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Vol. 15(7), pp. 199-206, 17 February, 2016
DOI: 10.5897/AJB2015.14936
Article Number: 4770CF157195
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Ascorbic acid content in leaves of Nightshade *(Solanum spp.)* and spider plant (*Cleome gynandra*) varieties grown under different fertilizer regimes in Western Kenya

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Received 21 August, 2015; Accepted 25 January, 2016

Vitamin C is an important micronutrient because of its antioxidant and health promoting properties. With the introduction and commercialization of improved African indigenous plants, few studies have examined the impact of leaf age or the nutrient status of the plants by fertilizer. This study sought to determine amounts of vitamin C using redox titration in mature and immature leaves of spider plant (*Cleome gynandra*) and black nightshade (*Solanum ssp*) grown in fields and subjected to various sources of fertilizers which were chicken manure to provide an organic source, Mavuno fertilizer to provide a conventional synthetic source and no fertilizer to serve as a control. Chicken manure led to the highest (167 mg/100 g) vitamin C content which was however not statistically significant from Mavuno fertilizer (150 mg/100 g) at P≤ 0.05 in the nightshade variety. The highest vitamin C with no fertilizer application was 105/100 g and 79 mg/100 g in SS-49 and UG-SF varieties respectively. Moreover, vitamin C content was highest in mature leaves than in immature ones whatever the kind of fertilization treatment applied. By recognizing the impact of leaf age and importance of providing adequate fertilization, farmers can produce higher yielding and more nutritious leafy greens.

Key words: Leaf age, vitamin C, fertilizers, plant nutrition, spider plant, nightshade

INTRODUCTION

Sub-Saharan countries are endowed with a variety of nutrient dense African Indigenous vegetables (AIVs) which are traditional vegetables with edible shoots, flowers and young leaves (Oji, 2009). In Kenya the most

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consumed of them is black nightshade, spider plant, and amaranthus (Kimiywe et al., 2007). Five black nightshade species reported by Maundu et al. (1999) to be common to Kenyans include *Solanum physalifolium, Solanum scarbrum, Solanum american, Solanum nigrum and Solanum villosum.* Of the five species, *S. nigrum* is the most popular one (Ondieki et al., 2011). African Nightshade *(S. nigrum)* is a highly valued indigenous vegetable and it is consumed for its flavorful and perceived healthy benefits. For instance, black nightshade leaves are consumed to manage diabetes, high blood pressure, anaemia, peptic ulcers, colds, coughs and sight problems (Kimiywe et al., 2007; Keding et al., 2007). Spider plant (*Cleome gynandra*) also called cat whiskers, is consumed as a side dish, herb or as a tasty relish (Chweya and Mnazwa, 1997).

However, hunger, micronutrients deficiencies and children malnutrition remain perennial problems in African developing countries and this due, in part, to low vegetable consumption. Moreover, World Health Organization (2005) data reveals that fruits and vegetable consumption in Sub-Saharan Africa (SSA) is below the recommended 400 g/day and Standing Committee on Nutrition (SCN, 2010) estimate that 60% of children in the African continent suffer from iron deficiency aneamia. In Kenya, fruits and vegetable consumption stands at 85.1 kg/person/annually (Afari-Sefa et al., 2012) and nearly 1.3 million persons are food insecure (Loewenberg, 2014).

African Indigenous vegetables production and consumption can be used to alleviate these food insecurity and micronutrient deficiencies (Mavengahama, 2013). In fact, some wild AIVs are capable of thriving during harsh climatic conditions while some food crops are incapable and AIVs also mature early, usually within 3 to 4 weeks and can be used to fight hunger before food crops maturation (Mavengahama, 2013). However, AIVs consumption declined in Kenya with the introduction of exotic vegetables that are less bitter hence preferred by younger generations. There is also shortage of knowledge regarding the nutritional importance of AIVs and the perception that AIVs are inferior compared to other foods. However, they, especially African nightshade and spider plant, contain micronutrients such as iron, protein, vitamin C, carotene, magnesium, calcium, fiber, flavonoids, terpenoids and phenols (Mibei et al., 2012; Yang and Keding, 2009). These micronutrients are essential for plant growth and human being, especially vitamin C which are synthesized only by plant but useful for human organism.

