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THE INFLUENCE OF MINERAL FERTILIZERS IN NUTRIENTS SUPPLEMENTATION AND QUALITATIVE CALYX PRODUCTION OF ROSELLE (*Hibiscus sabdariffa* L.)

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ABSTRACT

The effect of mineral fertilizer on the potentiality towards enhancing the nutritional quality of Hibiscus sabdariffa's calyx was investigated. The research was conducted between July to November, 2014 during the rainy season, at Research Farm of Kano University of Science and Technology, Wudil, Kano State. The results obtained indicated that, mineral fertilizer 100kg/ha had the higher compositions of essential elements (Na, K, Mg, Ca,), trace elements (Mn, Fe, Zn and Cu) and the proximate constituents (moisture, ash, crude protein and lipid), and then reduced the anti-nutrient content (tannin) drastically, where the F100 fertilizer recorded the least in the tannin content among other fertilizers (M 5.230> F75 4.443 > F100 4.190g/100g respectively). However, mineral fertilizer at a rate of 75kg/ha recorded highest in crude fiber, carbohydrate and calorific value (11.157%, 61.725%, and 321.023kcal respectively). This indicated that, mineral fertilizer was more proficient in influencing the calyx nutritional quality. It was however proven that, mineral fertilizer (N.P.K 15-15-15) at a rate of 100kg/ha was appropriate for the qualitative dietary calyx production.

Keywords: Calyx, Essential elements, Trace elements, Proximate composition, Calorific value

INTRODUCTION

Apart from nutrition and health importance, *Hibiscus sabdariffa* (Roselle) plays a key role in an income generation and subsistence among rural farmers in developing countries(Akanbi *et al*., 2010). The different parts of *Hibiscus sabdariffa*are the calyces, leaves and seeds which have been used for different purposes as vegetables, source of oils, refreshing drinks and food preserves, medicinal and health purposes(Akanbi *et al*., 2010). The leaves, seeds and calyces are valued for its nutritional and medicinal uses(Atta *et al*., 2013). The most exploited part of Roselle plant is its calyces which may be green, red or dark red (Schippers, 2000).The red and dark red calyces are utilized in producing drinks, jellies, sauces, preserves and tea(Atta *et al*., 2013). The calyces drink is receiving industrial attention internationally (Egharevba and Law- Ogbomo, 2007), and is a readily available and inexpensive source of vitamin C (Babajide *et al*., 2004). The Sudanese product is attractively bright red, very acidic and it is extremely popular in Germany, which imports most of the crop (Mohamed, *et al.,* 2012). Export prices for the 1992–1993 season for Sudanese, Chinese and Thai roselle was of the order of $US1700.00 (Mohamed, *et al.,* 2012).Germany and the U.S. are the main countries importing *Hibiscus sabdariffa*. They use *Hibiscus sabdariffa* in numerous products including herbal teas, herbal medicines, syrups and food coloring. Main importers in the U.S are Celestial Seasonings and Lipton, which are both tea companies. *Hibiscus sabdariffa* is also used in ready to serve beverages made by Knudson, Whole Foods and other food and beverage manufacturers (Plotto, 1999).

Demand is on the increase because of nutraceutics endowment of the natural food drink (Salmah *et al.,* 2003).Despite the substantial value of roselle in Nigeria, many constraints still limit the production and yield of the quality calyces and also seeds, by the farmers' in fields usually lower than what is being reported under experimental conditions and findings (Akanbi *et al*., 2010). This research work was designed to investigate the quality of cultivar *Hibiscus sabdariffa's* calyx when grown in the field by the use of conventional fertilizers. There is still little information on the effect of fertilizers on quality produce from the farm land. The objective of this work is to evaluate the effect of fertilizer use on proximate compositions, essential and non-essential nutrients for a quality calyx production. The aim of this work was to assess the effect of mineral fertilizer in nutrients supplementation and qualitative calyx production.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Kano University of Science and Technology, Wudil Research Farm Kano State, in Northern-Western Nigeria, (latitude $11^{o}51'28.05''$N, longitude 8^{0} $59'11.76''$E), and at an altitude of 430m above sea level. The area has mean annual rain fall of about 800mm with relative humidity of 75% during the rainy season and the annual temperature (25^{o}C -26^{o}C).

