

Original Research Article

Evaluating the Impact of Fertilisers and Elicitors on *Amaranthus cruentus* (L): Sustainable Fertilization and Elicitation Strategies for Enhanced Nutrition and Productivity

ABSTRACT

Amaranthus cruentus is a leafy vegetable that is widely grown and consumed in Nigeria. However, little effort has been made to focus on sustainable fertilization and enhance its micronutrient content. Addressing this issue could help alleviate micronutrient deficiencies in Nigeria.

In 2018, *Amaranthus cruentus* plants were grown in the screen house at the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria to assess the impact of fertilizers and elicitors on the growth, yield, and micronutrient composition of *Amaranthus cruentus*. The experiment comprised of compost manure (CP) applied at the rates of 5, 10, and 15 t/ha (CP₅, CP₁₀, and CP₁₅), poultry manure (PM) applied at equivalent rates (PM₅, PM₁₀, and PM₁₅), and NPK 15:15:15 fertilizer applied at the rates of 50, 100, and 150 kg/ha (NPK₅₀, NPK₁₀₀, and NPK₁₅₀), Methyl jasmonate and Salicylic acids applied each at 125, 250, 500 µmol/L (MejA₁₂₅, MejA₂₅₀, MejA₅₀₀; SA₁₂₅, SA₂₅₀, SA₅₀₀), with a control group receiving no treatment. The study used a completely randomized design with three replicates. Data were collected on plant height, number of leaves, leaf area, and stem girth. Harvesting was done six weeks after sowing, and fresh weight, dry matter was determined. Folate, iron and zinc, moisture contents, crude fiber, and ash contents were determined. Data were analysed with analysis of variance (ANOVA). Means were separated with Duncan Multiple Range Test (P=0.05).

A. cruentus plants treated with NPK₁₀₀ exhibited the highest plant height and leaf area. PM₁₀ treatment yielded the highest marketable yield (52.29 g/plant). SA₂₅₀ resulted in significantly higher folate content (250.20 µg/100) compared to the control. CP₁₀ treatment led to higher iron content (22.00 mg/100g) and iron concentration (17.30 mg/100g). Compost and poultry manure fertilizers at a rate of 10 t/ha and salicylic acid at 250 µmol/L can improve the nutritional quality of *A. cruentus*.

Keywords: [Amaranthus cruentus, growth, fertilizers, yield, micronutrients]

1. INTRODUCTION

The worldwide health emergency brought about by the Corona Pandemic coupled with the menace of hidden hunger on public health has shifted the attention of people globally to the nutritional value of their diet, particularly the micronutrients that are essential in boosting the body's immune system (1, 2). Therefore, the significance of agriculture, particularly in the production of high-quality food crops, has increased (3, 4). The agricultural farming system is now much more than just producing food; it is essential to supply food that promotes human health (5). This is because many people in developing countries continue to suffer from hidden hunger, despite increased crop yields (6,56,57,58). For example, there is a high prevalence of hidden hunger in Nigeria, as shown by the Global Hunger Index, which measures undernourishment, child underweight, and child mortality. Approximately 20% of African children are stunted, and 45% of deaths in under-fives are related to undernutrition (7). This affects cognitive development and negatively impacts future potential.

Hidden hunger results from insufficient intake of essential micronutrients, such as vitamins and minerals, owing to a lack of diversity in the diet, poor soil quality, and low micronutrient content in staple foods consumed in Africa (8, 6). People who rely mainly on staple foods, such as cereals, roots, and tubers, and consume few fruits and vegetables are at risk of hidden hunger because plants are the primary source of food and micronutrients for humans (9). The deficiency of certain nutrients in the soil may affect the quality of crops grown on such soil, which in turn could affect the health and well-being of people who consume such food crops (10).

Although several biofortification strategies, including breeding, metabolic engineering, and agronomic biofortification, have been adopted to enhance micronutrients in plants (11, 12), the choice of biofortification approach mostly depends on micronutrient deficiencies. Agronomic biofortification, which is a process of increasing micronutrient content in food

crops through agronomic approaches, such as the use of fertilizers and elicitors have the potential to improve crop productivity and nutritional quality by addressing micronutrient deficiencies because they can be easily adopted by farmers without the necessary technical skills required to carry out breeding and metabolic engineering (13, 14).

In addition, elicitors such as salicylic acid and methyl jasmonate are known to have an impact on the status of various metabolites and the general physiological states of crops. Thus, use of elicitors, serves as an alternative approach, sidestepping the substantial expenses associated with metabolic engineering (14).

The positive influence of fertilizer and elicitor application on yield, it also affects other aspects of human nutrition such as the quantity of vitamins and minerals in plants.

Furthermore, fertilizer application has been shown to enhance the nutritional quality of food crops in addition to increasing yield (15). However, more emphasis has been placed on optimum yields than on food quality (16). Thus, fertilizer usage should be directed towards promoting human health to achieve the goal of food security and healthy diets, leading to healthy lives in the face of an ever-increasing world population (17, 18). Although fertilizer use in agriculture is high because it has been shown to improve soil quality and crop productivity, the extent to which fertilizers can enhance changes in crop nutritional quality, particularly micronutrients such as zinc (Zn), iron (Fe) and folate in *Amaranthus cruentus*, has rarely been studied in Nigeria.

Moreover, over the past two decades, there has been increased interest in the promotion of African leafy vegetables as sources of micronutrients and bioactive compounds (19, 20). *Amaranth* leaves are rich in proteins and micronutrients, including iron, calcium, zinc, vitamin C, and vitamin A (21). However, research avenues continue to explore ways to increase yield. Although the yield has increased, micronutrients such as folate, iron, and zinc are still low. This trend might be linked to the adoption of high-yielding varieties and potential fertilizer misuse. Thus, leveraging agronomic approaches in terms of fertilizer usage would contribute to climate-smart agriculture, enhance the sustainability of agricultural systems, and improve human health. This study was designed to determine the effects of elicitors and inorganic and organic fertilizers on the growth and folate and mineral contents of *Amaranthus cruentus*.

