

Full Length Research Paper

Effect of reproductive phase on some micronutrients, anti-nutrients and toxic substances in *Vernonia amygdalina* grown in Minna, Niger State, Nigeria

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The chemical contents and compositions of vegetables are generally influenced during their developmental stages. It is for this reason that the research was conducted in pot experiment to investigate the effect of reproductive phase (fruiting) on anti-nutrients (soluble and total oxalates), toxic substances (cyanide and nitrate) and some micronutrients namely, vitamin C, β -carotene (provitamin A) and mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in *Vernonia amygdalina* grown on nitrogen and non-nitrogen treated soil. The leaves of *V. amygdalina* were harvested and analysed at vegetative phase (market maturity) and reproductive phase (fruiting). Results obtained showed that the levels of cyanide, nitrate, soluble and total oxalate, β -carotene and vitamin C were significantly elevated in the reproductive phase of *V. amygdalina* irrespective of soil nitrogen levels, except that the elevation in β -carotene concentration was only significant in control. The concentrations of Fe, Mg, Ca and K decreased during the reproductive phase, however, the reduction of Mg was observed only with the applied nitrogen fertilizer. The concentrations of Zn and Na were not significantly affected by the reproductive phase of *V. amygdalina*. The results conclude that phytotoxins are concentrated more at reproductive phase than at vegetative phase in *V. amygdalina*.

Key words: *Vernonia amygdalina*, anti-nutrients, toxic substances, micronutrients, vegetative phase, reproductive phase, soil nitrogen levels.

INTRODUCTION

Vernonia amygdalina is one of the leafy vegetables that have been used in an attempt to alleviate the problem of micronutrient malnutrition, prominent in tropical Africa (Ejoh et al., 2005). It is a perennial crop. The plant is up to 5 m 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 grown 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 is rich in several nutrients especially β -carotene and vitamin C, which are essential for human health. The vegetable also contains some minerals such as iron, phosphorus, calcium and potassium (Oshodi, 1992). Beside these nutrients, it has been reported that *V. amygdalina* contains some secondary metabolites such as dhurrin which is a cyanogenic glycoside, oxalate and other toxic substances which can affect health negatively at high concentration (Oke, 1966; Anderson, 1985; Schippers, 2000). However,

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Abbreviations: EA, Exchange acidity; EDTA, ethylenediaminetetraacetic acid; CEC, cation exchange capacity; RDA, recommended daily allowance.

the chemical composition 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. Against this background, this study is designed to investigate the influence of reproductive phase on the levels of micronutrients, antinutrients and toxic substances in the vegetable. This is the thrust of the work.

MATERIALS AND METHODS

The study area

The pot experiment was conducted 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 characterized by maritime. The geographical location of Minna is at 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. 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 characterized by dry air is between November and February (Osunde and Alkassoun, 1998).

Soil sampling and analysis

The soil used in this study was collected from Minna town. The soil has been 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 2 mm sieve. Sub-sample of the soil was subjected to routine soil analysis using procedure described by Juo (1979). The soil particle sizes were analyzed using hydrometer method. pH was determined potentiometrically in the water and 0.01M CaCl₂ solution in a 1: 2 soil/ liquid using a glass electrode pH meter and organic carbon by Walkley-Black method. Exchange acidity (EA) (H⁺ and Al³⁺) was determined by titration method (Juo, 1979). 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 ethylenediaminetetraacetic acid (EDTA) versenate titration method. Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method (Juo, 1979).

Sources of stem cuttings

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

Experimental design

Planting and nursery management

Two stem cuttings of *V. 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 adopted to determine the effect of vegetative phase (market maturity) and reproductive phase (fruiting) in control and nitrogen treated vegetable. Each treatment had 10 pots replicated three times. These 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. The insecticide Sherpa plus (Saro Agro Sciences) at 100 ml per 100 L of water was applied four weeks after planting to control insect infestation.

Fertilizer treatment

The fertilizer levels for vegetable are as follows:

F₁ (control): 0N, 30 mg P₂O₅/kg soil and 22 mg K₂O/kg soil;
F₂: 30 mgN/kg soil, 30 mg P₂O₅/kg soil and 22 mg K₂O/kg soil.