Experimental Design

The experimental design, consisted of a complete randomized block design with three replications, the distance between two consecutive blocks was 1m and 2m between replicates. The block was 3.6m long and 4.9m wide. All plots consisted of 6 rows of plants with 0.7m apart and 0.6m intra spacing within rows. The work was carried out between July to November, 2014 during the rainy season.

Crop establishment and Treatments

The black variety of *Hibiscus sabdariffa* plant was used for this research, organic manure was also applied uniformly after land preparation before sowing at a rate of 10 ton/ ha (T1), and the other treatments were NPK 15-15-15 mineral fertilizers at the rate of 75 kg N/ha (T2) and 100Kg N/ha (T3). The seeds were directly sown by digging in furrows after rainfall. The thinning was done to an intra-row spacing three weeks after planting. Shallow cultivation was observed; using hoe and hand pulling to keep the plants weed free, for about three times (Grubben, 2004).

Sample collection

The calyx was obtained from cultivated plots, where the four central rows of *Hibiscus sabdariffa* L. were sampled at 115 days after sowing (maturity/fruiting stage) from the farmlands. The collected samples were thoroughly separated into organs, rinsed with de-ionized water and the calyx evaporated at room temperature on a clean background paper with constant turning over to prevent fungal growth. The dried samples were grounded into a fine powder using pestle and mortar, and packed in an air tight plastic container for the analyses.

Chemical and reagents

All the chemicals and reagents used in this study were of analytical grade and are the products of Sigma - Aldrich.

i. Tannic acid standard; 0.05g tannic acid was dissolved in water and diluted to 500 cm^3 (1 cm^3=0.1mg tannic acid of the stock solution). This was prepared fresh.

ii. Folin-denis reagent; 50g of sodium tungstate was added to 375 cm^3 of water, followed by 10g phosphomolbdic acid and then 25 cm^3 orthophosphoric acid added and mixed together. This was also prepared a fresh.

iii. Mix indicator; 0.2g of methyl blue weighed into 100 cm^3 flask, also 0.4g methyl red added into another 100 cm^3 flask. Both were dissolved using ethanol and mixed in 200 cm^3 volumetric flask thoroughly.

iv. Calibration curve
The calibration curves prepared for each element were by serial dilution out of the stock solution. The standard and blank solutions were aspirated first in the instruments (Atomic Absorption and Flame Emission Spectrophotometers) for standardization, blanking and the establishment of calibration curves at a required wave length as; 283.3nm Pb, 279.5nm Mn, 3248nm Cu, 248.3nm Fe and 213.8nm for the Zn respectively (Allen *et al*., 1974).

Proximate Analysis

The recommended methods of the Association of Official Analytical chemists (AOAC, 1995) were used for determination of moisture, ash, crude lipid while, AOAC, (2010) method used for the determinations of crude fiber, nitrogen content and carbohydrate respectively.

Estimation of energy value

The calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57 respectively) used in the proximate analysis (Asibey-Berko and Tayie, 1999).

Determination of Tannin contents

Grounded sample of approximately 0.1gram was weighed into 100 cm^3 conical flask, 50ml of water then added, mixed and boiled for 1 hour using hot plate. The extract was filtered whilst warm through a No.44 filter paper into a 50 cm^3 volumetric flask. The filtered diluted to volume after cooling by running the water onto the extract residue on filter paper, same treatment did to water blank. Color was developed by pipetting a suitable aliquot (5 cm^3) of sample and also 0 to 3 cm^3 of the tannic acid ranging (0 to 0.3mg) tannic acid into 50cm^3 volumetric flask, at this point, samples and standard solutions were treated equally by adding water up to two-third full flask. 2.5 cm^3 folin-denis reagent and 10 cm^3 of Na_2CO_3 solution added then diluted to mark and mixed. The mixture kept in water bath at 25^{o}c for 20 minutes, where the optical density of standard read and obtained calibration curve for mg tannic acid from the samples at 760nm. Blank reading was also measured for subtraction from the sample.
Calculation for tannin content;