Indeed, vitamin C, also called ascorbic acid has many vital roles in many processes in the human body. It is a cofactor in many enzymatic reactions, acts as an antioxidant, maintains the flexibility of blood vessels improves blood circulation and facilitates iron absorption in the human body (Shokunbi et al., 2011; Lee and Kader, 2000).

These important roles played by ascorbate have necessitated the need to preserve and increase the amounts of the vitamin in fruits and vegetables. In fact, there are many factors that affect vitamin C in fruits and vegetables. Among them include oxygen, temperature and water (Mhina and Lyimo, 2013). So, ascorbic acid is easily oxidized in the presence of oxygen and moisture to dehydro ascorbic acid and further to diketogluconic acid which is irreversible and inactive (Kiremire et al., 2010). Prolonged exposure of ascorbic acid to high temperatures also converts it to diketogluconic acid which is physiologically inactive (Kiremire et al., 2010). Other factors that lead to vitamin C loss are trimming, extended storage time, chilling injury and physical damage (Lee and Kader, 2000).

To increase vitamin C in leafy vegetables, horticulturalists have resorted to use of both inorganic and organic fertilizers. For example, Gendy et al. (2012), in their study, used cattle manure and biofertilizers application to increase vitamin C amount and compared fresh yields of vegetables to when no fertilizers were used. Similarly, application of chicken manure (60 t/h) increased vegetable heights, market yield and leaf number (Masarirambi et al., 2012a). In another study, analysis of chicken manure revealed that it was higher in potassium and phosphorous than cattle manure and led to increased plant yield compared to inorganic/ chemical fertilizers (Masarirambi et al., 2012b). Nonetheless, increasing the overall amount of vitamin C by application of fertilizers does not reveal the vitamin's accumulation levels in the leaves. The study sought to establish whether different fertilizer sources impacts vitamin C accumulation in the edible leaves of nightshade and spider plant.

MATERIALS AND METHODS

Study area description

Field experiments were conducted at the University of Eldoret Agricultural Research Farm. The farm is located 9 km from Eldoret. The altitude of University of Eldoret is 2120 m above sea level while the latitude is 0° 34'N and the longitude is 35° 18'E. Eldoret receives an annual rainfall between 900 to 1300 mm. The mean range of temperature is 10 to 25°C in July and January respectively (Jaetzold and Schmidt, 1983). Soil fertility is low with a pH below 5.5. The soil class of Eldoret town is rhodic Ferralsols (FAO, 1994).

Experimental designs

Soil pH was determined as before and after planting using a procedure by Okalebo et al. (2002). The soil pH was adjusted to 5.5 from 4.90 before planting by liming. Lime was applied at the rate of 200 g per 8 m plot. Land was cultivated and soil prepared to fine tilth for direct sowing and then raised 10 cm above the ground. Spider plant (Figure 1) and nightshade (Figure 2) were planted using a spacing of 60 cm and 45 cm on 19th of December 2013 and harvested manually on 10th February 2014 at 9.00 am. Field experiments were conducted as a randomized complete block design with three replications.

Seeds were planted through direct seeding method. The land was

Figure 1. (a) Mature and (b) immature leaves used for vitamin C analysis in fields grown at the University of Eldoret, Eldoret, and western Kenya.

