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Effect of heading on some micronutrients, anti-nutrients and toxic substances in *Amaranthus cruentus* grown in Minna, Niger State

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ABSTRACT
Pot experiment was conducted to determine the effect of heading (fruiting) on antinutrients (soluble and total oxalates), toxic substances (cyanide and nitrate) and some micronutrients viz; vitamin C, β-carotene (provitamin A) and mineral elements (Fe, Mg, Cu, Zn, Ca Na and K) in *Amaranthus cruentus* grown in nitrogen and non-nitogen treated soil. The vegetable leaves were harvested at both market maturity (vegetative phase) and heading (reproductive phase) and were subjected to chemical analysis. Results obtained showed that the vitamin C, cyanide, soluble and total oxalates contents in the vegetable were significantly elevated ($p < 0.05$) during heading irrespective of soil nitrogen levels. Nitrate and β-carotene contents decreased significantly ($p < 0.05$) during heading except that the decrease in β-carotene was not significant in vegetable treated with nitrogen fertilizer. Similarly the result also revealed that the Mg, Zn, Ca and K content were not significantly affected with heading of the vegetable. However, the Fe content was increased, while the Ca and Na contents were reduced significantly ($p < 0.05$) with heading.The result concludes that harvesting of the vetagetatble at vegetative phase (market maturity) generally reduce the levels of most of the plant toxins and still conserve most of the micronutrients in an amount to meet our dietary requirements.

Keywords: *Amaranthus cruentus,* Heading, Market maturity, Micronutrients, Anti-nutrients, Toxic substances

INTRODUCTION

Amaranthus cruentus is an herbaceous annual leafy vegetable that can be produced for fresh market in 4 - 6 weeks after planting. It can be cultivated all the year round depending on the availability of water. This plant requires loamy to sandy loam soil for good yield and does well in soils with high organic matter content (Grubben, 1986). It is a widely distributed genus of short-lived herbs, occurring mostly in temperate and tropical regions. There are about 60 species of Amaranthus and several of these are cultivated as leafy vegetables, cereals or ornamental plants (Schippers, 2000; He, 2002; Dhellot *et al*., 2006). In Nigeria, amaranthus leaves combined with condiments are used to prepare sauce (Oke, 1983; Mepha *et al*., 2007; Akubugwo *et al*., 2007).

Amaranthus cruentus is rich in vitamins including β-carotene (precussor of vitamin A), vitamin B6, vitamin C, riboflavin, and folate, and dietary minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese (Makus, 1984; Makus and Davis, 1984; Sussan and Anne, 1988; Stallknecht and Schaeffer, 1993). This vegetable is also rich in lysine, an essential amino acid that is lacking in diets based on cereals and tubers (Schipper, 2000). However, the moderately high content of oxalic acid in the leaves of this vegetable inhibits the absorption of calcium and other mineral elements leading to the formation of kidney stone, oxalaneamia and electrolyte inbalance (Prien, 1991). *Amaranthus cruentus* under certain

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conditions have high nitrate content exceeding tolerable limit (Macrae *et al*., 1997). The vegetable is also known to contain some appreciable levels of cyanogenic glycoside which is a respiratory poison.

The levels of the nutrients and toxic substances in the vegetable are known to be influenced by stages of plant development. It is in this direction that the research was designed to investigate the effect of heading (fruiting) on the levels of some micronutrients, antinutrients and toxic substances in the vegetable. This was with the aim of determining the stage of plant development that the nutritional potential of this vegetable can be fully harnessed.

MATERIALS AND METHODS

Seeds

The seeds of jute mallow *(Amaranthus cruentus)* were obtained from Schools of Agriculture and Agricultural Technology's Farm/Nursery of Federal University of Technology, Minna.

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 rainfull is between April and October. During harmattan, dry desert wind blow between November and mid February while 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 of 1200 and 1300mm. About 90% of total annual rainfull 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 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 drying season from the field which has been under fallows for about four years. The bulked soil sample was passed through 2mm 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 EDTA versenate titration method (Juo, 1979). Total nitrogen was estimated by Macrokhjedal procedure and available phosphorus by Bray No 1 method (Juo, 1979).

Planting, experimental design and nursery management

About ten seeds of *Amaranthus cruentus* were planted in a polythene bag filled with 10.00kg of top soil and after emergence the seedlings were thinned to two plants per pot. 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 (Saro Agro Sciences) four weeks after planting at the rate of 100ml per 100 litres of water.

Fertilizer treatment

The fertilizer levels for this vegetable are stated below:

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

F_2: 37mgN/kg soil, 30mg P_2O_5/kg soil and 30mg K_2O/kg soil

Harvesting of the vegetable

The leaves of vegetable grown in pot experiment in control and nitrogen treated soil were harvested at vegetative phase (market maturity) and at reproductive phase (heading) of plant development. The level of nutrients, antinutrients and toxic substances in the leaves were then determined.