$$\text{Soluble tannin (\%)} = \frac{\text{C (mg) x extract volume (cm3)}}{\text{10xaliquot (ml) x sample wt (g)}}$$

Determination of oxalate content

According to the AOAC (1999) method, oxalate was determined by weighing 0.1 g of sample and mixed with 30 cm^3 of 1M HCl. Each mixture was then shaken in a water bath at 100^{0}C for 30 minutes. To each extract, 0.5ml of 5% calcium chloride was added and mixed thoroughly for the precipitation of calcium oxalate. The suspension was then centrifuged at 3000rpm for 15 minutes to separate the supernatant. The pallets were washed twice with 2 cm^3 of 0.35M NH_4OH and dissolved into a 0.5M H_2SO_4. The mixture was then titrated with 0.1M $KMnO_4$ solution while, temperature maintained at 60^{o}C. Where the end point was achieved as the faint violet color appeared and persisted for at least 15 seconds, and 1 cm^3 titer value equivalent to 2.2mg of oxalate.

Mineral Analysis

The mineral elements comprising calcium, magnesium, iron, zinc, copper, lead, zinc, sodium and potassium, were determined according to the method of Allen *et al.,* (1974) by atomic absorption spectrophotometer and flame emission spectrophotometer respectively, where their absorption compared with that of standards solutions and then the respective elements concentrations were determined.

Digestion of plant sample for elemental analysis

A 0.20-0.50g of oven dried plant sample was weighed into a 100ml kheldahl flask.1ml 60% $HClO_4$, 5ml HNO_3 and 0.5 ml concentrated H_2SO_4 were added, swirled gently and digested slowly at moderate heat and increased later for 15 minutes after white fumes appeared and cooled. The digests were then diluted and filtered using No.44 paper, into a 50ml volumetric flask and diluted to mark. Also, blank was treated the same way and then used for the elemental analysis using Atomic Absorption Spectrometry and Flame emission spectrometry (Allen *et al*, 1974).

Statistical analysis

All proximate, minerals and anti-nutrients parameters determinations were carried out in triplicates. The data generated from the experiments were subjected to statistical analysis using the Statistical Package for Social Science (SPSS) Version 16. Descriptive statistics, Anova and post hoc (LSD) also used to interpret the result obtained.