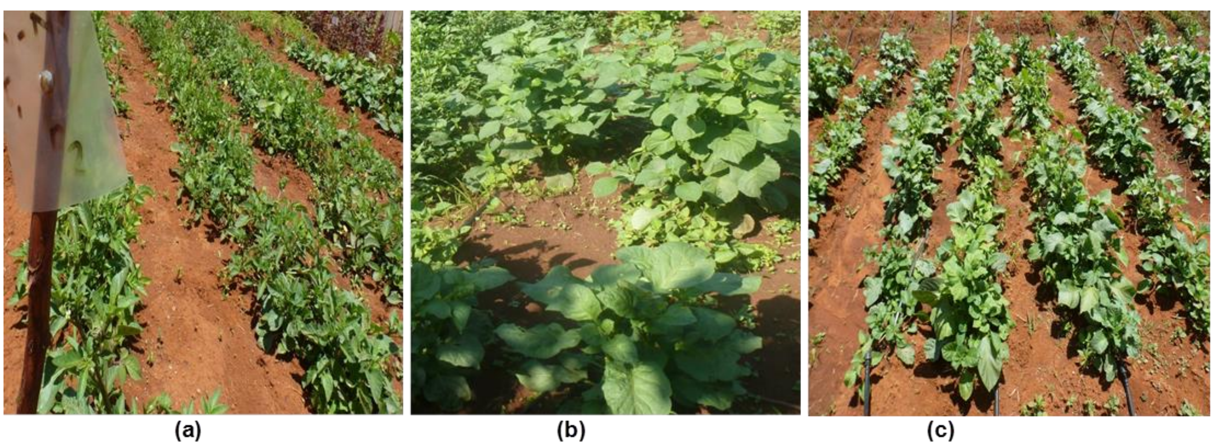

Figure 2. Pictures of three nightshade varieties. **(a)** Nightshade (local variety). **(b)** BG-16 variety. **(c)** SS- 49 variety grown at the University of Eldoret, Eldoret, and western Kenya.

subdivided into small plots of 4 m × 2 m in the main blocks. The subplots were separated by a margin of 0.5 m and 1 m within replicate blocks. Each block was separated by 1.5 m. Three varieties of black night shade (Figure 3) and spider plant species were planted using Mavuno fertilizer (a commercial fertilizer in Kenya), chicken manure and a control where (no fertilizer/manure was applied) at the rates of 0.4 g, 4.2 kg and 0.0 kg per 8 m^2 plot, respectively. Mavuno fertilizers contains 11 essential plant nutrients: primary nutrients are nitrogen (N), phosphrous (P), potassium (K); and other micronutrients such as calcium (Ca), sulphur (S), magnesium (Mg), zinc (Zn), copper (Cu), molybdenum (Mo), boron (Bo) and manganese (Mn). Plants that did not germinate were replanted after two weeks and weeding and harvesting was done manually. The experimental plots had drip irrigation installed to prevent the plants from suffering from water stress. The first harvesting was done 7 weeks and 4 days after planting on the 10th February 2014 at 9.00 am. Second harvesting was done at the pre-flowering stage 9 weeks after planting for spider plant and 10 weeks 4 days for Nightshade between 8.00 am and 9.00 am. Tissues from the second harvest were used for vitamin C analysis. Harvesting analysis was done at the pre-flowering stage during the second harvest. Harvesting was done by uprooting the whole plant above the soil surface. Ten to 15 plants were sampled in each plot to make a composite sample by uprooting the whole plant and

