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Full Length Research Paper

Effect of soil nitrogen levels on some micronutrients, antinutrients and toxic substances in *Corchorus olitorius* grown in Minna, Nigeria

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The nature of soil type is an important factor in the bioaccumulation of substances in plants. Application of nitrogen fertilizer is a common practice in vegetable farming. This study was therefore conducted to obtain information on the effect of soil nitrogen on the levels of oxalate, nitrate, cyanide, vitamins C and β-carotene (vitamin A precursor), and some mineral elements. Pot experiments were conducted to study the effect of soil nitrogen levels on the above substances including Fe, Mg, Zn, Cu, Ca, Na and K in *Corchorus olitorius*. Leaves of the vegetable were harvested at market maturity and fruiting and subjected to chemical analysis. Results obtained showed that the applied nitrogen fertilizer significantly ($p < 0.05$) elevated the nitrate content both at market maturity and fruiting. This is of particular interest because of its public health significance, nitrate being able to lead to human cancer. The cyanide, soluble and total oxalate contents were not significantly affected. The concentration of β-carotene increased significantly ($p < 0.05$) at market maturity, but not so at fruiting. Vitamin C content was reduced significantly ($p < 0.05$) at both stages of the plant development. There was significant ($p < 0.05$) reduction in Fe content at market maturity. Copper content was increased at market maturity but decreased at fruiting. The K content decreased significantly at both stages of plant development.

Key words: *Corchorus olitorius*, nitrogen level, antinutrients, toxic substances, micronutrients, market maturity, fruiting.

INTRODUCTION

The genus *Corchorus* consists of 50 - 60 species, of which about 30 are found in Africa. *Corchorus* is mainly known for its fibre product, jute and for its leafy vegetables (Schippers, 2000). Several species of *Corchorus* are used as vegetable, of which *Corchorus olitorius* is most frequently cultivated. *C. olitorius* called Jews mallow or jute mallow in English, “ayoyo” in Hausa and “ewedu” in Yoruba is popular as vegetable in both dry or semi-arid regions and in the humid areas of Africa. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. This vegetable does well in acid, neutral and basic (alkaline) soils (Facciola, 1990). *C. olitorius* is consumed as a health vegetable in Japan, because it contains abundant carotenoids, vitamin B1, B2, C and E, and minerals. On the other hand, accidental death of cattle has occurred when the cattle were fed vegetation containing the seeds, because the seeds contain cardiac glycoside (Shinobu et al., 2000).

The dark-green leaves of *C. olitirius* have varying proportion of Ca, Fe, β-carotene, vitamin C, fibre and protein required for health (Schippers, 2000; Adebanjo and Shopeju, 1993).

The nutritional potentials of this vegetable, like other leafy vegetables stem from the rich contents of these nutrients which are needed for normal metabolic activities

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of the body. The vegetable is also a good source of dietary fibres that are important for bowel movement. However, the presence of some inherent antinutrients and toxic substances has been a major obstacle in harnessing the full nutritional benefits of the vegetable. Many factors, including the nature and composition of soil affect the bioacumulation of chemical substances in plants. It is against this background that this study was undertaken to determine the effect of soil nitrogen levels on the bioaccumulation of oxalate, nitrate, cyanide, vitamin C and ß-carotene (vitamin A precursor), and the mineral elements Fe, Mg, Zn, Cu, Ca, Na and K in *C. olitorius*.

We report here that application of nitrogen fertilizer significantly elevates the nitrate content of *C. olitorius* leaves and regular consumption of nitrogen fertilized *C. olitorius* in this environment may be deleterious to health because of the carcinogenic nature of nitrate.

MATERIALS AND METHODS

The study area

Pot experiment was carried out 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 characterised by maritime air and rainfull is between April and October. During harmattan, dry desert wind blows between November and mid February while night temperature is very low. The geographical location of Minna is longitude 90 40' N and latitude 60 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 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 Alkassoum, 1988).

