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Effect of Nitrogen Fertilizer on the Levels of Some Nutrients, Anti-nutrients and Toxic Substances in *Hibiscus sabdariffa*

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ABSTRACT

The presence of antinutrients and toxic substances in vegetables limits the derivable benefits from vegetables. The levels of these substances in vegetables are influenced by the nature of soil in which the vegetables are grown. The effect of applied nitrogen fertilizer on the levels of some antinutrients and toxic substances is investigated with a view to determine the appropriateness or otherwise of the application of nitrogen fertilizer in growing vegetables. Pot experiments were conducted to determine the effect of soil nitrogen levels on soluble and total oxalates, cyanide, nitrate and some micronutrients namely, vitamin C, β-carotene (precursor of vitamin A) and mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in *Hibiscus sabdariffa*. The leaves of the vegetable were harvested and analysed at market maturity (vegetative phase) and fruiting (reproductive phase) of the plant development. Results obtained showed that the applied nitrogen fertilizer significantly elevated nitrate and β-carotene contents, while it decreases the levels of vitamin C, soluble and total oxalates in the vegetable. The levels of cyanide and mineral elements were not significantly affected by the applied nitrogen fertilizer.

Key words: *Hibiscus sabdariffa,* soil nitrogen levels, antinutrients, toxic substances, micronutrients, market maturity, fruiting

INTRODUCTION

Vegetables are rich sources of micronutirents, vitamins and minerals with leafy vegetables being the most important source of minerals (Lola, 2009). Roselle (*Hibiscus sabdariffa*) popularly called Yakuwa in Hausa belongs to the family of Malvaceae and is a popular vegetable in Indonesia, India, West Africa and many tropical regions (Fbabatunde, 2003; Tindal, 1986). The vegetable is widely grown in the North-eastern and middle belt regions of Nigeria (Akanya *et al.*, 1997). This plant has been found to thrive on a wide range of soil conditions. It can perform satisfactorily on relatively infertile soils but for economic purposes, a soil well supplied with organic materials and essential nutrients is important in the productions (Adanlawo and Ajibade, 2006; Tindal, 1986). It can tolerate relatively high temperature throughout the growing and fruiting periods. The plant requires an optimum rainfall of approximately 45-50 cm distributed over a 90-120 day growing period (Tindal, 1986). In Nigeria, two botanical varieties are recognised, the red variety in which the calyx is used for the preparation of sobo drink and the green variety which calyx and leaves are used in stew and sauces (Adanlawo and Ajibade, 2006; Duke, 1985;

Ojokoh, 2006). The leaves and calyx of the green variety are very rich in β-carotene, vitamin C and riboflavin with some major mineral elements (Babalola, 2000). Apart from the nutritional values, the plant is also of therapeutic value as extract of the calyx is used in the treatment of cardiovascular diseases and hypertension (Abo-Baker and Mostafa, 2011). Roselle like other leafy vegetables also contains appreciable levels of some antinutrients and toxic substance which have been shown to have negative effects on animal and human health at high concentrations (Ojokoh *et al*., 2002; Morton, 1987). For instance oxalate and phytate chelate minerals form complexes with proteins and thereby affects their nutrive value (Evans and Bandemer, 1967). Oxalate in combination with calcium form calcium oxalate which form precipitate in the kidney to form kidney stone (Aletor and Omodara, 1994; Osagie, 1998; Prohp *et al*., 2006). Higher levels of nitrate are responsible for methaemoglobineamia and cancer formation in human (Anjana *et al*., 2007). Cyanogenic glucosides also found in the vegetable are inhibitors of cytochrome oxidase enzyme and thereby acts as respiratory poison (Aletor, 1993).

The amount of nutrients, antinutrients and toxic substances in the vegetable beside other environmental factors, to a great extent depend on the soil nutrients content. Nitrogen and phosphorus are major limiting nutrients for plant growth and this explains the improvement in plant yield by external supply of these elements to soils deficient in them (Tena and Beyene, 2011). Thus this research was designed to study the effect of soil nitrogen levels as it affects the bioaccumulation of some nutrients (β-carotene, vitamin C, Fe, Cu, Mg, Zn, Ca, Na and K), antinutrients (soluble and total oxalates) and toxic substances (cyanide and nitrate) in *H. sabdariffa*.

MATERIALS AND METHODS

The study area: The pot experiment was carried out between 6th June and 18th December 2005 in the nursery of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State of Nigeria.