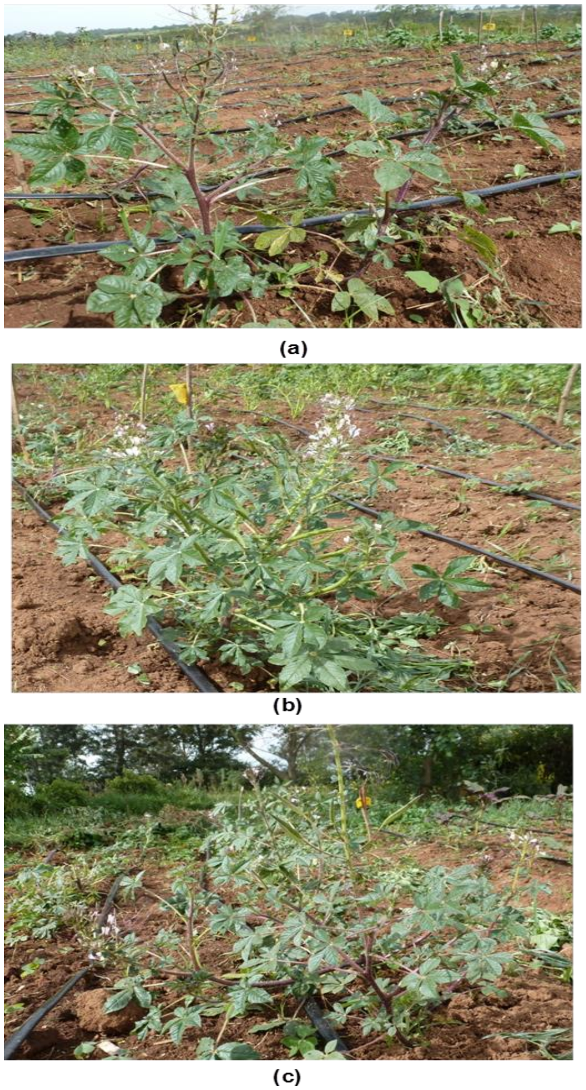

Figure 3. Pictures showing various photos of spider plant. **(a)** UG-SF-17. **(b)** local variety. **(c)** ML-SF-29 used in fields grown at the University of Eldoret, Eldoret, and western Kenya.

keeping in a cool box before being transported to the Chemistry Laboratory at the University of Eldoret. While in the lab, the petioles of the leaves were harvested manually from the plant stem. The dark green leaves located in the branch position were grouped as mature while the light green leaves from the apex were considered immature leaves (Figure 4).

The experimental model is, $y_{ij} = \mu + T_i + \beta_j + \varepsilon_{ij}$.

Where μ= population mean, T_i is the effect due to treatments (varieties and fertilizers), β_j= is the effect due replicates and ε_{ij} is the error that results from treatments and replicate interaction, y_{ij}= is vitamin C concentration in the leaves.

Vitamin C analysis

Vitamin C was analyzed as described by College of Science (2011) at the University of Canterbury. First, 100 g of fresh leaves were manually ground using mortar and pestle. The ground vegetable pulp was then strained in cheese cloth to obtain the vegetable juice. The extracted sample was diluted with distilled water and 0.2 g of potassium iodide was carefully weighed into a beaker of 100 ml. Next, 1.3 g of iodine was added into the same beaker and swirled to dissolve the iodine solution where it was then transferred into in to a one litre volumetric flask. This solution was then made to a litre using distilled water. Next, 20 ml of the sample solution was pipetted into a 250 ml conical flask and 150 ml of distilled water was added to the conical flask. Finally, 2 drops of starch solution previously made was added into the flask. Titration was done using 0.005 moles per litre solution of potassium iodine until a dark blue black color of starch-iodine complex was formed. Leaves from each plot were analyzed in triplicates and vitamin C results were expressed in mg/100 g.

$$I_2 \longrightarrow 2I^- + \text{dehydro ascorbic acid}$$

Data analysis

Data analysis was done using Portable SAS version 9.3. The effects of different fertilizer application on vitamin C were done at $P \leq 0.05$ using the general linear model (GLM) procedure. Treatments means of vitamin C were separated by least significance difference (*lsd*) at $P \leq 0.05$ and the interactions of varieties and fertilizers were generated in ANOVA.

RESULTS

Mean vitamin C of three nightshades and spider plant varieties under different fertilizers are presented in Table 1. In general, leaves of both nightshade and spider plant from control plots which received no fertilizer application had significantly ($P \leq 0.05$) lower vitamin C content compared to those from plots which received either chicken manure or mavuno fertilizer. Local night shade variety had the least vitamin C content compared to two improved varieties regardless of the type of fertilizer applied. This can be attributed to the inherent genetic differences.

Table 2 presents vitamin C amounts in spider plant varieties. Unlike in nightshade, there were no significant varietal differences in vitamin C contents in all the three spider plant varieties tested.

In all varieties of nightshade plant studied, mature leaves had significantly (($P \leq 0.05$) higher vitamin C than immature leaves (Figure 5).

