade generated substantially more leaves ($P < 0.05$) than Agyenkwa and Zamzam. There were no significant ($P > 0.05$) variations in the number of leaves in cowpea after iron administration on all sampling days. The interaction impact was substantial ($P < 0.05$) for sampling days. The significant variances detected in the number of leaves of the cowpea plant at 45 DAS indicate that the types had disparities in the number of foliage they generated. Also, selecting a variety will determine the number of leaves the plant can produce and subsequently affect the biomass of the plant. These differences could be attributed to differences in genes responsible for leaf production in these varieties. This finding conforms to the work of Ezeaku *et al.* (2015), who also observed significant differences among cowpea genotypes for their number of leaves. This quality will best suit cowpea production for feeding livestock and also for green manuring as more plant biomass would mean more feed and more source of nitrogen. The contrasting significant differences observed between the number of leaves and number of branches may be since, for Nketewade variety, which produced more leaves, it produced more nodes, which in turn produce leaves, as compared to the other varieties. This finding is in agreement with that of Ajeigbe *et al.* (2005); Kamara *et al.* (2010) and Ezeaku *et al.*

(2015) who identified some varieties to have significantly higher biomass yield and a good source of fodder to livestock.

Effect of Variety and Iron Fertilizer Application on Chlorophyll Content of Cowpea Leaves

Table 4: Effect of Variety and Iron Fertilizer Application on Chlorophyll Content of Cowpea Leaves

Variety	Chlorophyll Content (SPAD Values)			
	15 DAS	25 DAS	35 DAS	45 DAS
Agyenkwa	44.98	62.83	71.01	70.07
Nketewade	43.84	61.03	71.04	71.23
Zamzam	44.07	58.73	67.65	68.07
LSD (5%)	1.90	2.26	1.78	1.95
Rates (kg ha ⁻¹)				
0	43.83	61.26	69.51	68.02
1	44.07	61.71	70.91	69.73
2	45.48	59.86	68.79	70.04
3	43.20	60.55	69.78	69.48
4	44.91	60.92	70.51	71.68
LSD (5%)	2.46	2.91	2.30	2.52
CV (%)	5.74	4.96	3.41	3.74
p-value	0.356	0.762	0.833	0.281
(Var* Rates)				

DAS = Days After Sowing, CV = Coefficient of Variation, LSD = Least Significant Difference

The chlorophyll measurements showed that the cowpea varieties differed significantly ($P < 0.05$) on every sampling date except 15 DAS, with Zamzam consistently having higher chlorophyll levels than the other two varieties (see Table 4.4). Agyenkwa and Nketewade exhibited similar chlorophyll contents on all dates. Iron fertilization rates did not produce any significant effect ($P > 0.05$) at any sampling time, and there was no significant interaction between variety and fertilizer ($P > 0.05$). These results indicate that the varieties are inherently distinct in their chlorophyll production. This is a reflection of the fact that the varieties in

question have different photosynthetic abilities. Hence, it would be promising for one to use the chlorophyll content as one of the criteria for selecting a good cowpea variety (Freitas *et al.*, 2022). The variation may be due to inherent abilities in the cowpea varieties to control the formation of chlorophyll. The non-significant differences observed among the Fe application rates and the interaction between the varieties and Fe application rates for the chlorophyll content is an indication that the Fe did not influence the levels or chlorophyll content in the leaves of cowpea. This result contrasts with the observations of Lavres et al. (2016) and Chatterjee and Bandyopadhyay (2017), who reported that micronutrients, including nickel and molybdenum, enhance chlorophyll content in plant leaves. This contradiction could be due to the rate of Fe applied which may have been low to positively influence chlorophyll formation (Fouda and Abd-Elhamied, 2017).

Days to 50% Flowering and Physiological Maturity of Cowpea Plants

4.5 Effect of Variety and Iron Fertilizer Application on Days to 50% Flowering and Physiological Maturity of Cowpea

Varieties	D50F	DPM
Agyenkwa	36.20	55.47
Nketewade	36.60	56.40
Zamzam	37.47	56.53
LSD (5%)	0.38	0.44
Rates (kg ha ⁻¹)		
0	36.56	56.22
1	36.89	55.89
2	36.78	56.22
3	36.89	56.44
4	36.67	55.89
LSD (5%)	0.49	0.57
CV (%)	1.39	1.06
p-value(Var* Rates)	0.088	0.591

D50F = Days to 50% Flowering, DPM = Days to Physiological Maturity, LSD = Least Significant Difference

Table 5 shows that varietal differences for 50% flowering days were significant at a 5% level of probability. The Zamzam cultivar reached 50 % flowering the quickest, taking significantly fewer days ($P < 0.05$) than the other varieties. Agyenkwa flowered earlier than Nketewade, while iron fertilization had no significant impact ($P > 0.05$) on the time to 50 % flowering. Days to physiological maturity were similar in the varieties as well as in the different rates of fertilizer application (Table 5). The interaction between variety and fertilizer did not reach statistical significance ($P > 0.05$) for either trait. However, the notable differences in days to 50 % flowering and physiological maturity indicate that the varieties are distinctly different in how quickly they flower and mature. This also means that the varieties will require different times to attain flowering and physiological maturity and subsequently be meaningful to make selection among them for targeted purposes such as stress tolerance or evasion. The days to flowering and physiological maturity of cowpea were less influenced by the application of iron as the days to flowering as well as physiological maturity were not significantly affected by the rates of iron and the interaction between the varieties and iron application rates. A shorter time to flowering and maturity means shorter length of days to start formation of sinks and hence the plant would expend less dry matter and thus, channel more dry matter into formation of pods and grain filling. Shorter days to physiological maturity would also cause the variety to save more grain, as it would evade the attack of some field pests. The variation observed could stem from the distinct genetic backgrounds of the cowpea lines tested, and it aligns with earlier reports by Singh *et al.* (2011), Mawo *et al.* (2016) and Adams *et al.* (2017) who also found notable differences among improved genotypes.

