

Full Length Research Paper

The effects of applied nitrogen fertilizer and leaf positions on levels of micronutrients, anti-nutrients and toxic substances in *Amaranthus cruentus*

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Accepted 5 May, 2011

The presence of antinutrients and toxic substances in vegetables limits the nutritional benefits of vegetables. The effects of age of plant and application of nitrogen fertilizer on the bioaccumulation of antinutrients (oxalate), toxic substances (cyanide and nitrate), micronutrients (vitamin C, β -carotene - provitamin A) and mineral elements (Fe, Mg, Cu, Zn, Ca Na and K) in *Amaranthus cruentus* were investigated using pot experiment. Leaves were harvested at market maturity (vegetative phase) at three different leaf positions, basal (oldest), middle (younger) and upper (youngest) and were subjected to chemical analysis. Results obtained showed that cyanide, nitrate and oxalate were concentrated significantly in the basal and middle positions. The concentration of β -carotene, vitamin C and Zn were significantly higher in the leaves in the middle part than in the basal and upper leaves. Similarly, Fe, Mg, Cu and Na contents were significantly higher in the basal leaves than in the middle and upper leaves, while the concentration of K was higher in the younger leaves. We concluded that consumption of the vegetable leaves from the upper leaf position will provide the dietary requirements of the analysed micronutrients with significant reduction in the levels of oxalate, cyanide and nitrate and associated health problems.

Key words: *Amaranthus cruentus*, market maturity, micronutrients, anti-nutrients, toxic substances, vegetable, oxalate, nitrate, cyanide.

INTRODUCTION

Amaranthus cruentus is an herbaceous annual leafy vegetable that can be produced for fresh market in 4 to 6 weeks after planting. It can be cultivated all year round depending on availability of water. In Nigeria, *Amaranthus* leaves combined with condiments are used to prepare sauce (Akubugwo et al., 2007; Mepha et al., 2007; Oke, 1983).

A. cruentus is rich in vitamins including β -carotene (precursor of vitamin A), vitamin B6, vitamin C, riboflavin and folate, and dietary minerals such as calcium, iron,

magnesium, phosphorus, potassium, zinc, copper and manganese (Sussan and Anne, 1988). 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 and leads to the formation of kidney stone, oxalaneamia and electrolyte imbalance (Prien, 1991). The vegetable is also known to contain some appreciable levels of cyanogenic glycoside which is a respiratory poison.

Nitrogen (N) is a very important plant nutrient and it is a key yield determining factor in crop production (Ali, 2006) and this probably explains why it is the most abundant element in plants. It is a constituent of many

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macromolecules including protein, nucleic acids, hormones and chlorophyll (Ahmadil et al., 2010). It stimulates vegetative growth, producing large stems and leaves in plants. However, excess nitrogen reduces carbohydrate synthesis, lowers resistance to diseases (rust and downy mildew), lowers resistance to insect damage and reduces the biological value of plant protein (Hornick, 2010)

It is known that most leafy vegetables prefer nitrate to either ammonium or urea as their source of nitrogen (Chen et al., 2004; Gulser, 2005). Accumulation of nitrate in plants occurs when reduction of nitrate is much less than its uptake. *A. cruentus* under certain conditions has high nitrate content exceeding tolerable limit (Macrae et al., 1997). Although, nitrate accumulation is genetically controlled, a number of other factors have a modulatory effect. These factors include environment (light intensity, air temperature, soil temperature and moisture), fertilizer management and crop production practices (Maynard et al., 1976). The concentration of nitrate varies in different parts of the plant with the petiole containing several folds the amount in leaf blades (Pavlovic et al., 1996).

The levels of nutrients and toxic substances in the vegetable could therefore, be influenced by age of the leaves on the mother plant and the level of soil nitrogen. It is for this reason that this study was designed to investigate the effect of the interplay of applied nitrogen fertilizer and the three leaf positions (basal, middle and upper) on the levels of some micronutrients, antinutrients and toxic substances in the vegetable.

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, Nigeria.

Niger state has a Savannah 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 9°40' N and latitude 6°30' E. Minna lies in the Southern Guinea Savannah 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 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 in Minna and classified as inceptisol (FDARL, 1985). The bulk sample was collected during the dry season from the field which has been fallow for about four years. The bulk 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 CaCl₂ 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 Al³⁺) was determined by titration method. Exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1 N NH₄OA solution. Sodium and potassium were determined by flame emission spectrophotometry, while Mg and Ca were determined by E.D.T.A versenate titration method. Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No. 1 method. The composition of the soil used is presented in Table 1.