2. MATERIAL AND METHODS

A screen house trial was conducted at the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria, in 2016. The experiment comprised the following

treatments: organic manure (poultry manure and compost); compost manure applied at three rates (5 t/ha-CP₅, 10 t/ha-CP₁₀ and 15 t/ha- CP₁₅); poultry manure applied at three rates (5 t/ha-PM₅, 10 t/ha-PM₁₀ and 15 t/ha- PM₁₅); NPK 15:15:15 (inorganic fertilizer) applied at 50 kgN/ha- NPK₅₀, 100 kgN/ha -NPK₁₀₀, and 150 kgN/ha -NPK₁₅₀ and a control (no treatment), Methyl jasmonate(MejA) and Salicylic acid (SA) applied each at 125, 250, 500 µmol/L (MejA₁₂₅, MejA₂₅₀, MejA₅₀₀; SA₁₂₅, SA₂₅₀, SA₅₀₀, respectively). The experiment was conducted in a completely randomized design (CRD) with three replicates.

List 1 : Treatment composition

Treatments	Composition
NPK ₅₀	50 kg Nha ⁻¹ NPK
NPK ₁₀₀	100 kg Nha ⁻¹ NPK
NPK ₁₅₀	150 kg Nha ⁻¹ NPK
CP ₅	5 t-ha ⁻¹ Compost
CP ₁₀	10 t-ha ⁻¹ Compost
CP ₁₅	10 t-ha ⁻¹ Compost
PM ₅	5 t-ha ⁻¹ Poultry manure
PM ₁₀	10 t-ha ⁻¹ Poultry manure
PM ₁₅	15 t-ha ⁻¹ Poultry manure
MejA ₁₂₅	Methyl jasmonate applied at 125 µmol/L
MejA ₂₅₀	Methyl jasmonate applied at 250 µmol/L
MejA ₅₀₀	Methyl jasmonate applied at 500 µmol/L
SA ₁₂₅	Salicylic acid applied at 125 µmol/L
SA ₂₅₀	Salicylic acid applied at 250 µmol/L
SA ₅₀₀	Salicylic acid applied at 500 µmol/L
Control	No treatment

Amaranthus cruentus seeds were obtained from the National Institute of Horticultural Research (NIHORT) Ibadan. Cured Poultry manure was obtained from the Teaching and Research Farm of the University of Ibadan, Nigeria. NPK (15:15:15) was obtained from the Department of Agronomy at the University of Ibadan, Nigeria. Composting was performed from the shoots of *Tithonia diversifolia* (L) and fresh poultry manure, which were mixed in a ratio of 3:1 on a layer basis. The heap was left to decompose for three months with continuous turning and watering every week. The matured compost was air-dried and ground to ensure uniformity (22).

Forty-eight, 5 litre capacity polythene planting bags were filled with five-kilogram (5 kg) dry top soil collected at a depth of 0-15 cm at the crop garden of the Department of Crop Protection and Environmental Biology. Compost (5, 10, and 15 t/ha) and poultry manure (5, 10, and 15 t/ha) were applied to the soil in polythene bags one week before sowing, and two weeks after sowing, NPK was administered at 50, 100, and 150 kg N/ha two weeks after sowing. Crystal salicylic acid was first dissolved in 1 ml of absolute ethanol and added in drops to 1L of distilled water (ethanol/water - 1 /1000 v/v) and three drops of Tween 20 added as surfactant. Methyl jasmonate was added dropwise to 1L of distilled water (54, 55). Methyl jasmonates and Salicylic acids were each applied thrice at 3, 4 and 5 weeks after sowing at concentrations of 125, 250, 500 $\mu\text{mol/L}$. The experiment was laid out in a completely randomized design (CRD) with three replicates.

Amaranthus cruentus seeds were sown directly into each of the designated 48 planting bags, thinning was done to two plants per pot at 2 weeks after planting, and watering was done regularly to field capacity.

Data were collected starting two weeks after sowing at two weeks interval on the following growth parameters of *Amaranthus cruentus*: plant height, number of leaves, leaf area, and stem diameter. Shoot harvesting was performed six weeks after sowing, and yield parameters, such as shoot fresh weight and shoot dry matter, were determined. Folate (23, 24, 25), iron and zinc (26, 27), percentage moisture content, crude fiber, and ash content were determined in shoot dry matter using standard procedures (27). All data were analyzed by analysis of variance (ANOVA) using SAS software. Means were separated using Duncan's multiple range test ($P = .05$).

3. RESULTS AND DISCUSSION

In this study, both organic and inorganic fertilizers improved *Amaranthus cruentus* performance. Fertilizer application (organic and inorganic) has been shown to improve plant performance, which reconfirms the assertion that fertilizers are necessary for optimal plant performance (28, 29). Several researchers have affirmed the beneficial effects of fertilizers on the performance of *Amaranthus cruentus* (30)

3.1. Vegetative Parameters

3.1.1 Plant height (cm)

The effect of fertilizer application on the height of *Amaranthus cruentus* was significantly (Table 1). The tallest plants (52.70 cm) were observed in NPK₁₀₀ treated plants, which were comparable heights of *Amaranthus cruentus* treated with PM₁₀, PM₁₅, CP₁₀, and CP₁₅. The height of plants treated with organic manure (poultry manure and compost) compared favorably with inorganic amendments (NPK₅₀, NPK₁₀₀), suggesting that although organic manure may release of nutrients slowly, it can still provide adequate nutrient for plant growth, and contains secondary nutrients that promote overall plant growth and development (31, 32).