Effect of Variety and Iron Fertilizer Application Rates on Crop Growth Rate of Cowpea Plant

Table 6: Effect of Variety and Iron Fertilizer Application Rates on Crop Growth Rate of Cowpea Plant

Varieties	Crop Growth Rate ($\text{gm}^{-2}\text{day}^{-1}$)		
	20-30 DAS	30 - 40 DAS	40 - 50 DAS
Agyenkwa	0.50	0.86	1.61
Nketewade	0.50	0.72	2.20
Zamzam	0.44	1.04	2.03
LSD (5%)	0.15	0.31	0.72

Rates (kg ha ⁻¹)			
0	0.45	1.00	1.50
1	0.54	0.70	2.09
2	0.50	0.76	2.07
3	0.39	0.82	2.10
4	0.52	1.07	1.97
LSD (5%)	0.19	0.40	0.93
CV (%)	40.63	47.19	49.74
p-value (Var* Rates)	0.584	0.971	0.955

DAS = Days After Sowing, CV = Coefficient of Variation, LSD = Least Significant Difference

The crop growth rate measured at 30, 40, and 50 days after sowing (DAS) indicated that neither fertilizer application rates nor variety had a significant effect ($P > 0.05$) across all sampling periods (Table 6). The non-significant differences observed for the crop growth rate of the cowpea varieties imply that varieties had similar rates of growth and that selecting a variety based on the crop growth rate would prove futile. This also means that the varieties were similar in terms of their dry matter accumulation. This could be ascribed to the growth pattern or habit (i.e. determinate or indeterminate nature) of the varieties used in this study, which is consistent with the findings from the work of Haruna and Usman (2013). The non-significant effect of the iron application rates used in this research and the interaction between the varieties and iron application rates on the growth rate of cowpea implies that the rate of cowpea growth was not affected by the iron application rate and that iron application to cowpea would not result in any significant increase in rate of dry matter accumulation. This, however, disagrees with the findings from the work of Poonia *et al.* (2022) who recorded significant dry matter accumulation. This contradiction maybe due to the rates of iron application used in the two studies.

Effect of Variety, Iron Application Rate and the Interaction Effect on Biological Nitrogen Fixation

Table 7: Effect of Variety, Iron Application Rate and the Interaction Effect on Biological Nitrogen Fixation

Variety	Nitrogen Fixed (kg ha ⁻¹)
Agyenkwa	14.28±1.71
Nketewade	20.58±2.16
Zamzam	7.88±1.89
LSD	3.65
Rates (kg ha ⁻¹)	
0	10.06±2.13
1	12.67±4.00
2	15.75±4.36
3	16.90±1.23
4	15.85±2.76
LSD	4.71
CV%	34.26
P-value (Var*Rates)	9.26E-05

CV = Coefficient of Variation, LSD = Least Significant Difference

The results for biologically fixed nitrogen revealed significant variation ($P < 0.05$) among the cowpea cultivars in their capacity to fix atmospheric nitrogen. Additionally, nitrogen fixation was significantly affected ($P < 0.05$) by both iron application rates and the interaction between variety and fertilizer rate. In comparison to the Agyenkwa and Zamzam types, Nketewade had the maximum nitrogen fixed, which differed considerably ($P < 0.05$). Although the 3 kg ha⁻¹ iron application rate resulted in the highest nitrogen fixation, it was significantly different from the control ($P < 0.05$) but did not differ significantly ($P > 0.05$) from the 2 kg ha⁻¹ and 4 kg ha⁻¹ rates. Under 1 kg ha⁻¹ iron application, the Nketewade variety exhibited significantly higher nitrogen fixation than both Agyenkwa and Zamzam. The varieties varied in their capacity to establish symbiotic relationships with native soil rhizobia and to absorb nitrogen from the atmosphere, as evidenced by the notable variations found in the number of nodules, effective nodules, and biological nitrogen fixation (BNF). The highest nodulation recorded by Zamzam variety is an indication that, this variety would readily form symbiotic association with soil-borne nitrogen fixing bacteria to help fix atmospheric nitrogen. This feature would be more relevant for green manuring as more nitrogen would be needed in the tissue of cowpea prior to use as green manure. As a result, green manuring would help lessen the burden on inorganic fertilizers and also enrich the soil biome. This finding conforms to the results of Singh and

Usha (2003) who also observed significant differences among cowpea genotypes. The significantly higher BNF of Nketewade implies Nketewade would be most efficient at fixing atmospheric nitrogen into the soil. It would also be more efficient to use Nketewade as a green manure since it has a higher BNF potential.

