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Research Article

INFLUENCE OF APPLIED NITROGEN FERTILIZER ON THE BIOACCUMULATION OF MICRONUTRIENTS, ANTI-NUTRIENTS AND TOXIC SUBSTANCES IN *TELFAIRIA OCCIDENTALIS* (FLUTED PUMPKIN).

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ABSTRACT: The chemical contents and compositions of plants are influenced by the physical and chemical properties of the soils on which they were grown. It is on this grounds that pot experiment was carried out to investigate the effect of soil nitrogen levels on antinutrients (soluble and total oxalates), toxic substances (cyanide and 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 *Telfairia occidentalis*. The leaves of *Telfairia occidentalis* were harvested at market maturity (vegetative phase) and fruiting (reproductive phase) and they were subjected to chemical analysis. Results obtained showed that the applied nitrogen fertilizer significantly ($p < 0.05$) increased the cyanide and nitrate concentrations in *Telfairia occidentalis* in both phases of plant development. The soluble and total oxalate concentrations in the vegetable were not signinificantly affected by the applied nitrogen in both stages of its development. Similarly, the increasing effect of nitrogen fertilizer on β-carotene content in *Telfairia occidentalis* was not significant at vegetative phase, but its concentration was increased significantly ($p < 0.05$) at reproductive phase. Vitamin C concentration in *Telfairia occidentalis* was reduced significantly ($p < 0.05$) with the applied nitrogen fertilizer irrespective of the phase of its development. The concentrations of Mg, Ca, Na and K were not signicantly affected by the applied nitrogen fertilizer, however, the levels of Zn and Cu in *Telfairia occidentalis* were increased significantly at market maturity and fruiting, respectively.While the concentration of Fe in the vegetable increased significantly at both stages of plant development. The result concludes that the application of nitrogen fertilizer for the cultivation of *Telfairia occidentalis* increases the concentration of plant totoxins and reduces the nutritional quality of the vegetable.
Keywords: *Telfairia occidentalis*, soil nitrogen levels, oxalates, nitrate, cyanide, micronutrients.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*) popularly called “ugwu” by Igbos, is a creeping vegetative shrub that spread low across the ground with large lobed leave, and long twisting tendrils (Syndenham, 1985; Horsfall and Spiff, 2005; Christian, 2006; Ojiako and Igwe, 2008). Nkang *et al.* (2003) reported that this vegetable is of commercial importance grown across the low land humid tropic in West Africa (Nigeria, Ghana and Sierra Leone being the major producers). Fluted pumpkin prefers a loose, friable ample humus and shaded position. Nitrogen is essential for adequate vegetation and should ideally be given in the form of manure (Schippers, 2000). The leaves and seeds are widely eaten as they are good sources of minerals (potassium, magnesium, sodium, phosphorus and iron), β-carotene, vitamins, fibres, fats (Schipper, 2000; Nkang *et al*, 2003; Christian, 2006; Ogbadoyi *et al.*, 2011).

Harvesting of fluted pumpkin takes place 120 - 150 days after planting. The seed contains 13% of oil (Okoli and Nyanayo, 1988) and is used for cooking (Horsefall and Spiff, 2005).

Telfairia occidentalis like other leafy vegetables also contains appreciable levels of some antinutrients and toxic substance which has been shown to have negative effect on animal and human health at high concentrations (Morton, 1987; Ojokoh *et al*., 2002; Ogbadoyi *et al*., 2011; Musa *et al.*, 2011). Ekpedema *et al.* (2000) reported the presence of antinutritional factors (oxalate and phytate) and other toxic substances in the leaves and seeds of *Telfairia occidentalis*.
Various cultural practices like optimum N, P and K fertilizer levels required for adequate vegetative growth and yield in *Telfairia occidentalis* has been established. However, the effect of these optimal fertilizer levels on the nutrients, antinutrients and toxic substances has not been determined. The present study is therefore, designed to investigate the influence of soil fertility in terms of N levels on the accumulation of some micronutrients and phytotoxins in the leaves of *Telfairia occidentalis*.