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 described 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 concentration in the samples was determined by 2, 6-dichlorophenol indophenols method of Eleri and Hughes (1983). While β-carotene concentration was determined by ethanol and petroleum ether extraction method as described by Musa et al. (2010).

Statistical analysis

T-test was used to determine the effect of vegetative and reproductive phases on the concentrations of the selected micronutrients and antinutrients in *V. amygdalina*.

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 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 cation exchange capacity (CEC) is moderate while base saturation percentage is high. Soil pH indicates that the soil is slightly acidic.

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 ₂ O)	6.51
pH (in 0.1 M C _a Cl 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.

Table 2. Effect of reproductive phase on antinutrients and vitamins content in *V. amygdalina*.

Anti-nutrients and vitamins	Stage of analysis	
	Vegetative phase	Reproductive phase
Cyanide (mg/kg DW), Control	358.00 ± 57.00 ^a	894.00 ± 108.00 ^b
Cyanide (mg/kg DW), nitrogen applied	426.00 ± 78.00 ^a	994.00 ± 102.00 ^b
Nitrate (mg/kg DW), control	276.00 ± 47.00 ^a	3394.00 ± 772.00 ^b
Nitrate (mg/kg DW), nitrogen applied	891.00 ± 228.00 ^a	4246.00 ± 568.00 ^b
Soluble oxalate (g/100g DW), control	1.80 ± 0.03 ^a	2.33 ± 0.11 ^b
Soluble oxalate (g/100g DW), nitrogen applied	1.97 ± 0.09 ^a	2.18 ± 0.04 ^a
Total oxalate (g/100g DW), control	2.23 ± 0.09 ^a	3.30 ± 0.14 ^b
Total oxalate (g/100g DW), nitrogen applied	2.51 ± 0.8 ^a	3.04 ± 0.06 ^b
β-carotene (μg/100g FW), control	11170.00 ± 667.00 ^a	13731.00 ± 427.00 ^b
β-carotene (μg/100g FW), nitrogen applied	14318.00 ± 582.00 ^a	14422.12 ± 610.00 ^a
Vitamin C (mg/100g FW), control	12.08 ± 1.10 ^a	14.75 ± 0.76 ^b
Vitamin C (mg/100g FW), Nitrogen applied	9.00 ± 0.70 ^a	13.08 ± 1.10 ^b

DW, dry weight; FW, fresh weight; control; no nitrogen applied. Values represent means of nine determinations. Mean values carrying the same superscripts across rows do not differ significantly from each other (P > 0.05).

Effect of reproductive phase on antinutrients and vitamins content

The investigation of the effects of reproductive phase on cyanide concentration in *V. amygdalina* showed that the cyanide content in the vegetable increased significantly during this stage of plant development irrespective of nitrogen levels. The mean values of cyanide in *V. amygdalina* at reproductive phase in control (894.00 ±

108.00 mg/kg) and nitrogen applied (994.00 ± 102.00 mg/kg) were significantly elevated compared to level at the vegetative phase (358.00 ± 57.00 mg/kg and 426.00 ± 78.00 mg/kg respectively (Table 2).

The concentration of nitrate also increased significantly during reproductive phase in control and nitrogen fertilized *V. amygdalina*. The amount of nitrate at vegetative and reproductive phases in control were 276.00 ± 47.00 mg/kg and 3394.00 ± 772.00 mg/kg while

Table 3. Effect of reproductive phase on minerals content in *Vernonia amygdalina*.

Minerals	Stage of analysis	
	Vegetative phase	Reproductive phase
Fe (mg/kg) , control	23.30 ± 4.50 ^b	10.64 ± 0.79 ^a
Fe (mg/kg) , nitrogen applied	25.84 ± 2.20 ^b	10.93 ± 1.10 ^a
Mg (mg/kg), control	18.94 ± 0.50 ^a	18.30 ± 0.29 ^a
Mg (mg/kg), nitrogen applied	20.53 ± 0.57 ^b	18.06 ± 0.50 ^a
Zn (mg/kg), control	0.06 ± 0.02 ^a	0.04 ± 0.01 ^a
Zn (mg/kg), nitrogen applied	0.03 ± 0.01 ^a	0.02 ± 0.01 ^a
Cu (mg/kg), control	3.35 ± 0.98 ^a	1.12 ± 0.42 ^a
Cu (mg/kg), nitrogen applied	2.00 ± 0.62 ^a	1.80 ± 0.54 ^a
Ca (mg/kg), control	18.58 ± 2.10 ^b	15.90 ± 2.10 ^a
Ca (mg/kg), nitrogen applied	18.53 ± 2.40 ^b	12.30 ± 1.70 ^a
Na (mg/kg), control	4.53 ± 0.39 ^a	5.52 ± 1.00 ^a
Na (mg/kg), nitrogen applied	4.37 ± 0.69 ^a	5.49 ± 0.55 ^a
K (mg/kg), control	167.50 ± 9.10 ^b	142.60 ± 6.50 ^a
K (mg/kg), nitrogen applied	174.30 ± 20.00 ^b	108.90 ± 8.30 ^a