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 (Juo, 1979). The soil particle sizes were analyzed as described by Juo (1979), pH was determined potentiometrically in water and 0.01M 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 1N 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 results of soil analyses are presented in Table 1

Seeds

The seeds of jute mallow (*C. olitorius*) 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 ten seeds of *C. olitorius* were planted 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 below:

F1 (control): 0 N, 30 mg P_2O_5 /kg soil and 30 mg K_2O /kg soil
F2: 30 mgN/kg soil, 30 mg P_2O_5 /kg soil and 30 mg K_2O /kg soil

Sample analysis

Both soluble and total oxalate content in the vegetable leaves were determined by titrimetric method (Oke, 1966). The nitrate content in the test samples was determined by colourimetric method (Sjoberg and Alanko, 1994). Alkaline picrate method was used to analyse the cyanide content in the test samples (Ikediobi et al., 1980). The mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in the samples were determined by using atomic absorption spectrophotometer (Alpha 4A AAS) and flame photometer (Jenway PFP7) method (Ezeonu et al., 2002), while ascorbic acid content in the samples was determined by 2, 6-dichlorophenol indophenols method (Eleri and Hughes, 1983). β-carotene content on the other hand was determined by ethanol and petroleum ether extraction method as described briefly below. 2.0 grammes of Na_2SO_4 was added to 10.0 g of vegetable leaves and ground in a mortar with pestle. The ground leaves were extracted with 100 cm^3 of hot 95% ethanol for 30 min in hot water bath. The extract obtained was filtered and measured. Water was added to the extract to bring the percentage of the ethanol extract to 85%. The 85% ethanol extract was cooled in a cold water bath for some minutes. After cooling, the ethanol extract was put inside a separating funnel and 30 cm^3 of petroleum ether was added and the mixture shaken. The separating funnel was clamped to the retort stand for some time to allow the solution to settle down into layers. The bottom layer containing ethanol was collected into the beaker while the top layer of the petroleum ether was stored in 250 cm^3 conical flask. The ethanol layer in the beaker was re-extracted twice with 10 cm^3 of petroleum ether. The ether layers from the re-extraction was added to the original petroleum extract in the conical flask and re-extracted with 50 cm^3 of 85% ethanol in order to remove any xanthophylls which may be present. The top petroleum ether layer which contained β-carotene was collected, measured and the volume noted.

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

The concentration of β-carotene was calculated thus:

$$A = E\% \times C \times l$$

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	7.60
Clay (%)		6.51
pH (in H_2O)		5.25
pH (in 0.1 M $CaCl_2$)		0.83
Organic Carbon (%)		1.43
Organic Matter (%)		0.05
Total nitrogen (%)		
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.

Where, A = absorbance of the sample; E% = extinction coefficient of β-carotene; l = path length (usually 1.0 cm).

Statistical analysis

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

Results

Physical and chemical properties of soil

Result of analyses of the soil used for pot experiment is presented in Table 1. The texture class of the soil is sandy loam. 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 (FDALR, 1985; FAO, 1984; Black, 1965).

Effect of soil nitrogen levels on antinutrients and vitamins

Cyanide level

The investigation of the effects of soil nitrogen levels on cyanide concentrations showed that application of nitrogen fertilizer had no significant effects ($p > 0.05$) on cyanide content irrespective of the stage of plant development. The mean values for controls at market maturity (663.00 ± 47.00 mg/kg) and fruiting (1325.00 ± 48.00 mg/kg) were not significantly different from the values (618.00 ± 106.00 and 1220.00 ± 142.00 mg/kg respectively) for vegetables grown on nitrogen fertilized soil (Table 2).

Nitrate level

Nitrate levels were significantly ($p < 0.05$) increased with application of nitrogen fertilizer. The mean nitrate concentrations in vegetables planted on nitrogen fertilized soils at market maturity (2717.00 ± 370.00 mg/kg) and fruiting (3500.00 ± 54.00 mg/kg) were significantly elevated as compared to level of controls (2028.00 ± 412.00 and 230.00 ± 35.00 mg/kg respectively) as shown in Table 2.

