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Full Length Research Paper

Effect of fruiting on micronutrients, antinutrients and toxic substances in *Telfairia occidentalis* grown in Minna, Niger State, Nigeria

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A pot experiment was conducted to determine the effect of fruiting on antinutrients (soluble and total oxalates), toxic substances (cyanide and nitrate) and some micronutrients namely: vitamin C, β-carotene (provitamin A) and mineral elements [(sodium (Na), iron (Fe), magnesium (Mg), copper (Cu), Zinc (Zn), calcium (Ca) potassium (K)] in *Telfairia occidentalis* grown in nitrogen and non-nitrogen treated soil. Vegetable leaves were harvested at both market maturity (vegetative phase) and fruiting (reproductive phase), and were subjected to chemical analysis. Results showed that the cyanide and total oxalate concentrations were significantly higher at fruiting stage of vegetables grown on both control and nitrogen applied soil, while the nitrate and β-carotene concentration in *T. occidentalis* were significantly reduced irrespective of the soil nitrogen levels. Fruiting however, had no significant effect on soluble oxalate and vitamin C concentration in *T. occidentalis* grown under control and nitrogen treatment conditiond. The results also revealed that while Fe and Mg contents were increased, K and Cu content decreased significantly with fruiting in both control and nitrogen fertilization treatment. Similarly, the Na content in the vegetable was decreased significantly only when nitrogen fertilizer was used. The levels of Zn and Ca were not affected by fruiting. We conclude that consumption of the leaves of *T. occidentalis* at vegetative phase (market maturity) reduces the levels of most of the plant toxins and still retain most of the micronutrients in an amount to meet nutritional requirements.

Key words: *Telfairia occidentalis,* anti-nutrients, toxic substances, micronutrients, market maturity and fruiting, soil nitrogen levels.

INTRODUCTION

Telfairia occidentalis, commonly known as fluted pumpkin, is a creeping vegetative shrub that spreads low on the ground with large lobed leave and long twisting tendrils (Horsfall and Spill, 2005; Christian, 2006; Ojiako and Igwe, 2008). The vegetable prefers to grow on loose, friable a humus-rich land under shade. Nitrogen is essential for adequate vegetation and manure is the ideal form of organic nitrogen fertilizer (Schippers, 2000). Harvesting of fluted pumpkin takes place 120 - 150 days after planting. The vegetable is of commercial importance grown across the low land humid tropic in West Africa with Nigeria, Ghana and Sierra Leone being the major producers (Nkang et al., 2003). Leaves and seeds of *T. occidentalis* are widely comsumed, providing a good source of minerals (potassium, magnesium, sodium, phosphorus and iron), vitamins, fibres, fats (Schipper, 2000; Nkang et al., 2003; Christian, 2006). Seeds contain

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Abbreviation: ADI, acceptable daily intake.

13% of oil (Okoli and Nyanayo, 1988) and is used for cooking (Horsefall and Spiff, 2005).

T. occidentalis like other leafy vegetables also contains considerable levels of certain antinutrients and toxic substances which have been shown to have negative effect on animal and human health at high concentrations (Morton, 1987; Ekpedema et al., 2000; Ojokoh et al., 2002). For instance, oxalate, in addition to the formation of kidney stone, it also reduces the bioavailability of mineral elements (Nakata, 2003; Shingeru et al., 2003; Okon and Akpanyung, 2005; Antia et al., 2006; Proph et al., 2006). Cyanide is a potent respiratory poison (Ames et al., 1981, Ellenborn and Barcelonx, 1988) while nitrate causes methaemoglobinemia and cancer (Galler, 1997; Waclaw and Stefan, 2004; Oyesom and Okoh, 2006; Anjana et al., 2007). However, the content of nutrients and toxic substances in this vegetable like others are known to be influenced by their developmental phases. It is in this direction that the research was designed to determine the effect of vegetative phase (market maturity) and reproductive phase (fruiting) on some nutrients, antinutrients and toxic substances in the vegetable with a view to determine the stage of the plant development that the derivable nutrients can be fully exploited.