Physical and chemical properties of soil

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; FDARL, 1985).

Seeds

The seeds of *A. cruentus* were obtained from School of Agriculture and Agricultural Technology's Farm/Nursery of Federal University of Technology, Minna.

Planting, experimental design and nursery management

Ten seeds of *A. cruentus* were sown in a polythene bag filled with 10.00 kg 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 four weeks after planting at the rate of 100 ml per 100 L of water.

Fertilizer treatment

The fertilizer levels for this vegetable are stated as: F₁ (control): 0N, 30 mg P₂O₅/kg soil and 30 mg K₂O/kg soil; F₂: 37 mgN/kg soil, 30 mg P₂O₅/kg soil and 30 mg K₂O/kg soil.

Harvesting of the vegetable

Leaves were harvested at vegetative phase (market maturity) of plant development at three different leaf positions including, basal, middle and upper. The levels of nutrients, antinutrients and toxic substances in the leaves from these different leaf regions were then determined.

Analytical procedure

Oxalate determination

Both soluble and total oxalates in the fresh and processed samples

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

Parameter	Value
Sand (%)	74.40
Silt (%)	18.00
Clay (%)	7.60
pH (in H ₂ O)	6.51
pH (in 0.1M CaCl 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.

were determined by titrimetric method of Oke 1966).

Determination of nitrate level

The nitrate content in the test samples was determined by the colourimetric method as described by Sjoberg and Alanka (1994).

Cyanide analysis

Alkaline picrate method of Ikediobi et al. (1980) was used to analyse the cyanide content in the test samples.

Analysis of mineral elements

The mineral elements (Fe, Cu, Mg, Na and K) in samples were determined according to the method of Ezeonu et al. (2002).

Determination of ascorbic acid

The ascorbic acid content in the samples was determined by 2, 6-dichlorophenol indophenols method of Eleri and Hughes (1983).

Determination of β -carotene

β -Carotene was determined by ethanol and petroleum ether extraction method as described briefly further. Two grammes of Na₂SO₄ was added to 10.0 g of vegetable leaves and ground in a mortar. The ground vegetables were extracted with 100 cm³ 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 cm³ 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 cm³ conical flask. The ethanol layer in the beaker was re-extracted twice with 10 cm³ of petroleum ether. The ether layers of re-extraction were added to the original petroleum ether extract in the conical flask and re-extracted with 50 cm³ 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 is the absorbance of the sample; E% is the extinction coefficient of β -carotene and l is the path length (usually 1.0 cm).

Statistical analysis

Analysis of variance (ANOVA) was carried out using statistical package Minitab to determine the variation between treatments (three levels of age of plant leaves). The Duncan's multiple range test (DMRT) was used for the comparison of mean.

RESULTS

Cyanide content

Analysis of results obtained showed that cyanide content was significantly highest ($p < 0.05$) in basal (258.38 \pm

Table 2. Effect of leaf position and applied nitrogen fertilizer on antinutrients and vitamins content in *A. cruentus* at market maturity stage.

Antinutrient and vitamin	Leaf position		
	Basal leaves	Middle leaves	Upper leaves
Cyanide (mg/kg DW), control	258.38 ± 2.76 ^c	219.34 ± 5.38 ^b	188.18 ± 20.33 ^a
Cyanide (mg/kg DW), nitrogen applied	261.90 ± 20.95 ^b	288.09 ± 6.18 ^b	223.10 ± 1.27 ^a
Nitrate (g/kg DW), control	15.43 ± 0.28 ^b	26.99 ± 0.49 ^c	10.71 ± 0.36 ^a
Nitrate (g/kg DW), nitrogen applied	24.35 ± 0.38 ^b	30.05 ± 0.09 ^c	15.83 ± 0.61 ^a
Soluble oxalate (g/100 g DW), control	2.58 ± 0.03 ^c	2.32 ± 0.02 ^b	2.22 ± 0.04 ^a
Soluble oxalate (g/100 g DW), nitrogen applied	3.92 ± 0.28 ^b	2.75 ± 0.20 ^a	2.67 ± 0.32 ^a
Total oxalate (g/100 g DW), control	4.92 ± 0.11 ^c	3.81 ± 0.16 ^b	2.52 ± 0.19 ^a
Total oxalate (g/100 g DW), nitrogen applied.	4.53 ± 0.17 ^b	4.93 ± 0.16 ^b	3.37 ± 0.28 ^a
β-carotene (mg/100 g FW), control	7.185 ± 0.69 ^{ab}	8.70 ± 0.16 ^b	6.46 ± 0.10 ^a
β-carotene (mg/100 g FW), nitrogen applied	6.03 ± 0.1 ^a	11.03 ± 0.93 ^b	7.05 ± 0.18 ^a
Vitamin C (mg/100 g FW), control	76.75 ± 10.75 ^a	91.64 ± 10.37 ^b	68.18 ± 9.42 ^a
Vitamin C (mg/100 g FW), nitrogen applied	84.34 ± 9.92 ^a	108.84 ± 17.42 ^b	90.78 ± 15.04 ^a