Level of oxalate

The applied nitrogen fertilizer had no significant effect on soluble and total oxalate content in both vegetative and reproductive phases of the plant. The mean soluble oxalate concentrations of control and test samples at maturity were 1.68 ± 0.14 and 2.18 ± 0.21g/100 g while at fruiting the values obtained were 6.82 ± 0.48 and 5.96 ± 0.29 g/100 g. Similarly, the mean values of total oxalate in control and nitrogen fertilized *C. olitorius* at market maturity were 3.15 ± 0.10 g/100 g and 3.20 ± 0.19 g/100 g while the values obtained at fruiting were 8.39 ± 0.54 g/100 g and 7.45 ± 0.2 g/100 g (Table 2).

Table 2. Effect of soil nitrogen levels on antinutrients and vitamin content in *Corchorus olitorius.*

Antinutrients and vitamins analysed at market maturity and fruiting stages	Nitrogen levels	
	Control (no of nitrogen applied)	Nitrogen applied
Cyanide at market maturity (mg/kg DW)	663.00 ± 47.00a	618.00 ± 106.00a
Cyanide at fruiting (mg/kg DW)	1325.00 ± 48.00a	1220.00 ± 142.00a
Nitrate at market maturity (mg/kg DW)	2028.00 ± 412.00a	2717.00 ± 370.00b
Nitrate at fruiting (mg/kg DW)	250.00 ± 35.00a	350.00 ± 54.00b
Soluble oxalate at market maturity (g/100 g DW)	1.68 ± 0.14a	2.18 ± 0.21a
Soluble oxalate at fruiting (g/100g DW)	6.82 ± 0.48a	5.96 ± 0.29a
Total oxalate at market maturity (g/100 g DW)	3.15 ± 0.10a	3.20 ± 0.19a
Total oxalate at fruiting (g/100 g DW)	8.39 ± 0.54a	7.45 ± 0.20a
β-carotene at market maturity (µg/100 g FW)	2626.00 ± 198.00a	10260.00 ± 581.00b
β-carotene at fruiting (µg/100 g FW)	10278.00 ± 1034.00a	11678.00 ± 639.00a
Vitamin C at market maturity (mg/100 g FW)	101.70 ± 7.30b	86.00 ± 8.60a
Vitamin C at fruiting (mg/100 g FW)	46.77 ± 2.7b	37.37 ± 2.30a

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).

β-carotene level

The mean β-carotene levels in control and nitrogen fertilized plants at market maturity were 2626.00 ± 198.00 µg/100 g and 10260.00 ± 581.00 µg/100 g while at fruiting the values obtained were 10278.00 ± 1034.00 µg/100 g and 11678.00 ± 639.00 µg/100 g. Data analysis showed that the applied nitrogen significantly ($p < 0.05$) elevated the provitamin content at maturity while no significant variation was recorded at fruiting (Table 2).

Level of vitamin C

Results obtained from the determination of vitamin C content showed that the applied nitrogen fertilizer significantly decreased ($p < 0.05$) the vitamin content at both market maturity and fruiting stages of the plant development. The levels of the vitamin obtained in control and nitrogen treated samples at market maturity were 101.70 ± 7.30 mg/100g and 86.00 ± 8.60 mg/100 g while the corresponding values at fruiting were 47.77 ± 2.70 mg/100 g and 37.37 ± 2.30 g/100 g (Table 2).

Effect of soil nitrogen levels on the level of mineral elements

The determination of effect of soil nitrogen levels on mineral content in control and nitrogen treated *C. olitorius* showed that nitrogen fertilizer significantly ($p < 0.05$) reduced the Fe content of plant in vegetative phase but had no significant effect on the mineral content at reproductive phase. The mean Fe concentrations in control and nitrogen treated vegetable at maturity were 17.97 ± 1.90 mg/kg and 10.29 ± 0.90 mg/kg while the corresponding values obtained at heading were 7.31 ± 1.10 mg/kg and 8.74 ± 0.86 mg/kg (Table 3).

