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Effect of flowering on the concentrations of some plant toxins and micronutrients in the leaf of *Vernonia amygdalina* (Bitter leaf)

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Abstract

The developmental stages of plants generally influenced their chemical constituents and compositions as the physiology and biochemical reactions in plants vary with their developmental phases. It is against this back ground that the experiment was conducted to evaluate the influence of flowering on the concentrations of some plant toxins (cyanide, nitrate, soluble and total oxalates) and micronutrients (vitamin C, β-carotene (Provitamin A) and mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in the leaf of *Vernonia amygdalina* grown on nitrogen and non-nitrogen treated soil. The leaves of *V. amygdalina* were harvested and analysed at market maturity and flowering. The concentrations of nitrate, cyanide, β-carotene and mineral elements were determined by spectrophotometric method while the concentrations of vitamin C, soluble and total oxalates were evaluated by titrimetric method. The results showed that the concentrations of cyanide, nitrate, soluble and total oxalate, β-carotene and vitamin C increased significantly ($p < 0.05$) in the leaves of *V. amygdalina* during flowering irrespective of soil nitrogen levels, except that the elevation in β-carotene content was only significant in control. The concentrations of Fe, Mg, Ca and K decreased significantly ($p < 0.05$) in the leaves of *V. amygdalina* during the flowering; however, significant reduction in the concentration of Mg was observed only when nitrogen fertilizer was applied. The concentrations of Zn and Na were not significantly ($p > 0.05$) affected during flowering of *V. amygdalina*. The study concludes that the plant toxins are concentrated more during flowering than at at market maturity in *V. amygdalina*.

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Introduction

Vernonia amygdalina (Bitter leaf) is one of the leafy vegetables that can be used in an attempt to alleviate the problem of micronutrient malnutrition, prominent in tropical Africa (Ejoh *et al.,* 2005; Musa *et al*., 2011a). It is a perennial crop and some bushes are known to have been in continuous production for up to 7 years. The plant is up to 5m high, with abovate to oblanceolate leaves with the widest part below the middle. This species is frequently found in gardens (Schippers, 2000) and commonly found in Nigeria, Cameroon, Gabon and Congo (Democratic Republic). *V. amygdalina* is generally raised by stem cutting that are usually planted at an angle of 45° to obtain fast sprouting (Schippers, 2000). This leafy vegetable is relatively inexpensive and it is rich in several nutrients especially β-carotene and vitamin C, which are essential for human health. The vegetable also provides some minerals such as iron, phosphorus, calcium and potassium (Oshodi, 1992). Beside these nutrients, it has been reported that *V. amygdalina* accumulates some secondary metabolities such as dhurrin which is a cyanogenic glycoside, oxalate and other toxic substances which can affect human health negatively at high concentration (Oke, 1966; Anderson, 1985; Schippers, 2000; Musa *et al*., 2011a). However, the chemical compositions and content of nutrients and toxic substances in *V. amygdalina* like in other vegetables are known to be influenced by the stages of plant development. It is for this reason this study is conducted to investigate the influence of flowering on the concentrations of some plant toxins and micronutrients in the leaf of *V. amygdalina*.

Materials and methods

Study Area

Pot experiment was conducted in the nursery of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State of Nigeria.

The geographical location of Minna is at longitude 9° 40'N and latitude 6° 30'E, the city lies in the Southern Guinea Savannah zone of Nigeria and has a sub - humid semi arid tropical climate. The raining season is between April and October. About 90% of the total rainfall occurs between the month of June and September. The mean annual rainfall is in the range of 1200 – 1300 mm. The temperature of this zone rarely falls below 22°C with peaks temperature of 40°C in February/March and 30°C in November/December. Wet season average temperature is about 29°C. The Dry season occurs between November and March while harmattan which is chacterised by dry air is between November and February (Osunde and Alkassoun, 1998).

Soil Sampling and Analysis

The soil used in this study was obtained from Minna town. The soil is classified as Inceptisol (FDALR, 1985). The bulked sample was collected during the drying season from the field which has been under fallow for about four years. The bulked soil sample was passed through 2mm sieve. Sub-sample of the soil was analysed for its physicochemical properties 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. Exchange acidity (E.A H^+ and Al^{3+}) was determined by titration method (Juo, 1979). Exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1N NH_4OA solution. Sodium and potassium were determined by flame emission spectrophotometry while Mg and Ca were determined by E.D.T.A versenate titration method. Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method (Juo, 1979).