MATERIALS AND METHODS

The study area

The pot experiment was carried out between 6th June and 18th December 2009 in the nursery of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State of Nigeria.

Niger state has a savana climate characterised by maritime air, and the rainfall season is from April to October. During harmattan, dry desert wind blow between November and mid February when night temperature is very low. The geographical location of Minna is longitude 9° 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 at 1200 - 1300 mm. About 90% of total annual rainfall occurs in the months of June and September. Temperature rarely falls below 22°C with peaks of 40 and 30°C in February/March and November/December respectively. In the wet season the average temperature is about 29°C (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 (FDARL, 1985). The bulked sample was collected during the drying season from the field which had 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 the 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 (Juo, 1979). Exchange acidity (E.A H^+ and Al^{3+}) was determined by titration method (Jou, 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 (Jou, 1979). Total nitrogen was estimated by Macrokjedal 1979).

Sources of seeds

Seeds of fluted pumpkin (*T. occidentalis*) were obtained from Schools of Agriculture and Agricultural Technology's Farm/Nursery of Federal University of Technology, Minna.

Planting, experimental design and nursery management

About two seeds of *T. occidentalis* were planted in a polythene bag filled with 20.00 kg of top soil. After emergence the seedlings were thinned to one plant per pot. The factorial design was adopted to determine the effect of vegetative phase (market maturity) and reproductive phase (fruiting) in control and nitrogen treated plots. Each treatment had 10 pots replicated three times. This gave a total of 60 pots for the vegetable. The seedlings were watered twice daily (in the 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 100 ml per 100 l 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 at the recommended dose specific for the vegetable. Basal application of P_2O_2 and K_2O in the form of single super phosphate and murate of potash were applied at recommended rate respectively. The details of the fertilizer treatments are as follow:

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

F_2: 30 mgN/kg soil, 30 mg P_2O_5/kg soil and 22 mg K_2O/kg soil

Harvesting of the vegetable

The leaves of vegetable in control and nitrogen treated soil were harvested at vegetative phase (market maturity) and at reproductive phase (fruiting) of plant development and were then used for chemical analysis.

Analytical procedure

Both soluble and total oxalates in the samples 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 Alanka (1994). Alkaline picrate method of Ikediobi et al. (1980) was used to analyze 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). The ascorbic acid concentration in the samples was determined by 2, 6-dichlorophenol indophenols method of Eleri and Hughes (1983), while β-carotene concentration was determined by ethanol and petroleum ether extraction method as described by Musa et al.

Table 1. Some Physical and chemical properties of the soil (0 – 20 cm) used for pot experiment.

Parameter	Value
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.

(2010).

Statistical analysis

T-test was used to determine the effect of vegetative and reproductive phases in *T. occidentalis* on the parameters under investigation.

RESULTS

Physical and chemical properties of soil

Results of analyses of the soil used for pot experiment are 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 fruiting on antinutrients and vitamins content

The results obtained from the determination of effect of fruiting on cyanide content in *T. occidentalis* showed that fruiting significantly elevated the cyanide content of the vegetable in the control and nitrogen applied. The mean cyanide concentrations at market maturity and fruiting in control were 438.00 ± 37.00 and 771.00 ± 21.00 mg/kg while the values obtained with the application of nitrogen fertilizer were 699.00 ± 48.00 and 885.00 ± 37.00 mg/kg (Table 2).

Analysis of nitrate content in the vegetable showed that fruiting significantly decreased its content in the vegetable irrespective of the soil nitrogen levels. The mean values of nitrate at market maturity for controls (550.00 ± 95.00 mg/kg) and nitrogen applied (696.00 ± 117.00 mg/kg) were significantly higher than values at fruiting (45.10 ± 15.00 and 34.90 ± 5.10 mg/kg, respectively) (Table 2).