Niger state has a Savanna climate characterised by maritime air and rainfall is between April and October. During harmattan, dry desert wind blows between November and mid February while night temperature is very low. The geographical location of Minna is longitude 90°40' N and latitude 6° 30' E. Minna lies in the Southern Guinea Savanna zone of Nigeria and has a sub-humid semi arid tropical climate with mean annual precipitation of 1200 and 1300 mm. About 90% of total annual rainfall occurs between the months of June and September. Temperature rarely falls below 22°C with peaks of 40°C and 30°C in February/March and November/December, respectively. Wet season temperature average is about 29°C (Osunde and Alkassoum, 1998).

Soil sampling and analysis: The soil used in this study was collected from Minna. The soil has been classified as Inseptisol (FDALR, 1985). The bulked sample was collected during the drying season from the field which has been under fallows for about four years. The bulked soil sample was passed through 2 mm sieve. Sub-sample of the soil was subjected to routine soil analysis using the procedure described by Juo (1979). The soil particle sizes were analyzed using hydrometer method, pH was determined potentiometrically in water and 0.01 M $CaCl_2$ solution in a 1: 2 soil/liquid using a glass electrode pH meter and organic carbon by Walkey-Black method. Exchange acidity (E.A H^+ and Al^{3+}) was determined by titration method. Exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1 N NH_4OA solution. Sodium and potassium were

Table 1: Some physical and chemical properties of the soil (0-20 cm) used for pot experiment

Parameters	Values
Sand (%)	74.40
Sand (%)	74.40
Silt (%)	18.00
Clay (%)	7.60
pH (in H_2O)	6.51
pH (in 0.1 M C_aCI_2)	5.25
Organic carbon (%)	0.83
Organic matter (%)	1.43
Total nitrogen (%)	0.05
Available phosphorus (mg kg^{-1})	6.69
K (cmol kg^{-1})	0.92
Na (cmol kg^{-1})	0.68
Mg (cmol kg^{-1})	4.80
Ca (cmol kg^{-1})	8.00
E. A (H^+ +AL^{3+}) (cmol kg^{-1})	1.50
CEC (cmol kg^{-1})	15.90
Base saturation (%)	90.57
Texture class	Sandy loam

Values represent means of triplicate determinations

determined by flame emission spectrophotometry while Mg and Ca were determined by EDTA versenate titration method. Total nitrogen was estimated by Macro-Kjedal procedure and available phosphorus by Bray No. 1 method. The results of soil analyses are presented in Table 1.

Seeds: The seeds of roselle (*Hibiscus sabdariffa*) were obtained from School of Agriculture and Agricultural Technology's Farm/Nursery of Federal University of Technology, Minna.

Planting, experimental design and nursery management: About ten seeds of roselle were planted in a polythene bag filled with 10 kg of top soil. Following emergence, the seedlings were thinned to two plants per pot. The Complete Randomised Design (CRD) was adopted, using two treatments namely; two levels of soil fertility. Each treatment had 10 pots replicated three times. This gave a total of 60 pots. The seedlings were watered twice daily (mornings and evenings) using watering can and weeded regularly. The experimental area and the surroundings were kept clean to prevent harbouring of pest. The pots were lifted from time to time to prevent the roots of the plants from growing out of the container. Insects were controlled using Sherpa plus four weeks after planting at the rate of 100 mL per 100 L of water.

Fertilizer treatment: The fertilizer treatment for the vegetable was N fertilization at two levels. The first level was control (no N fertilization) and the second level was N fertilization at the recommended dose specific for the vegetable. Basal application of P_2O_2 and K_2O in form of single super phosphate and muriate of potash were applied at recommended rate, respectively. The details of the fertilizer treatments are as follow:

F_1 (control): 0 N, 40 mg P_2O_5 kg^{-1} soil and 40 mg K_2O kg^{-1} soil
F_2: 40 mg N kg^{-1} soil, 40 mg P_2O_5 kg^{-1} soil and 40 mg K_2O kg^{-1} soil