MATERIALS AND METHODS

The study area

The pot experiment was conducted in the nursery of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State of Nigeria.
Niger State has a Savannah climate characterized by maritime. The geographical location of Minna is at longitude 9º 40'N and latitude 6º 30'E. Minna lies in the Southern Guinea Savannah zone of Nigeria and has a sub - humid semi arid tropical climate. The raining season is between April and October. About 90% of the total rainfall occurs between the month of June and September. The mean annual rainfall is in the range of 1200 – 1300 mm. The temperature of this zone rarely falls below 22ºC with peaks temperature of 40ºC in February /March and 30ºC in November /December. Wet season average temperature is about 29^{0}C. The Dry season occurs between November and March while harmattan which is chacterised by dry air is between November and February (Osunde and Alkassoun, 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 dry 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 procedure described by Juo (1979). The soil particle sizes were analyzed using hydrometer method; pH was determined potentiometrically in the water and 0.01M $CaCl_2$ solution in a 1: 2 soil/ liquid using a glass electrode pH meter and organic carbon by Walkey-Black method (Juo, 1979). Exchange acidity (E.A H^+ and Al^{3+}) was determined by titration method (Juo, 1979). Exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1N NH_4OA solution. Sodium and potassium were determined by flame emission spectrophotometry while Mg and Ca were determined by E.D.T.A versenate titration method (Juo, 1979). Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method (Juo, 1979).

Sources of Seeds

The seeds of fluted pumpkin *(Telfairia occidentalis)* were obtained from Schools of Agriculture and Agricultural Technology's Nursery of Federal University of Technology, Minna.

Planting, experimental design and nursery management

Exactly two seeds of *Telfairia occidentalis* were planted in a polythene bag filled with 20.00 kg of top soil and after germination the seedlings were thinned to one plant per pot. The factorial design was adopted to determine the effect nitrogen fertilizer at vegetative and reproductive phases in *Telfairia occidentalis*.
Each treatment had 10 pots replicated three times. This gave a total of 60 pots for *Telfairia occidentalis*.The seedlings were watered twice daily (morning and evening) 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 (Saro Agro Sciences) four weeks after planting at the rate of 5 ml per 5 litres of water.

Fertilizer treatment

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. Basal application of of P_2O_5 and K_2O in the form of single super phosphate and murate of potash was done. The details of fertilizer treatments are as follow:

F_1 (control): 0N, 30mg P_2O_5/kg soil and 22mg K_2O/kg soil
F_2: 30mgN/kg soil, 30mg P_2O_5/kg soil and 22mg K_2O/kg soil

Harvesting

The leaves of *Telfairia occidentalis* grown in pot experiment in control and nitrogen treated soil were harvested at market maturity and fruiting.

Sample analysis

The soluble and total oxalates concentration in the leaves of *Telfairia occidentalis* were determined by titrimetric method of Oke (1966). Nitrate concentration in the samples was determined by the colourimetric method (Sjoberg and Alanka, 1994). Alkaline picrate method was used to analyse the cyanide concentration (Ikediobi *et al*., 1980). The mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in samples were determined according to the method of Ezeonu *et al*. (2002). The ascorbic acid concentration was determined by 2, 6-dichlorophenol indophenols method (Eleri and Hughes, 1983) while the estimation of β-carotene was done by ethanol and petroleum ether extraction method (Musa *et al*., 2010).

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 analysis 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 nitrogen fertilizer on antinutrients and vitamins content

The results obtained from the determination of cyanide concentration in *Telfairia occidentalis* indicated that the applied nitrogen fertilizer significantly ($p < 0.05$) increased the cyanide concentration in the vegetable irrespective of the stage of plant development. The mean values of cyanide in control and nitrogen fertilized *Telfairia occidentalis* at vegetative phase were 438.00 ± 37.00 mg/kg and 699.00 ± 48.00 mg/kg while the values obtained at reproductive phase were 771.00 ± 21.00 mg/kg and 885.00 ± 37.00 mg/kg (Table 2).

The applied nitrogen fertilizer also increased nitrate concentration significantly ($p < 0.05$) in *Telfairia occidentalis* at vegetative and reproductive phases. The mean values of nitrate in the control and nitrogen applied vegetable at market maturity were 550.00 ± 95.00 mg/kg and 696.00 ± 117.00 mg/kg while the corresponding values obtained at fruiting were 34.90.00 ± 5.1.00 mg/kg and 45.10 ± 15.00 mg/kg (Table 2).

Application of nitrogen fertilizer had no significant effect ($p>0.05$) on soluble and total oxalate concentrations at both stages of the plant development. (Table 2).