Results from the analysis of Mg, Zn, Ca and Na levels in control and test sample revealed that the applied nitrogen fertilizer had no significant effect on the mineral contents (Table 3).

The mean Cu concentrations of control and nitrogen fertilized plants at maturity were 13.50 ± 3.80 mg/kg and 6.59 ± 0.84 mg/kg while at fruiting the values obtained were 2.00 ± 0.31 mg/kg and 6.00 ± 1.90 g/kg. The results revealed that with application of nitrogen fertilizer there is a significant ($p < 0.05$) decrease in the mineral element content at market maturity while at fruiting the applied nitrogen fertilizer significantly increased the mineral content of the vegetable (Table 3).

Similarly, the K content of the vegetable significantly increased ($p < 0.05$) with nitrogen fertilizer in both stages of the plant development. The mean K content in *C. olitorius* planted on nitrogen fertilized soils at maturity (166.89 ± 2..00 mg/kg) and fruiting (123.00 ± 9.90 mg/kg) were significantly lower as compared with the levels of controls (217.90 ± 26.00 mg/kg and 194.00 ± 7.80 mg/kg respectively) as shown in Table 3.

DISCUSSION

The observed significant elevation in nitrate level

Table 3. Effect of soil nitrogen levels on mineral content in *Corchorus olitorius*.

Mineral analysed at market maturity and fruiting stages	Nitrogen levels	
	Control (No nitrogen applied)	Nitrogen applied
Fe at Market maturity (mg/kg)	17.97 ± 1.90b	10.29 ± 0.90a
Fe at fruiting (mg/kg)	7.31 ± 1.10a	8.74 ± 0.86a
Mg at market maturity (mg/kg)	18.73 ± 0.46a	18.74 ± 0.69a
Mg at fruiting (mg/kg)	15.19 ± 0.99a	17.10 ± 0.35a
Zn at market maturity (mg/kg)	0.03 ± 0.01a	0.02 ± 0.01a
Zn at Fruiting (mg/kg)	0.02 ± 0.01a	0.04 ± 0.01a
Cu at market maturity (mg/kg)	13.50 ± 3.80b	6.59 ± 2.84a
Cu at fruiting (mg/kg)	2.00 ± 0.31a	6.00 ± 1.90b
Ca at market maturity (mg/kg)	15.41 ± 1.80a	11.15 ± 1.50a
Ca at fruiting (mg/kg)	16.97 ± 1.70a	16.65 ± 1.80a
Na at market maturity (mg/kg)	6.67 ± 0.24a	5.64 ± 0.33a
Na at fruiting (mg/kg)	4.63 ± 0.31a	4.66 ± 0.18a
K at market maturity (mg/kg)	217.90 ± 26.00b	166.89 ± 12.00a
K at fruiting (mg/kg)	194.00 ± 7.80b	123.00 ± 9.90a