DW, Dry weight; FW, fresh weight; control, no nitrogen applied. Values represent means of triplicate determinations. Row mean values carrying the same superscripts do not differ significantly from each other ($P > 0.05$).

2.76 mg/kg) followed by middle (219.34 ± 5.38 mg/kg) and lowest in upper (188.18 ± 20.33 mg/kg) leaves in control samples (Table 2). Vegetable with applied nitrogen had no significant difference ($p > 0.05$) in cyanide content between basal (261.90 ± 20.95 mg/kg) and middle (288.09 ± 6.18 mg/kg) leaves. However, the two leaf positions were significantly ($p < 0.05$) higher in the cyanide content than in upper leaf region (Table 2).

Nitrate level

The nitrate levels in the vegetable indicated that the nitrate content was significantly ($p < 0.05$) highest in the middle leaf position followed by basal region and lowest in upper leaf position irrespective of the fertilizer levels. The nitrate concentrations in the basal, middle and upper leaves in control were 15.43 ± 0.28 , 26.99 ± 0.49 and 10.71 ± 0.36 g/kg, respectively. The corresponding values obtained with the application of nitrogen fertilizer were 24.34 ± 0.37 , 30.06 ± 0.09 and 15.83 ± 0.61 g/kg.

Oxalate content

The levels of soluble oxalate in the three leaf positions in control sample were in the following order: Basal (2.58 ± 0.03 g/100 g) > Middle (2.32 ± 0.02 g/100 g) > Upper (2.22 ± 0.04 g/100 g). With the application of nitrogen fertilizer, no significant difference was observed in the antinutrient contents between middle (2.75 ± 0.20 g/100 g) and upper (2.67 ± 0.32 g/100 g) leaf regions. However, the two leaf positions were significantly ($p < 0.05$) lower in the antinutrient content than the basal (3.92 ± 0.28 g/100 g) leaf position. The total oxalate concentration in control

sample was significantly highest in the basal position (4.92 ± 0.11 g/100 g) closely followed by the middle position (3.81 ± 0.16 g/100 g) and least in the upper leaf position (2.52 ± 0.19 g/100 g). However, when the plant received nitrogen fertilizer, no significant difference in total oxalate content was observed between basal (4.53 ± 0.17 g/100 g) and middle (4.93 ± 0.16 g/100 g) leaf positions. The two leaf positions had significantly ($p < 0.05$) higher content of oxalate than the upper (3.37 ± 0.28 ng/100 ng) leaf region (Table 1).

Level of β-carotene

There was no significant ($p > 0.05$) difference in β-carotene content between basal (7.19 ± 0.69 mg/100 g) and middle (8.70 ± 0.16 mg/100 g) leaves and basal and upper (6.46 ± 0.10 mg/100 g) leaves. However, middle leaf position was significantly ($p < 0.05$) higher in the provitamin than leaves in the upper position in the control samples. When the plant received nitrogen fertilizer, middle (11.03 ± 0.93 mg/100 g) leaf region was significantly ($p < 0.05$) higher in β-carotene content than the upper (7.05 ± 0.18 mg/100 g) and basal (6.03 ± 0.14 mg/100 g) leaf positions (Table 2).

Vitamin C level

Results from analysis of vitamin C showed that there was no significant difference ($p > 0.05$) in the vitamin C content between basal and upper leaves. However, both leaf positions were significantly ($p < 0.05$) lower in vitamin C content than leaves in the middle region irrespective of nitrogen levels.

Table 3. Effect of leaf position on minerals content in *A. cruentus* at market maturity stage.