SA₂₅₀ resulted in the highest plant height (49.80 cm) when compared among elicitor treatments (Table 1), similar to the heights obtained in plants foliar sprayed with MejA₅₀₀, and in plant foliar sprayed SA₅₀₀, while the lowest plant height (37.62 cm) was obtained in the control. Salicylic acid plays important role in plant defense against environmental stress which in turn leads to healthy plants and better allocation of nutrients for plant growth (34)

3.1.2 Number of leaves

PM₁₀ produced the highest number of leaves (22.70), which was similar to the number of leaves obtained under PM₁₅, CP₁₀, CP₅, NPK₁₀₀, and MejA₅₀₀. SA₅₀₀ produced more leaves than the control (16.00), whereas plants sprayed with SA₁₂₅ and SA₅₀₀ produced leaves that were not more than those of the control (Table 1). The increase in the number of leaves might be because fertilizers provide the necessary nutrients for leaf development, especially nitrogen, which is essential for leaf development (35,38). This indicates that the NPK, compost, and poultry manure used in this study contained adequate Nitrogen to enhance leaf growth. Methyl jasmonate Salicylic acid enhance plant ability to withstand external stress which in turn leads to healthy plants and better allocation of nutrients leaf production (34)

3.1.3 Stem girth (cm)

Similarly, the highest stem girth (3.05 cm) was obtained in *Amaranthus cruentus* treated with PM₁₅, which was superior to the control (2.01 cm), but not more than the values obtained in CP₁₀, CP₁₅, PM₁₀, and PM₅, and was closely followed by *Amaranthus cruentus* that received

NPK₁₀₀ when compared to the control (Table 1). Since N, P and K is essential for plant growth, the availability of these nutrients for plant use might have influenced stem girth (35) In elicitor treatments, the highest stem girth (2.60 cm) obtained in plants sprayed SA₂₅₀ which was similar to all the rates of either SA or MejA applied (34)

3.1.4 Leaf area (cm²)

All fertilizer treatments produced leaf area values that were significantly higher than the control, with the highest leaf area (65.05 cm²) obtained in *Amaranthus cruentus* amended with NPK₁₀₀, and it was not more than the leaf area obtained in PM₁₅, PM₁₀, PM₅, CP₁₀ and NPK₁₅₀, respectively (Table 1). In elicitor treatments, *Amaranthus cruentus* sprayed with SA₂₅₀ had the highest leaf area (58.35 cm²) and was similar to the leaf area obtained in plants sprayed with SA₅₀₀, SA₁₂₅, and MejA₅₀₀ (Table 1). The increased leaf area observed as a result of application of poultry manure (PM₅, PM₁₀, PM₁₅), compost (CP₁₀) or NPK (NPK₁₀₀, NPK₁₅₀) may be attributed to the favorable soil conditions created by the organic matter components of poultry manure and compost. Meanwhile, the effects by NPK may be due to the availability of nitrogen, phosphorus and potassium, which are readily available for plant uptake and contribute to plant overall growth (32, 37). MejA and SA interacts with other hormones such as Auxin and cytokinin in the plant, this balance interaction enhances leaf initiation and growth (36)

Table 1: Effects of fertilisers and elicitors on the vegetative parameters of *Amaranthus cruentus* in the screen house at 6 weeks after sowing

Treatments	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area(cm ²)
CP ₅	44.40c	20.60ab	2.42bcd	56.45bc
CP ₁₀	50.76ab	20.75ab	3.00a	60.67a
CP ₁₅	51.55a	19.00bc	2.70abc	55.75bc
PM ₅	47.20bc	18.00cd	2.50abc	61.80a
PM ₁₀	50.40ab	22.70a	2.73abc	64.93a
PM ₁₅	51.89a	20.90ab	3.05a	60.75a
NPK ₅₀	49.60ab	17.00cd	2.20bcd	52.18c
NPK ₁₀₀	52.70a	19.50bc	2.54abc	65.05a
NPK ₁₅₀	50.00ab	20.00bc	2.45abc	62.39a
MejA ₁₂₅	39.55fg	19.30bc	2.20bcd	47.49e
MejA ₂₅₀	44.30de	18.75bcd	2.33bcd	50.81cde
MejA ₅₀₀	48.65abc	21.60abc	2.59abc	54.45c

SA ₁₂₅	44.90bcd	18.00bcd	2.40bcd	52.20d
SA ₂₅₀	49.80ab	20.10abc	2.60abc	58.35bc
SA ₅₀₀	45.25cd	17.50bcde	2.45abc	57.99bc
Control	37.62d	16.00d	2.01d	47.35d

Means with identical letters in each column are statistically significant (P = .05).

3.2 Yield Parameters

3.2.1 Fresh weight (g/plant)

The marketable yield (fresh weight) of *Amaranthus cruentus* was significantly influenced by fertilizer treatments (Table 2). Applied poultry manure rates (PM₅, PM₁₀, PM₁₅) enhanced fresh weight compared to control, and were comparable to fresh weight achieved in *Amaranthus* treated with CP₁₅ or NPK₁₅₀. These yield increases associated with poultry manure could be attributed to the rich nutrient profile and slow-release pattern of poultry manure and compost. Their slow release ensures a prolonged supply of nutrients necessary for plant growth and development (32). SA₂₅₀ showed an increase that was comparable to PM₁₀. This effect could be attributed to the ability of SA to improve solubility and nutrient absorption might have enhanced yield (34,39).

3.2.2 Dry weight (g/plant)

Application of poultry manure rates of PM₅, PM₁₀, and PM₁₅ significantly increased dry weight of *Amaranthus cruentus* compared to control. These effects on dry weight were comparable to dry weight achieved in *Amaranthus* treated with CP₁₀, having higher effects than NPK-fertilized plants. The application of organic manure has been shown to improve the organic matter content of soil, which enhance nutrient availability, and also promote root development, which enable plants to adequately absorb nutrients from the soil leading to better plant productivity and overall plant performance. Poultry manure or compost also provides essential nutrients, such as nitrogen, phosphorus, potassium and other secondary nutrient which are crucial for plant growth and development, ultimately improving dry matter accumulation (31,32,33). The highest dry matter (3.85 g) of *Amaranthus cruentus* in the elicitor treatments was obtained in plants treated with MejA₅₀₀, which was not more than the values obtained for all salicylic acids applied (Table 2). The effects of MejA and Salicylic acid on dry matter accumulation may be due to their ability to influence nutrient uptake (34,39).

