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Evaluating the Effect of Fertilizers on Physiological Growth, Chemical, Bioactive Components and Secondary Metabolites in *Vigna unguiculata* (L.)

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Abstract

Objective: *Vigna unguiculatata (L.)* (Cowpea) is an African indigenous protein-dense crop that most sub-Saharan Africans rely on for food and nutrition security.

Material and Methods: The current study assessed the effect of different levels of poultry manure fertilizer (10, 30, 60 and 90 kg F/ha) and nitrogen fertilizer (30, 45, 60 and 90 kg N/ha) on the growth, yield, mineral composition, bioactive compounds and secondary metabolites in the legume crop *Vigna unguiculata* (L.). At the end of 15 weeks, 90 kg F/ha and 45 kg N/ha enhanced the growth, yield, mineral composition, production of ascorbic acid, total phenolic and FRAP assay, this informed our decision for the selection of this treatments (45kgN/ha, 90kgF/ha and 0kg/ha control) for further analyses of secondary metabolites.

Results: Poultry manure and nitrogen fertilizer improved the mineral compositions of cowpea leaves, and different accumulation trends were noted depending on different application levels, with poultry manure responding well.

Conclusion: Thus, the application of organic poultry manure at 90 kg F/ha for cowpea cultivation should potentially be recommended in the Mpumalanga Province.

Introduction

Vigna unguiculatata (L.) (Cowpea) is an African indigenous protein-dense crop that most sub-Saharan Africans rely on for food and nutrition security. This crop is climate-friendly, drought, pest, and disease tolerant [1]. Cowpea is the third most-produced legume pulse grain after chickpea (*Cicer arietinum*) and the common dry bean (*Phaseolus vulgaris*) [2]. Cowpea is not only an affordable crop to most rural dwellers but also critical in alleviating hidden hunger. The leaves are endowed with appreciable levels of essential amino acids; including histidine, tryptophan, threonine, isoleucine, methionine, leucine, valine, lysine and phenylalanine, and the non-essential amino acids, such as glutamate, alanine, glycine, proline, cysteine, serine, tyrosine and aspartate [3]. Additionally, it is important to include the leaves of cowpea in Africa's cuisine because of their nutritional values, since they contain appreciable amounts of protein, minerals (phosphorus, iron and calcium), functional compounds (carotenoids, amino acids

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and polyphenols), as well as vitamins (folate, thiamin, riboflavin, provitamin A and vitamin C) [4]. The leaves of cowpea are protein-rich, and this ranges from 21.5 to 43.7%, which is comparable to that of kale (36.8%), Brussel sprouts (34.1%), mustard greens (34%) and spinach (38.2%) [3].

Cowpea leaf consumption has been linked to a decreased risk of obesity and type two diabetes mellitus [1]. Furthermore, the dietary phenolic compounds has been shown to inactivate the carbohydrate digestive enzymes glucosidase and amylase while activating suitable antihyperglycemic agents [1, 5]. These enzymes are important in regulating glucose in the blood and obesity by reducing the reabsorption of glucose in the intestines [5]. Crops need adequate nutritional constituents in the right proportions and at the right time for healthy growth and optimum yields. Organic and inorganic fertilizers are therefore supplied to the crops to meet these crop requirements. The application of fertilizers has also been shown to enhance crop nutritional quality [4]. But the report of Dumas et al. [6] showed that organic fertilizers improved plant antioxidant activity while inorganic fertilizers had the opposite effect. Higher crop yield has been reported in fields where manures were applied compared to inorganic sources at the same phosphorus level [7]. Organic fertilizers are normally endowed with elevated levels of plant growth available essential nutrients. In addition, to improve soil physical properties, green manure, poultry, pigeon, cattle, goat, rabbit, and general farmyard manure are recommended [8].

About 232 and 239 million people from Africa suffer from micronutrient and protein-calorie malnutrition, indicating a severe food and nutrition insecurity scenario [9]. Rampant and incessant El Nino caused droughts, poor soil fertility, long season crops that cannot tolerate long spells without moisture, and pests and diseases are some of the major causes of hunger, food, and nutrition insecurity in Africa [10]. Fertilizers are often the answer to soil nutrition problems. However, in Africa, most smallholder farmers are poor and therefore have no access to them. African smallholder farmers apply an average of 8.8 kg NPK fertilizer per hectare [11]. Fertilizers play an important role in increasing crop production [12]. Although synthetic fertilizers boost soil fertility and improve soil productivity leading to increased yields, the effects on the environment and the soil over time are rather negative [13]. But organic fertilizers have the ability not only to boost the soil's nutritional capacity but also to boost productivity and improve soil properties over time. Soil conditions, including pH, hydraulic conductivity, water holding capacity, soil bulk density and infiltration rate, can be improved by adding cow manure and improving nutrition for plant root absorption [14].