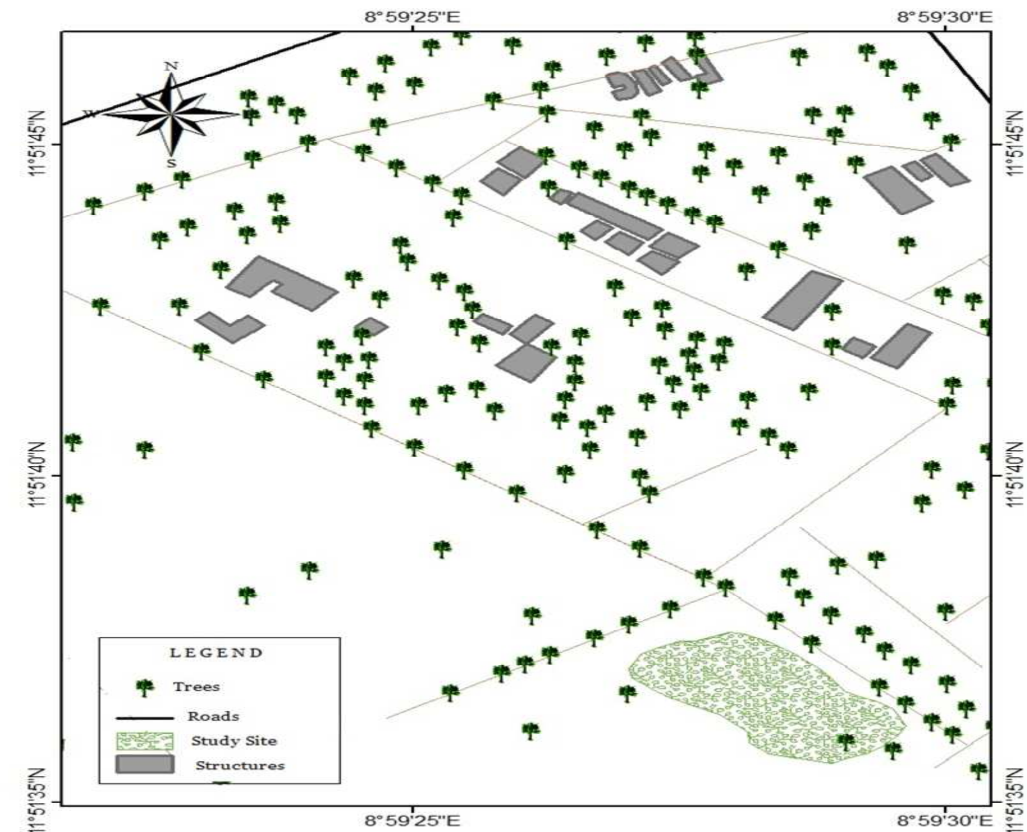

Figure 3.1: Map of KUST Research Farm, Gaya indicating the sampling site

SOURCE: GIS Unit, Dept. of Geography KUST, Wudil (2017)

RESULTS AND DISCUSSION

The analysis of proximate and minerals composition provides information on proximate constituents; moisture, ash, crude fat, crude protein, crude fiber, carbohydrate and calorific values as presented in Table 1 and mineral compositions including essential and trace elements at Table 2 and 3, where Table 4 showed the anti-nutrients (tannin and oxalate contents) from the *Hibiscus sabdariffa's* calyx grown under mineral and organic fertilizers.

Proximate composition

The mineral fertilizer at a rate of 100kg/hectare (F100); recorded with the higher contents in most of the proximate constituents except, crude fiber, carbohydrate and calorific value (Table 1).

The moisture content among the fertilizers observed to be increased with an increase in the levels of mineral fertilizer where calyx from F100 was the paramount in moisture over the other fertilizers used.

Mineral fertilizer at a rate of 75kg/hectare (F75); observed to be greater in the crude fiber content, carbohydrate and calorific value and statistically found significantly different to organic fertilizer in calorific value at ($p<0.05$), owing to this verdict, F75 fertilizer could almost certainly be regarded the appropriate fertilizer concentration needed for calyx production purposely to be used for natural energy drinks and beverages, which justified the work of Gyllaspy *et al*. (1993) who reported that, N fertilizer availability could affect the sink function of fruit (that is its quality) and this play a role in the control of carbohydrate accumulation. On the other hand, Hellal and Mahfouz (2011), reported the increase of volatile oil yield with an increase in the N fertilizer level from dill plant.

Henceforward, the effect of mineral fertilizers also recorded in this investigation indicated that, mineral fertilizer F100 was significantly found different in proteins content when compared to F75 fertilizers, and the mineral fertilizer F75 was consequently found significantly different in the carbohydrate and calorific value at ($p>0.05$) in comparison to other fertilizers studied. This relatively authenticated the statement of Drake and Fellman (1987), and that of Stefano *et al*. (2004) who reported that, mineral fertilizer may be applied to produce crop fruit and seed that will conform to the consumer's demand. Owing to this fact, the mineral fertilizers (F100 and F75) could be considered the appropriate fertilizers for the enhancement of dietary calyx production especially for protein, carbohydrate and calorific value which are the fundamental parameters for malnourishment amelioration. This will however, be substantial information to our local farmers for the production of the nutritionally valuable calyx. Meanwhile, the other constituents like, moisture content, as a factor that gives freshness to fruit and the dry matter content, crude fat that determines the free fatty lipids of a product which is the healthiest oil when compared to other source, and then the ash content as the mineral content index in biota, were however, found to be higher with F100 fertilizer. But, the difference that exists among the fertilizers was found to be insignificant ($p>0.05$).