Sources of Cuttings

The stem cuttings of *V. amygdalina* were obtained from Schools of Agriculture and Agricultural Technology Experimental Farm of Federal University of Technology, Minna.

Planting, Experimental Design and Nursery Management

Two stem cuttings of *Vernonia amygdalina* were planted in a polythene bag filled with 20.00 kg of top soil and after prouting they were thinned to one plant per pot.

The factorial design was used to evaluate the effect of market maturity and flowering in control and nitrogen treated vegetable. Each treatment had 10 pots replicated three times. This gave a total of 60 pots for the vegetable. The seedlings were watered twice daily (morning and evening) using watering can and weeded regularly. The experimental area and the surroundings were kept clean to prevent harbouring of pest. The pots were lifted from time to time to prevent the roots of the plants from growing out of the container. Insects were controlled using Sherpa plus (Saro Agro Sciences) four weeks after planting at the rate of 5 ml per 5 litres of water.

Fertilizer Treatment

Two levels nitrogen fertilizer levels (F_1 and F_2) were applied for the research and were the levels recommended for *V. amygdalina* (NIHORT, 1983).

Harvesting of the Vegetable

The leaves of *V. amygdalina* in control and nitrogen treated soil were harvested at vegetative and reproductive phases of plant development and were then used for chemical analysis.

Sample analysis

Both soluble and total oxalates in the samples were determined by titrimetric method of Oke, (1966). The nitrate concentration in the test samples was determined by the colourimetric method as decribed by Sjoberg and Alanko, 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 analysed by 2, 6-dichlorophenol indophenols method of Jones and Hughes (1983). Whereas β-carotene concentration was evaluated by ethanol and petroleum ether extraction method as described by Musa *et al.* (2010).

Statistical Analysis

T-test was carried out using Minitab 17 statistical package to determine the influence of flowering on concentrations of the plant toxins and micronutrients in the leaves of *V. amygdalina*

Results

Physical and Chemical Properties of Soil

Table 1 below shows the result of physicochemical of the soil used for pot experiment.

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

Parameters	Values
Sand (%)	73.6
Silt (%)	18.5
Clay (%)	7.62
pH (in H_2O)	6.54
pH (in 0.1M C_aCI_2)	5.35
Organic Carbon (%)	0.84
Organic Matter (%)	1.33
Total nitrogen (%)	0.06
Available phosphorus (mg/kg)	6.68
K (cmol/kg)	0.82
Na (cmol/kg)	0.65
Mg (cmol/kg)	4.9
Ca (cmol/kg)	8.1
E. A (H^+ +AL^{3+})(cmol/kg)	1.51
CEC (cmol/kg)	15.92
Base saturation (%)	90.67
Texture class	sandy loam

*Values represent means of triplicate determinations.

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).

Table 2. Effect of flowering on plant toxins and vitamins concentration in *V. amygdalina*.

Plant toxins and vitamins	Stage of analysis	
	Market maturity	flowering
Cyanide (mg/kg DW), Control	349.00 ± 55.00[a]	889.00 ± 110.00[b]
Cyanide (mg/kg DW) , Nitrogen applied	430.00 ± 69.00[a]	992.00 ± 100.00[b]
Nitrate (mg/kg DW), Control	280.00 ± 39.00[a]	3482.00 ± 690.00[b]
Nitrate (mg/kg DW), Nitrogen applied	879.00 ± 207.00[a]	4305.00 ± 517.00[b]
Soluble oxalate (g/100g DW), Control	1.68 ± 0.02[a]	2.40 ± 0.10[b]
Soluble oxalate (g/100g DW), Nitrogen applied	1.89 ± 0.07[a]	2.09 ± 0.03[a]
Total oxalate (g/100g DW), Control	2.19 ± 0.08[a]	3.40 ± 0.12[b]
Total oxalate (g/100g DW), Nitrogen applied	2.35 ± 0.06[a]	3.26 ± 0.04[b]
β-carotene (μg/100g FW), Control	10897.00 ± 576.00[a]	12842.00 ± 342.00[b]
β-carotene (μg/100g FW), Nitrogen applied	13228.00 ± 472.00[a]	13313. 127 ± 520.00[a]
Vitamin C (mg/100g FW), Control	13.07 ± 1.40[a]	17.80 ± 0.69[b]
Vitamin C (mg/100g FW), Nitrogen applied	10.02 ± 0.50[a]	15.06 ± 1.20[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 ($P > 0.05$).