4.8 Effect of Variety and Fertilizers Application Rates on Grain Yield Parameters

Table 8: Effect of Variety and Fertilizers Application Rates on Grain Yield Parameters

Variety	Weight of Pod (g)	Pods per Plant	Seeds per Pod
Agyenkwa	92.11	15.35	9.73
Nketewade	113.8	17.11	10.24
Zamzam	114.57	17.56	10.17
LSD (5%)	9.3	1.7	0.54
Rates (kg/ha-1)			
0	86.33	14.8	9.26
1	97.47	12.73	10.26
2	99.11	16.24	9.92
3	115.33	16.2	10.02
4	135.89	23.38	10.78
LSD (5%)	12.01	2.2	0.7
CV (%)	11.64	13.64	7.21
p-value	0.03	0.003	0.208
(Var* Rates)			

CV = Coefficient of Variation, LSD = Least Significant Difference

The considerable differences in grain yield among the varieties indicate that they possess distinct yield potentials. The significantly higher yield recorded for Zamzam and Nketewade is an indication that these varieties would produce higher yield to improve the productivity of cowpea and subsequently the return of cowpea producers in SSA. This could also indicate that Zamzam had a better sink capacity, allowing it to store more dry matter while having much less leaves and chlorophyll content than Nketewade and Agyenkwa. This might also be concluded from the large disparities in pod weight and number of pods per plant across the types, with Zamzam having significantly more pods per plant than Agyenkwa. This agrees with the findings from the work of El Naim and Jabereldar (2010). However, the number of seeds per pod cannot be used as a good indication of grain yield in cowpea as variety did not significantly influence this parameter. This finding contradicts that of Mawo *et al.* (2016), Dugje *et al.* (2009) and Singh *et al.* (2011). The similarity in seeds per pod could be explained as a narrow genetic variation among the genotypes for such trait.

Iron (Fe) application influenced grain yield of cowpea as this recorded significance. This implies, addition of iron to the nutrient requirements of cowpea would increase the productivity of cowpea in SSA. However, selection of specific varieties in combination with different iron application rates would not enhance the productivity of cowpea since this did not record significant differences. A higher yield would be obtained by addition of 4 kg ha⁻¹ of iron to the nutrient requirements of cowpea. This result is consistent with the findings of Shil et al. (2007) and Poonia et al. (2022), who reported that the application of micronutrients, including boron, molybdenum, and iron, can enhance crop yield. Although Fe application did not improve the growth rate and other growth attributes of cowpea, it improved the yield of cowpea. These effects may be attributed to the role of iron in enhancing the uptake of other essential nutrients, such as phosphorus and potassium, which are critical for sink development (pod and seed formation), thereby increasing pod weight as well as pod and seed numbers (Bohra et al., 2006). This observation aligns with the findings of Lavres et al. (2016). Nadergholi et al. (2011) also reported that iron significantly influences reproductive organs, including stamens and pollen. In the present study, higher iron application rates, particularly 4 kg ha⁻¹, resulted in greater pod and seed numbers compared with the untreated control across all varieties (Agyenkwa, Nketewade, and Zamzam). On average, iron application led to increases of 15.79 % in pod number and 26.66 % in grain yield, consistent with the results of Desta et al. (2015), who reported increases of 36.2 % and 10.9 % for these parameters, respectively.

Effect of Variety and Fertilizers Application Rates on Grain Yield and Hundred Seed Weight

Table 9: Effect of Variety and Fertilizers Application Rates on Grain Yield and Hundred Seed Weight

Variety	100 Seed Weight (g)	Grain Yield (kg ha ⁻¹)
Agyenkwa	16.21	1776.39
Nketewade	16.78	1925.97
Zamzam	18.68	1963.75
LSD(5%)	0.62	155.03
Rates (kg ha ⁻¹)		
0	15.32	1556.71
1	16.17	1649.31
2	16.45	1889.58
3	18.14	2047.22
4	20.03	2300.69
LSD (5%)	0.8	200.14
CV (%)	4.81	10.97
p-value (Var*Rates)	0.000	0.286

CV = Coefficient of Variation, LSD = Least Significant Difference

The Zamzam variety produced the highest average seed weight, which was significantly greater ($P < 0.05$) than that of the other varieties. Iron fertilization also markedly influenced seed weight (Table 9), with the 4 kg ha^{-1} rate eliciting the strongest response and significantly outperforming all other treatments ($P < 0.05$). In contrast, the 1 kg ha^{-1} and 2 kg ha^{-1} rates resulted in the smallest increases. As presented in Table 9, both cowpea variety and iron application rate had a significant effect ($P < 0.05$) on grain yield. The Zamzam variety has considerably greater grain output ($P < 0.05$) than the Agyenkwa variety alone. Fertilizer rate of 4 kg ha^{-1} resulted in significantly ($P < 0.05$) better grain output compared to other treatments. The effects of 2 and 3 kg ha^{-1} treatments were comparable, with either outperforming 1 kg ha^{-1} and control treatments. Iron (Fe) application influenced grain yield of cowpea as this recorded significance. This implies, addition of iron to the nutrient requirements of cowpea would increase the productivity of cowpea in SSA. However, the selection of specific varieties in combination with different iron application rates would not enhance the productivity of cowpeas since this did not record significant differences. A higher yield would be obtained by addition of 4 kg ha^{-1} of iron to the nutrient requirements of cowpeas. This result is consistent with the findings of Shil et al. (2007) and Poonia et al. (2022), who reported that crop yield can be enhanced through the application of micronutrients such as boron, molybdenum, and iron. While iron fertilization did not significantly affect cowpea growth rate or other vegetative attributes, it markedly improved yield. This effect may be attributed to iron's role in facilitating the uptake of other essential nutrients, including phosphorus and potassium, which are critical for sink development, leading to increased pod weight and greater pod and seed numbers (Bohra et al., 2006). These observations are in agreement with Lavres et al. (2016). Additionally, Nadergholi et al. (2011) reported that iron significantly influences reproductive structures, including stamens and pollen. In the present study, higher iron application rates, particularly 4 kg ha^{-1} , produced greater pod and seed numbers compared with untreated plots across all varieties (Agyenkwa, Nketewade, and Zamzam). On average, iron application resulted in 15.79 % and 26.66 % increases in pod number and grain yield, respectively, corroborating the findings of Desta et al. (2015), who observed 36.2 % and 10.9 % increases for these parameters.