Analytical procedure

Both soluble and total oxalates in the fresh and processed 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 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). The ascorbic acid content in the samples was determined by 2, 6-dichlorophenol indophenols method of Eleri and Hughes (1983) as described briefly. A known weight (2.0 g) of the samples of fresh and processed leaves of the vegetables were put in the mortar and 15 cm^3 of metaphosphoric acid/acetic acid mixture added to the vegetable and ground with pieces of glasses. The extract obtained was decanted and filtered into 100 cm^3 volumetric flask. This extraction was repeated with another 10 cm^3 of metaphosphoric acid/acetic acid mixtures and the residues washed with distilled water. Both the second extract and washed solution were added to the first extract in the 100 cm^3 volumetric flask and the volumes made up to the marked level with distilled water. The prepared indophenol was standardized with 5.0 cm^3 of freshly prepared standard ascorbic acid. 5.0 cm^3 of the filtered aliquots of the sample was then titrated against the standardized indophenol and the end point was reached when a faint permanent pink colour was observed. The titre value obtained was used to calculate the actual concentration of ascorbic acid present in the samples.

β-carotene was determined by the ethanol and petroleum ether extraction method as described briefly. A known amount (2.0 g) 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 minutes 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 30cm^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 10cm^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 50cm^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. The optical density 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 using the expression:

$$A = E^{\circ} \times C \times l$$

Where A = absorbance of the sample

E° = extinction coefficient of β-carotene

l = path length (usually 1.0cm).

Statistical analysis

T-test was used to determine the effect of vegetative and reproductive phases in *Amaranthus cruentus*.

RESULTS

The texture class of the soil is sandy loam indicating that the water holding capacity is moderate (Table 1). 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 (CEC) is moderate while base saturation percentage is high. The soil pH indicates that the soil is slightly acidic

The investigation of the effect of heading on cyanide concentration in *Amaranthus cruentus* showed that the cyanide content of the vegetable increased significantly ($P<0.05$) during heading irrespective of nitrogen levels (Table 2). The nitrate content of the vegetable significantly decreased ($p < 0.05$) at heading however, with the application of nitrogen fertilizer, heading had no significant effect ($p > 0.05$) on the nitrate content of the vegetable (Table 2). The mean soluble oxalate concentrations at market maturity and heading in control were 3.11 ± 0.22g/100g and 3.86 ± 0.25g/100g while the values obtained with the application of nitrogen fertilizer were 2.37 ± 0.05g/100g and 3.67 ± 0.18g/100g respectively. This data showed that fruiting significantly elevated ($p < 0.05$) the soluble oxalate content of the vegetable in the control and the applied nitrogen (Table 2). Similarly, the total oxalate contents of the vegetable significantly increased ($p < 0.05$) the levels of the antinutrient content at heading irrespective of the soil nitrogen levels (Table 2. The investigation of the effects of headings on β-carotene content in *Amaranthus cruentus* revealed that heading has significant decreasing effects on the provitamin content of the vegetable irrespective of the nitrogen levels. The vitamin C content of the vegetable at heading (reproductive phase) was also significantly increased as compared to the values obtained at market maturity (vegetative phase) irrespective of the soil nitrogen levels (Table 2).

The determinations of the effects of heading on Fe concentrations in *Amaranthus cruentus* showed that the Fe content of the vegetable increased significantly during heading irrespective of nitrogen levels (Table 3). The results obtained from the analysis of Mg, Zn, Ca and K indicated heading (reproductive phase) had no significant effect on the minerals content in control and nitrogen fertilized vegetable (Table 3). The concentrations of Cu obtained in the studied vegetable decreased significantly during heading in both control and nitrogen applied. Similarly, results obtained from the analysis of Na in the vegetable also showed that heading has a decreasing effect on the mineral content in control and nitrogen treated vegetable (Table 3).

DISCUSSION

Significant increase in cyanide content in *Amaranthus cruentus* during heading (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 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).

Table 1: Some physical and chemical properties of the soil (0-20 cm) 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 loamy

*Values represent means of triplicate determinations

Table 2: Effect of heading on antinutrients and vitamins content in *Amaranthus cruentus*