However, some constituents like the ash content, crude protein and fat content were virtually found to be lowest with F75 fertilizers, which further indicated that organic fertilizer 10tonnes/hectare (M) and mineral fertilizer F100 have shown level of equality and are the best nutrients yielding fertilizers as per these nutrients are to supplemented in the calyx.

Minerals composition

The F100 fertilizer was, found to be predominantly higher in the mineral compositions (both essential and trace elements) among other fertilizers as presented in (Table 2 and 3). Some of these elements were observed to be increase with an increased in the mineral fertilizer levels such as: Mg, Ca, Fe, Zn and Cu. This is in conformity with the work of (Akanbi *et al*. 2010 and Ibrahim *et al*., 2014) who demonstrated an increase in dry matter accumulation on calyx with an increase in N fertilizer level. Gyllaspy *et al*, (1993) also reported the same thing from tomato and okra while, Hellal and Mahfouz (2011) revealed synonymous finding from dill herb plant. The result that obtained in this work however, possessed additional information to that of (Oloyede *et al*,2012), who reported an increase in the percentage concentration of nutrients and antioxidants in pumpkin seeds significantly by mineral fertilizer level at a rate below 100kg/ha but, observed a depressed outcome with fertilizer rates above 100 kg/ha at ($p>0.05$). However, elements like Na, K, Mn, and Zn, recorded to be higher with F100 fertilizer though, the difference that exists among the fertilizers was found insignificant at ($p>0.05$), this is an indication that mineral fertilizer in calyx production is able to enhanced the nutrients quality optimally for the market value if other study will be conducted elsewhere. Beside, poultry manure could be substituted for economic reasons, according to Michael *et al*., (2010) and Ammanabo and Musa (2012) on vegetables quality, yield and production.

The results obtained for the minerals analysis were grossly adequate to meet the Recommended Daily Allowance (RDA) minimum requirement, except the essential element sodium concentration as compared to RDA value, and their content however, varied with an individual fertilizer used in the calyx (Table 2). The Na content in most of the fertilizers obtained were below the RDA of (500mg/day), it must however be noted that excessive consumption in Na increase calcium loss in urine and also contribute to hypertension in some people (Ilelaboye *et al*., 2013 and Wardlaw and Kessel, 2002). Therefore, the low level of Na in the yielded calyx makes it suitable for use in a Na restricted diets (FNB, 1997). The K concentration was found to be within a safe RDA value of 2000mg/day as set by the (RDA, 1989). Ca concentration from all the fertilizers studied, were obtained adequate to meet the Recommended Daily Allowance (RDA) of 800mg/day for adults (Table 2). However, the Ca concentration observed to be increases with an increase in the fertilizer concentration. Therefore, the Ca content obtained in the calyx could be suggested for vegetarians as the alternative dietary source to meet their needs for calcium (Mepba, *et al*., 2007). However, Mg was found to be increase with an increased in the level of fertilizers (Table 2). The concentrations of Mg recorded were above the RDA of 400mg/day for (men) 19-30years old and 310mg/day for (women) 19-30 years old (FNB, 1997), of all fertilizers used. The essential elements content implicated that calyx produce in this study is able to be used for the diet enrichment as per this nutrients are concern.

Table 3 however, revealed the trace elements nutrients compositions (Mn, Fe, Zn and Cu), where the Mn contents were recorded at optimum value for each fertilizer compared to the tolerable daily intake of 12mg/day, also within the permissible limit of 600mg/kg (FAO/WHO, 2001) for a healthy living.