Studies conducted on soluble oxalate and vitamin C content in *T. occidentalis* showed that fruiting had no significant effect on the levels of this compound in control and nitrogen fertilized vegetable (Table 2). However, the results obtained from the analysis of total oxalate showed that fruiting significantly increased the antinutrient content of the vegetable irrespective of the soil nitrogen levels. The mean values of total oxalate at fruiting in control (3.20 ± 0.09 g/100 g) and nitrogen applied (2.82 ± 0.04 g/100 g) were significantly higher compared to levels at market maturity (2.22 ± 0.07 and 2.21 ± 0.16 g/100 g, respectively) (Table 2).

The investigation of the effect of fruiting on β-carotene content in the vegetable revealed that fruiting led to significant decrease in the provitamin content of the vegetable, irrespective of the nitrogen levels. The mean values of β-carotene at market maturity for controls (15501.00 ± 591.00 µg/100 g) and nitrogen applied (17602.00 ± 1009.00 µg/100 g) were significantly higher

Table 2. Effect of fruiting on antinutrients and vitamins content in *Telfairia occidentalis.*

Antinutrients and vitamins	Stage of analysis	
	Market maturity	Fruiting
Cyanide (mg/kg DW), Control	438.00 ± 37.00[a]	771.00 ± 21.00[b]
Cyanide (mg/kg DW) , Nitrogen applied	699.00 ± 48.00[a]	885.00 ± 37.00[b]
Nitrate (mg/kg DW), Control	550.00 ± 95.00[b]	45.10 ± 15.60[a]
Nitrate (mg/kg DW), Nitrogen applied	696.00 ± 117.00[b]	34.90 ± 5.10[a]
Soluble oxalate (g/100g DW), Control	1.82 ± 0.14[a]	2.03 ± 0.06[a]
Soluble oxalate (g/100g DW), Nitrogen applied	1.64 ± 0.06[a]	1.65 ± 0.09[a]
Total oxalate (g/100g DW), Control	2.22 ± 0.07[a]	3.20 ± 0.09[b]
Total oxalate (g/100g DW), Nitrogen applied	2.21 ± 0.16[a]	2.82 ± 0.04[b]
β-carotene (µg/100g FW), Control	15501.00 ± 591.00[b]	10381.00 ± 724.00[a]
β-carotene (µg/100g FW), Nitrogen applied	17602.00 ± 1009.00[b]	12150.00 ± 920.00[a]
Vitamin C (mg/100g FW), Control	208.40 ± 7.50[a]	224.60 ± 11.00[a]
Vitamin C (mg/100g FW), Nitrogen applied	191.60 ± 13.00[a]	186.30 ± 11.00[a]

DW = Dry weight, FW = Fresh weight, Control = No nitrogen applied. Values represent means of nine determinations. Row mean values carrying the same superscripts do not differ significantly from each other (P > 0.05).

than values at fruiting (10381.00 ± 724.00 µg/100 g and 12150.00 ± 920.0 µg/100 g, respectively) (Table 2).

Effect of fruiting on content of mineral elements

The determination of effect of fruiting on Fe content in *T. occidentalis* showed that Fe content of the vegetable increased significantly during fruiting, irrespective of nitrogen levels. The mean values of the mineral in vegetable at fruiting in control (17.84 ± 2.40 mg/kg) and nitrogen applied (28.19 ± 2.40 mg/kg) were significantly higher compared to level at market maturity (10.00 ± 0.92 mg/kg and 13.63 ± 1.30 mg/kg, respectively) (Table 3).

Results obtained from the analysis of Mg showed fruiting of the vegetable significantly increased the mineral content, irrespective of the nitrogen levels. The amount of Mg in the vegetable at fruiting for control (23.13 ± 0.75 mg/kg) and nitrogen applied (23.59 ± 1.00 mg/kg) was significantly higher compared to that obtained at market maturity (19.90 ± 1.00 mg/kg and 20.34 ± 0.80 mg/kg, respectively) (Table 3).