Effect of Flowering on Plant Toxins and Vitamins Content

The investigation of the effects of flowering on the concentrations of plant toxins and vitamins in *V. amydalina* is presented in Table 2. The results showed that the concentrations of cyanide, nitrate, total oxalate and vitamin C in the leaves of the vegetable increased significantly ($p < 0.05$) during this stage of plant development irrespective of soil nitrogen levels.

Similary, the results also showed that the soluble oxalate and β-carotene contents in the leaves of *V. amygdalina* increased significantly ($p < 0.05$) during flowering without application of nitrogen fertilizer, however, with the application of nitrogen fertilizer no significant variations in concentrations of these parameters were observed between the two stages of plant development.

Effect of Flowering on Mineral Elements Content

The result obtained from the determination of effects of flowering on mineral element contents in *V. amygdalina* is shown in Table 3. The concentrations of Fe, Ca and K in the leaves of the vegetable decreased significantly ($p < 0.05$) during flowering irrespective of the soil nitrogen levels.

Similary, analysis of Mg content in *V. amygdalina* showed that the concentration of the mineral element in the vegetable during flowering was not significantly different from that of market maturity in control, however, when the plant was treated with nitrogen fertilizer, the Mg content decreased significantly ($p < 0.05$) during flowering.

The concentrations of Zn, Cu, and Na in leaves of *V. amygdalina* were not significantly affected during flowering in both control and nitrogen applied.

Discussion

Concentrations of Plant Toxins

Significant high concentration of cyanide in *V. amydalina* during flowering compared with values at market maturity corroborates the report of of Musa (2010) and Musa *et al.* (2011a, b). The authors observed that the cyanide content in the leaves of *Telfairia occidentalis* and *Corchorus olitorius* is higher at the reproductive phase than the vegetative phase. The increase may likely be that during reproductive phase, the gene responsible for the synthesis of cyanogenic glycoside may be activated by some hormonal action connected 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; Musa 2010: Musa *et al.*, 2011a, b).

Table 3. Effect of flowering on minerals content in *V. amygdalina*.

Minerals	Stage of analysis	
	Market maturity	Flowering
Fe (mg/kg) , Control	25.40 ± 3.20^{b}	11.20 ± 1.00^{a}
Fe (mg/kg) , Nitrogen applied	27.84 ± 3.00^{b}	12.30 ± 1.80^{a}
Mg (mg/kg), Control	17.80 ± 0.40^{a}	18.30 ± 0.29^{a}
Mg (mg/kg), Nitrogen applied	18.35 ± 0.50^{b}	15.02 ± 0.32^{a}
Zn (mg/kg), Control	0.05 ± 0.02^{a}	0.04 ± 0.01^{a}
Zn (mg/kg), Nitrogen applied	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
Cu (mg/kg), Control	2.40 ± 1.00^{a}	1.36 ± 0.31^{a}
Cu (mg/kg), Nitrogen applied	1.91 ± 0.57^{a}	1.89 ± 0.60^{a}
Ca (mg/kg), Control	19.40 ± 1.90^{b}	16.60 ± 1.80^{a}
Ca (mg/kg), Nitrogen applied	19.22 ± 2.00^{b}	13.50 ± 2.10^{a}
Na (mg/kg), Control	4.64 ± 0.27^{a}	5.70 ± 1.10^{a}
Na (mg/kg), Nitrogen applied	4.28 ± 0.70^{a}	5.23 ± 0.35^{a}
K (mg/kg), Control	170.30 ± 8.00^{b}	142.60 ± 6.50^{a}
K (mg/kg), Nitrogen applied	182.30 ± 22.00^{b}	114.70 ± 6.20^{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 ($P > 0.05$).

