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Studies on the production of tigernut (*Cyperus esculentus L.*) in Southeastern Nigeria, II: Biochemical quality of tigernut in response to seven complementary fertilizer treatments.

§Baiyeri Kayode Paul, Okonkwo Onyinye Joy, Akachukwu Judith Ihechiluru & Ezugwu Uchenna Immaculata

Department of Crop Science, University of Nigeria, Nsukka, Nigeria.

Corresponding author: Baiyeri Kayode Paul. Email address: paul.baiyeri@unn.edu.ng

Abstract:

Part I of this series showed significant influence of fertilizer on the growth and yield of tigernut. This paper documents the effects of these complementary fertilizers on the nutritional quality of the crop. Thus, the effects of seven fertilizer treatments on the nutritional quality of tigernut were studied. Proximate, mineral, vitamins and phytochemical contents of tigernut were evaluated across the fertilizer treatments. Most of the mineral contents were significantly ($p < 0.05$) influenced by the fertilizer combination except for magnesium (suggesting non-responsiveness of this mineral to fertilizer treatment). On the proximate contents, ash and moisture were statistically similar while other components varied significantly. The effect of fertilizer treatment on the vitamin content was only significant on vitamins B6 and C while for the phytochemical content, only phytates and tannin were significantly ($p < 0.05$) influenced by fertilizer. It was conclusive that combined application of organic and inorganic fertilizer increased the carbohydrate, fibre, vitamin C, tannin and most of the mineral contents of tigernut tubers. Findings reported herewith therefore validate the application of complementary fertilizer treatment for growing tigernut in this region because of enhanced nutritional quality of the tigernut seeds.

Keywords: Fertilizer use, Tigernut tubers, Biochemical quality, *Cyperus esculentus* L

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INTRODUCTION

Tigernut (*Cyperus esculentus*) is considered an underutilised tuber of the family Cyperaceae that produces rhizomes from the base of the tuber (Devries and Feuke, 1999). The tigernut plant is erect and forms a complex, shallow underground system comprising of fine fibrous roots and thin scaly rhizomes. These rhizomes store proteins, starches and other nutrients, which contribute to the formation of many tubers and golden-brown flower head (Abdelkader *et al.,* 2017). Tigernuts can be grown annually or perennially by seeds or by corm like solid bulbs at the base of a leaf fascicle or by rhizomes (Lowe and Whitewell, 2000). The tubers are rich in minerals such as phosphorus, potassium, calcium, magnesium and iron. Additionally, the tubers contain vitamin E, C and a good quantity of vitamin B (Maduka and Ire, 2018). Advancement has been made towards enhancing the phytochemical, mineral, vitamin and proximate quality even though it's an ancient crop. Major nutritional challenges can be addressed by harnessing the economic and nutritional benefit of these tubers. Nutritional composition of tigernut tubers exhibits some distinct features, between other tubers and nuts (Sanchez-Zapata *et al.,* 2012). The nutritional value of tigernuts and their products are dependent on various factors including varieties, soil conditions, growth environment, cultivation techniques and storage conditions (Nina *et al.,* 2019). The quality of any agricultural produce is influenced by the prevailing growth environment of which the soil fertility variables are major determinants (Wills *et al.,* 1998; Lundergardh and Martensson, 2003). The percentage composition of moisture, carbohydrate, fiber, protein, and other substances including minerals in tigernut is an indication of its quality. During the growth stage of plant, some essential nutrients are required for healthy growth and development. Application of fertilizers ensures that plant receives the necessary nutrient required for optimal growth. Fertilizers contain essential nutrients required by the plant, including nitrogen, potassium and phosphorus. Manure is a slow-release fertilizer, and serves as a valuable source of nutrients and organic matter, which can contribute to enhanced productivity and sustainable production (Mugwira, 1979; Baiyeri and Tenkuano, 2007). The combined application of manure alongside inorganic fertilizers facilitates the prompt release of nutrients needed for plant growth (Mohammed, 2002). Fertilizers therefore play a crucial role in tigernut cultivation but there's limited knowledge regarding whether the application of these fertilizers could influence the nutritional components of the tubers.

This study aims to evaluate the biochemical quality of tigernut tubers in response to seven complementary fertilizers. The specific objective of this study was to determine the proximate, mineral, vitamin and phytochemical contents of tigernut in response to the seven complementary fertilizer treatments.