Analysis of Zn and Ca in the vegetable showed that fruiting had no significant effect on contents of these mineral elements in control and nitrogen treated vegetable (Table 3). The determination of Cu in the vegetable showed that fruiting significantly decreased the Cu content in control and nitrogen applied. The concentrations of Cu in the vegetable at market maturity and fruiting in control were 3.66 ± 0.12 mg/kg and 1.30 ± 0.51 mg/kg, respectively, while the corresponding values obtained with the application of nitrogen fertilizer were 8.78 ± 0.72 mg/kg and 2.66 ± 0.53 mg/kg, respectively (Table 3).

Results from the analysis of Na in *T. occidentalis* showed that fruiting had no significant effect on the mineral content of the vegetable in the control. However, when plant received nitrogen fruiting significantly decreased the mineral content. The mean values of the mineral obtained at market maturity and fruiting of the vegetable in control were 5.40 ± 0.54 and 4.59 ± 0.38 mg/kg, while the corresponding values obtained when nitrogen fertilizer was applied were 5.21 ± 0.16 and 3.71 ± 0.2 mg/kg, respectively (Table 3).

Results obtained from the analysis of K in the studied vegetable showed that fruiting significantly decreased the mineral content of the vegetable, irrespective of the nitrogen levels. The mean values of the mineral at market maturity for controls (117.40 ± 3.40 mg/kg) and nitrogen applied (112.90 ± 7.30 mg/kg) were significantly higher than values (62.47 ± 3.00mg/kg and 67.40 ± 3.80mg/kg respectively) at fruiting (Table 3).

DISCUSSION

Significant increase in cyanide content in *Telfairia occidentalis* during fruiting compared with values at market maturity is in agreement with the report of Cleveland and Soleri (1991) and Carmen et al. (2007). The authors independently observed that the cyanide content in the leaves of Crucifers and cassava increase

Table 3. Effect of fruiting on minerals content in *Telfairia occidentalis.*

Minerals	Stage of analysis	
	Market maturity	Fruiting
Fe (mg/kg) , Control	10.00 ± 0.92[a]	17.84 ± 2.40[b]
Fe (mg/kg) , Nitrogen applied	13.36 ± 1.30[a]	28.19 ± 2.40[b]
Mg (mg/kg), Control	19.00 ± 1.00[a]	23.13 ± 0.75[b]
Mg (mg/kg), Nitrogen applied	20.34 ± 0.80[a]	23.59 ± 1.00[b]
Zn (mg/kg), Control	0.03 ± 0.01[a]	0.01 ± 0.01[a]
Zn (mg/kg), Nitrogen applied	0.05 ± 0.01[a]	0.02 ± 0.01[a]
Cu (mg/kg), Control	3.66 ± 0.12[b]	1.30 ± 0.51[a]
Cu (mg/kg), Nitrogen applied	8.78 ± 0.72[b]	2.66 ± 0.53[a]
Ca (mg/kg), Control	17.26 ± 3.20[a]	16.27 ± 2.40[a]
Ca (mg/kg), Nitrogen applied	20.90 ± 3.40[a]	18.95 ± 2.00[a]
Na (mg/kg), Control	5.40 ± 0.54[a]	4.59 ± 0.38[a]
Na (mg/kg), Nitrogen applied	5.21 ± 0.16[b]	3.71 ± 0.29[a]
K (mg/kg), Control	117.40 ± 3.40[b]	62.47 ± 3.00[a]
K (mg/kg), Nitrogen applied	112.90 ± 7.30[b]	67.40 ± 3.80[a]

Control = No nitrogen applied. Values represent means of nine determinations. Row mean values carrying the same superscripts do not differ significantly from each other (P > 0.05).

with the age of the plants, respectively. The reason for the increase may likely be that during fruiting, the gene responsible for the synthesis of cyanogenic glycoside may be triggered by some hormonal action associated with fruit initiation and development to produce more of the compound for onward translocation into the fruiting body. This observation is likely to be correct since one of the functions of cyanogenic glycoside in some plants is to protect the plants and their products from predators in order to ensure the continuity of their generation (Peter and Birger, 2002).