Mineral	Leaf position		
	Basal leaves	Middle leaves	Upper leaves
Fe (mg/kg), control	34.58 ± 1.85 ^a	33.50 ± 1.71 ^a	32.50 ± 6.19 ^a
Fe (mg/kg), nitrogen applied	27.53 ± 3.37 ^b	23.67 ± 2.20 ^a	22.53 ± 5.33 ^a
Mg (mg/kg), control	28.49 ± 0.71 ^c	26.98 ± 1.15 ^b	23.32 ± 0.59 ^a
Mg (mg/kg), nitrogen applied	30.52 ± 1.40 ^c	27.96 ± 1.09 ^b	22.88 ± 1.24 ^a
Zn (mg/kg), control	0.07 ± 0.04 ^a	0.11 ± 0.03 ^b	0.07 ± 0.03 ^a
Zn (mg/kg), nitrogen applied	0.05 ± 0.02 ^a	0.10 ± 0.02 ^b	0.04 ± 0.02 ^a
Cu (mg/kg), control	5.79 ± 0.14 ^b	5.24 ± 2.17 ^b	2.63 ± 1.57 ^a
Cu (mg/kg), nitrogen applied	6.08 ± 2.58 ^a	4.99 ± 2.11 ^a	4.09 ± 1.24 ^a
Ca (mg/kg), control	30.89 ± 2.52 ^a	30.49 ± 1.64 ^a	29.46 ± 1.52 ^a
Ca (mg/kg), nitrogen applied	29.87 ± 1.28 ^a	29.65 ± 1.03 ^a	28.96 ± 0.49 ^a
Na (mg/kg), control	14.33 ± 1.15 ^b	11.90 ± 3.52 ^{ab}	8.24 ± 0.99 ^a
Na (mg/kg), nitrogen applied	16.47 ± 2.03 ^b	10.74 ± 2.61 ^{ab}	7.90 ± 0.53 ^a
K (mg/kg), control	174.95 ± 42.32 ^a	219.87 ± 17.78 ^b	234.10 ± 66.10 ^b
K (mg/kg), nitrogen applied	177.09 ± 65.20 ^a	231.61 ± 67.66 ^b	249.34 ± 78.83 ^b

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

Level of mineral elements at market maturity

Determination of the effect of leaf positions on mineral content in *A. cruentus* at market maturity showed that leaf position had no significant effect on the Fe content in the leaves in the control samples. But when the plant received nitrogen fertilizer no significant difference in the mineral content was observed between middle (23.67 ± 2.20 mg/kg) and upper (22.53 ± 5.33 mg/kg) leaves though, basal leaves (27.53 ± 3.37 mg/kg) had significant ($p < 0.05$) higher content of mineral than the two leaf positions (Table 2).

Irrespective of nitrogen fertilizer levels, the levels of Mg was in the order: basal > middle > upper leaves (Table 3). Results obtained from analysis of Zn in control and nitrogen fertilized samples indicated that there were no significant difference in the mineral content between basal and upper leaves. However, middle leaves had significantly higher content of the minerals than the leaves obtained from the two other leaf position (Table 3).

There was no significant difference in the Cu content between basal (5.79 ± 0.14 mg/kg) and middle (5.24 ± 2.17 mg/kg) leaves. The mineral content in the two leaf positions were significantly ($p < 0.05$) elevated compared with the upper leaf (2.63 ± 1.57 mg/kg) region in the control sample. With the application of nitrogen fertilizer, leaf positions had no significant effect on the mineral content in the leaves (Table 2). Similarly, results from the analysis of Ca showed leaf positions had no significant effect on the mineral contents in the vegetable. The determination of K content in the vegetable showed that there was no significant difference in the mineral element content between middle and upper leaves. However, the two leaf regions were significantly higher in the mineral

than the basal leaf region irrespective of soil nitrogen levels (Table 3). The level of Na in the middle leaves was not significantly different from the levels in the basal and middle leaves, but basal leaves had significantly ($p < 0.05$) higher content of the mineral than upper leaves in control and nitrogen treated vegetable (Table 3).

DISCUSSION

In line with documented report (Carmen et al., 2007; Cleaveland and Soleri, 1991), the level of cyanide in the basal leaves is significantly higher than the cyanide level in the upper leaves at market maturity. These results however, disagree with the report of Richard (1991), who observed that the level of this respiratory poison is concentrated in younger leaves of sorghum than the older leaves. The cyanide content of leaves from different positions in vegetables may therefore, be a function of many factors including cultivars and environmental factors.