Plant tissues analysis: Both soluble and total oxalates content in the vegetable leaves were determined by titrimetric method of Oke (1966). The nitrate content in the test samples was determined by the colourimetric method as decribed by Sjoberg and Alanko (1994). Alkaline picrate method of Ikediobi *et al.* (1980) was used to analyse the cyanide content in the test samples. The mineral elements (Fe, Cu, Mg, Na and K) in samples were determined according to the method of Ezeonu *et al.* (2002) while ascorbic acid content in the samples was determined by 2, 6-dichlorophenol indophenols method of Jones and Hughes (1983). β-carotene content on the other hand was determined by ethanol and petroleum ether extraction method as described briefly. Two gram of Na_2SO_4 was added to 10.0 g of vegetable leaves and ground in mortar. The ground vegetables were extracted with 100 cm^3 of hot 95% ethanol for 30 min in hot water bath. The extract obtained was filtered and measured. Water was added to the extract to bring the percentage of the ethanol extract to 85%. The 85% ethanol extract was cooled in a cold water bath for some minutes. After cooling, the ethanol extract was put inside separating funnel and 30 cm^3 of petroleum ether was added and the mixture shaken. The separating funnel was clamped to the retort stand for some time to allow the solution to settle down into layers. The bottom layer containing ethanol was collected into the beaker while the top layer of the petroleum ether was stored in 250 cm^3 conical flask. The ethanol layer in the beaker was re-extracted twice with 10 cm^3 of petroleum ether. The ether layers of re-extraction was added to the original petroleum extract in the conical flask and re-extracted with 50 cm^3 of 85% ethanol in order to remove any xanthophylls which may be present. The top petroleum ether layer which contained β-carotene was collected, measured and the volume noted.

Lastly, the optical density (OD) of the final petroleum ether extract was determined at the wave length of 450 nm with spectrophotometer using petroleum ether as blank.

The concentration of β-carotene was calculated thus:

$$A = E^{\%} \times C \times L$$

Where:

A = Absorbance of the sample
$E^{\%}$ = Extinction coefficient of β-carotene
L = Path length (usually 1.0 cm)

Statistical analysis: T-test was used to determine the effect of soil fertility using two levels of nitrogen fertilizer on the level of the parameters under investigation.

RESULTS

Physical and chemical properties of soil: Result of analyses of the soil used for pot experiment is presented in Table 1. The texture class of the soil is sandy loam indicating that the water holding capacity is moderate. The organic matter content, total nitrogen and available phosphorus are low. Sodium and calcium contents are moderate while magnesium and potassium contents are high. The CEC (Cation Exchange Capacity) is moderate while base saturation percentage is high. Soil pH indicates that the soil is slightly acidic (FAO, 1984; Black, 1985; FDALR, 1985).

Effect of soil nitrogen levels on antinutrients and vitamins content: The determination of the effects of soil nitrogen levels on cyanide concentrations in *Hibiscus sabdariffa* showed that the applied nitrogen fertilizer had no significant effect on cyanide content of the studied vegetable irrespective of the stage of plant development. The mean values for controls at market maturity (459.60±21.00 mg kg^{-1}) and fruiting (390.20±32.00 mg kg^{-1}) were not significantly different from the values (419.50±21.00 and 410.60±26.00 mg kg^{-1}, respectively) for vegetables grown on nitrogen fertilized soil (Table 2).

The mean nitrate contents in the vegetable grown on nitrogen treated soils at market maturity (101.90±26.00 mg kg^{-1}) and (344.40±29.00 mg kg^{-1}) were significantly higher when compared to the level of controls (85.00±28.00 and 285.20±23.00 mg kg^{-1}, respectively) as shown in Table 2.

The soluble oxalate concentrations of control and nitrogen fertilized *Hibiscus sabdariffa* at market maturity were 1.62±0.05 g/100 g and 1.37±0.05 g/100 g while at fruiting the values obtained were 2.93±0.15 g/100 g and 1.77±0.07 g/100 g. The results showed that the application of nitrogen fertilizer significantly ($p<0.05$) decreased the soluble oxalate content of the vegetable irrespective of stage of plant development (Table 2). Similarly the applied nitrogen fertilizer significantly ($p<0.05$) decreased the total oxalate content of the vegetable in both stages of plant development. The mean values of the antinutrient recorded in the control and test *Hibiscus sabdariffa* at market maturity were 2.08±0.07 g/100 g and 1.92±0.04 g kg^{-1} while the corresponding values recorded at fruiting were 4.04±0.27 g/100 g and 3.22±0.20 g/100 g, respectively (Table 2).

The mean **β**-carotene contents of control and test plants at market maturity were 5405.00±433.00 and 7067.00±266.00 μg/100 g while at fruiting the values obtained were 6123.00±379.00 and 6481.00±406.00 μg/100 g. Data analysis showed that with application of nitrogen fertilizer there is a significant elevation ($p<0.05$) in the provitamin content at market maturity while no significant variation was recorded at fruiting (Table 2).