MATERIALS AND METHODS

Experimental site: The pot experiment was conducted at the Department of Crop Science Teaching and Research farm, University of Nigeria, Nsukka, Enugu state, Nigeria. The study spanned through August and December 2022. The freshly harvested tubers were analyzed for the nutritional quality at the Simuch Scientifics Analytical Laboratory, Nsukka, Enugu state, Nigeria.

Treatment application

Seven fertilizer combinations were used, which included; NPK 15:15:15(300kg/ha), NPK 20:10:10 (300kg/ha), Poultry manure (5t/ha), NPK 15:15:15 (150kg/ha) + 3t/ha PM, NPK 20:10:10 (150kg/ha) + 3t/ha PM, K-Nitrate + Ca-Nitrate (100kg/ha) + 2.5t/ha PM and control. The treatments were applied two weeks after transplanting.

Data Collection:

The tigernut tubers were harvested for each fertilizer treatment, ground and replicated into two samples for laboratory analysis. The processed samples were analyzed for proximate, minerals, vitamin and phytochemical composition following the standard analytical procedures.

Determination of proximate content

Proximate quality was determined using modified standard methods of Association of Official Analytical Chemist (2005). The proximate quality comprises ash, carbohydrate (CHO), fat, fiber, moisture and protein.

Ash content was determined by heating a silica dish up to 600^{o}C, cooled in desiccators and weighed. A 2 g ground sample was weighed into the silica dish in a muffle furnace and heated at 600 ^{0}C for 3hours. The sample was allowed to cool and weighed, and then percentage ash was calculated using the formula below:

$$\% \text{ Ash} = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

Crude protein was determined using the micro - Kjeldhal method. A 1 g of the sample was poured into a 50 ml Kjeldahl flask with conc. H_2SO_4 and a pinch of catalyst and heated until the mixture turns to green. After it has cleared, the mixture was heated for 2 minutes more and allowed to cool. A 10 ml of distilled water was added to avoid caking and the volume made up to 50 ml. A 10ml of the digested sample was transferred to the Kjeldahl apparatus with a receiver flask (50 ml) containing 20 ml of boric acid indicator solution through a funnel stop cork and distillation commenced. The distillate was collected (35 ml) through the condenser tips and titrated with 0.01M HCL to pink colour, and percentage protein calculated using the formular below:

$$\% \text{ Nitrogen} = \frac{\text{Titer value} \times 14.1 \times 0.01 \times 100 \times 50}{1000 \times 1g \times 10ml}$$

$$\% \text{ Protein} = \% Nitrogen \times 6.25$$ (where 6.25 is a constant)

Crude fat content was determined using the Soxhlet method. An extraction flask was washed and dried in hot air oven for 30minutes and allowed to cool in desiccators and weighed as B. 2g of the ground sample as A was weighed and transferred into an extraction thimble and dropped inside the Soxhlet extractor. 200ml of petroleum ether was added and the apparatus set up to run for 4 hours. The thimble and ether were recovered at the end of the extraction. The oil collected in the flask was dried at 100^o C in an oven and the flask is weighed with the oil as C. Percentage fat is calculated as:

$$\% \text{ Fat} = \frac{C-B}{A} \times \frac{100}{1}$$

Crude fiber was determined using the Weende method. **A** 2g of the grounded sample was added to 150 ml of 0.128 M pre-heated H_2SO_4 in 400 ml beaker and allowed to boil for 30 minutes, cooled and filtered. The residue was washed trice with hot water. A 150 ml of 0.128 pre- heated KOH was added and heated to boiling point with some drops of antifoaming agent. It was left to boil slowly for 30 minutes and filtered. The residue was washed three times with hot water and another three times with acetone. Dried at 103 ^{0}C with crucible in an oven for 1 hour and weighed (W_2). Ashed at 500 ^{0}C and weighed again (W_3). Crude fiber is calculated thus:

$$\% \text{ Fiber} = \frac{w2-w3}{w1} \times \frac{100}{1}$$

Determination of moisture content: Five grams (5g) of the grounded sample was dried to a constant weight at 600 ^{0}c in a hot air circulating oven. The moisture content was calculated as the difference in weight after drying as shown below:

$$\frac{w2-w3}{w1} \times \frac{100}{1}$$

Where w_1 = initial weight of empty crucible
W_2 = weight of crucible + sample before drying
W_3 = Final weight of crucible + sample after drying. Carbohydrate was determined by subtracting the summation of Ash, fat, fiber, moisture and protein values from 100 for each treatment used.