The soil's physical and biological properties are adversely affected in the long term by the application of synthetic fertilizers even though these fertilizers boost soil productivity and increase crop yield [12, 15]. The pollution of water bodies, soil and the air has also been linked to synthetic fertilizer applications. However, to counter the effects of synthetic fertilizers, organic fertilizers or manures are often recommended [16]. Organic fertilizers, including organic industrial waste, plant waste, and composts, have immense potential in sustainable agricultural production [17]. Secondary metabolites such as antioxidants in basil, tomatoes, amaranth, lettuce and garlic are affected by the availability of nitrogen in the soil [18]. Also, synthetic fertilizers and organic fertilizers have been shown to positively influence cowpea growth and yield [19]. The need to develop environmentally friendly and sustainable agricultural practices is urgent given the toxic effects synthetic fertilizers cause on soils over time, including the ever-rising reduced fertility of African soils. New technologies that are affordable to poor resource smallholder farmers need to be developed specifically to improve the nutritional quality of crops without compromising sustainable cultivation for posterity. Organic manures improve the soil's physical and chemical structures while improving the crop's vegetative growth and yield in cowpea, and these are important aspects in sustainable crop production [19]. Improved rhizosphere soil characteristics due to the application of organic manures mean positive and improved root growth and, therefore, a better-quality crop [20]. In addition, when the organic matter decomposes, the supply of nutrients to the crop also improves, leading to increased crop nutrient uptake hence the production of a highly nutritious crop. But the lower nitrogen levels present in the substrate have been shown to positively affect phenolic and antioxidant capacity in vegetables cultivated using

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organic fertilizers [21]. Therefore, this study aimed to compare the influence of different levels of organic (poultry manure) and inorganic N (nitrogen) fertilizer on the growth, yield, mineral composition, quality and secondary metabolites of cowpea leaves cultivated in the Mpumalanga Province.

Materials and Methods

Plant material and growth condition

Seed of cowpea (*V. unguiculata* L.) planted in Southern African region, were obtained from smallholder farmers from Ga-Manyaka village in Limpopo, under Department of Agriculture. A field sprinkler irrigated trial was conducted between October and December 2020 at the Faculty of Agriculture and Natural sciences experimental farm at the University of Mpumalanga, in South Africa and located at 25.4371° S 30.9818°E. The cowpea seeds were directly planted in the soil. However, ten soil samples were randomly collected at 20 cm depth using a soil auger from each site for analysis before planting. The soil's chemical and physical properties (Table 1) were favourable for cowpea production. Using the split-plot design cowpea seeds were planted, the experiment was laid out in a Randomized Complete Block Design(RCBD) with four replicates and four N levels (30, 45, 60 and 90 kg N/ha) as inorganic treatments and four poultry manure levels (10, 30, 60 and 90 kg F/ha) on 6.3 m^2 plots. RCBD is the standard design for agricultural experiments where similar experimental units are grouped into blocks or replicates. Pathogen-free poultry manure or feeds (F) were collected from birds grown in controlled systems for commercial use. Two seeds were sown per planting station at a depth of 2cm in 2 rows per plot with 60 × 20 cm in-row and inter-row spacing. Sprinkler irrigation was applied every two days after transplanting due to the high soil drainage in the study area. Poultry manure was spread evenly over the plot and incorporated into the soil with tiller two weeks before planting to allow decomposition. Nitrogen fertilizer was applied in the form of Limestone Ammonium Nitrate (28% N), which was supplied before transplanting and four weeks after transplanting as a top dressing. Because the soil analysis showed enough of the mineral in the soil, no additional potassium was supplied. Granular superphosphate (10.5% P) was broadcast on all experimental plots at an identical rate of 20 kg/ha before planting to meet the phosphorus needs of the soils. Recommended agronomic applications were followed during the trial. Mean temperatures ranged between 33.56 and 3.96°C (maximum and minimum respectively) for the trial duration.

Table 1: *Physical and chemical properties of soil analysis and poultry manure*

Sample	Soil	Sample:	Poultry Manure
pH	6.04	pH	7.1
EC (mS/cm)	4.2	EC (Ms/cm)	5.2
Moisture (%)	15	Moisture (%)	34.4
Ash (g/kg)	-	Ash (g/kg)	218
Organic Matter (g/kg)	-	Organic Matter (g/kg)	806
P (Bray1)	15.6	P (%)	1.22
K (g/kg)	35	K (%)	0.07
Na (g/kg)	5	Na (%)	0.27
Ca (g/kg)	200	Ca (%)	2.51
Mg (g/kg)	53	Mg (%)	1.08
Fe (g/kg)	16.25	Fe (ppm)	1.07
Mn (mg/kg)	40.41	Mn (%)	0.11
Zn (mg/kg)	6.28	Zn (ppm)	793
B (g/kg)		B (ppm)	34
Al (g/kg)	3	Al (%)	1.50
Ca/Mg	3.77	N (%)	1.88
Ca + Mg/K	7.23	S (%)	0.40
-	-	Mo (ppm)	1.55
Cu (g/kg)	1.15	Cu (ppm)	151

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Growth parameters data collection

The growth parameters, including plant height (mm), were measured using a measuring tape from ten plants growing and randomly selected from the central rows. The number of leaves were also recorded from each experimental unit and to measure relative chlorophyll content (SPAD value) a SPAD-502 (MINOLTA CO. LTD, JAPAN) meter was used at harvest according to method reported by Maboko and Du Plooy, [22]. Five plants per plot were selected two and four weeks after the application to estimate the shoot dry matter. The plants were uprooted, and their roots removed, and the leafy parts placed in sampling bags. The Plants were then washed on arrival at the laboratory and dried to a constant weight in an oven at 80°C for 48 hours. The average biomass dry weight was calculated [23].