4.10 Effect of Variety and Iron Fertilizer Application Rates on Mineral (Ultimate) Composition of Cowpea Grain

Table 10: Effect of Variety and Iron Fertilizer Application Rates on Mineral (Ultimate) Composition of Cowpea Grain

Variety	Iron (mgkg ⁻¹)		Phosphorus	Potassium	Calcium	Magnesium	Sodium	Zinc	Total Nitrogen (%)	
	Pod shells	Grain							Residue	Grain
Agyenkwa	473.64±32.6	216.58±23.5	0.85±0.04	2.94±0.04	0.43±0.02	0.16±0.01	0.06±0.00	13.49±0.50	1.63±0.06	4.01±0.11
Nketewade	426.38±26.6	198.47±10.5	0.90±0.02	2.91±0.07	0.49±0.02	0.18±0.01	0.06±0.00	15.76±1.15	2.63±0.07	3.64±0.19
Zamzam	353.14±11.4	189.07±10.2	0.85±0.01	2.98±0.03	0.48±0.02	0.16±0.00	0.07±0.00	12.78±0.32	1.41±0.06	3.59±0.13
LSD (5%) Rates (kg ha ⁻¹)	13.64	0.80	0.00	0.02	0.00	0.01	0.00	0.51	0.04	0.34
0	321.77±12.0	137.81±4.8	0.82±0.02	2.86±0.02	0.41±0.02	0.18±0.00	0.05±0.00	12.11±0.54	1.91±0.31	3.56±0.09
1	337.67±10.3	168.82±4.9	0.77±0.04	2.85±0.06	0.47±0.02	0.18±0.01	0.06±0.00	12.57±0.25	1.79±0.22	3.43±0.06
2	412.91±11.8	201.11±2.3	0.95±0.02	3.00±0.09	0.43±0.04	0.16±0.01	0.07±0.01	15.87±1.93	1.89±0.21	3.90±0.30

3	479.55±35.0	209.22±4.5	0.88±0.03	3.12±0.0	0.51±0.03	0.16±0.01	0.07±0.00	14.23±0.49	1.99±0.15	3.91±0.17
				4						
4	536.70±34.7	289.90±23.2	0.91±0.01	2.90±0.0	0.51±0.02	0.14±0.01	0.06±0.00	15.28±0.67	1.87±0.13	3.92±0.24
				3						
LSD(5%)	17.62	10.30	0.01	0.03	0.01	0.01	0.001	0.66	0.05	0.44
CV (%)	4.37	5.30	0.65	0.92	1.38	4.09	2.73	4.86	2.59	12.09
p-value	3.52E-10	3.05E-12	<2e-16	<2e-16	<2e-16	2.12E-12	<2e-16	1.75E-15	<2e-16	0.016
(Var*Rates										
)										

Values are mean ± se, CV = Coefficient of Variation, LSD = Least Significant Difference

Mineral concentrations differed significantly ($P < 0.05$) among the cowpea varieties evaluated in this study (Table 10). The results also indicated that pod shells contained higher iron levels compared with the seeds. For the concentration of iron in the pod shells and in the grain, Agyenkwa had the highest concentration of iron. These were much greater ($P < 0.05$) than those of Nketewade and Zamzam, the lowest varieties. Nketewade also had the highest concentration of Phosphorus, Calcium and Zinc which were significantly ($P < 0.05$) different from those had by Agyenkwa and Zamzam varieties. However, the calcium concentration in Nketewade was not substantially different ($P > 0.05$) from that of the Zamzam type. Zamzam exhibited much higher potassium and salt values than Nketewade and Agyenkwa. Nketewade had the highest concentration of total nitrogen in the residue, considerably different from Agyenkwa and Zamzam ($P < 0.05$). Despite this, the Agyenkwa cultivar has the greatest concentration of total nitrogen in its grain. This was considerably different ($P < 0.05$) from that of Nketewade and Zamzam. (Table 10).

Table 9 shows significant ($P < 0.05$) differences in mineral concentrations following foliar iron application, with the exception of total nitrogen in the grain. Plots receiving 4 kg ha^{-1} iron exhibited the highest iron concentrations in both pod shells and grains ($P < 0.05$) compared with other treatments, while iron levels in grains from the 2 kg ha^{-1} and 3 kg ha^{-1} treatments were statistically similar. Phosphorus and zinc concentrations were significantly greater ($P < 0.05$) in plots treated with 2 kg ha^{-1} iron compared with the 0 kg ha^{-1} and 1 kg ha^{-1} treatments; however, zinc content in the 4 kg ha^{-1} treatment did not differ significantly ($P > 0.05$) from that in the 2 kg ha^{-1} plots. The 3 kg ha^{-1} treatment produced the highest potassium, calcium, and sodium concentrations, which were significantly greater than in the 0 kg ha^{-1} and 1 kg ha^{-1} treatments. Calcium levels in the 3 kg ha^{-1} and 4 kg ha^{-1} treatments were similar, whereas sodium and potassium differed significantly ($P < 0.05$) between these two rates. Magnesium concentrations were highest in the control and 1 kg ha^{-1} treatments, both significantly higher ($P < 0.05$) than in the 4 kg ha^{-1} treatment, which had the lowest levels. For total nitrogen in cowpea residues, the 3 kg ha^{-1} treatment produced the highest concentrations, significantly exceeding those in the control, 1 kg ha^{-1} , and 4 kg ha^{-1} treatments. In contrast, total nitrogen in the grain did not differ significantly across treatments ($P > 0.05$) (Table 10).