Determination of vitamin

Vitamin content was determined using the modified method of AOAC (2005). Vitamin contents are vitamin A (carotenoid), Vitamin B2, Vitamin B6, Vitamin C.

Vitamin A was determined by macerating 1 g of sample with 20 ml of petroleum ether for 10 minutes and allowed to stand for 1 hour with intermittent shaking at every 10 minutes. It was centrifuged for 5 minutes and 3 ml of the supernatant transferred into triplicate test tubes. It was left to evaporate to dryness and residue re-dissolved with 0.2 ml of acetic anhydride/chloroform 1:1 and 2 ml of 50 % trichloroacetic acid (TCA) in chloroform. The absorbance of the resulting solution was then taken at wavelength of 620 nm at 15 seconds and 30 seconds against the corresponding blank. The vitamin concentration of the sample will be calculated thus;

$$\text{Conc. (mg)} = \frac{Abs \times DF \times Volume\ of\ curvette}{E}$$

Where Abs = Absorbance
DF = Dilution factor
E = Extinction coefficient
Vitamin C was determined by macerating 1 g of sample with 20 ml of 0.4% oxalic acid for 10min and centrifuged for 5minutes. A 5 ml of the supernatant was transferred into triplicate test tubes to which 2 ml of 2,6-dichlorophenol indophenols (12 mg/l) had been added and then mixed thoroughly by shaking. The absorbance of the resulting solution was taken at wavelength of 520 nm at 15 seconds and 30 seconds against the corresponding blank. The vitamin concentration of the samples was calculated as;

Conc. (mg) = $\frac{Abs \times DF \times Volume of cuvette}{\varepsilon}$

Where Abs = Absorbance; DF = Dilution factor
E = Extinction coefficient.
Vitamin B2 was determined by extracting 5 ml of the sample with 100 ml of 50 % hydrogen peroxide (H_2O_2) and allowed to stand for 30 minutes. A 2ml of 40 % sodium sulphate was added to make it up to 50 ml mark. The

Vitamin B6 was determined by extracting 5 ml of the sample with 100 ml of 50 % hydrogen peroxide (H_2O_2) and allowed to stand for 30 minutes. Thereafter, 2 ml of 40 % sodium sulphate was added to make it up to the 50 ml mark. The absorbance was measured at 510 nm in a spectrophotometer. The vitamin concentration of the sample was calculated thus:

Conc. (mg) = $\frac{Abs \times DF \times Volume of cuvette}{\varepsilon}$

Where Abs = Absorbance
DF = Dilution factor
E = Extinction coefficient

Determination of phytochemicals

This comprises of flavonoids, phenol, phytates and tannin. Tannin was determined by weighing 2.0 g of the sample into a 50 ml conical flask and 10 ml of 2 M HCL was added and shaked plus 20 ml of water. The content was filtered into 50 ml volumetric flask and made up to 50 ml. A 5 ml of the solution was pipetted into the test tube and 5 ml of water as control into another test tube, A 3ml of $FeCl_3$ in 0.1 m HCL and 3 ml of 0.008 ($K_3Fe(CN)_6$) was added to each test tube respectively. It was allowed to stand for 30 seconds and absorbance read at 720 nm. The readings were plotted against the concentrations of the various standards.

% Tannin = $\frac{An}{As}$ x C x $\frac{100}{W}$ x $\frac{Vf}{Va}$

Where An = Absorbance of test sample
As= Absorbance of standard solution
C = Concentration of standard solution
W =Weight of sample
Vf = Total volume of extract
Va = Volume of extract analyzed
Flavonoids was determined by weighing 10 g of the sample to 250 ml beaker and dissolved with 70 ml of water at room temperature and left for 15 minutes. A 6.0g of activated charcoal was added and mixed properly. It was left to stand for 30 minutes and filtered into a 400 ml beaker using a vacuum, 60 ml fritted glass funnel

absorbance was measured at 510 nm in a spectrophotometer. The vitamin concentration of the sample was calculated as:

Conc. (mg) = $\frac{Abs \times DF \times Volume of cuvette}{\varepsilon}$

Where Abs = Absorbance
DF = Dilution factor
E = Extinction coefficient

containing asbestos pad. Two drops of HCl were added and left to evaporate on a steam bath to about 40 ml and quantitatively transferred to a 50 ml volumetric flask and made up with water. Further dilutions could be made with water. Read the absorbance of the sample at 233 nm.