Number of nodules and seeds per pod

When the plants reached 50% flowering, five were randomly and gently uprooted from the middle row. The roots were separated from the shoots. The soil particles were shaken off, washed the roots in water, and carefully removed and counted the nodules. Seeds from the pods from the same plants per treatment were carefully shelled and counted.

Mineral analysis

Five plants per plot were selected, and the fourth-youngest leaf from the growing point was used to determine Nitrogen (N), potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Zinc (Zn) and Iron (Fe) concentrations of cowpea leaves. This is due to the fact that the youngest leaves are most consumable parts by rural communities as they are tender and highly nutritious. The fourth youngest leaf was pluck from the growing tip from five different plants, and Na, Mg, Ca, Fe, Zn, K, P and N determined by oven drying the leaves 70°C for 48 hrs and grinding and passing through a 1 mm sieve. To determine N, a Carlo Erba NA 1500 C/N/S analyzer purchased from Thermo Scientific (Milan, Italy) was used to analyze the dry milled samples according to the method of Jimenez and Ladha [24]. The ICP-OES (Liberty Series II Model; Varian, Mulgrave, Australia) was used spectrometrically to determine Na, Zn, K, Mg, Ca, Fe and P in acid digested leaf samples on a dry weight basis.

Ascorbic acid analysis

The spectrometric method of Ncayiyana et al. [25] was used to determine the Ascorbic acid (AA) content in cowpea leaves. About one gram of fresh leaf samples was vortexed for 1 min after mixing with 5 ml of HPO_3 (4.5 g/100 ml). The mixture was then centrifuged (3000 G/ 5 min) and transferred into a volumetric flask, and this process was repeated twice. An adjustment was made to the extracts to a volume of 20 ml with meta-phosphoric acid, after which 300 µl of 50 g/L trichloroacetic acids were added to 200 µl of the extract. This mixture was centrifuged like the extract and 100 µL 2,4-dinitro-phenylhydrazine reagent added to 300 µl of the supernatant, and the resultant mixture was heated at 60ºC for an hour and cooled in an ice bath for 5 min. Sulphuric acid (400 µL 15.75 M) was added to this mixture and allowed to cool for 20 min at temperature of 25°C, using a V-530 photometer purchased from Jasco GmbH (Germany) the absorbance was read at 520 nm.

Total phenolic analysis

The cowpea leaf samples were snap-frozen, and method described by Singleton et al. [26] using Folin–Ciocalteu (FC) assay was used to determine the total phenolics. Leaf samples (5 g) were incubated at 37°C for 30 min after mixing with 10 ml of HCl (1.0 mol/L) in a centrifuge tube. For alkaline hydrolysis and to obtain the bound phenolic fraction, 10 ml sodium hydroxide (2.0 mol/L in 75% methanol) was added to the mixture. The samples were then mixed with 10 ml meta-phosphoric acid (0.56 mol/L) following 30 min of incubation at 37°C. An acetone/ water mixture (1:1) amounting to 10 ml was then added at the end of the extraction process. Centrifugation of the mixture then followed (3000 g/ 5 min), after which 10 mil of deionized water, 2 ml sample and 2 ml FC reagent were all mixed in a volumetric flask and then allowed to stand at ambient temperature for 5-8 min. Seventy percent sodium carbonate solution (20 ml) was added to the mixture. Deionised deionized water was added to the volume and allowed to stand at ambient temperatures for two hours after mixing the entire solution. The UV-1800

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spectrophotometer (Shimadzu, Kyoto, Japan) was used to determine the total phenolics against gallic acid (mg/L GAE) after filtering the sample aliquots through 0.45 µm Whatman filter paper (WHA2200070). To construct a standard curve, a 10 mg/L - 5 g/L range of standards was used.

Trolox Equivalent Antioxidant Capacity (TEAC) FRAP Assay

A microplate reader purchased from CLARIOstar Plus BMG Labtec (South Africa) was used to conduct the FRAP assay on 0.2 g snap-frozen cowpea leaf samples according to the method of Mpai et al. [27]. About 220 µL of FRAP reagent solution was mixed with 15 µL leaf extract aliquot and added to the wells. The absorbance was determined at 593 nm, and a Trolox standard curve was constructed to calculate the reducing antioxidant capacity. The assay results were then expressed as µmol TEAC/100 g [27].