Similarly, Vitamin C concentration in the three spider plant varieties was also found to be significantly higher in the mature leaves than in immature ones (Figure 6). The vitamin C content in immature leaves of ML-SF-29 was slightly lower compared to UG-SF-17 and local night shade variety. Mature leaves had approximately one and a half times more vitamin C than immature leaves.

DISCUSSION

Application of the organic fertilizer source, chicken

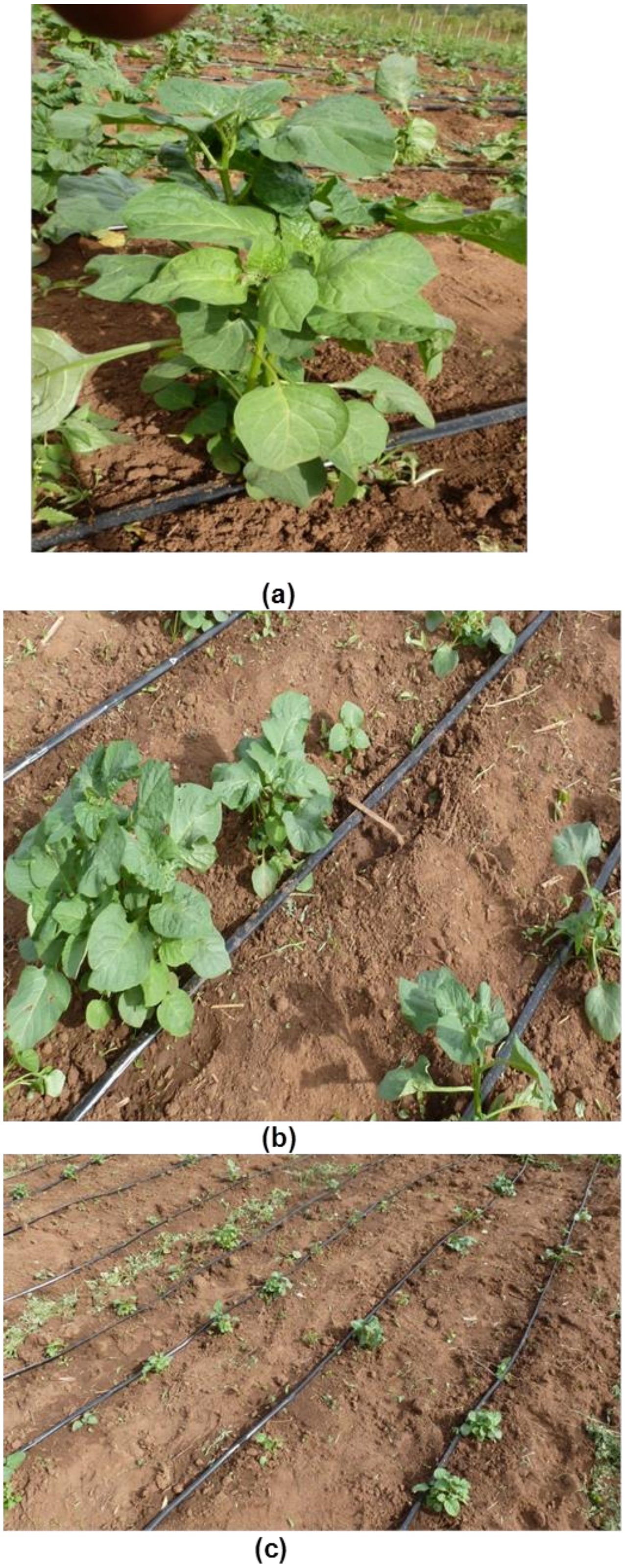

Figure 4. Pictures of black nightshade plants- BG-16 grown (a) with chicken manure (b) with mavuno fertilizer and (c) without fertilizer grown under inorganic and organic fertilizer sources at the University of Eldoret Research Farm, Western Kenya.