The higher concentration of nitrate at flowering than at market maturity in *V. amygdalina* could suggest that during reproductive of the vegetable, the activity of enzyme, nitrate reductase which is responsible for the conversion of nitrate to protein may be reduced or inactive due to some physiological and biochemical changes linked with stages involved in fruits development and thereby leading to the accumulation of nitrate in the leaves of the vegetable (Musa *et al.*, 2011a; Musa *et al.*, 2011b). Noggle and Fritz (2006) observed that change in protein profile during reproductive phase in some plants may suggest that some enzymes probably disappear or become inactive during this stage of plant development (Musa, 2010; Musa *et al.*, 2011a, b).

Higher concentrations of soluble and total oxalates in the leaves of *V. amygdalina* at flowering than at market maturity corroborates the findings of (Waldemar *et al.*, 2005; Noggle and Fritz, 2006;

Musa *et al*., 2011a, b; c, d; Musa *et al*., 2012) to the effect that many substances, such as the so - called secondary plant substances (secondary metabolites) accumulate in tissues and organs during aging.

The higher concentrations of the plant toxins (cyanide, nitrate and oxalates) in the leaves of *V. amygdalina* at flowering compared to at market maturity could suggest that consumption the vegetable during flowering could delivered toxic levels of these plant toxins to the body with associated health problems. For instance cyanide causes respiratory poison; oxalate is responsible for kidney stones while nitrate is a culprit for methaemoglobineamia and cancer in man.

Concentrations of Vitamins

The increase in the concentration of β-carotene in the leaves of *V. amygdalina* during flowering in control contradict submission of Barros *et al.* (2007a, b, c, d) and Musa *et al.* (2011a) who reported that the provitamin A content decreased during reproductive in mushroom, *Lactarius piperatus* and *Telfairia occidentalis*, respectively. They putforward that the reduction in the concentration of the compound in the vegetables may be attributed to the possible translocation of some of its contents to the developing fruits and a decline in the concentration and activity of light absorbing pigments (including caroteniods) following senescence, brought about by flower formation, seed develoment and maturation (Noggle and Fritz, 2006).

The discrepancies observed in this study from the reports of these authors suggest that the influence of developmental stages on β-carotene concentration in the plants may depend on cultivars and other environmental factors. Although the concentration of provitamin A that helps to maintain good sight and prevent certain diseases of the eyes is significantly increased during flowering, its concentration at market maturity in *V. amygdalina* can provide enough of vitamin A to meet the adult recommended daily allowance (RDA). Therefore, this increase in β-carotene alone does not justify for the inclusion of this popular leaf vegetable in our meal during flowering.

The significant increase in the vitamin C concentration during flowering of *V. amygdalina* 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 concentration 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 disparity 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, 2003). The significantly higher concentration of this water soluble vitamin that has anti-infective properties, promote wound healing, acts as antioxidant and boosting of immune system at flowing does not confer much of nutritional advantage of the vitamin than at the market maturity. This is because the concentration of the vitamin C in the leaves of *V. amygdalina* at both stages of its development were lower than recommended daily allowance of 60 mg (Olaofe, 1992; George, 1999) if 100g of the leaves of the vegetable are consumed. However, considering the crucial roles of this water soluble vitamin in health and the associated diseases emanating from its deficiency, supplementation of the vitamin from other sources (such as from pharmaceutical or fruits) in either cases will be necessary.

Concentrations of Mineral Elements

The decrease in the concentration of some mineral elements (Fe, Mg, Ca and K) in *V. amygdalina* during flowering compare to higher contents at market maturity corroborates with the finding of Noggle and Fritz (2006), to the effect that during reproductive phase, 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 reproductive phase.

Conclusion

The study concludes that the concentrations of the plant toxins were elevated while the mineral elements (Fe, Mg, Ca and K) were reduced in the leaves of *V. amygdalina* during flowering than at market maturing.

Therefore keeping away from the consumption of the vegetable at flowering state of plant development will reduce the public health problems connected with high ingestion of cyanide (respiratory poison), nitrate (cancer, metheamoglobinemia) and oxalate (kidney stone) and will also improve the availability of the mineral elements and nutritional quality of the vegetable.

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