Concentration = $\frac{Abs \times 50}{1000}$ = mg/100g flavonoid

Phytate was determined by weighing 0.5 g of the sample into 500 ml round bottomed flask and extracted with 100 ml of 2.4 % HCl for 1 hour at room temperature, decanted and filtered. Pipette 5 ml of the filtrate and dilute to 25 ml with water. Add 15 ml of 0.7 M sodium chloride. Read the absorbance at 520 nm. Prepare a standard curve with phytic acid and blank.

Total phenol content was determined using the Foline-ciocalten reagent method. A 1 m extract was mixed with 0.5 ml foline-ciocalten reagent previously diluted with 7 ml de-ionized water. It was left to stand for 3 min, at 25 ^{0}C. 2 ml of 0.2 ml of saturated sodium carbonate solution was added. The mixture was allowed to stand for another 120 mins. Read absorbance at 725 nm. Gallic acid was used as standard and for the calibration curve. The total phenolic content of the extract was calculated in form of Gallic acid equivalent.

C = C1 x V/M

Where C = total phenolic compound in mg/gm of the extract.
C1 = concentration of garlic and (established via calibration curve)
V = Volume of the extract (ml)
M = weight of extract in grams

Determination of mineral

The ash content of the sample obtained as the remains of inorganic residue after the organic residues were burnt away in the furnace at 650 ^{0}C was dissolved in 30 % hydrochloric acid. The solution was filtered and used for the

determination of minerals. Other minerals were determined with Atomic Absorption Spectrophotometer (AAS); each element was determined from the solution prepared from ash content. The sample were introduced into the Atomic Absorption Spectrophotometer using the appropriate lamp set for each element and standards, the concentration of the elements in the sample were obtained.

Statistical Analysis

The data measured were subjected to Analysis of Variance (ANOVA) using the procedures outlined for a one-way design (completely randomized design) using Genstat12.0. Fischer's Least Significance Difference (F-LSD) at 5 % probability level was used to determine the differences between means.

RESULTS

Most of the proximate quality measured was significantly ($p < 0.05$) influenced by the fertilizer treatments except for ash and moisture (Table 1). Plants without fertilizer treatment had high protein content, NPK 20:10:10 (300 kg/ha) had higher moisture and ash content while NPK 15:15:15 (150 kg/ha) + 3 t/ha PM had high carbohydrate (CHO) content. Ash content was statistically similar in values in NPK 15:15:15 (300 kg/ha) and poultry manure. NPK 15:15:15 (150 kg/ha) + 3 t/ha PM had the least fat, moisture and protein while NPK 20:10:10 (300 kg/ha) and NPK 15:15:15 (300 kg/ha) gave the least carbohydrate and fibre content respectively. Except for Mg, most of the mineral content studied was significantly ($p < 0.05$) influenced by the fertilizer treatment (Table 2). NPK 20:10:10 (150 kg/ha) +3 t/ha PM had high Ca content at 3.760 mg/100 mg while the other treatment was statistically similar in values. Plant without fertilizer treatment had high K, Zn and Mg content. NPK 15:15:15 (150 kg/ha) + 3 t/ha PM gave the least Fe, K, and P while poultry manure (5 t/ha) had the least Zn content. For the vitamin, only B6 and C were significantly influenced. From Table 3, K- nitrate + Ca- nitrate (100 kg/ha) + 2.5 t/ha PM had high vitamin C content, while vitamin B2, B6 and carotenoids in plant without fertilizer treatment. Carotenoids content was statistically similar in all fertilizer combinations except plant without fertilizer treatment. The least vitamin B2 and vitamin B6 content were obtained in NPK 20:10:10 (300 kg/ha) while NPK 15:15:15 (150 kg/ha) + 3 t/ha PM gave the least vitamin C content.