Table 2: Effects of inorganic and organic fertilizers on chlorophyll contents, shoot fresh and dry weights of *Amaranthus cruentus* at 6 weeks after sowing

Treatments	Chlorophyll (SPAD)	Fresh weight (g/plant)	Dry matter (g)
CP ₅	34.60bcd	40.00d	3.10bc
CP ₁₀	35.70abc	45.00cd	3.32ab
CP ₁₅	36.20abc	48.00abc	2.17e
PM ₅	34.00cde	51.35a	3.24ab
PM ₁₀	35.20abc	52.29a	4.50a
PM ₁₅	30.50de	48.70abc	3.90ab
NPK ₅₀	35.00bcd	45.00cd	2.90c
NPK ₁₀₀	36.90abc	47.30bc	3.20bc
NPK ₁₅₀	40.00ab	49.48ab	3.22bc
MejA ₁₂₅	34.60bcd	35.44ef	2.48cde
MejA ₂₅₀	30.50de	34.00ef	2.71cde
MejA ₅₀₀	34.70cde	40.00de	3.85a
SA ₁₂₅	37.50abc	41.00d	3.35ab
SA ₂₅₀	40.20a	49.00ab	3.76ab
SA ₅₀₀	35.50bcd	48.62b	3.80a
Control	32.40de	31.30e	1.60d

Means with identical letters in each column are statistically significant ($P = .05$).

3.3 Quality Parameters

3.3.1 Chlorophyll (SPAD)

Chlorophyll content was slightly enhanced with the application of fertilizers, especially inorganic fertilizers, which showed an approximately 23% increase in plants treated with NPK₁₅₀ when compared to the control, and compost, which showed only a 12% increase in plants treated with CP₁₅ when compared to the control (Table 2). The elicitors significantly

influenced the chlorophyll content of *Amaranthus cruentus* with the highest chlorophyll content (40.20 SPAD) when compared to the control (32.40 SPAD) obtained in plants treated with SA₂₅₀ (Table 2). NPK application enhanced the chlorophyll content of *Amaranthus cruentus* compared to that of the control. This assertion was also observed (32), who showed that plants that received inorganic fertilizers had a higher chlorophyll content than those that received organic fertilizers. (42) also showed that NPK fertilizers improve the chlorophyll content and yield of *Amaranthus hybridus*. This assertion was corroborated by (43), who observed improved growth and chlorophyll content in *Amaranthus tricolor*. The increased chlorophyll content observed might be due to ability of SA and MeJA to improve photosynthetic efficiency of plant (44).

3.3.2 Folate concentration ($\mu\text{g}/100\text{ g}$)

The effects of fertilizers on folate concentrations in *Amaranthus cruentus* varied across the treatments. Although NPK₁₀₀ and PM₁₀ resulted in significantly higher folate contents (190.05, 185.06 $\mu\text{g}/100\text{ g}$, respectively) than the control (140.10 $\mu\text{g}/100\text{ g}$), all other fertilizer rates did not enhance folate concentration in *Amaranthus cruentus*; in elicitor treatments, the highest folate concentration (250.20 $\mu\text{g}/100\text{ g}$) in plant tissue was higher in plants sprayed with SA₂₅₀, whereas MeJA₅₀₀ gave the highest folate concentration (186.10 $\mu\text{g}/100\text{ g}$). However, methyl jasmonate treatment resulted in lower folate concentrations than SA treatments. The higher folate concentration obtained in plants treated with NPK might be due to the presence of nitrogen, which is a precursor for the glutamate link necessary for folate synthesis in plants (39,40). Moreover, the synthesis of most vitamins is determined by the presence of an amino acid precursor that has been linked to nitrogen metabolism in plants. As Salicylic acid enhances the absorption of nutrients from the soil, nitrogen absorption from the soil might have been enhanced as well (38)

3.3.3 Zinc concentration (mg/100g)

A higher zinc concentration (3.86 mg/100 g) in *Amaranthus cruentus* was obtained under CP₁₀ when compared to the control. The result was similar to 3.40 mg/100 g obtained under NPK₁₀₀. The increased zinc concentration might be attributed to the ability of compost to improve the structure of the soil and increase the availability of nutrients to plants, and nutrient absorption. Compost has been shown to promote root development, leading to better soil exploration and zinc uptake. Additionally, the organic acids produced by compost enhance solubility of zinc ions which leads to better zinc absorption. The nitrogen content in NPK promote overall plant growth such as leaf and root growth, which in turn leads to better

zinc absorption. Adequate P levels also support better zinc utilization, while K regulates water balance, leading to enhanced Zn transport within the plant (33,41).

Zinc concentration (3.70 mg/100 g) was higher in plants sprayed with SA₂₅₀ than in those sprayed with SA₅₀₀ (3.69 mg/kg) (Table 2). While SA effects might be due to influence nutrient transporters and uptake processes of nutrients, this suggests that SA can enhance the plant's ability to regulate the uptake and distribution of micronutrients, thereby improving plant health and stress tolerance (38)

3.3.4 Iron concentration (mg/100g)

The highest iron concentration (22.00 mg/100 g) was obtained in plants treated with CP₁₀, which was significantly higher than that in the control, and was similar to that obtained in plants treated with CP₅ and PM₅ (Table 3). Studies have also shown that organic fertilizers can increase the nutrient content of leafy vegetables by enhancing the soil organic matter, pH, cation exchange capacity, and microbial activity, which can improve the solubility and mobility of these micronutrients in the soil (45). Foliar application of elicitors significantly increased iron concentration in *Amaranthus cruentus* when compared to the control (Table 4.). The highest value of iron (218.60 mg/kg) was obtained in SA₁₂₅ and was not significantly different from that in SA₂₅₀, MeJA₅₀₀, and MeJA₂₅₀. MeJA and SA enhance the absorption of micronutrients in plants (46). This suggests that SA can enhance the ability of plants to regulate micronutrient uptake and distribution, thereby improving plant health and stress tolerance (38,44).