This validates the Mn contents obtained in this study plant as a good source of Mn, where F100 fertilizer yielded the highest value. Manganese is crucial and very important to human physiology, which is also significant for haemoglobin formation. More so, Fe contents of all the fertilizers used in this study were found adequate as compared against RDA of 8mg Fe/day for (men 19 years and older), and then 18mg/day for (girl and women 11 to 56years) (FNB,2001). Moreover, the Fe concentration was observed to be increase with an increase in the fertilizer level, where the Food and Nutrition Board (NRC, 1980) recommends 10mg Fe/day for children between (1 to 10 years). Additionally, Zinc was also found to be adequate with all fertilizers in the calyx when compared to Zn RDA of 15mg/day for men and 12mg/day for women. Hence, the calyx consumption will correct zinc deficiency in diet, and the F100 fertilizer will be the best in the content yield. Copper was however, obtained below its RDA of 12mg/day (Dickson, *et al.*, 2012), but is still within the permissible limit of 20mg/Kg (WHO/FAO, 2001). This is an indication that the calyx fruit is safe and free from Cu toxicity, rather is a good source of dietary Cu which is an essential nutrient that can help in the development and progression of a number of disease states, including cardiovascular disease and diabetes if found deficient. Deficits of this nutrient during pregnancy can result in gross structural malformations in the foetus, and persistent neurological and immunological abnormalities in the offspring (Uriu-Adams and Keen, 2005). The poisonous Pb content observed to be at a higher concentration, which is evidence that the study site was too proximate to busy road that need to be considered in the cultivation of this plant.

Anti-nutrients composition

These are very important parameters in the dietary plant harvest and fertilization. Oxalate content was found to increase with an increase in mineral fertilizer level in the soil. It has a ranged from 8.867g to 9.418g/100g Table 4, in which M fertilizer had the least content and F100 was the highest, the difference that exist amongst fertilizers was found to be insignificant ($p>0.05$). On comparing the levels of an oxalate content obtained in this work to those of the green and variegated cultivars of sorrel (14.7 and 24.4g/100g) as Tuazon and Savage (2013) reported, the values were relatively higher to our finding and, were all exceeded the WHO tolerable limit of 0.256g/100g. In relation to that, Ilelaboye *et al.* (2013) reported a significant reduction of 31.26% to 49.24% in oxalate content by blanching and further reduction of 39.22% to 54.42% was achieved when the blanched vegetables were processed to vegetables' soups. This indicated that, raw sample of oxalate content in calyx can equally be removed by boiling process before been used as a diet.

The tannin content however recorded to be decrease with an increased in the fertilizer levels ranging between 4.190 to 5.230%, where F100 fertilizer had the least value and M was the highest. Tannin content also was below WHO permissible limit of 76-90g/kg (Alekor, 1995). Ilelaboye *et al*, 2013, also demonstrated that blanching and cooking significantly ($P>0.05$) reduced the tannin contents of green vegetables. The tannin content could however, be reduce in cooking by leaching for their being phenolic compounds and water soluble in nature (Kumar *et al.*, 1979 and Uzogara, *et al*,1990). This is an indication that, anti-nutrients factors obtained in this study plant (calyx) will have no any toxicity effect to its dietary value.

Table 1; Percentage proximate compositions and calorific value of calyx grown under mineral and organic fertilizer levels

Treatments/ Parameters	% Moisture	% Ash	%Crude protein	%Crude fat	%Crude fiber	%Carbohydrate	Calorific value (kcal/100g)
M	7.433± 0.131	6.965± 0.296	3.069± 0.320 **ab**	11.813± 0.644	10.280± 0.326	60.440± 1.177 **b**	309.302± 3.796 **b**
F75	7.524± 0.449	6.581± 0.054	2.986± 0.975 **b**	10.028± 2.337	11.157± 0.924	61.725± 3.375 **a**	321.023± 4.851 **a**
F100	8.032± 0.266	7.075± 1.749	3.335± 1.312 **a**	14.094± 2.272	10.967± 0.837	56.497± 1.591 **b**	301.622± 6.859 **b**

All data were mean ± standard deviation of triplicate determinations, Mean within a column of parameters followed by unlike letter(s) are significantly different using LSD at 5% level of significance. M= manure tonnes/ha, F75=75 Kg of fertilizer/ha and F100=100 Kg of fertilizer/ha.