Iron deficiency is a critical public health concern, often linked to inadequate dietary iron intake (Bouis and Welch, 2010; Cakmak et al., 2010). The significant variation observed among the cowpea varieties in mineral concentrations, as revealed by the chemical analyses, suggests that

these varieties differ in their capacity to accumulate essential minerals for human and animal nutrition. This could be explained by the inherent potential of the various varieties to accumulate/store minerals. This finding conforms with those from the work of Addo *et al.* (2016) who indicated significant concentrations in the mineral constituents of some improved cowpea varieties. The higher values of iron recorded in the pod shells relative to the seed is an indication that, the pod shells stored a higher concentration of iron compared to the grain. This could be because, iron, aside from calcium and potassium, is a vital cation for solidification of organic materials. This finding, however, contradicts that of Desta *et al.* (2015) who observed higher concentrations of Fe in the grain of Faba bean. For consumption of fresh green pods, higher levels of iron would be made available compared to dry beans. Also, for animal feed, the dry pod shells could be added to the diet of animals to supplement their iron requirements. The pod shells could also be saved and used as bedding materials or mulch. After decomposition, this could make iron available for plant use.

The significantly higher levels of iron recorded for seeds of Agyenkwa variety is an indication that, this variety would contain higher concentrations of iron and would be very important in a biofortification programme to supplement the iron requirements in the meals of humans, thus fighting hidden hunger. Aside the higher concentration of iron in the seeds of Agyenkwa, it had a higher percentage total nitrogen (TN) which further confers a higher protein content compared to the other varieties. But for the significantly lower yield of Agyenkwa variety compared to Zamzam variety, Agyenkwa would have been a perfect genotype for fighting hidden hunger. This requires that the variety be improved to make up for the yield difference. This result is consistent with the findings of Freitas *et al.* (2022), who identified cowpea genotypes with high iron concentrations capable of providing over 30 % of the recommended daily intake. The significant differences observed among the Fe application rates for the concentration of the minerals in the ultimate chemical analysis, except for the TN in grain implies, the application of iron would significantly improve the concentrations of these minerals but not the TN in the grain. Also, the significant differences observed for the proximate nutrient composition except for the protein content, is an indication that application of iron would also enhance the nutrient composition of cowpea grain. This observation could be because iron aids the absorption of certain minerals such as zinc and potassium. This finding is per that of Burton *et al.* (1998) and Aciksoz *et al.* (2011) who indicated that iron application significantly improved the concentration of minerals including iron in the grain. On average, the application of Fe enhanced the concentration of Fe in grain by 57.65%. In wheat, foliar

spray of FeSO_4 improved grain Fe concentration by about 28% in China (Zhang *et al.*, 2010) and 21% in Iran, (Pahlavan-Rad and Pessarakli, (2009).

The non-significant effect recorded for the TN in grain implies the application of Fe would not enhance the concentration of total nitrogen in the grain of cowpea. This contradicts earlier results in this work which indicated significance of Fe application on BNF. This contradiction could be due to the fact that, the nitrogen fixed from the atmosphere was extensively stored in the biomass of the cowpea plant. As such there was no efficient translocation of N into the seeds during grain filling. This finding is in agreement with the work of Aciksoz *et al.* (2011). This finding, however, disagrees with the the work of Desta *et al.* (2015) who indicated that micronutrient application enhanced the concentration of nitrogen in the grain of faba bean. Higher rates of iron (4 kg ha^{-1}) not only enriched the concentration of iron in the grain but also improved absorption of minerals and enriched the concentration of these minerals such as calcium and zinc. These findings suggest that efforts to biofortify cowpea with iron may concurrently enhance the accumulation of other minerals, such as calcium and zinc. This contrasts with the results of Aciksoz *et al.* (2011), who reported a reduction in zinc concentration in wheat grain following iron application. Conversely, Marschner (2002) noted that calcium is primarily deposited in the vegetative tissues of maturing plants due to its limited phloem mobility.

4.11 Effect of Variety on Proximate Nutrients Composition of Cowpea Grain

Table 11: Effect of Variety on Proximate Nutrients Composition of Cowpea Grain

Variety	Fat	Fibre Percentage (%)	Ash	Moisture	Protein	NFE- Carbohydrates
Agyenkwa	4.52±0.30	2.61±0.12	2.65±0.21	6.16±0.12	25.04±0.70	59.02±0.95 b
Nketewade	3.81±0.27	3.21±0.21	2.93±0.07	6.83±0.17	22.75±1.19	60.47±1.16
Zamzam	3.11±0.07	2.44±0.06	2.43±0.14	6.91±0.23	22.45±0.79	62.67±0.88
LSD (5%)	0.14	0.12	0.06	0.35	2.12	2.17
Rates (kg ha^{-1})						
0	3.06±0.04	3.08±0.40	2.87±0.18	6.30±0.14	22.28±0.58	62.40±0.99