Predominant secondary metabolites Profile

Using the methods of Managa et al. (2020) and Ndou et al. (2019), the secondary metabolites were extracted from the leaves as follows: Fifty milligrams of snap frozen *B. pilosa and C. gynandra* leaves were extracted in 70:30 (v/v) ethanol/ water solution and centrifuged at 1000 X g for 20 min at 4 °C (Hermle Z326k, Hermle Labortechnik GmbH, Wehingen, Germany) before ultrasonication for 30 min. A 0.22-µm polytetrafluorethylene filter was then used to filter the supernatants. A Quadrupole 120 time-of-flight (QTOF) mass spectrometer UPLC-QTOF/MS (Waters, Milford, MA, USA) was used to identify and quantify predominant secondary metabolites. An ACQUITY UPLC BEH C18 column (2.1 × 100 mm i.d., 1.7 × 10^{-6} m; Waters) was used for all analyses. The mobile phase was composed of acetonitrile (A) and 0.1% formic acid, v/v (B), with the following gradient elution: 0–8 min, 95–80% A; 8–12 min, 80–70% A; 12–15 min, 70–65 A; 15–18 min, 65% A; 18–21 min, 65–20% A; 21–23 min, 20–5% A; 23–24 min, 5% A; 25–30 min, 95% A. The flow rate of the mobile phase was 0.4 mL/min and the temperatures of the column and autosampler were maintained at 30 and 10°C, respectively. Data was processed using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) [27, 28]. Functions 1 (unfragmented channel) and 2 (fragmented channel) of the Waters MSe data were processed by MSDial to produce MS1 and MS2 spectra as well as extracted ion chromatograms with associated peak height intensity data. Since calibration standards are not available for the majority of these compounds, the peak height intensity was converted to concentration in a semi-quantitative manner by interpolation off a calibration curve for catechin acquired under the same instrumental conditions. Each deconvoluted feature (alignment in MSDial), together with its associated MS1 and MS spectra was exported from MSDial to MSFinder. Based on the accurate mass elemental compositions, possible compounds were identified from the listed databases and then subjected to in-silico fragmentation. According to the spectral match between the in-silico and measured spectra, a score (out of 10) is assigned to each of the possible compound matches with the highest score being accepted as the most likely (assuming a score of at least 4).

Data Analysis

The data were subjected to ANOVA using the GENSTAT 12th edition software. The least significant difference was used to compare the means, and the means were treated as significantly different at $p < 0.05$.

Effect of different levels of organic and inorganic fertilizer on growth parameters of cowpea

The chemical composition of the organic fertilizers (Feeds/F) in the current study revealed high nitrogen content (1.88 %) in poultry manures (Table 1). As shown in Table 2, there was a noticeable increase in all the growth parameters in all the treatments compared to the control. All the growth parameters were affected by fertilizer source and rate of application. Poultry manure significantly ($p<0.05$) increased the number of pods, plant height, seeds per plant and number of nodules, while nitrogen fertilizer significantly increased the number of leaves per plant, shoot fresh and dry weight. The current study results show that plant height increased with increasing fertilizer application, and significantly higher plant height was obtained at 90 kg F/ha rate of application (Table

2). Plant height ranged from 104.44 to 120.05 mm (poultry manure fertilizer) and from 103.11 to 116.62 mm (nitrogen fertilizer). The control significantly lowered plant height (104.68 mm) while 90 kg F/ha increased the plant height to 120.05 mm. The results from the current study are in agreement with results reported by Islam et al. [29] who reported that the total height of bush bean, winged bean and yard long bean was significantly higher in the vermicompost (20%) treatment compared to traditional compost (20%) and farm practice treatments. It has been suggested that probably vermicompost added humic acid to the soil, which subsequently enhanced plant growth [30].

A significantly ($p<0.05$) higher leaf number was observed in plants cultivated under 45 kg N/ha compared to the control and other treatments. The present study results also showed 90 kg F/ha showed the highest harvest on the number of pods and seeds per pod per plant (yield). The control significantly ($p<0.05$) lowered the number of nodules per plant, and 90 kg F/ha significantly ($p<0.05$) increased the number of nodules per plant. The results from the present study are in contrast with results reported by Yoganathan et al. [31], who reported that the highest grain yield of cowpea was obtained by combined application of poultry manure and inorganic nitrogen fertilizer than a single application of poultry manure and inorganic nitrogen fertilizer. These results agree with results reported by Akanbi and Togun [32], who reported a substantial decrease in plant growth parameters when soil is deficient in nutrients, particularly nitrogen, as they are often essential for the growth, development protoplasm and chlorophyll formation of the plant. Abebe et al. [33] also reported that the application of 4 t/ ha poultry manure enhanced pod formation, height, and yield in cowpeas. These results may apply to other leguminous crops. Cowpea is one of the most important legume plants in sub-Saharan countries. Its tender leaves and pods are edible, highly nutritious and rich in phytonutrients and mostly consumed to eradicate hunger and malnutrition in rural communities. Results from the current study are similar to results noted by Ayoola and Makinde [34], who observed that the yield of vegetable cowpea significantly improved with the addition of organic fertilizers. The current study show that plants cultivated under 45 kg N/ha had significantly increased fresh and dry shoot weight compared to the other treatments. In the current study, the chlorophyll content of the leaves varied significantly. The results indicate that plants cultivated under 60 and 90 kg N/ha possessed a lowest chlorophyll content than the other treatments. Poultry manure contains nitrogen that is essential for the growth of leaves, as it enhances vegetative growth, which is very crucial, especially in plants where leaves are the source of food. For instance, in case of cabbages and kales, it is a nutrient essential in the formation of the chlorophyll molecule, giving the leaf its deep green colour and ensuring that they grow in a healthy manner [35, 36]. Poultry manure also contains potassium, an essential element in the formation of chlorophyll, hence the high chlorophyll content in cowpea leaves treated with poultry manure. The results showed a strong positive correlation between leaf chlorophyll, plant height, number of leaves, number of pods, number of nodules, and the cowpea shoot fresh weight, as shown in Table 2.