The determination of vitamin C content in *Hibiscus sabdariffa* showed that the applied nitrogen fertilizer significantly decreased the vitamin content at both stages of plant development. The mean values recorded in the test and control vegetable at market maturity were 12.51±1.10 mg/100 and 15.39±1.30 mg kg^{-1} while at fruiting the values recorded were 13.08±0.77 mg/100 g and 16.08±0.82 mg/100 g, respectively (Table 2).

Table 2: Effect of soil nitrogen levels on antinutrients and vitamins content in *Hibiscus sabdariffa*

Anti nutrients and vitamins analysed at market maturity and fruiting stages	Nitrogen levels	
	Control (No nitrogen applied)	Nitrogen applied
Cyanide at market maturity (mg kg^{-1} DW)	459.60±21.00^{a}	419.50±21.00^{a}
Cyanide at fruiting (mg kg^{-1} DW)	390.20±32.00^{a}	410.60±26.00^{a}
Nitrate at market maturity (mg kg^{-1} DW)	85.00±28.00^{a}	101.90±26.00^{b}
Nitrate at fruiting (mg kg^{-1} DW)	285.20±23.00^{a}	344.40±29.00^{b}
Soluble oxalate at market maturity (g/100 g DW)	1.62±0.04^{b}	1.37±0.05^{a}
Soluble oxalate at fruiting (g/100 g DW)	2.93±0.15^{b}	1.77±0.15^{a}
Total oxalate at market maturity (g/100 g DW)	2.08±0.07^{b}	1.92±0.04^{a}
Total oxalate at fruiting (g/100 g DW)	4.04±0.27^{b}	3.22±0.20^{a}
β-carotene at market maturity (μg/100 g FW)	5405.00±433.00^{a}	7067.00±266.00^{b}
β-carotene at fruiting (μg/100 g FW)	6123.00±379.00^{a}	6481.00±406.00^{a}
Vitamin C at market maturity (mg/100 g FW)	15.39±1.30^{b}	12.51±1.10^{a}
Vitamin C at fruiting (mg/100 g FW)	16.08±0.82^{b}	13.08±0.77^{a}

DW = Dry weight, FW = Fresh weight. Values represent means of nine determinations. Mean values carrying the same superscripts within a row do not differ significantly from each other ($p>0.05$)

Table 3: Effect of soil nitrogen levels on mineral content in *Hibiscus sabdariffa*

Mineral analysed at market maturity and fruiting stages	Nitrogen levels	
	Control (No nitrogen applied)	Nitrogen applied
Fe at market maturity (mg kg^{-1})	33.17±1.60^a	31.77±2.90^a
Fe at frniting (mg kg^{-1})	28.44±1.50^a	35.20±3.50^a
Mg at market maturity (mg kg^{-1})	18.78±1.80^a	20.79±1.10^a
Mg at heading (mg kg^{-1})	17.99±0.26^a	17.19±0.37^a
Zn at market maturity (mg kg^{-1})	0.04±0.01^a	0.03±0.01^a
Zn at fruiting (mg kg^{-1})	0.03±0.01^a	0.03±0.01^a
Cu at market maturity (mg kg^{-1})	2.07±0.36a	2.89±0.47^a
Cu at fruiting (mg kg^{-1})	2.28±0.37^a	1.88±0.25^a
Ca at market maturity (mg kg^{-1})	24.14±0.98^a	24.81±0.86^a
Ca at fruiting (mg kg^{-1})	24.31±1.00^a	22.52±1.30^a
Na at market maturity (mg kg^{-1})	2.80±0.18^a	3.07±0.14^a
Na at fruiting (mg kg^{-1})	3.17±0.14^a	2.83±0.12^a
K at market maturity (mg kg^{-1})	39.18±2.50^a	38.65±0.94^a
K at frniting (mg kg^{-1})	36.01±2.90^a	42.33±2.80^a

Values represent means of tripple determinations. Mean values carrying the same superscripts within a row do not differ significantly from each other ($p>0.05$)

Effect of soil nitrogen levels on mineral elements content: Analysis of Fe Mg, Zn, Cu, Ca, Na and K were conducted in control and in *Hibiscus sabdariffa* grown on soil treated with nitrogen with a view of determining the effect soil nitrogen levels on the mineral contents of the vegetable. The results obtained showed that the applied nitrogen fertilizer had no significant effect on the mineral contents of the vegetable irrespective of the stage of plant development (Table 3).