Table 2; Essential elements concentrations of calyx grown under mineral and organic fertilizer levels

Treatment/ Elements	M(mg/Kg)	F75(mg/Kg)	F100(mg/Kg)	RDA
Na	118.356±0.252	102.342±0.265	142.433±0.100	500
Mg	3801.449±1.724**ab**	4569.806±5.148**a**	4927.246±3.077**a**	400
K	14964.375±0.361	13718.690±0.416	17492.187±0.815	2000
Ca	18457.987±22.742**b**	19146.253±10.204**b**	22252.735±4.299**a**	800

All data were mean ± standard deviation of triplicate determinations, Mean within a row of minerals followed by unlike letter(s) are significantly different using LSD at 5% level of significance. M; manure 10 tonnes/ha, F75; 75 Kg of fertilizer and F100; 100 Kg of fertilizer.

Table 3; Trace elements compositions of calyx grown under mineral and organic fertilizer levels

Sample/ Elements	M(mg/kg)	F75(mg/kg)	F100(mg/kg)	RDA
Mn	518.251±0.108	463.280±0.689	521.139±0.641	12
Fe	105.955±0.054 **b**	122.934±0.097 **b**	205.662±0.515 **a**	8
Zn	43.740±0.021	48.143±0.074	59.995±0.085	15
Pb	23.607±0.040	22.803±0.008	24.201±0.003	-
Cu	4.105±0.006 **b**	4.397±0.004 **ab**	5.738±0.010 **a**	10

All data were mean ± standard deviation of triplicate determinations, Mean within a row of elements followed by unlike letter(s) are significantly different using LSD at 5% level of significance. M;manure 10tonnes/ha, F75; 75 Kg of fertilizer and F100; 100 Kg of fertilizer.

Table 4; Anti-nutrients contents of calyx grown under mineral and organic fertilizer levels

Treatments/ Parameters	Oxalate (g/100g)	Tannin (g/100g)
M	8.867±1.422	5.230±3.012
F75	8.481±1.771	4.443±0.377
F100	9.428±1.714	4.190±0.346
WHO (permissible limit)	0.256	7.6 - 9

All data were mean ± standard deviation of triplicate determinations, that indicated Mean within a column of components are insignificantly different using LSD at 5% level of significance. M; manure 10tonnes/ha, F75;75 Kg of fertilizer and F100; 100 Kg of fertilizer.

CONCLUSION

The findings of this study indicated that, mineral fertilizer (F100) was the best fertilizer for protein, and the essential elements supplementation in calyx especially Mg and Ca, and then trace elements Fe and Cu, more so, the moisture content. Moreover, the mineral fertilizer (F75) was recorded excellent for energy sourcing nutrients (carbohydrate and calorific value), also crude fiber. Additionally, mineral fertilizer level had shown linear effect on nutrients like, Ca, Mn, Fe, Zn, Cu, protein and crude fat content, which relatively found increases with an increase of mineral fertilizer concentration. On this basis the nutrients that recorded significantly higher with a particular fertilizer in the calyx can be regarded the apt fertilizer for the optimum yield of that nutrients. Besides, the anti-nutrients contents noted down in this study were at non detrimental level, where the tannin content found to be decreases with an increase of mineral fertilizer concentration and are within the permissible limit of (7.6-9g), while oxalate contents were within the control level as can be remove by normal boiling process undergoes before used as diet. The Pb concentration can therefore be prevented if *Hibiscus sabdariffa* plant will be grown away from the road site.

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