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

the corresponding values obtained with the application of nitrogen fertilizer were 891.00 ± 228.00 mg/kg and 4246.00 ± 568.00 mg/kg (Table 2).

The mean soluble oxalate concentrations at vegetative and reproductive phases in control were 1.80 ± 0.03 g/100g and 2.33 ± 0.11 g/100g respectively, while the equivalent values obtained with the application of nitrogen fertilizer were 1.97 ± 0.09 and 2.18 ± 0.04 g/100g. Data analysis showed that reproductive phase of the plant development significantly elevated the soluble oxalate content in the vegetable in the control, however with the application of nitrogen fertilizer no significant variations in the oxalate levels was observed between the two stages of plant development (Table 2). Similarly the determination of total oxalate concentration in *V. amygdalina* showed that the level of the anti-nutrient increased significantly during the reproductive phase irrespective of the nitrogen levels. The mean values of total oxalate at reproductive phase in control (3.30 ± 0.14 g/100g) and nitrogen applied (3.04 ± 0.06 g/100g) were significantly higher compared to the levels at vegetative phase (2.23 ± 0.09 and 2.51 ± 0.08 g/100g respectively (Table 2).

The mean β -carotene concentrations at vegetative and reproductive phases in control were 11170.00 ± 667.00 and 13731.00 ± 427.00 μ g/100g while the corresponding values obtained with the application of nitrogen fertilizer 14318.33 ± 582.00 and 14422.18 ± 610.00 μ g/100g. Analysis of the data showed that the concentration of β -carotene in the vegetable increased significantly during reproductive phase in control, however with the application of nitrogen fertilizer, no significant variation was observed in the β -carotene content between the two

stages of the plant development (Table 2).

Similarly, the vitamin C concentration in the leaves of *V. amygdalina* increased significantly during reproductive phase irrespective of the soil nitrogen levels. The amount of the vitamin in the vegetable at reproductive phase for control (14.75 ± 0.70 mg/100g) and nitrogen applied (13.08 ± 0.62 mg/100g) was significantly elevated compared to level obtained at vegetative phase (12.08 ± 1.10 and 9.07 ± 0.76 mg/100g respectively); this is shown in Table 2.

Effect of reproductive phase on mineral elements content

The results obtained from the investigation of effect of reproductive phase on Fe concentration in *V. amygdalina* indicated that this phase of plant development significantly reduced the mineral content of the vegetable irrespective of the nitrogen levels. The mean values of Fe at vegetative phase for controls (23.30 ± 4.50 mg/kg) and nitrogen applied (25.84 ± 2.20 mg/kg) were significantly higher than values (10.64 ± 0.79 and 10.93 ± 1.10 mg/kg respectively) at reproductive phase (Table 3).

Analysis of Mg in *V. amygdalina* showed that the concentration of the mineral was not significantly affected by the two stages of plant development in control. However, when the plant was treated with nitrogen fertilizer, the Mg content decreased significantly during the reproductive phase. The mean values of the mineral obtained at vegetative and reproductive phases in control were 18.94 ± 0.50 and 18.30 ± 0.29 mg/kg while the corresponding values obtained when nitrogen fertilizer

was applied were 20.53 ± 0.57 and 18.06 ± 0.50 mg/kg respectively (Table 3).