The applied nitrogen fertilizer significantly ($p < 0.05$) elevated the β-carotene concentration in *Telfairia occidentalis* at market maturity and had no significant ($p > 0.05$) effect on its concentration at fruiting. The amount of β-carotene concentration in control and nitrogen treated *Telfairia occidentalis* at market maturity were 15501.00 ± 591.00 µg/100 g and 17600 ± 1009 µg/100 g while the corresponding values at fruiting were 10381.00 ± 395.79 g/100g and 12150.00 ± 276.00 µg/100 g (Table 2).

The vitamin C concentration in control and nitrogen applied *Telfairia occidentalis* at market maturity were 208.40 ± 7.50 mg/100 g and 191.60 ± 13.00 mg/100g while the values obtained at fruiting were 224.60 ± 11.00 mg/100 g and 186.30 ±11.00 mg/100 g. The results showed that the application of nitrogen fertilizer significantly decreased ($p < 0.05$) the vitamin C concentration in *Telfairia occidentalis* at both stages of plant development (Table 2).

Effect of nitrogen fertilizer on mineral elements content

The application of nitrogen fertilizer significantly ($p < 0.05$) increased Fe concentration in *Telfairia occidentalis* irrespective of the phase of its development. The mean value of Fe concentrations in nitrogen applied vegetable at market maturity (13.63 ± 1.30 mg/kg) and fruiting (28.19 ± 2.40 mg/kg) were significantly higher when compared with the values of controls (10.76 ± 0.92 mg/kg and 17.84 ± 2.40 mg/kg respectively), (Table 3).

Table 1: Some Physical and Chemical Properties of the Soil (0 – 20cm) Used for Pot Experiment

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

*Values represent means of triplicate determinations.

The applied nitrogen fertilizer had no significant effect on Mg, Ca, Na and K concentrations of the vegetable at both stages of plant development (Table 3).

There was a significant ($p < 0.05$) increase in Zn concentration at market maturity with nitrogen fertilization while no significant variation was observed at fruiting (Table 3).

Table 2: Effect nitrogen fertilizer on antinutrients and vitamins content in *Telfairia occidentalis*

Antinutrients and vitamins analysed at market maturity and fruiting stage	Nitrogen levels	
	Control (No. nitrogen applied)	Nitrogen applied
Cyanide at market maturity (mg/kg DW)	438.00 ± 37.00[a]	699.00 ± 48.00[b]
Cyanide at fruiting (mg/kg DW)	771.00 ± 21.00[a]	885.00 ± 37.00[b]
Nitrate at market maturity (mg/kg DW)	550.00 ± 95.00[a]	696.00 ± 117.00[b]
Nitrate at fruiting (mg/kg DW)	34.90 ± 5.10[a]	45.90 ± 15.00[b]
Soluble at market maturity (g/100 g DW)	1.64 ± 0.06a	1.82 ± 0.14[a]
Soluble at fruiting (g/100 g DW)	1.65 ± 0.09a	2.03 ± 0.06[a]
Total oxalate at market maturity (g/100 g DW)	2.18 ± 0.07a	2.01 ± 0.16a
Total oxalate at fruiting (g/100 g DW)	3.20 ± 0.09a	2.82 ± 0.04a
β-carotene at market maturity (µg/100 g FW)	15501.00 ± 591.00a	17600.30 ± 100.00b
β-carotene at fruiting (µg/100 g FW)	10381.00 ± 395.79a	12150.00 ± 276.00a
Vitamin C at market maturity (mg/100 g FW)	208.40 ± 7.50b	191.60 ± 13.00a
Vitamin C at fruiting (mg/100 g FW)	224.60 ± 11.00b	186.30 ± 11.00a

DW = Dry weight, FW = Fresh weight, values represent means of nine determinations. Row mean values carrying the same superscripts do not differ significantly from each other ($P > 0.05$).