On the phytochemicals studied (Table 4), the treatment applied had no significant effect on the flavonoids and phenol content. Tanin content was recorded high in NPK 15:15:15 (150 kg) +3 t/ha PM while NPK 20:10:10 (300 kg/ha) had high flavonoids and phytates content. Poultry manure (5 t/ha) had the least flavonoids, phenol and tannin

Table 1: Effect of Seven Fertilizer Combinations on Proximate Content (%) of Tigernut tubers

Treatment	Ash	CHO	Fat	Fibre	Moisture	Protein
Control	3.007	77.285	5.255	1.250	4.008	9.195
NPK 15:15:15(300kg/ha)	2.502	76.763	6.502	1.100	5.252	7.880
NPK 20:10:10(300kg/ha)	4.010	74.295	6.008	2.877	5.805	7.002
Poultry manure (PM)(5t/ha)	2.505	76.683	7.502	1.300	5.008	7.002
NPK 15:15:15(150kg/ha) + 3t/ha PM	2.600	82.942	4.750	1.375	3.505	4.820
NPK 20:10:10(150kg/ha) + 3t/ha PM	3.507	75.850	5.498	2.650	5.053	7.442
K-Nitrate + Ca-Nitrate (100kg/ha)+2.5t/ha PM	3.003	74.303	5.008	5.507	4.303	7.877
LSD(0.05)	NS	4.523	1.342	1.430	NS	1.222

NS= non-significant

Table 2: Effect of seven fertilizer combinations on mineral content (mg/100 g) of tigernut tubers

Treatment	Ca	Fe	K	Mg	P	Zn
Control	2.505	0.102	0.672	1.945	11.515	0.237
NPK 15:15:15(300kg/ha)	2.502	0.095	0.568	1.915	13.053	0.190
NPK 20:10:10(300kg/ha)	2.505	0.105	0.475	1.703	8.547	0.210
Poultry manure (PM)(5t/ha)	2.502	0.062	0.455	1.640	10.630	0.143
NPK 15:15:15(150kg/ha) + 3t/ha PM	2.502	0.035	0.105	1.647	4.642	0.158
NPK 20:10:10(150kg/ha) + 3t/ha PM	3.760	0.103	0.415	1.317	8.947	0.152
K-Nitrate + Ca-Nitrate (100kg/ha)+2.5t/ha PM	2.502	0.115	0.300	1.770	9.200	0.165
LSD (0.05)	0.802	0.039	0.178	NS	2.624	0.032

NS= non-significant

Table 3: Effect of seven fertilizer combinations on vitamins content (mg/100g) of tigernut tubers

Treatment	Vitamin B2	Vitamin B6	Vitamin C	Carotenoids
Control	1.823	14.888	8.742	0.025
NPK 15:15:15(300kg/ha)	1.448	14.470	11.055	0.010
NPK 20:10:10(300kg/ha)	1.057	10.582	9.137	0.010
Poultry manure (PM) (5t/ha)	1.363	10.828	10.325	0.010
NPK 15:15:15(150kg/ha) + 3t/ha PM	1.748	11.078	8.617	0.015
NPK 20:10:10(150kg/ha) + 3t/ha PM	1.778	12.282	8.748	0.020
K-Nitrate +Ca-Nitrate (100kg/ha) +2.5t/ha PM	1.480	11.262	15.592	0.015
LSD (0.05)	NS	2.081	2.542	NS

NS= non-significant

Table 4: Effect of seven fertilizer combinations on phytochemicals content (mg/100g) of tigernut tubers

Treatment	Flavonoids	Phenol	Phytates	Tanin
Control	0.090	9.320	18.270	9.273
NPK 15:15:15(300kg/ha)	0.120	7.570	17.037	7.545
NPK 20:10:10(300kg/ha)	0.135	0.460	25.008	7.357
Poultry manure (PM)(5t/ha)	0.065	1.170	12.652	5.673
NPK 15:15:15(150kg/ha) + 3t/ha PM	0.080	4.770	13.545	12.482
NPK 20:10:10(150kg/ha) + 3t/ha PM	0.120	10.270	7.693	6.853
K-Nitrate + Ca-Nitrate (100kg/ha)+2.5t/ha PM	0.130	1.772	5.855	6.595
LSD(0.05)	NS	NS	6.702	2.204

NS= non-significant

DISCUSSION

This study on biochemical qualities of tiger nuts in response to seven fertilizer treatments revealed that the carbohydrate content was found higher in tubers grown with NPK 15:15:15 (150 kg/ha) + 3 t/ha poultry manure. This might be due to an increase in nitrogen, which contributed in enhancing foliage development and stimulated chlorophyll formation. Fertilizer application increased the supply of nitrogen to the plants, leading to an increase in the number of leaves and photosynthetic surface. This in turn, increased photosynthetic activities and physiological processes leading to production of more assimilates which significantly increased the chemical composition of plants (Alabi, 2006;

Amujoyegbe *et al*., 2007; Lawal and Rahman, 2007).