Table 2: *Effect of organic manure and inorganic N fertilizers on cowpea growth, yield and chlorophyll content*

Treatment	Plant height (mm)	Number of leaves /plant	Number of pods/ plant	Number of seeds/pods/ plant	Number of nodules/ plant	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf chlorophyll
0 kg/ha	104.68^{a}	330^{a}	330^{b}	324^{a}	304^{a}	330^{a}	78^{a}	25.4^{b}
10 kg F/ha	104.44^{a}	349^{a}	357^{c}	364^{b}	379^{b}	349^{a}	80^{a}	26.5^{b}
30 kg F/ha	107.85^{a}	338^{a}	342^{bc}	383^{bc}	359^{c}	338^{a}	83^{a}	25.8^{b}
60 kg F/ha	108.89^{b}	339^{a}	387^{d}	385^{c}	381^{b}	339^{a}	80^{a}	24.7^{bc}
90 kg F/ha	120.05^{c}	373^{b}	404^{e}	415^{d}	399^{d}	373^{b}	96^{b}	27.4^{a}
30 kg N/ha	103.11^{a}	383^{b}	331^{b}	341^{a}	365^{bc}	383^{b}	76^{a}	23.9^{c}
45 kg N/ha	113.73^{b}	430^{c}	327^{b}	375^{bc}	355^{c}	430^{c}	106^{c}	26.5^{b}
60 kg N/ha	113.53^{b}	328^{a}	310^{b}	357^{b}	381^{b}	328^{a}	84^{a}	22.1^{d}
90 kg N/ha	116.62^{b}	361^{b}	306^{a}	385^{c}	376^{bc}	361^{b}	98^{b}	22.7^{d}

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P-value	0,75	0,00	5,66	5,32	3,09	0,00	0,00	0,00

Different letters down the column represent significant differences at p<0. 05

Table 3: *Correlation between leaf chlorophyll, plant height, number of leaves, number of pods, number of nodules and shoot fresh weight.*

Parameter	Leaf chlorophyll	Plant Height (cm)	Number of Leaves/plant	Number of pods/plant	Number of Nodules/ plant	Shoot fresh weight (g)
Leaf chlorophyll	1					
Plant Height (mm)	-0,029600645	1				
Number of Leaves/plant	0,125623084	-0,014871702	1			
Number of pods/plant	-0,071685417	-0,097981791	-0,131270075	1		
Number of Nodules/ plant	-0,116447957	0,021560472	-0,181752768	0,021898332	1	
Shoot fresh weight (g)	0,025172055	-0,029009093	-0,11453019	0,203445276	0,162197535	1

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Effect of different levels of organic and inorganic fertilizers on the mineral composition of cowpea

As shown in Table 4, cowpea leaves in the current study are endowed with a wide variety of macro and micro elemental nutrients as affected by organic and inorganic fertilizers. In this study, poultry manure and nitrogen fertilizer improved leaf mineral constituents in cowpea and variations were reported depending on different levels of application with poultry manure responding well. Applying 90 kg F/ha led to a significant (p<0.05) increase in cowpea leaves' Mg, N, Ca, Fe, Na, and Zn concentrations. The K concentration of cowpea leaves did not differ significantly between the treatments, while P concentration was significantly lower (p<0.05) in control (0 kg/ha). At a daily rate of 470 mg, the consumption of Mg reportedly ameliorates diarrhoea in adults, while 1200 and 384 mg/ day ameliorates duodenal cancers and congestive heart failure, respectively [37]. This shows that the consumption of a single cup of cowpea leaves per day can alleviate these conditions. Iron from animal sources is less bio-available than that derived from plants [37]. This also potentially shows the ability of this crop in the fight against anaemia in Sub-Saharan Africa (SSA). Potassium is well known for preventing osteopenia, osteoporosis, bone fracture, bone breakage, and its role in bone density improvement, but P and Ca also play crucial teeth and bone-strengthening role [38]. Although Ca is well known for kidney stone prevention in adults, this mineral also collaborates with Mg, K, and fibre to reduce high blood pressure [39]. Therefore, the consumption of cowpea leaves could potentially assist in skeletal formation and strengthening and repair and maintain a healthy circulatory system. Ncayiyana et al. [25] reported that an increase in synthetic N from 0 – 120 kg/ ha did not significantly change the K and P levels in onion bulbs, although organic fertilizer led to an increase in these minerals. The previous authors' findings are in contrast with those of our study. Unfortunately, in SSA, mineral and micronutrient deficiencies continue to be leading problems [40]. Underutilized leafy vegetables have contributed immensely to Ethiopia's food bank while providing a cheaper and more available food and nutrition security and numerous health benefits to the poor and marginalized communities [41, 42]. In Nigeria, the contribution of fiber, specific hormone receptors, minerals, vitamins, protein and energy promotion of health and the prevention of some cancers have been linked with the consumption of leafy vegetables [37]. Our findings reveal and document the rich mineral and proximate contents found from cowpea leaves' consumption.