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

reaffirms earlier reports in the literature (Richard, 1991; Yang, 1992; Chweya, 1993; Oladele, et al., 1997; Muramoto, 1999; Waclaw and Stefan, 2004; Kansal et al., 2005; Anjana and Muhammad, 2006; Anjana et al., 2007; Carmenet al., 2007). This is very disturbing because of the public health implications, nitrate being carcinogenic in humans. Plants require nitrogen for normal growth and for the synthesis of proteins. However if nitrogen is applied in excess of what the plant requires for protein production, the excess is accumulated as nitrates and stored predominantly in the green leafy part of the plant (Virginia, 2001). Vegetables may be classified as high or low nitrate content. *C. olitorius*, by the classification of JECFA (2003) and Anjana et al. (2007) is a high nitrate (nitrate levels of 1000-4000 mg/kg) vegetable based on the level of nitrate in the control samples. Transformation of ingested nitrate produces carcinogenic compounds. Therefore the increased nitrate levels brought about by addition of nitrogen fertilizer to the soil has serious health implications. If the vegetable must be grown on fertilizer to obtain optimum yield, then care must be taken to reduce the amount of fertilizer used. Vegetable farmers should be cautious in the application of nitrogen fertilizer to minimize the risk of ingesting nitrate to a level that could result to cancer and/or methaemoglobinaemia. Appropriate steps should be taken in the processing of harvested leaves such as boiling and then discarding the decoction resulting from the boiling before consumption. This is known to significantly reduce the levels of nitrate in vegetables to acceptable levels (Abakr and Ragaa, 1996; Waclaw and Stefan, 2004; Anjana and Muhammed, 2006; Anjana et al., 2007).

The increase in β-carotene content observed in the vegetable may probably be 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). Even though the concentration of β-carotene increases with the applied nitrogen fertilizer, the provitamin A content in the control sample can provide enough of vitamin A to meet adult recommended daily allowance (RDA). This therefore does not constitute a significant advantage that justifies the use of nitrogen fertilizer in growing *C. olitorius*.

Our results that nitrogen fertilizer had no significant effect on the levels of cyanide and oxalate of Corchorus olitorius are contrary to the findings of Richard (1991), Chweya (1993), Kriedeman (1964), Jones and Ford (1972), Peter and Birger (2002), Singh (2005), Rolinda and Ma (2008) who reported that application of nitrogen fertilizer significantly increased cyanide content and decreased the level of oxalate in vegetables. Applied nitrogen fertilizer is believed to stimulate the enzymatic conversion of tyrosine to p - hydroxymandelonitrile which ultimately leads to increase in the biosynthesis of cyanogenic glycoside (Peter and Birger, 2002). Anions have been reported to generally reduce the levels of oxalate since they compete with oxalate for cations and depress its synthesis Singh (2005). The variations observed in this study from the reports of these authors suggest that the influence of nitrogen fertilizer on cyanide and oxalate content may depend on cultivars and other environmental factors.

The significant lowering of vitamin C level in *C. olitorius* treated with nitrogen fertilizer is in harmony with the finding of Chweya (1993), Virginia (2001) and Mozafar (2005). Decrease in vitamin C content results from the increase in protein production and decreased carbohydrate formation following the nitrogen treatment since vitamin C production is tightly linked to carbohydrate metabolism Virginia (2001). From the point of view of the physiological importance of vitamin C, it is disadvantageous to add nitrogen fertilizer to the soil in the cultivation of this vegetable.

There are conflicting reports in the literature on the effect of nitrogen fertilizer on the levels of mineral elements in vegetables (Chweya, 1993; Kansal et al., (2005; Ojeniyi and Adeniyan, 1999; Tarfa et al., 2001; Safaa and Abd El Fattah, 2007). The variations arise due to differences in cultivars and environmental factors, such as season of the year, temperature, length of day, light intensity and chemical and physical properties of the soil (Singh, 2005; Takebe et al., 1995; Chweya and Nameus, 1997; Grazyna and Waldemar, 1999; Aliyu and Morufu, 2006; Lisiewska et al., 2006; Rickman et al., 2007).

Our results showed decreases in the levels of Fe, Cu and K. While the effect of nitrogen fertilizer on the levels of mineral elements may be affected by a variety of factors, the fact that in some instances, it may lead to decreases in the levels of mineral elements, as is the case in the present study, points to the overwhelming disadvantages of it use and calls for caution in its application for the cultivation of vegetables

Conclusion

Application of nitrogen fertilizer to the soil in the cultivation of *C. olitorius* increased the nitrate content of the vegetable. Levels of vitamin C and Fe, Cu, and K decreased considerably on application of nitrogen fertilizer.

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