The addition of nitrogen fertilizer resulted in increased cyanide levels in all the leaf positions. Generally, plants growing under high nitrogen levels have high levels of cyanogenic glucosides and this explains why addition of nitrogen fertilizer resulted in higher levels of cyanide.

The significantly higher nitrate content in basal leaves followed by the middle and then, least in the upper leaves in *A. cruentus* may indicate that nitrate content in leaves of these vegetables increased with leaf age. This result is in line with the report of Anjana et al. (2007) who attributed the higher accumulation of nitrate content in older leaves than younger ones to lower activity of nitrate reductase enzyme in the former than in the later. This enzyme is responsible for the reduction of nitrogen to

amino acid used for protein synthesis. Low activity of the enzyme in the basal leaves reduce the rate of protein formation from nitrogen and this may favour the accumulation of nitrogen and its subsequent oxidation to nitrate in the affected leaf regions. This finding is likely to be correct since the same author has also reported that nitrate had a significant negative correlation with nitrate reductase activity. In general, the higher and lower nitrate contents observed in the different leaf positions may be due to the lower and higher nitrate reductase activity, respectively, in those leaf regions.

The generally highest level of soluble and total oxalates in basal leaves followed by middle and least in the upper leaf region in *A. cruentus*, is in harmony with the submission of Ekpедema et al. (2000) and Beis et al. (2007) that the oxalates (soluble and total) were higher in older leaves than younger ones in *Telfairia occidentalis* and *Spinacia oleracea*, respectively. The reason for this observation could be that the older leaves are fully matured with optimum metabolic activity leading to the production of oxalates. The results however, disagree with the finding of Bassey et al. (2004) and Oscarson and Savarge (2007). These authors independently observed that oxalate contents in younger leaves were slightly higher than in the older leaves in *Diplazium sammatil* and *Colocasia esculenta*, respectively. It therefore follows that one or two experiments of this nature can not be used to make a general statement on the expected levels of antinutrients and toxic substances in vegetables.

The significantly higher level of β -carotene in the middle than upper leaves in *A. cruentus*, agrees with the report of Bergquist et al. (2007) and Mou and Ryder (2007) to the effect that β -carotene content was highest in the oldest leaves than the younger ones in baby spinach and lettuce. Significantly, higher content of the provitamin A in the middle than basal leaves in this study contrasts the results of Bergquist et al. (2007).

The significant higher Fe and Cu contents in the basal than the upper leaves in nitrogen treated and control vegetable, respectively concur with the findings of Taiz and Zeiger (2002), Hochmuth et al. (2004) and Bassey et al. (2004), who reported that these minerals in plants are generally higher in older leaves than the younger ones. Taiz and Zeiger (2002) and Hochmuth et al. (2004), attributed the higher level of these minerals in the older leaves compared with the younger leaves to their immobile nature in the plant. The low mobility of Fe in the plant is probably due to its precipitation in the older leaves as insoluble oxides or phosphates or to the formation of complexes with phytoferritin, an iron-binding protein found in the leaf and other plant parts (Oh et al., 1996; Taiz and Zeiger, 2002).

The observed higher level of K and Zn in middle than basal leaves justifies the highly mobile nature of these mineral elements (Taiz and Zeiger, 2002). Since these minerals can be mobilized readily to younger leaves, the concentration appears to be higher in the younger leaf

regions than the basal leaf position (Taiz and Zeiger, 2002; Hochmuth et al., 2004). Although, Taiz and Zeiger (2002) and Hochmuth et al. (2004) reported that Mg is a highly mobile mineral element in plants, the results obtained in this study revealed that the mineral content was significantly higher in the basal than in the upper leaves in *A. cruentus*. This observation may likely suggest that besides the degree of mobility of the mineral element that are known to influence their translocation into the different leaf positions on the plant, other factors such as cultivar and some unknown factors could equally influence the nutrient distributions in the plants. The hidden factors may be responsible for the observed variations.

Conclusions

In conclusion, in order to reduce the health problems associated with antinutrients and toxic substances in the vegetable, we recommend the consumption of younger leaves, as they had lower levels of these phytotoxins than the older leaves and can still provide enough nutrients to meet the body's requirements. By this, the nutritional potential of this commonly consumed leafy vegetable can be fully harnessed.

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