Application of nitrogen fertilizer had no significant effect on the Cu concentration in *Telfairia occidentalis* at market maturity, however, at fruiting, the concentration of the mineral element increased significantly ($p < 0.05$). The mean values in the control and nitrogen treated vegetable at market maturity were 3.67 ± 0.12 mg/kg and 2.66 ± 0.53 mg/kg while at fruiting the values obtained were 1.30 ± 0.51 mg/kg and 8.78 ± 0.72 mg/kg (Table 3)

.Table 3: Effect of nitrogen fertilizer on mineral contents in *Telfairia occidentalis*

Mineral analysed at market maturity and fruiting stages.	Nitrogen levels	
	Control (No nitrogen applied)	Nitrogen applied
Fe at market maturity (mg/kg)	10.76 ± 0.92[a]	13.63 ± 1.30[b]
Fe at fruiting (mg/kg)	17.84 ± 2.40[a]	28.19 ± 2.40[b]
Mg at market maturity (mg/kg)	19.90 ± 1.00[a]	20.34 ± 0.80a
Mg at fruiting (mg/kg)	23.13 ± 2.24[a]	23.59 ± 3.06a
Zn at market maturity (mg/kg)	0.03 ± 0.01a	0.05 ± 0.01b
Zn at fruiting (mg/kg)	0.01 ± 0.01a	0.02 ± 0.01a
Cu at market maturity (mg/kg)	3.67 ± 0.12a	2.66 ± 0.53a
Cu at fruiting (mg/kg)	1.30 ± 0.51a	8.78 ± 0.72b
Ca at market maturity (mg/kg)	17.76 ± 3.20a	16.27 ± 2.40a
Ca at fruiting (mg/kg)	16.27 ± 2.00a	14.95 ± 1.40a
Na at market maturity (mg/kg)	5.40 ± 0.54a	5.21 ± 0.16a
Na at fruiting (mg/kg)	4.59 ± 0.38a	3.71 ± 0.29a
K at market maturity (mg/kg)	117.40 ± 3.40a	112.90 ± 7.40a
K at fruiting (mg/kg)	62.47 ± 3.00a	67.40 ± 3.80a

Values represent means of nine determinations. Row mean values carrying the same superscripts do not differ significantly from each other ($P > 0.05$).

DISCUSSION

The significantly higher cyanide, nitrate and β-carotene concentrations in the *Telfairia occidentalis* grown on soil treated with nitrogen compared with the control is in accordance with the report of the following authors (Kriedenman, 1964; Jones and Ford, 1972; Richard, 1991; Yang, 1992; Chweya, 1993; Oladele *et al*., 1997; Muramoto, 1999; Peter and Birger 2002; Waclaw and Stefan, 2004; Kansal *et al*. (2005); Carmen *et al*., 2007; Anjana and Muhammad, 2006; Anjana *et al*., 2007; Rolinda and Ma, 2008; Musa *et al*., 2010; Musa *et al*., 2011). Virginia (2001) stressed that plants require nitrogen for normal growth and protein synthesis however, if nitrogen is applied in excess of what the plant requires for protein formation, the excess is accumulated as nitrate and stored predominantly in the green leafy part of the plant. Peter and Birger (2002) stated that the increased in cyanide cocentration following the application of nitrogen fertilizer is that the applied nitrogen stimulates the enzymatic conversion of tyrosine to p - hydroxymandelonitrile which ultimately lead to increase in the biosynthesis of cyanogenic glycoside. The higher concentration of these phytotoxins (cyanide and nitrate) is enough warning signinal why we must take adequate precauctions when growing our vegetables with synthetic fertilizers because of the negative effect of these plant toxins in our health. For instance, cyanide is a respiratory poison while nitrate is responsible for cancer and methaemoglobinemia in human and other animals at higher concentrations. This study thus suggest that the attendant health problems associated with high levels of cyanide and nitrate can be reduced or minimised by careful application of nitrogen fertilizer at levels much lower the current dose or possible its use is completely avoided (Musa *et al*., 2010; Musa *et el*. 2011).

Similarly, the in increase in β-carotene may be probably due to elevation in the content and activity of chlorophyll and associated light absorbing pigments (including caroteniods) following the application of nitrogen fertilizer (Taiz and Zeiger, 2002; Havling *et al*., 2006). Though the concentration of the provitamin A that helps to maintain good sight and act as a powerful antioxidant is increased in *Telfairia occidentalis* with application of synthetic nitrogen fertilizer, its concentration in controls can provide enough of vitamin A to meet the adult remmended daily allowance of 900 µg.