This finding aligns with the study done by Oliveira *et al*., (2002) who reported an increase in starch content of *Dioscorea cayennensis* with addition of mineral fertilizers of N, P and K. This is likely attributed to the fertilizer impact on chlorophyll formation, photosynthesis and leaf formation, which can lead to higher starch accumulation in the plants. The tubers with high moisture content were those fertilized with NPK 20:10:10 (300 kg/ha), followed by NPK 15:15:15 (300 kg/ha). This could be due to the fact that application of nitrogenous fertilizer increased the moisture content of tuber crop by influencing the water uptake and retention capabilities. This is in consonance with Kareem *et al*., (2020) who reported that the moisture content of sweet potato could be increased through the application of NPK fertilizers. The observation that plants grown without fertilizer had the highest protein content might be due to the balance in soil nutrients which guaranteed even absorption of nutrients without antagonistic interaction that may occur when inorganic fertilizer is used (Kareem *et al*., 2020). Potassium nitrate and calcium nitrate significantly increased the fiber content of the tubers than other fertilizers applied. However, the overall low fiber content of the tubers may be due to the seed size of the tubers harvested (Dr. S. I. Umeh, personal communication).

On the mineral content, the higher phosphorus associated with NPK 15:15:15 (300 kg/ha) is likely due to the phosphorus supplied by the NPK fertilizer while NPK 20:10:10 (300 kg/ha) + 3 t/ha PM had higher calcium content which may be due to the calcium supplied by the poultry manure. Mohammed *et al*., (2021) reported that attention should be paid to fertilization with nitrogen and calcium where nitrogen and calcium influence the concentration of various plant minerals in maize grains. This explained why the combination of NPK and poultry manure fertilizer led to an increase in the soil nitrogen, which could have contributed to the high calcium content.

From the study, vitamin C and vitamin B6 were influenced by the fertiliser treatments applied. Vitamin C content was higher in K-nitrate + Ca-nitrate (100 kg/ha) + 2.5 t/ha PM which conforms to the study that increased application of potassium nitrate increased the vitamin C content of potato tubers (Moawiya *et al*., 2016). Vitamin C is well known for its role as a natural anti-oxidant agent with rich sources of metabolites (Bvenura *et al*., 2014). Plants grown without fertilizer had the highest vitamin B6 content, which could also be probably because of the balance in the soil nutrients. Flavonoids and phytates content were higher in plants grown with NPK 20:10:10 (300 kg/ha), which may be probably due to the high nitrogen in the fertilizer, used. Tanin was also higher in NPK 15:15:15 (150 kg/ha) + 3 t/ha PM, phenols were found higher in NPK 20:10:10 (15 0kg/ha) + 3 t/ha PM which can also be attributed to the combined effect of nitrogen found in poultry manure and in the inorganic fertilizer. This conforms to the study by Chen *et al*., (2021) which suggested that nitrogen fertilizers can have a significant impact on the level of total phenols and flavonoids found in *Allium fistulom L.* It was evident that the combined application of inorganic and organic fertilizer significantly increased the carbohydrate, fiber, vitamin C, tannin and most of the mineral content of tigernut tubers and hence improved the nutritional quality of tigernut tubers.

CONCLUSION

Evidences from papers II and I justify the need for ample application of organic and inorganic fertilizer for the growth, yield and nutritional quality of tiger nut grown in southeastern Nigeria. Within the limits of the two experiments, application of NPK 15:15:15 (300 kg/ha) or NPK 20:10:10 (150 kg/ha) + 3 t/ha PM was most appropriate for growth and yield but NPK 15:15:15 at 150 kg/ha plus 3 t/ha PM enhanced better nutritional quality of tiger nuts planted in Nsukka. These recommendations might be appropriate for similar environments to Nsukka. However further fertilizer trials across time and space are suggested in southeastern Nigeria.

Author contribution

This research is the result of collaborative efforts by all the authors. KPB developed the concept, designed and guided the conduct of the experiment and reviewed and corrected the manuscript. AJI, OO and EUI conducted the field trials, collected the data, carried out the analyses and developed the draft manuscript.

Conflict of Interest

Authors have no conflict of interest to declare.

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