Table 4: *Effect of different levels of organic manure and inorganic N fertiliser applications on mineral composition of cowpea*

Treatment	N (%)	K (%)	P (%)	Ca (%)	Mg (%)	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
0 kg/ha	2.88^{b}	3.68	0.54^{d}	0.34^{ab}	0.23^{b}	583.80^{a}	47.84^{a}	87.89^{c}
10 kg F/ha	2.75^{bc}	3.67	0.60^{bc}	0.33^{ab}	0.22^{bc}	494.40^{abcd}	44.05^{a}	92.68^{b}
30 kg F/ha	2.77^{bc}	3.68	0.67^{ab}	0.33^{ab}	0.21^{bc}	477.00^{bcd}	39.94^{b}	89.88^{bc}
60 kg F/ha	2.72^{bc}	3.66	0.68^{ab}	0.32^{b}	0.20^{bc}	535.80^{abc}	47.38^{a}	88.35^{bc}
90 kg F/ha	3.11^{a}	3.67	0.63^{b}	0.36^{a}	0.25^{a}	549.80^{ab}	42.75^{ab}	98.87^{a}
30 kg N/ha	2.89^{b}	3.65	0.64^{b}	0.31^{c}	0.23b	507.00^{abcd}	40.78^{b}	92.13^{b}
45 kg N/ha	2.70^{bc}	3.66	0.60^{bc}	0.36^{a}	0.21^{bc}	554.00^{ab}	43.07^{ab}	91.56^{b}
60 kg N/ha	2.82^{b}	3.69	0.62^{bc}	0.35^{ab}	0.22^{b}	449.30^{cd}	42.75^{ab}	93.42^{b}
90 kg N/ha	2.75^{bc}	3.67	0.64^{b}	0.34^{ab}	0.21^{bc}	440.50^{cd}	47.56^{a}	98.25^{a}
P-value	0.315	NS	0.06	0.044	0.223	95.22	0.036	0.899

Different letters down the column represent significant differences at p<0. 05

Effect of different levels of organic and inorganic fertilizers on bioactive compounds of cowpea

The results of the current study show that ascorbic acid (AA) increased with increasing fertilizer application and significantly lowered (p<0.05) in the control treatment (0 kg/ha) (Fig 1). The antioxidant activity using the FRAP assay was significantly higher at 90 kg F/ha than the other treatments (Fig 2). The total phenolic contents were significantly higher at 90 kg F/ha (Fig 3). Various sources and rates of fertilizer influenced the AA, FRAP assay and total phenolics. Organic and inorganic fertilizer increased the accumulation of AA, phenolics and antioxidant activity in cowpea. The application of 90 kg F/ha increased total phenolics (227 mg/L Gallic DW) and FRAP assays (8.68 µm Fe (II)/g dry weight. Gallic acid has a high scavenging activity and has anti-diabetic, albuminuria, psoriasis and external haemorrhoids activity [43]. The anticancer properties of gallic acid and its cancer cell growth inhibition activity are widely reported [44, 45]. The role of gallic acid in cancer prevention has been reported [46]. Therefore, the current study indicate that *Vigna unguiculata* L. fertilized with organic supplements can enhance its anticancer and antimicrobial activities. However, in contrast to the current study, [47] found that basil leaves cultivated at lower than recommended rates possessed higher phenolic compounds. In addition, Onyango et al. [40] report showed a general decrease in phenolic compounds with increasing nitrogen application but depending on the source and rate of application. Ryan et al. [48] noted that different plants have different phenolic reservoirs in different parts like in the roots, shoot, and leaves. Results from the current study agree with results reported by Faller and Fialho [49], who reported that NPK reduces antioxidant levels, but organic fertilizers increase its levels all shown that phenolic had anticancer activities that can inhibit cancer cell growth.