1	4.72±0.48	2.91±0.15	3.08±0.10	6.59±0.11	21.45±0.40	61.26±0.86 a
2	3.96±0.46	2.72±0.10	2.71±0.13	6.42±0.18	24.37±1.90	59.82±2.37
3	3.48±0.07	2.48±0.19	2.83±0.07	7.30±0.31	24.45±1.08	59.45±0.74
4	3.83±0.28	2.58±0.05	1.86±0.20	6.55±0.36	24.52±1.50	60.66±1.49
LSD	0.19	0.15	0.08	0.45	2.73	2.80
CV (%)	5.05	5.69	2.94	7.00	12.09	4.78
p-value	1.25E-15	4.47E-14	2.20E-16	0.001	0.016	0.001
(Var*Rates)						

Values are mean ± se, CV = Coefficient of Variation, LSD = Least Significant Difference

Analysis of variance for the proximate composition revealed significant differences ($P < 0.05$) among the cowpea varieties (Table 11). The Agyenkwa variety exhibited the highest fat and protein contents, which were significantly greater ($P < 0.05$) than those of Zamzam and Nketewade, while the latter two varieties had statistically similar protein levels. Nketewade showed the highest fiber and ash contents, significantly exceeding those of Zamzam and Agyenkwa. For nitrogen-free extract (NFE) carbohydrates, Zamzam recorded the highest value, significantly differing ($P < 0.05$) from both Nketewade and Agyenkwa. Although Zamzam also had the highest moisture content, which was significantly higher ($P < 0.05$) than that of Agyenkwa, it did not differ significantly ($P > 0.05$) from Nketewade (Table 4.10). Regarding the influence of iron application rates, significant effects ($P < 0.05$) were observed for all proximate nutrients except protein and NFE carbohydrates (Table 10).

Iron application significantly influenced the proximate composition of cowpea ($P < 0.05$). Foliar application at 1 kg ha^{-1} resulted in the highest fat and ash contents compared with other rates, whereas the control exhibited the greatest fiber content, significantly exceeding all iron-treated plots ($P < 0.05$). Moisture content was highest under the 3 kg ha^{-1} iron treatment, significantly differing from the other application rates, while all remaining treatment means were statistically comparable.

Analysis of variance further revealed significant variation ($P < 0.05$) among cowpea varieties (Table 4.12). The Agyenkwa variety had the highest fat and protein contents, which were significantly greater than those of Zamzam and Nketewade, the latter two being statistically similar in protein content. Nketewade recorded the highest fiber and ash contents, significantly exceeding those of Zamzam and Agyenkwa. For nitrogen-free extract (NFE) carbohydrates, Zamzam had the highest values, significantly differing from both Nketewade and Agyenkwa.

Although Zamzam also had the highest moisture content, it was significantly greater than Agyenkwa ($P < 0.05$) but not significantly different from Nketewade ($P > 0.05$).

Overall, iron application rates significantly affected all proximate nutrients except protein and NFE carbohydrates ($P < 0.05$). Specifically, fat and ash contents were maximized at 1 kg ha^{-1} , fiber was highest in the control, and moisture content peaked at 3 kg ha^{-1} , with all other treatment means showing no significant differences.

CONCLUSION

This study highlights the potential of agronomic biofortification as a practical and sustainable approach to alleviating iron deficiency, particularly in Sub-Saharan Africa. The results indicate that optimized iron application enhances not only the mineral content but also the yield and nutritional quality of cowpea, a staple crop critical to food security and nutrition in Ghana and other regions. Notably, foliar application of 4 kg ha^{-1} iron produced the highest iron concentrations in both grains and pods, substantially improving their dietary value. Among the varieties tested, Agyenkwa demonstrated superior iron content and nitrogen-fixing capacity, identifying it as a promising candidate for targeted biofortification programs. These findings support the broader adoption of foliar iron fertilization as an immediate strategy to combat “hidden hunger” in developing countries. While conventional and genetic breeding approaches remain important for long-term biofortification, the accessibility and effectiveness of agronomic interventions make them indispensable in current food systems. Future studies should focus on optimizing the trade-off between yield and nutrient density, as well as evaluating the scalability of these interventions across diverse agroecological zones. Incorporating such strategies into agricultural policies could substantially improve public health outcomes and advance the development of more resilient, nutrient-rich food systems, aligning with global food and nutrition objectives.

ACKNOWLEDGEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors sincerely acknowledge the staff of the CSIR–Crops Research Institute, Kumasi, Ghana, and the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, for their technical support, guidance, and assistance during the execution of this project and the preparation of the manuscript.

DECLARATIONS

Ethics and Consent to Participate

The cowpea plant materials used in this study were cultivated for experimental purposes at the CSIR-Crops Research Institute, Fumesua, Ashanti Region, Ghana. The plant materials comprised the released cowpea varieties Zamzam, Nketewade, and Agyenkwa, which were sourced from the CSIR-Crops Research Institute. The collection, cultivation, and use of the plant materials complied with applicable institutional and national guidelines for agricultural research. No human participants or animals were involved in this study.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

This research received no external funding.

Authors' Contributions

A.B. conceived and designed the study, conducted the field experiment, collected and analysed the data, and prepared the initial manuscript. R.A. contributed to the study design, data analysis and interpretation, and manuscript preparation and revision. J.S.-A. provided academic supervision and contributed to the study design, interpretation of results, and critical revision of the manuscript. K.A. contributed to the provision and characterization of the cowpea varieties and supported the field and laboratory aspects of the study. G.A. contributed to the experimental work, data collection and laboratory analyses. All authors read and approved the final manuscript.