Table 3: Effects of different rates of fertilisers on folate, mineral of *Amaranthus cruentus* grown in the screen house at 6 weeks after sowing

Treatments	Folate	Zinc (mg/100g)	Iron (mg/100g)
	($\mu\text{g}/100\text{g}$)		
CP ₅	148.32bcd	3.39ab	20.50a
CP ₁₀	150.54bcd	3.86a	22.00a
CP ₁₅	135.12d	2.91ef	18.00bcd
PM ₅	144.61d	2.55fgh	19.00abcd
PM ₁₀	185.06ab	3.14def	16.65def
PM ₁₅	160.00bcd	2.83f	13.90ef
NPK ₅₀	158.40bcd	2.94defg	16.94def
NPK ₁₀₀	190.05a	3.40abcd	17.30cde
NPK ₁₅₀	181.00ab	3.28bcde	15.10e
MejA ₁₂₅	151.28de	3.00def	16.72de
MejA ₂₅₀	175.60cde	2.98efg	18.00bcd
MejA ₅₀₀	186.10cde	3.23cde	20.00ab
SA ₁₂₅	245.15ab	3.00def	21.86ab
SA ₂₅₀	250.20a	3.70abc	19.50abc
SA ₅₀₀	239.40ab	3.69abc	15.20de
Control	140.10d	2.250h	14.34fg

Means with identical letters in each column are statistically significant ($P = .05$).

3.4 Correlation

The SPAD chlorophyll values of *Amaranthus cruentus* had a low correlation with its fresh weight (28%), whereas folate was 45%, zinc was 53%, and iron was 22% (Table 4). Fresh weight was positively correlated with folate (38%), and dry matter was positively correlated with folate (63%) (Table 4). Low iron and zinc levels can impair the synthesis of chlorophyll; thus, high iron levels enhance chlorophyll levels and are essential for chlorophyll synthesis.

Thus, a positive correlation between high folate levels in plants with high chlorophyll levels and the overall health of plants is ensured, which might lead to higher folate synthesis. Dry matter accumulation represents the total biomass of the plant. Chlorophyll is crucial for photosynthesis, and contributes to biomass production. Chlorophyll content has been positively correlated with iron and zinc levels in plants. are important for chlorophyll synthesis. Thus, a positive correlation indicates efficient photosynthesis, which leads to better plant development. A high chlorophyll content contributes to efficient photosynthesis, leading to increased fresh weight and overall plant vigor. Elevated chlorophyll levels enhance photosynthetic efficiency, resulting in greater dry matter accumulation and biomass (47, 48)

3.5 Percentage Contribution

Table 5 shows the percentage contribution of the micronutrients obtained in this study with their corresponding Recommended Daily Allowance (RDA) values. *Amaranthus cruentus* treated with NPK₁₀₀ had the highest percentage (31.68%) contribution to the recommended daily folate intake, followed by PM₁₀, whereas *A. cruentus* treated with CP₁₀ had the highest percent contribution of iron and zinc. *Amaranthus cruentus* treated with NPK₁₀₀ had the highest percentage (31.68%) contribution to the recommended daily folate intake of 600 µg/100 g for pregnant women, followed by PM₁₀, and *Amaranthus cruentus* treated with CP₁₀ had the highest percentage contribution of iron and zinc to the daily intake of 15 mg/day for both minerals. These results suggest that organic and inorganic fertilizers are effective for enhancing human nutrition and health. Furthermore, as folate, iron, and zinc levels in all samples exceeded 20% of the daily intake, *Amaranthus cruentus* can be considered a rich source of micronutrients and a potential crop for alleviating hidden hunger in Africa.

3.6 Proximate concentration % (Crude fibre, Ash and moisture content)

The proximate concentration of *Amaranthus cruentus* also improved with fertilizer application (Table 6). The highest crude fiber content (12.55%) was obtained under PM₁₅ and was similar to the crude fiber obtained at all fertilizer rates applied. The ash content was only slightly affected by fertilizer treatments with NPK₁₀₀, giving the highest ash content (10.91%) and was not significantly better than the values obtained in *Amaranthus cruentus* (AC) under PM₁₀, CP₁₀, CP₁₅, PM₁₀, and PM₁₅. A higher moisture content (13.60%) in *Amaranthus cruentus* was obtained under PM₁₅ than in the control and was comparable to the moisture content obtained under PM₅, PM₁₀, and CP₁₀. The application of compost and poultry manure enhanced the crude fiber level in *Amaranthus cruentus*. This increase is likely to be

attributed to the availability of nutrients for plant use in the compost and poultry manure. Additionally, improved soil conditions created by organic matter component of compost and poultry manure facilitated better nutrient uptake and efficient translocation throughout the plant.

However, the application of NPK fertilizer enhanced the crude ash content of *Amaranthus cruentus*, although this effect was comparable to the effects of poultry manure and compost on crude ash. The results obtained for crude fiber, moisture, and ash were similar to those reported by (49, 50), who found higher crude fiber and ash contents in organic fertilizer treatments. However, (51) observed differing effects where poultry-grown *Amaranthus cruentus*, *Amaranthus hybridus*, and *Amaranthus deflexus* had higher ash content, while higher crude fiber was obtained in poultry-grown *Amaranthus cruentus* while NPK₁₀₀ enhanced higher crude fiber in *Amaranthus hybridus* and *Amaranthus deflexus*. SA₂₅₀ and MejA₅₀₀ improved crude fiber content and were comparable to PM₁₅ effects on crude fiber, while SA₁₂₅ showed higher effects on ash; the effects were comparable to the effects on ash observed in NPK₁₀₀, and higher moisture content was obtained in MejA₅₀₀, which was lower than the moisture content obtained in PM₁₅. This suggests that SA can enhance the uptake and distribution of essential nutrients, thereby improving performance and leading to improved crude fiber, ash, and moisture content in the plant (34).