DISCUSSION

The insignificant effect of nitrogen fertilizer on cyanide and nitrate content in *Hibiscus sabdariffa* is at variance with the report of Richard (1991), Chweya (1993), Peter and Birger (2002), Mozolewski and Smoczynski (2004), Anjana *et al.* (2007), Boroujerdnia *et al.* (2007) and Wobeto *et al.* (2007). These authors reported that application of nitrogen fertilizer to the soil elevated the nitrate and cyanide content of the plants that were grown on them. Worthington (2001) stressed that plants require nitrogen for normal growth and for the synthesis of proteins. Nitrogen is one of the major elements that are essential for plant growth and development (Mostafa and Abo-Baker, 2010; Suriharn *et al.*, 2011; Undie *et al.*, 2012). However, if nitrogen is applied in excess of what the plant requires for protein production, the excess is accumulated as nitrates and stored predominantly in the green leafy part of the plant. Thus the insignificant difference in the nitrate content recorded between nitrogen applied *Hibiscus sabdariffa* and the control, may infer that, the amount of nitrogen supplied to the vegetable is adequate for optimum utilization for normal growth and protein formation or that the control has enough nitrogen for normal growth and protein formation for the particular species.

Similarly, Peter and Birger (2002) further stated that increase in cyanide content following nitrogen fertilization is because the applied nitrogen stimulates the enzymatic conversion of tyrosine to p-hydroxymandelonitrile which ultimately lead to increase in the biosynthesis of cyanogenic glycoside. It therefore, follows that the variation of this result from the findings of these authors may suggest that the bioaccumulation of cyanide in response to the applied nitrogen varies from one cultivar to another.

Significant higher β-carotene content obtained in response to the applied nitrogen fertilizer than in controls in the vegetable is in line with the observations of Chweya (1993) and Kansal *et al.* (2005) that nitrogen fertilizer increased the provitamin content of the vegetable. The reason for this increase may be probably due to elevation in the content and activity of chlorophyll and associated light absorbing pigments (including carotenoids) following the application of nitrogen fertilizer (Taiz and Zeiger, 2002; Havling *et al.*, 2006).

The generally lower levels of vitamin C in the vegetables, soluble and total oxalates in *Hibiscus sabdariffa* grown in soil supplied with nitrogen fertilizer compared with the control indicate that the applied nitrogen fertilizer significantly reduced the levels of these compounds in the vegetables (Chweya, 1993; Worthington, 2001; Mozafar, 2005; Singh, 2005). Worthington (2001) further stressed that the observed decrease in vitamin C content resulted from the increase in protein production and decrease in carbohydrate production following the application of nitrogen fertilizer. Because vitamin C is formed from carbohydrates, its synthesis is also reduced. Singh(2005) stated that not only nitrogen fertilizers decrease the oxalate content of the vegetables but anions generally reduce the levels of the antinutrients since they compete with oxalate for cations and depress the oxalate synthesis. The decrease in oxalate concentration in the vegetables following nitrogen fertilization may be attributed to decreasing effect of nitrogen on vitamin C content, since oxalates are synthesised via vitamin C (Streeter, 2005.). This present results also revealed that nitrogen fertilizer had no significant effect on the studied mineral elements (Fe, Mg, Zn, Ca, Cu, Na and K). It may be possible to increase the levels of these minerals in the vegetable through integrated nutrient management using appropriate combinations of organic and inorganic fertilizers as this is known to increase nutrient yield (Abd El-Lattief, 2011; Singh *et al.*, 2011).

CONCLUSION

Even though nitrate and β-carotene contents in the vegetable are elevated with nitrogen fertilizer, the nitrate contents in the test vegetable are still within the tolerable levels. The β-carotene contents in the controls are high enough to meet the adult recommended daily allowance of 900 μg of vitamin A. The level of vitamin C decreased significantly with nitrogen fertilizer, however, the vitamin content in both control and nitrogen treated vegetable are still lower than adult recommended dietary allowance of 60 mg. Thus in either case, supplementation of the vitamin from other sources is required. However, the significantly higher soluble and total oxalates content in controls compared with nitrogen treated vegetable may encourage the application of this recommended nitrogen fertilizer when growing *Hibiscus sabdariffa* on this soil type. This practice will reduce oxalate toxicosis associated with high level of oxalates in the food.

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