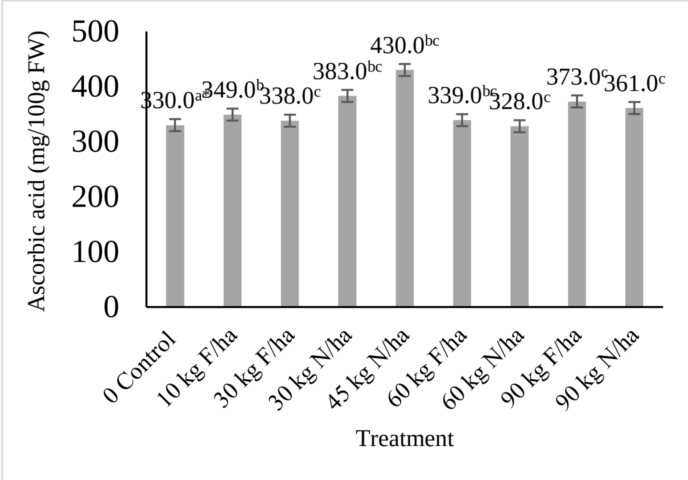

Figure 1: *Effect poultry manure and nitrogen fertilizer on ascorbic acid content of cowpea*

*Bars with the same superscripts are not significantly different (P<0.05)

Table 5: *Identification of secondary metabolites present in cowpea leaves subjected to organic and inorganic fertilizer by a UPLC–QTOF/MS data in negative ionization mode.*

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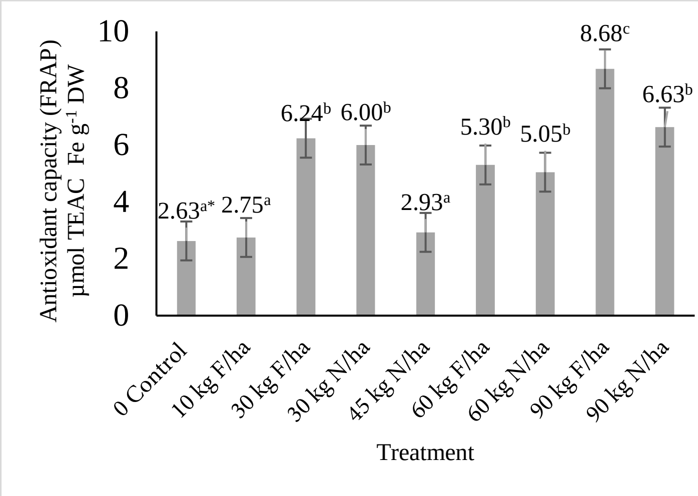

Figure 2: *Effect poultry manure and nitrogen fertilizer on Antioxidant capacity of cowpea*

*Bars with the same superscripts are not significantly different (P<0.05)

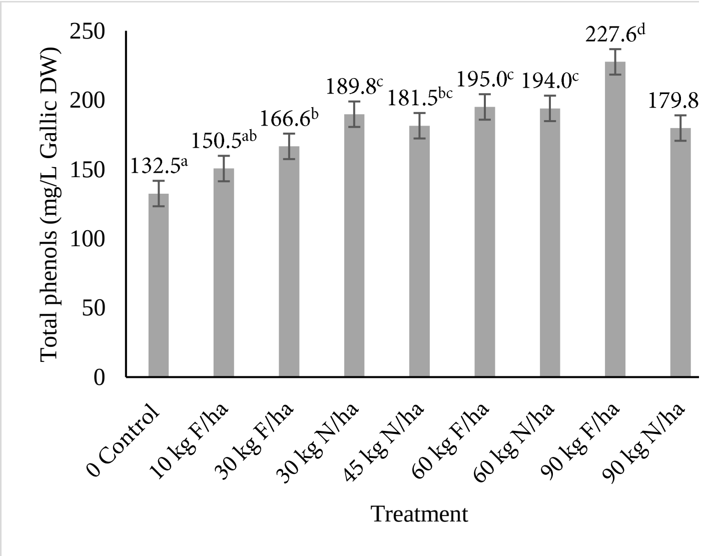

Figure 3 *Effect poultry manure and nitrogen fertilizer on Total phenol content of cowpea*

*Bars with the same superscripts are not significantly different (P<0.05)

Quantification of Non-Targeted secondary Metabolites in cowpea

Compound identification of the cowpea leaves treated with organic and inorganic fertilizer was carried out by UPLC-TOF-MS/MS. The negative ionization mode was ideal, since it has been testified to be more sensitive for the detection of polyphenols and has lower limits of detection compared to the positive mode of analysis [50]. Thirteen major peaks were identified or tentatively identified according to their retention times and fragment ions in the

Specific plant natural product database, as shown in Table 5 and Fig. 4. With regards to antifungal agents, compound at peak 1 were deemed relevant. Peak 1 was characterized as Phaseollidin, displaying the parent ion with m/z 323.131 at 10.122 min. Peak 4 was tentatively identified as a coumarin derivative, as 2-O-p-Coumaroyltartronic acid, which was reported to be predominant compound in cowpea leaves with highest concentration (260.973, 245.162 and 197.888 mg/kg DW) in cowpea leaves treated with 45kgN/ha, 0kg/ha and 90kgF/ha respectfully at m/z 265.034. Peak 10 was tentatively identified a kaempferol derivative, Kaempferol 3-sophorotrioside, which displayed the deprotonated ion [M-H]- at m/z 771.197. Kaempferol derivatives has many pharmacological activities including antioxidant, , antimicrobial, antiinflammatory antidiabetic, and anticancer activities. The anticancer activity of kaempferol has been reported in cancer cells from different organs including breast, pancreatic cancer, gastric, ovarian, lung, and blood cancers [51].