Table 4. Correlation matrix between SPAD chlorophyll, fresh shoot weight, dry shoot weight, folate, zinc and iron values in *Amaranthus cruentus*

	Chlorophyll (SPAD)	Fresh weight (g/plant)	Dry matter (g)	Folate (µg/100g)	Zinc (mg/100g)	Iron (mg/100g)
Chlorophyll (SPAD)	1.000					
Fresh weight (g/plant)	0.426	1.000				
Dry matter (g)	0.223	0.611	1.000			
Folate (µg/100g)	0.405	0.168	0.470	1.000		
Zinc (mg/kg)	0.532	0.325	0.428	0.471	1.000	
Iron (mg/kg)	0.283	-0.057	0.068	0.222	0.384	1.000

Table 5. Comparison of effects of the treatments on folate, zinc and iron contents of *Amaranthus cruentus* RDA of 600 µg/100g of folate, and 15.0 mg/day of iron and zinc

Treatments	Folate µ/100g	Zinc (mg/100g)	Iron (mg/100g)	Contribution to RDA value (%)
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					Zinc (mg/100g)	Iron (mg/100g)
CP ₅	148.32	3.39	20.50	24.72	22.59	136.67
CP ₁₀	150.54	3.86	22.00	25.09	25.71	146.67
CP ₁₅	135.12	2.92	18.00	22.52	19.45	120.01
PM ₅	144.61	2.55	19.00	24.10	17.00	126.67
PM ₁₀	185.06	3.14	16.65	30.84	20.93	111.00
PM ₁₅	160.00	2.83	13.90	26.67	18.89	92.67
NPK ₅₀	158.40	2.94	16.94	26.40	19.63	112.93
NPK ₁₀₀	190.05	3.40	17.30	31.68	22.69	115.33
NPK ₁₅₀	149.00	3.28	15.10	24.83	21.85	100.67
MejA ₁₂₅	151.28	3.00	16.72	25.21	20.00	111.47
MejA ₂₅₀	175.6	2.98	18.00	29.27	19.87	120.00
MejA ₅₀₀	186.1	3.23	20.00	31.02	21.52	133.33
SA ₁₂₅	245.15	3.00	21.86	40.86	20.00	145.73
SA ₂₅₀	250.2	3.70	19.50	41.70	24.67	130.00
SA ₅₀₀	239.4	3.70	15.21	39.90	24.63	101.40
Control	140.1	2.25	14.34	23.35	15.00	95.60

*Percent contribution calculated with RDA of 600 µg/100g of folate, and 15.0 mg/day of iron and zinc

Table 6: Effects of fertilisers on crude fibre, ash and moisture composition of *Amaranthus cruentus* at 6weeks after sowing in the screen house

Treatments	Crude Fibers (%)	Crude Ash (%)	Moisture content (%)
CP ₅	11.35a	9.32cde	11.15bcd
CP ₁₀	11.88ab	10.01abc	13.34a
CP ₁₅	10.96abc	10.50ab	11.04bcd
PM ₅	8.01d	9.00de	13.35a
PM ₁₀	11.92a	10.62ab	12.51ab
PM ₁₅	12.55a	10.15ab	13.60a
NPK ₅₀	10.98abc	8.60e	11.65bc
NPK ₁₀₀	11.01ab	10.91a	12.00b
NPK ₁₅₀	11.25ab	9.48bcd	11.37bc
MejA ₁₂₅	10.41bcd	10.26a	11.01bc
MejA ₂₅₀	10.18bcd	10.01bc	10.95cde

MejA ₅₀₀	11.00ab	9.45bcde	11.20bc
SA ₁₂₅	10.30bcd	10.77a	10.90cdef
SA ₂₅₀	11.15ab	9.14cde	11.13bc
SA ₅₀₀	9.40cde	9.85bc	9.84def
Control	8.00d	9.01de	10.13d

Means with identical letters in each column are statistically significant ($P = .05$).

4. CONCLUSIONS

The application of NPK fertilizer at the rate of 100 kg N/ha had the highest significant impact on the plant height and leaf area of *Amaranthus cruentus*, and were comparable to those achieved with poultry manure and compost manure. Poultry manure was found to enhance the highest number of leaves, fresh weight, and dry matter content, while salicylic acid at the rate of 250 $\mu\text{mol/L}$ was found to be the best for enhancing folate content in *Amaranthus cruentus*. Based on these findings, it is recommended that farmers use poultry manure at the rate of 10 t/ha as an alternative to NPK fertilizer at 100 kg N/ha, in order to mitigate the negative effects of inorganic fertilizers on the ecosystem. Additionally, salicylic acid at the rate of 250 $\mu\text{mol/L}$ is recommended for farmers interested in enhancing folate content in *Amaranthus cruentus*. It is crucial to balance nutrient inputs with ecological sustainability and to explore strategies such as the use of poultry manure, compost, and judicious application of salicylic acid to promote healthy growth and nutritional quality in *Amaranthus cruentus*.

Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp Allergy. 2002;32(4):489-98.

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REFERENCES

1. Calder PC. Nutrition, immunity and COVID-19. *BMJ Nutrition Prev. Health.* 2020; 3(1):74-92
2. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections. *Nutrients.* 2020;12(4):1181.
3. Otsuka K. Food Security and the Role of Agriculture in Economic Development. *Asian Development Review.* 2013; 30(1): 1-16.
4. Wegren SK, Elvestad C. Russia's Food Self-Sufficiency and Food Security: An Assessment. *Post-Communist Economies.* 2018; 30(5):565-587.
5. Gali SP, Subhashini M, Meenatchi R. Food Security Through Farming Subsistence Crops. In *Food Production, Diversity, and Safety Under Climate Change.* Cham: Springer Nature Switzerland. 2024; 2:63-76.
6. Food and Agriculture Organization (FAO). *The State of Food Insecurity in the World Meeting the 2015 international hunger targets: taking stock of uneven progress, 2015.* Food and Agriculture Organization of the United Nations, Rome. Accessed 26 May 2018. Available: <https://www.fao.org/documents/card/en?details=c2cda20d-ebeb-4467-8a94-038087fe0f6e>
7. World health statistics. *Monitoring health for SDGs, sustainable development goals* (Geneva: World Health Organization, 2022). Accessed 6 January 2023. Available: <https://www.who.int/publications/i/item/9789240051157>.
8. Von Grebmer ASK, Biriol E, Wiesmann D. *Global Hunger Index: the challenge of hidden hunger.* In [IFPRI Welthungerhilfe, and Concern Worldwide, editor]. Bonn, Washington, D.C., and Dublin. 2014.
9. Joy EJM, Stein AJ, Young SD, Ander EL, Watts MJ, Broadley MR. *Agricultural Value Chains in Developing Countries: A Framework for Analysis.* *Agriculture and Human Values.* 2015; 32(4): 705-725.