Antinutrients and vitamins	**Stage of analysis**	
	Market maturity	**Heading**
Cyanide (mg/kg DW), Control	223.10 ± 12.00[a]	308.70 ± 19.00[b]
Cyanide (mg/kg DW) , Nitrogen applied	256.50 ± 9.50[a]	435.00 ± 117.00[b]
Nitrate (g/kg DW), Control	17.71 ± 2.42[b]	7.62 ± 1. 00[a]
Nitrate (g/kg DW), Nitrogen applied	23.41 ± 2.07[a]	18.72 ± 3.40[a]
Soluble oxalate (g/100g DW), Control	3.11 ± 0.22[a]	3.86 ± 0.25[b]
Soluble oxalate (g/100g DW), Nitrogen applied	2.37 ± 0.05[a]	3.67 ± 0.18[b]
Total oxalate (g/100g DW), Control	4.40 ± 0.19[a]	5.27 ± 0.24[b]
Total oxalate (g/100g DW), Nitrogen applied	3.75 ± 0.35[a]	5.04 ± 0.22[b]
β-carotene (mg/100g FW), Control	7.45 ± 0.47[b]	2.48 ± 0.33[a]
β-carotene (mg/100g FW), Nitrogen applied	8.04 ± 0.87[b]	4.86 ± 0.57[a]
Vitamin C (mg/100g FW), Control	94.60 ± 5.60[a]	160.50 ± 7.10[b]
Vitamin C (mg/100g FW), Nitrogen applied	78.90 ± 4.50[a]	149.90 ± 8.20[b]

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 ($P > 0.05$).

Table 3: Effect of heading on minerals content in *Amaranthus cruentus*

Minerals	**Stage of analysis**	
	Market maturity	**Heading**
Fe (mg/kg), Control	33.53 ± 1.20[a]	39.76 ± 2.30[b]
Fe (mg/kg) , Nitrogen applied	24.58 ± 1.30[a]	45.70 ± 6.50[b]
Mg (mg/kg), Control	26.25 ± 0.81[a]	25.54 ± 0.94[a]
Mg (mg/kg), Nitrogen applied	27.12 ± 1.20[a]	25.98 ± 1.00[a]
Zn (mg/kg), Control	0.08 ± 0.01[a]	0.05 ± 0.01[a]
Zn (mg/kg), Nitrogen applied	0.06 ± 0.01[a]	0.06 ± 0.01[a]
Cu (mg/kg), Control	4.55 ± 0.66[b]	2.78 ± 0.70[a]
Cu (mg/kg), Nitrogen applied	5.05 ± 0.66[b]	2.94 ± 0.55[a]
Ca (mg/kg), Control	30.28 ± 0.60[a]	29.49 ± 0.55[a]
Ca (mg/kg), Nitrogen applied	29.49 ± 0.32[a]	30.60 ± 0.40[a]
Na (mg/kg), Control	11.49 ± 1.10[b]	8.81 ± 0.42[a]
Na (mg/kg), Nitrogen applied	11.70 ± 1.40[b]	8.19 ± 0.40[a]
	209.70 ± 16.00[a]	254.30 ± 22.00[a]
K (mg/kg), Control	219.30 ± 22.00[a]	223.90 ± 24.00[a]
K (mg/kg), Nitrogen applied		

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)

The significantly lower nitrate content in *Amaranthus cruentus* at heading (reproductive phase) compared to market maturity (vegetative phase) 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 heading of the vegetable may spell two things; firstly that during this stage of plant development, 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 nitrate 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 the report of Noggle and Fritz (2006) that during the stage of fruit development, metabolites for cellular synthesis and the growth substances are translocated to the developing fruits from the leaves, stems, and roots. They further stressed that growing fruit is an active sink that diverts and draws water and solutes from other regions of the plant.

Higher oxalates (soluble and total) content observed at heading than at market maturity of the vegetable concur with the finding of Waldemar *et al* (2005) that older plant had higher oxalates than the younger plant 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 content during heading in *Amaranthus cruentus* agrees with the report of Barros *et al* (2007a) and Barros *et al* (2007b) 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 vegetables 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 caroteniods) following senescence induced by fruit formation and maturation (Noggle and Fritz, 2006).

The increase in the vitamin C content during heading in *Amaranthus cruentus* though contrary to the observations of Zofia *et al* (2006) and Bergquist *et al* (2007), agreed with the submission of Chweya (1993) and Chweya and Nameus (1997) that vitamin C content increased significantly with plant age in *Gynandropsis gynandra* and *Cleome gynandra* respectively. Barros *et al*. (2007b) reported the same trend of results in *Lactarius piperatus* that vitamin C content in *Lactarius piperatus* was highest at maturity and lowest at immaturity stage. The variations in the vitamin C content reported by different authors during this stage of plant development may have resulted from differences in cultivar (Guillermo *et al*., 2005; Signh, 2005; Aliyu and Morufu, 2006; Weerakkody, 2006).

The observed significant decrease in some of mineral elements (Cu, Na) observed in the vegetable during heading compare 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 the leaves, stems, and roots to the 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.

The elevated levels of Fe in vegetable leaves during heading may likely indicate that the possible physiological and biochemical changes during fruit initiation and development could lead to an increase

uptake of the mineral 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.

In conclusion, harvesting of the vetagetatble at vegetative phase generally reduce the levels of most of the plant toxins and still conserve some of the micronutrients in an amount to meet the dietary requirements. This will reduce the health problems associated with high levels of antinutrients and toxic substances in the vegetables and improve the general well being of individual. This will in turn lead to increase in productivity.

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