The significantly lower nitrate content in *T. occidentails* at fruiting compared to market maturity is in line with report of Richard (1991) and Brown (1993) that young plant in the vegetative stage generally contains more nitrate than mature plants of the same species. Shigeru et al. (2003), Waldemar et al. (2005) and Carmen et al. (2007) also found the same trend in setaria grasses, *Anethum graveolens* and cassava leaves, respectively. This decrease in the nitrate content during fruiting of the vegetables may spell two things: firstly that during fruiting there could be an increase in the activity of nitrate reductase enzyme leading to an increase in amino acids and proteins required for fruiting and seeds development. This observation is likely to be correct since there is a report of significant negative correlation between nitrate content in the plant and nitrogen reductase activity (Anjana et al., 2007). Secondly, there is likelihood of the translocation of some of nitrate contents in the leaves during fruiting to the developing fruits. This observation is supported by Noggle and Fritz (2006). According to these authors during fruit development, metabolites for cellular synthesis and the growth substances are translocated to the developing fruits from the leaves, stems, and roots. These authors further stressed that growing fruit is an active sink that diverts and draws water and solutes from other regions of the plant. Even though the nitrate content in the vegetable decreases with fruiting, its content at market maturity (vegetative phase) is lower than the acceptable daily intake (ADI) of 3.65 mg/kg for 60 kg adult body weight (219.00 mg/day) if 100g samples are consumed per day. The decrease in this compound alone could not justify the inclusion of *T. occidentalis* in our meal during fruiting.

Higher total oxalate content observed at fruiting than at market maturity in *T. occidentalis* concurs with the finding of Waldemar et al. (2005), that older plants had higher oxalates than the younger ones in *Anethum graveolens.* The reason for this could be that many substances, such as the so called secondary plant substances (secondary metabolites) accumulate in tissues and organs during aging (Noggle and Fritz, 2006).

Decrease in β-carotene concentration during fruiting in *T. occidentalis* agrees with the report of Barros et al. (2007a, b) that the provitamin A content decreased in mature fruiting body of mushroom and *Lactarius*

piperatus. The likely reason for the decrease of the compound in the vegetable may be due to the possible translocation of some of its content to the developing fruits, and a decline in the content and activity of chlorophyll and associated light absorbing pigments (including carotenoids) following senescence induced by fruit formation and maturation (Noggle and Fritz, 2006).

The insignificant differences observed in the vitamin C content between market maturity and fruiting stage in *T. occidentalis* may imply that translocation of vitamin C into the developing fruit may not have significant effect on its content in the leaves of the vegetables.

The elevated levels of Fe and Mg in the vegetable leaves during fruiting may likely indicate that the possible physiological and biochemical changes during fruit initiation and development could lead to an increased uptake of the minerals from the soil by the plant for an onward translocation into the fruiting body. This observation is likely to be true since Noggle and Fritz (2006) concluded that the chemical composition of fruit at maturity reflect the presence of materials translocated from other parts of the plant as well as materials formed by metabolic activities of the fruit tissues. While the significant lower levels of Na and K in the vegetable during fruiting compared to higher values at market maturity is in line with finding of Noggle and Fritz (2006), to the effect that during fruit initiation and development, some metabolites for cellular synthesis and growth substances are translocated from leaves, stems, and roots to developing fruits. Lanyasunya et al. (2007) observed that the rapid uptake of mineral by plants during early growth and the gradual dilution that occurs as plant matures would have been responsible for the decrease in some of the mineral content during fruiting.

Conclusion

The derivable nutritional benefits of *Telfairia occidentalis* can be fully harnessed by inclusion of the vegetable in our meal at vegetative phase. This practice will reduce the health problems associated with high intake of cyanide (respiratory paralysis) and oxalate (kidney stone) in the vegetable, and therefore contribute to the improvement of health condition of individual and the entire population in general.

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