10. White PJ, Broadley MR. Biofortification of crops with seven mineral elements often lacking in human diets—iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytology*. 2009; 182:49–84.
11. Cakmak I, Kutman UB. Agronomic biofortification of cereals with zinc: a review. *Eur. J. Soil Sci.* 2017; 69 (1):172–180.
12. Van Der Straeten D, Bhullar NK, De Steur, H, Gruissem, W, MacKenzie, D, Pfeiffer, W. Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nat. Commun.* 2021;1 (1):1–10.
13. Cakmak I. *Agronomic Biofortification*; International Food Policy Research Institute: Washington, DC, USA. 2014; 16: 56–97.
14. Khandaker L, Masumakond ASMG, Oba S. Foliar application of salicylic acid improved the growth, yield and leaves bioactive compounds in Red Amaranth (*Amaranthus tricolor* L.). *Vegetable Crops Research Bulletin*. 2011; 74: 77-86.
15. Vanlauwe B, Descheemaeker K, Giller KE, Huising J, Merckx R, Nziguheba G, Wendt J, Zingore S. Integrated soil fertility management in sub-Saharan Africa: unravelling local adaptation. *SOIL*. 2015; 1:491-508.
16. Gould J. A world of insecurity. *Nature*. 2017; 544: S6 – S7.
17. Stewart WM, Roberts TL. Food security and the role of fertilizer in supporting it. *Proc Engin.* 2012; 46:76–82.
18. Van der Velde M, Folberth C, Balkovic J, Ciaïș P, Fritz S, Janssens IA, Obersteiner M, See L, Skalsky R, Xiong W, Peñuelas J. African crop yield reductions due to increasingly unbalanced nitrogen to phosphorus consumption. *Glob Change Biol.* 2014; 20:1278–88.
19. Maseko Innocent, Tafadzwanashe Mabhaudhi, Samson Tesfay, Hintsia Tesfamicael Araya, Melake Fezzehazion, Christian Phillipus, Du Plooy. *African Leafy Vegetables: A Review of Status, Production and Utilization in South Africa*. *Sustainability*. 2018; 10: 1-16.
20. Mavengahama S, McLachlan M, de Clercq W. The role of wild vegetable species in household food security in maize based subsistence cropping systems. *Food Secur.* 2013, 5, 103–122.
21. Aregheore EM. Nutritive value and inherent anti-nutritive factors in four indigenous edible leafy vegetables in human nutrition in Nigeria. *Journal of Food Resource Science*. 2012; 1: 1–14.
22. Adejumo SA, Togun AO, Adediran JA. Comparative study of different rates of compost made from Mexican sunflower (*Tithonia diversifolia*) and cassava peels on maize growth on lead contaminated soil. *Journal of Agricultural Science and Technology*. 2013; 3: 216 – 225.
23. A.O.A.C. *Methods of Analysis for nutritional labelling*. Edited by Sullivan, D. M. and Carpenter, D. E. Association of Official Analytical Chemists International, Arlington Virginia, USA. 1993
24. Sethi PD. *Quantitative analysis drug pharmaceutical formulation*. CBs Publishers and distributors, Darya Ganj, New Delhi. 2006. ISBN 13:978-8123905600, 643pp.
25. Ashok Kumar CK, Divya Sree MS, Joshna A, Mohana Lakshmi S, Satheesh Kumar DA. Review on South Indian edible leafy vegetables. *Journal of Global Trends in Pharmaceutical Sciences*. 2013; 4 (4): 1248-1256.
26. Horneck DA, Hanson D. Determination of potassium and sodium by flame Emission In: *hand book of reference methods for plant analysis*. 1998.pp 153- 155.

27. A.O.A.C. Official Methods of Analysis of the Association of Official Agriculture Chemists. Published by Association of Official Agriculture Chemists, 13th Ed. Washington, D.C., USA. 2005.
28. Ritche Hannah, Roser Max. Micronutrient deficiency. 2017. Accessed 5 May 2021. Available: <https://ourworld.org/micronutrient-deficiency>
29. Ibrahim U, Rabo M. Performance of Amaranth (*Amaranthus Cruentus*) As Influenced by Npk Fertilizer and Organic Liquid Fertilizer at Samaru, Northern Guinea Savanna of Nigeria. *Fudma Journal Of Sciences*. 2023; (7):175- 181.
30. Anjali K, Topno S, Kerketta A. Effect of Different Organic and Inorganic Fertilizers on Growth, Yield and Quality of Amaranthus under Polyhouse Condition (*Amaranthus cruentus*) cv. NSC 999IUS. *International Journal of Environment and Climate Change*. 2022; 12(11): 1647 – 1653.
31. Suwor PS, Jeakkhajorn, Kramchote S. Effects of different compost manures application on growth of lettuces (*Lactuca sativa* L.). *International Journal of Agricultural Technology*. 2020; 16(5):1257-1266.
32. Islam MM, Karim AJM, Jahiruddin M, Majid NM, Miah MG, Ahmed MM, Hakim MA. Effects of organic manure and chemical fertilizers on crops in the radish-stem Amaranth-Indian spinach cropping pattern in homestead area. *Australian journal of crop science*. 2011;5(11):1370-1378. 21.
33. Meena Lokendra, Ram Bharose, Arun Alfred David, Tarence Thomas, Neha Toppo, Harsh Kumar. Effect of NPK and Zinc Inorganic Fertilizers on Soil Health, Growth and Yield of Chickpea (*Cicer Arietinum* L.). *International Journal of Plant & Soil Science*. 2023; 35 (17): 155-61.
34. Wang Y, Mostafa S, Zeng W, Jin B. Function and mechanism of jasmonic acid in plant responses to abiotic and biotic stresses. *Int J Mol Sci*. 2021; 22(16):8568
35. Law-Ogbomo KE, Ajayi SO. Growth and yield performance of *Amaranthus cruentus* influenced by planting density and poultry manure application. *Notudae Botanicae Horticultural Agrobotanici*. 2009; 37 (1): 195-199.
36. Ali B. Salicylic acid: an efficient elicitor of secondary metabolite production in plants. *Biocatal Agric Biotechnol*. 2021; 31:101884
37. Olowoake AA. 2014. Influence of organic, mineral and organomineral fertilizers on growth, yield, and soil properties in grain amaranth (*Amaranthus cruentus*. L). *Journal of Organics*. 2014; 15 (5):1-11.
38. Wang M, Shen Q, Xu G, Guo S. New Insight into the Strategy for Nitrogen Metabolism in Plant Cells. *International review of cell and molecular biology*. 2014; 310: 1-37.
39. Zhao JL, Zhou LG, Wu JY. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. *Appl Microbiol Biotechnol*. 2010; 87:137–144
40. Hou S, Men Y, Zhang Y, Zhao K, Ma G, Li H, Han Y, Sun Z. Role of miRNAs in regulation of SA-mediated upregulation of genes involved in folate and methionine metabolism in foxtail millet. *Front Plant Sci*. 2022;13:1023764.
41. Akamine H, Ohshiro M, Hossain M. A. Effects Of N, P And K Fertilizers On Edible Amaranth (*Amaranthus* Spp.) Grown On The Red Soil Of Okinawa. *Applied Ecology And Environmental Research*. 2021; 19(3):2333-2346.
42. Pelinganga OM, Mphosi MS. Optimum NPK fertilizer requirement for *Amaranthus hybridus* leafy vegetable under greenhouse conditions. *Research on Crops*. 2019; 20 (2): 353 -356.