REFERENCES

- Abbas, G., Khan, M. Q., Khan, M. J., Hussain, F., & Hussain, I. (2009). Effect of iron on the growth and yield contributing parameters of wheat (*Triticum aestivum* L.). *Journal of Animal and Plant Sciences*, 19(3), 135–139.
- Aciksoz, S. B., Yazici, A., Ozturk, L., & Cakmak, I. (2011). Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. *Plant and Soil*, 349(1–2), 215–225. <https://doi.org/10.1007/s11104-011-0863-2>
- Adams, B., Osekre, E., & Amoah, S. (2018). Evaluation of Cowpea (*Vigna unguiculata* L.) Genotypes' Growth and Yield Performance and Resistance to the Cowpea Seed Beetle, *Callosobruchus maculatus* F. *Journal of Experimental Agriculture International*, 19(5), 19.
- Ajeigde, H. A., Singh, B. B., & Osenj, T. O. (2005). Cowpea-cereal intercrop productivity in the Sudan savanna zone of Nigeria as affected by planting pattern, crop variety and pest management. *African crop science journal*, 13(4), 269–279.
- Amarakoon, D., Thavarajah, D., McPhee, K., & Thavarajah, P. (2012). Iron-, zinc-, and magnesium-rich field peas (*Pisum sativum* L.) with naturally low phytic acid: A potential food-based solution to global micronutrient malnutrition. *Journal of Food Composition and Analysis*, 27(1), 8–13. <https://doi.org/10.1016/j.jfca.2012.05.007>
- Animasaun, D. A., Oyedele, S., Mustapha, O. T., & Azeez, M. A. (2015). Genetic Variability Study Among Ten Cultivars of Cowpea (*Vigna unguiculata* L. Walp) Using Morpho-agronomic Traits and Nutritional Composition. *Journal of Agricultural Sciences*, 10(2), 119. <https://doi.org/10.4038/jas.v10i2.8057>
- Annobil Bisiw Gabriel (2018). Assessing the impact of mixed solar dryer on the quality of heap fermented cocoa beans. In Thesis submitted to Kwame Nkrumah University of Science and Technology. Kwame Nkrumah University of Science and Technology.
- AOAC (1990). Official Methods of Analysis of the Association of Official Analytical Chemists. (4th Ed. Williams, S.) *Association of Analytical Chemists*, USA.
- Bloem, J.F., Trytsman, G., & Smith, H.J. (2009). Biological nitrogen fixation in resource-poor agriculture in South Africa. *Symbiosis*, 48, 18–24.

- Bouis, H. E., & Welch, R. M. (2010). Biofortification a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, 50, S-20.
- Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V., & Pfeiffer, W. H. (2011). Biofortification: a new tool to reduce micronutrient malnutrition. *Food and nutrition bulletin*, 32(1_suppl1), S31–S40.
- Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., & Fatokun, C. (2019). Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding*, 138(4), 415–424.
- Cakmak, I. (2014). Agronomic biofortification. Conference brief# 8. In Proceedings of the 2nd global conference on biofortification: getting nutritious foods to people, Rwanda.
- Cakmak, I., Pfeiffer, W. H., & McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. *Cereal chemistry*, 87(1), 10–20.
- Carvalho, A. F. U., De Sousa, N. M., Farias, D. F., Da Rocha-Bezerra, L. C. B., Da Silva, R. M. P., Viana, M. P., Gouveia, S. T., Sampaio, S. S., De Sousa, M. B., De Lima, G. P. G., De Moraes, S. M., Barros, C. C., & Filho, F. R. F. (2012). Nutritional ranking of 30 Brazilian genotypes of cowpeas including determination of antioxidant capacity and vitamins. *Journal of Food Composition and Analysis*, 26(1-2), 81-88. <https://doi.org/10.1016/j.jfca.2012.01.005>
- Devi, C. B., Kushwaha, A., & Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea (*Vigna unguiculata*). *Journal of food science and technology*, 52(10), 6821–6827. <https://doi.org/10.1007/s13197-015-1832-1>
- Dugje, I. Y., Omoigui, L. O., Ekeleme, F., Kamara, A. Y., & Ajeigbe, H. (2009). Farmers' guide to cowpea production in West Africa. IITA, Ibadan, Nigeria, 20, 12–14.
- FAO. (2021). Food and Agriculture Organization of the United Nations. Rome. URL: <http://faostat.fao.org>

- Frankenberger Jr, W. T., & Abdelmagid, H. M. (1985). Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into soil. *Plant and Soil*, 257–271.
- Freitas, T. K. T., Gomes, F. D. O., Araújo, M. D. S., Silva, I. C. V., Silva, D. J. S., Damasceno-Silva, K. J., & Rocha, M. D. M. (2022). Potential of cowpea genotypes for nutrient biofortification and cooking quality. *Revista Ciência Agronômica*, 53, e20218048.
- García-Oliveira, A. L., Chander, S., Ortiz, R., Menkir, A., & Gedil, M. (2018). Genetic basis and breeding perspectives of grain iron and zinc enrichment in cereals. *Frontiers in Plant Science*, 9, 937.
- Hemmati, A. (2022). The effects of rhizobium strains on drought resistance, yield and yield components of common bean in dryland conditions. *Water Productivity Journal*, 2(1), 95–110.
- Hoekenga, O. W., Lung'aho, M. G., Tako, E., Kochian, L. V., & Glahn, R. (2011). Iron biofortification of maize grain. *Plant Genetic Resources: Characterization and Utilization*, 1–3.
- Lavres, J., Castro Franco, G., & de Sousa Câmara, G. M. (2016). Soybean seed treatment with nickel improves biological nitrogen fixation and urease activity. *Frontiers in Environmental Science*, 4, 37.
- Márquez-Quiroz, C., De-La-cruz-Lázaro, E., Osorio-Osorio, R., & Sánchez-Chávez, E. (2015). Biofortification of cowpea beans with iron: Iron's influence on mineral content and yield. *Journal of Soil Science and Plant Nutrition*, 15(4), 839–847. <https://doi.org/10.4067/S0718-95162015005000058>
- Mfeka, N., Mulidzi, R. A., & Lewu, F. B. (2019). Growth and yield parameters of three cowpea (*Vigna unguiculata* L. Walp) lines as affected by planting date and zinc application rate. *South African Journal of Science*, 115(1–2), 1–9.
- MoFA. (2019). Agriculture in Ghana: Facts and Figures (2018). Ministry of Food and Agriculture–Ghana.
- Pahlavan-Rad, M. R., & Pessarakli, M. (2009). Response of wheat plants to zinc, iron, and manganese applications and uptake and concentration of zinc, iron, and