Thus this increase in β-carotene alone is not a good reason to encourage the application of nitrogen fertilizer for the cultivation of this vegetable, bearing in mind the attendant health problems associated with high concentrations of cyanide and nitrate.
The insignificant effect of nitrogen fertilizer on soluble and total oxalates concentration in *Telfairia occidentalis* observed in this study is invariance with the findings of Chweya (1993) and Singh (2005). These authors reported that nitrogen fertilizers decreased the oxalate content in *Cleome gynandra* and *Chemopodium amaranthicolor*, respectively. Singh (2005) attributed the decrease due to the fact that nitrogen and anions generally reduced the levels of the antinutrients since they compete with oxalate for cations and depressed the oxalate synthesis. However, the observed variation in this work from those of the previous works could suggest that the effect of nitrogen fertilizer on oxalate concentration may vary from cultivar to cultivars and other environmental factors. However, this variation is subject to further study.
The significantly lower concentration of vitamin C in *Telfairia occidentalis* following the application of inorganic nitrogen fertilizer is in harmony with the finding of the following researchers (Chweya, 1993; Virginia, 2001; Mozafar, 2005; Musa *et al*., 2010; Musa *et al*., 2011) to the effect that the application of nitrogen fertilizer significantly reduced the leaf vitamin C content in the vegetables. Virginia (2001) further stressed that the observed decreased in vitamin C concentration results from the increase in protein production and decrease in carbohydrate production following the application of nitrogen fertilizer. Since vitamin C is formed from carbohydrates, its synthesis also reduces. Consindering the pivotal of this water soluble vitamin in human health, application nitrogen fertilizer in growing *Telfairia occidentalis* for human consumption as leafy vegetable should not be encouraged as this practice decrease the vitamin concentration significantly. Thus comsumers may be denied of the full derivable nutritional benefits from the vegetable.

The significantly higher levels of Fe, Zn, and Cu in nitrogen applied *Telfairia occidentalis* agreed with the findings of other workers. Ojeniyi and Adeniyan (1999), Tarfa *et al*. (2001), Kansal *et al.* (2005), Safaa and Abd El Fattah (2007) reported that nitrogen fertilizer increased the mineral content in maize leaf, *Spinacea oleracea* and lettuce plants respectively. Safaa and Abd El Fattah (2007) attributed the increase in the mineral content (especially Fe) to increase in the levels of chlorophyll following the application of nitrogen fertilizer. The observations by these authors however, disagreed with the results of Chweya (1993) who reported that application of nitrogen fertilizer to *Gynandropsis gynandra* decreases the Fe content of the vegetable and has no significant effect on the Ca and Na content. The observed variations in the mineral content in this vegetable from the report of other authors following the application of nitrogen fertilizer could be as a result of differences in cultivars and environmental factors (Chweya and Nameus, 1997; Grazyna and Waldemar, 1999; Bolanle *et al*., 2004; Singh, 2005; Aliyu and Morufu, 2006; Lisiewska *et al*., 2006; Rickman *et al*., 2007). This increased concentration of Zn and Cu in the *Telfairia occidentalis* treated with nitrogen fertilizer has also no much of nutritional advantage of the minerals than the controls. This is because concentration of these mineral elements in *Telfairia occidentalis* in both soil nitrogen levels were lower than the recommended daily allowance of 10 – 15 mg/kg and 1.3 – 3.0 mg/kg for Zn and Cu respectively, (Titz et al., 1994) if 100g of the vegetable were consumed. Thus considering important roles of these mineral elements in metabolism, majorly as cofactors for many enzymatic reactions, supplementation of the elements from other sources in either case will be apparently necessary. Even with the Fe that the concentration increased significantly with applied nitrogen fertilizer, at both market maturity and fruiting, it was only at fruiting that the concentration of Fe can meet the recommended daily allowance of 18 mg/day (Titz et al., 1994). However, Musa *et al*. 2011 had reported unsafe high levels of some phytotoxins in *Telfairia occidentalis* at reproductive phase and adviced the consumption of the vegetable at vegetative phase rather than at reproductive phase.

CONCLUSSION

This study concludes that growing of *Telfairia occidentalis* with application of nitrogen fertilizer increase the concentrations of phytotoxins (particularly cyanide and nitrate) and reduce the nutritional quality of the vegetable. The study therefore suggest that application of nitrogen fertilizer for growing *Telfairia occidentalis* should be avoided, however, where this practice is inevitable it should be done with cautions because of the health problems associated with high ingestion of these plant toxins.

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