The levels of Zn, Cu, and Na in *V. amygdalina* were not significantly affected by its reproductive phase in control and nitrogen applied (Table 3). However the concentration of Ca in the vegetable was significantly decreased during reproductive phase in control and nitrogen treated. The concentration of the mineral recorded at vegetative and reproductive phases of *V. amygdalina* in control were 18.58 ± 2.10 and 15.90 ± 2.10 mg/kg while the corresponding values obtained when nitrogen fertilizer was applied were 18.53 ± 2.40 and 12.30 ± 1.70 mg/kg respectively (Table 3).

Similarly, the results obtained from the analysis of K content in *V. amygdalina* also followed the same trend as found in the analysis of Ca. The mean values of K at vegetative phase for control (167.50 ± 9.10 mg/kg) and nitrogen applied (174.30 ± 20.00 mg/kg) were significantly higher ($p < 0.05$) than the values (142.60 ± 6.50 and 108.90 ± 8.30 mg/kg respectively) at reproductive phase (Table 3).

DISCUSSION

Significant increase in cyanide concentration in *V. amygdalina* during reproductive phase compared with values at vegetative phase is in agreement with the report of Cleveland and Soleri (1991). 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). The higher nitrate content at reproductive phase than at vegetative phase in *V. amygdalina* may imply that during fruits formation in the vegetables, the activity of nitrate reductase enzyme responsible for the conversion of nitrate to protein may be reduced or brought down to a halt due to some physiological and biochemical changes that are likely to be associated with fruits development and thereby resulting in the accumulation of nitrate in the vegetables. Noggle and Fritz (2006) reported that change in protein profile during fruiting in some plants may suggest that some enzymes probably disappear or become inactive during this stage of plant development.

Higher oxalates (soluble and total) content observed at reproductive phase than at vegetative phase in *V. amygdalina* 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). The significantly high levels of these phytotoxins (cyanide, nitrate and oxalates) at reproductive phase compared to their values at vegetative phase may suggest that, this vegetable is not keen to be eaten at reproductive phase as it evolve higher level of these harmful substances to scare the predators. These results are of concern because of the health problems associated with high intake of these toxic substances. For instance cyanide causes respiratory poison; oxalate is responsible for kidney stones while nitrate is a culprit for methaemoglobineamia and cancer in man.

Our results showed that, the level of β -carotene increase in *V. amygdalina* during the reproductive phase in control, is contrary to the findings of Barros et al. (2007a, b) who observed that the provitamin A concentration 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 concentration to the developing fruits and a decline in the concentration and activity of light absorbing pigments (including carotenoids) following senescence brought about by fruit formation 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. Though the concentration of provitamin A that helps to maintain good sight and prevent certain diseases of the eyes is significantly increased during the reproductive phase, its concentration at the vegetative phase in *Vernonia amygdalina* can provide enough of vitamin A to meet the adult recommended daily allowance (RDA). Thus this increase in β -carotene alone does not give a good reason for the inclusion of this popular leaf vegetable in our meal at the reproductive phase.

The significant increase in the vitamin C concentration during the reproductive phase in *V. amygdalina* though divergent 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; Singh, 2005; Aliyu and

Morufu, 2006; Weerakkody, 2006). The significantly high level of this water soluble vitamin that has anti-infective properties, promote wound healing, acts as antioxidant and boosting of immune system at reproductive phase do not have much of nutritional advantage of the vitamin than at the vegetative phase. This is because the contraction of vitamin C in *V. amygdalina* at both stages of its development were lower than recommended daily allowance of 60 mg (Olaofe, 1992; George, 1999) if 100 g of the leaves of the vegetable are consumed. Thus considering the pivotal roles of this water soluble vitamin in health and the associated diseases resulting from its deficiency supplementation of the vitamin from other sources (such as from pharmaceutical or fruits) in either cases will be necessary.

Observed significant decrease in some of mineral elements (Fe, Mg, Ca and K) in *V. amygdalina* during reproductive phase compared to higher values at vegetative phase is in line with the finding of Noggle and Fritz (2006) 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.

Conclusion

This study indicated that the concentration of anti-nutrients and toxic substances were elevated while the mineral elements (Fe, Mg, Ca and K) were reduced during the reproductive phase of *V. amygdalina*. Thus avoiding the consumption of the vegetable at reproductive phase will reduce the public health problems associated with high intake of nitrate (methemoglobinemia, cancer), oxalate (kidney stone) and cyanide (respiratory poison).

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