- manganese in wheat grains. *Communications in Soil Science and Plant Analysis*, 40(7–8), 1322–1332.
- Pal, V., Singh, G., & Dhaliwal, S. S. (2019). Agronomic biofortification of chickpea with zinc and iron through application of zinc and urea. *Communications in Soil Science and Plant Analysis*, 50(15), 1864–1877.
<https://doi.org/10.1080/00103624.2019.1648490>
- Phattarakul, N., Rerkasem, B., Li, L.J., Wu, L.H., Zou, C.Q., Ram, H., Sohu, V.S., Kang, B.S., Surek, H., Kalayci, M., & Yazici, A. (2012). Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant and Soil*, 361, 131–141.
- Poelman, M., Strak, M., Schmitz, O., Hoek, G., Karssenbergh, D., Helbich, M., Ntarladima, A.M., Bots, M., Brunekreef, B., Grobbee, R., & Dijst, M. (2018). Relations between the residential fast-food environment and the individual risk of cardiovascular diseases in The Netherlands: A nationwide follow-up study. *European journal of preventive cardiology*, 25(13), 1397–1405.
- Poonia, T., Bhunia, S. R., & Choudhary, R. (2022). Effect of iron fertilization on growth, yield and economics of groundnut (*Arachis hypogaea* L.). *International Journal of Economic Plants*, 9(Feb, 1), 038–044.
- Ramos, D.P., Tavares, T.C.D.O., Sousa, S.A.D., Nascimento, V.L., Martinez, R.A.S., Chagas Junior, A.F., & Fidelis, R.R. (2020). Agronomic biofortification of cowpea with selenium by foliar fertilization: effect of doses in three cultivars. *Journal of Plant Nutrition*, 43(4), 538–547.
- Ruel, M. T., & Alderman, H. (2013). Nutrition-sensitive interventions and programmes: How can they help to accelerate progress in improving maternal and child nutrition? *The Lancet*, 382(9891), 536–551.
- Salih, H. O. (2013). Effect of Foliar Fertilization of Fe, B, and Zn on nutrient concentration and seed protein of Cowpea (*Vigna unguiculata*). *IOSR Journal of Agriculture and Veterinary Science*, 6(3), 42–46. <https://doi.org/10.9790/2380-0634246>
- Shil, N. C., Noor, S., & Hossain, M. A. (2007). Effects of boron and molybdenum on the yield of chickpea. *Journal of Agriculture & Rural Development*, 17–24.

- Singh, A., Baoule, A. L., Ahmed, H. G., Dikko, A. U., Aliyu, U., Sokoto, M. B., & Haliru, B. (2011). Influence of phosphorus on the performance of cowpea (*Vigna unguiculata* (L) Walp.) varieties in the Sudan savanna of Nigeria. *Agricultural Sciences*, 2(03), 313.
- Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S., & Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Research*, 84(1–2), 169–177. [https://doi.org/10.1016/S0378-4290\(03\)00148-5](https://doi.org/10.1016/S0378-4290(03)00148-5)
- Singh, B., & Usha, K. (2003). Nodulation and symbiotic nitrogen fixation of cowpea genotypes as affected by fertilizer nitrogen. *Journal of Plant Nutrition*, 26(2), 463–473.
- Singh, U., Praharaj, C. S., Chaturvedi, S. K., & Bohra, A. (2016). Biofortification: Introduction, approaches, limitations, and challenges. *Biofortification of Food Crops*, 3–18.
- Teklu, D., Gashu, D., Joy, E. J. M., Lark, R. M., Bailey, E. H., Wilson, L., Amede, T., & Broadley, M. R. (2023). Impact of zinc and iron agronomic biofortification on grain mineral concentration of finger millet varieties as affected by location and slope. *Frontiers in Nutrition*, 10(May), 1–9. <https://doi.org/10.3389/fnut.2023.1159833>
- Viera-Vargas, M.S., Souto, C.M., Urquiaga, S., & Boddey, R.M., (1995). Quantification of the contribution of N₂ fixation to tropical forage legumes and transfer to associated grass. *Soil Biology and Biochemistry*, 27(9), .1193-1200
- Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, 55(396), 353–364. <https://doi.org/10.1093/jxb/erh064>
- Zhang, Y., Shi, R., Rezaul, K. M., Zhang, F., & Zou, C. (2010). Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. *Journal of Agricultural and Food Chemistry*, 58(23), 12268–12274.