43. Riesty OS, Siswanti DU. Effect of biofertilizer on growth and metaxylem diameter of *Amaranthus tricolor* L. in salinity stress condition. *Biogenesis: Jurnal Ilmiah Biologi*. 2021; 9(2):178-88.
44. Khan MI, Fatma M, Per TS, Anjum NA, Khan NA. Salicylic Acid-Induced Abiotic Stress Tolerance and Underlying Mechanisms in Plants. *Frontiers in Plant Science*. 2015;
45. Mofunanya A, Ebigwai J, Belo OS, Egbe AO. Comparative study of the effects of organic and inorganic fertilizer on nutritional composition of *Amaranthus spinosus*. *Asian journal of plant sciences*. 2015;14(1):34-39
46. Chenjia Shen, Yanjun Yang, Kaidong Liu, Lei Zhang, Hong Guo, Tao Sun, Huizhong Wang, Involvement of endogenous salicylic acid in iron-deficiency responses in *Arabidopsis*, *Journal of Experimental Botany*. 2016;67 (14): 4179–4193.
47. Kalaji HM, Oukarroum A, Alexandrov V, Kouzmanova M, Brestic M, Zivcak M, et al. Identification of Nutrient Deficiency in Maize and Tomato Plants by in Vivo Chlorophyll a Fluorescence Measurements. *Plant Physiol. Biochem*. 2014, 81, 16–25.
48. Szerement J, Szatanik-Kloc A, Mokrzycki J, Mierzwa-Hersztek M. Agronomic Biofortification with Se, Zn, and Fe: An Effective Strategy to Enhance Crop Nutritional Quality and Stress Defense—A Review. *J. Soil Sci. Plant Nut*. 2021; 22, 1129–1159.
49. Dada OA, Imade F, Anifowose EM. Growth and proximate composition of *Amaranthus cruentus* L. on poor soil amended with compost and arbuscular mycorrhiza fungi. *International Journal of Recycle Organic Waste Agriculture*. 2017; 6: 195 – 202.
50. Adedeji JA, Idowu- Agida OO, Awogbade AI, Oni OO, Oladejo LF, Oladosu BO. Effect of different organic fertilisers on yield and quality of *Amaranthus hybridus*. *International journal of research and scientific innovation*. 2019; 6 (5): 2321- 2705.
51. Ashraf MY, Oyediji S, Animasaun DA, Bello AA, Agboola OO. Effect of NPK and Poultry Manure on Growth, Yield, and Proximate Composition of Three Amaranths. *Journal of Botany*. 2014
52. Lee EK, Zhang X, Adler PR, Kleppel GS, Romeiko XX. Spatial and temporally explicit life cycle global warming, eutrophication, and acidification impacts from crop production in the US midwest. *J Clean Produc*. 2020; 242:118645.
53. MacDonald GK, Bennett EM, Potter PA, Ramankutty N. Agronomic phosphorus imbalances across the world's croplands. *PNAS*. 2021; 108:3086–91.
54. Shettel NL, Balke NL. Plant growth response to several allelopathic chemicals. *Weed Science*. 1983; 31: 293 – 298.
55. Reuveni R, Agapov V, Raviv M. Effects of foliar sprays of phosphates on powdery mildew of roses. *Journal of Phytopathology*. 1994;142: 331 – 337.
56. Pittelkow, Fábio Kempim, Aloisio Bianchini, Pedro Silvério Xavier Pereira, Luana Maria de Rossi Belufi, Maria das Graças de Assis Bianchini, and Rodrigo Pengo Rosa. 2019. "Phosphate Fertilization in Amaranth Culture Cv. BRS Alegria in Second Harvest in the Cerrado". *Journal of Experimental Agriculture International* 39 (4):1-9. <https://doi.org/10.9734/jeai/2019/v39i430339>.
57. Sarangi, Swati Sulagna, Ira Sarma, Sailen Gogoi, and Aradhana Barooah. 2023. "Effect of Organic Nutrients and Bio Fertilizers on Soil Parameters and Nutritional

Content of Amaranth". International Journal of Environment and Climate Change 13 (11):4326-30. <https://doi.org/10.9734/ijecc/2023/v13i113613>.

58.

Onyango, C. M., Harbinson, J., Imungi, J. K., Shibairo, S. S., & van Kooten, O. (2012). Influence of organic and mineral fertilization on germination, leaf nitrogen, nitrate accumulation and yield of vegetable amaranth. Journal of plant nutrition, 35(3), 342-365.

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