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Peak	Retention time (min)	Tentative Identification	Ontology	Acquired m/z	Theoretical M/z	Error (ppm)	Adducts	0kg/ha (mg/kg DW)	45kgN/ha (mg/kg DW)	90kgF/ha (mg/kg DW)
1	10.122	Phaseollidin	Pterocarpans	323.131	324.136708	5.374	M-H	50.284	55.203	44.922
2	11.899	trans-o-Coumaric acid 2-glucoside	Phenolic glycosides	325.095	326.100716	1.73	M-H	31.786	32.506	28.237
3	12.115	Methyl 6-O-galloyl-beta-D-glucopyranoside	Galloyl esters	345.079	346.090545	-9.289	M-H	5.621	7.422	49.141
4	12.454	2-O-p-Coumaroyltartronic acid	Coumaric acid esters	265.034	266.043201	-5.379	M-H	245.162	260.973	197.888
5	14.338	amphipaniculoside E	Fatty acyl glycosides of mono- and disaccharides	435.223	436.231396	-2.581	M-H	61.831	63.05	37.447
6	14.8	Ochrocarpin C	-	405.129	406.142187	-12.057	M-H	22.624	21.631	28.154
7	15.37	8-Hydroxypinoresinol 4-glucoside	Lignan glycosides	535.185	536.189925	4.784	M-H	6.966	6.596	1.332
8	16.077	Quercetin 3-(2Gal-apiosylrobinobioside)	Flavonoid-3-O-glycosides	741.187	742.196192	-4.988	M-H	53.896	55.395	21.573
9	16.383	Hypochoeroside A	Germacranolides and derivatives	427.196	428.205181	-3.622	M=H	44.835	46.864	33.374
10	16.601	Kaempferol 3-sophorotrioside	Flavonoid-3-O-glycosides	771.197	772.206757	-2.508	M-H	50.19	49.43	30.023
11	16.767	Quercetin 3-lathyroside	Flavonoid-3-O-glycosides	595.13	596.138283	-0.189	M-H	101.383	104.995	78.361
12	17.732	Isorhamnetin 3-glucosyl-(1->6)-galactoside	Flavonoid-3-O-glycosides	639.158	640.164498	2.549	M-H	74.458	78.817	48.805
13	18.822	Quercetin 3-O-(6''-acetyl-glucoside)	Flavonoid-3-O-glycosides	505.101	506.106589	3.677	M-H	12.08	13.159	5.357

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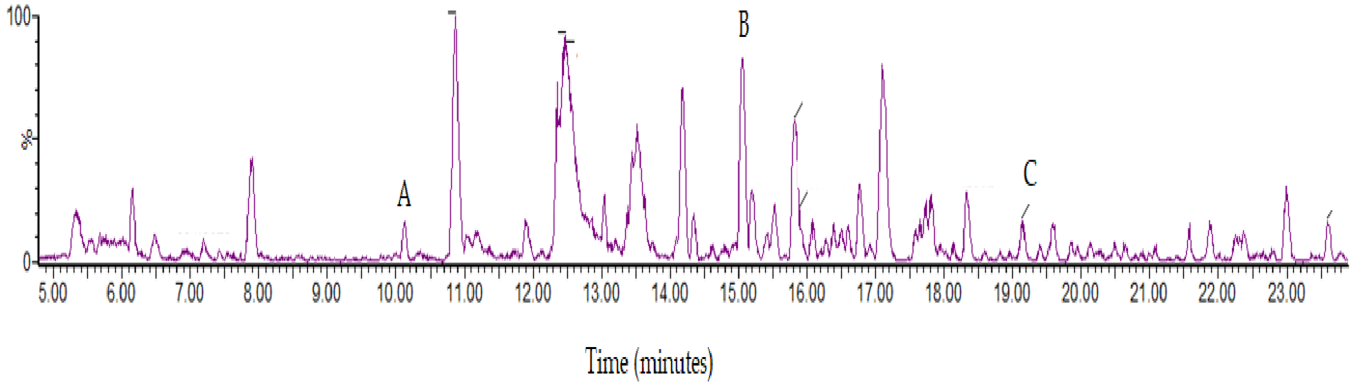

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Figure 4: *Chromatogram profile of Cowpea leaves; for peak identification see Table 5*

Conclusion

The rate of organic manure and inorganic fertilizer influenced the growth, development, yield, mineral composition, bioactive constitutes and secondary metabolites of cowpea planted in Mpumalanga Province. The current study's findings indicate the potential of organic fertilizers in sustainable agriculture and the environment. Synthetic fertilizers can potentially be replaced with organic poultry fertilizers for soil, crop, and human health. Therefore, the consumption of cowpea leaves in Mpumalanga province of South Africa, can drastically improve food and nutrition security, more so, the rampant mineral deficiencies in both children and adults. Application of organic poultry manure at 90 kg F/ha for cowpea cultivation could be recommended in the Mpumalanga Province.

Data Availability

The data used to support the findings of this study may be released upon application to the first author, who can be contacted at Wilfred.Mbeng@ump.ac.za

Ethical Approval

The study did not include any living objects to be studied; therefore, no ethical approval was needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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