manure led to higher vitamin C content in the leaves but was not statistically different from Mavuno fertilizer in most of the varieties studied. Plants grown with chicken manure were observed to have bigger leaves and higher vigour than those grown under Mavuno fertilizer and in plots that had no fertilizer. This can be attributed to higher nutrient density of chicken manure and its ability to hold more water and nutrients as well as serve as a slower release of nutrients. Also, the soil pH was amended to about 5.5 from 4.9. At soil pH of 5.5 nutrient uptake for plant growth is enhanced. Application of either Mavuno fertilizer or chicken manure application led to increase vitamin C in the harvested plant tissues. In a separate study, Gendy et al. (2012) also found that application of cattle manure and biofertilizers at 30 m^3 per fed increased vitamin C amounts in Roselle (hibiscus) plants. Improved root development from the application of manure was reported to have increased nutrient uptake from soil for plant growth and development (Gendy et al., 2012).

From this study, the increased vitamin C in the three nightshade and spider plant varieties can be attributed to interaction effects between variety. Materechera and Seeiso (2012) have also reported that interactions of fertilizers and plants increased plant height unlike where no manure/fertilizer application is done.

The RDA for lactating and pregnant mothers in the USA is 85 and 120 mg respectively (National Institute of Health, 2013). Further, the RDA for an adult female and male above 19 years is 75 and 90 mg respectively. Results from this study suggest that consumption of adequate amounts of improved varieties of nightshade and spider plant has the potential to meet vitamin C for all the age groups because they can provide up to 167 and 136 mg respectively. These leafy vegetables can meet vitamin C demands for such age groups.

Another significant finding of this study is that the amount of vitamin C was higher in mature than immature leaves. This is because the immature leaves are physiologically active than mature ones. The young leaves have more demand for vitamin C and cannot accumulate enough vitamin C to meet their physiological processes. In contrast, mature leaves have higher capability to synthesize ascorbic acid but their utilization rates are lower. This difference in requirement between the mature and immature leaves for ascorbate is the reason for ascorbic acid translocation from the source of manufacture to the immature leaves that have high demand for the vitamin. Nutrients are always stored in the mature leaves and are translocated to the young ones for growth and development. Young leaves have not formed storage organs for nutrients storage. Consequently, nutrient translocation is greater in young than in old leaves. In the mature leaves, vitamin C exists in its active form but is converted into the inactive dehydroascorbic acid (DHAA) in immature leaves, a process that is reversible. Furthermore, Lee and Kader (2000) found out that ascorbic acid oxidase is found in

Table 1. Fertilizer effects on vitamin C (mg/100 g) content of nightshade plant varieties, field grown at the University of Eldoret Research Farm, Eldoret, Kenya.

Fertilizer	Nightshade varieties		
	SS-49	***BG-16***	***LV-NS[1]***
No fertilizer (F0)	105.3[d]	102.0[d]	96.3[d]
Mavuno fertilizer (F1)	150.0[b]	149.7[b]	120.67[c]
Chicken manure (F2)	160.3[ab]	167.0[a]	125.0[c]

*R^2=0.94, C.V= 6.23; LV-NS[1] = refers to local variety of nightshade; BG-16 = refers to improved varieties of nightshade; SS-49 = refers to improved varieties of nightshade. Means with the same letter within the same vegetable species do not differ at P≤ 0.05.

Table 2. Fertilizer effects on vitamin C (mg/100 g) content of spider plant varieties, field grown at the University of Eldoret Research Farm, Eldoret, Kenya.

Fertilizer	Spider plant varieties		
	UG-SF	**ML-SF-29**	**LV-SP[1]**
No fertilizer (F0)	79.7[c]	65.7[c]	68.6[c]
Mavuno fertilizer (F1)	125.7[b]	119.7[b]	135.0[ab]
Chicken manure (F2)	136.0[a]	130.7[b]	134.3[b]

*R^2=0.86; C.V=12.25; LV-SP[1]= refers to local variety of spider plant; UG-SF = refers to improved spider plant variety; ML-SF-29 = improved spider plant variety. Means with the same letter within the same vegetable species do not differ at P≤ 0.05.

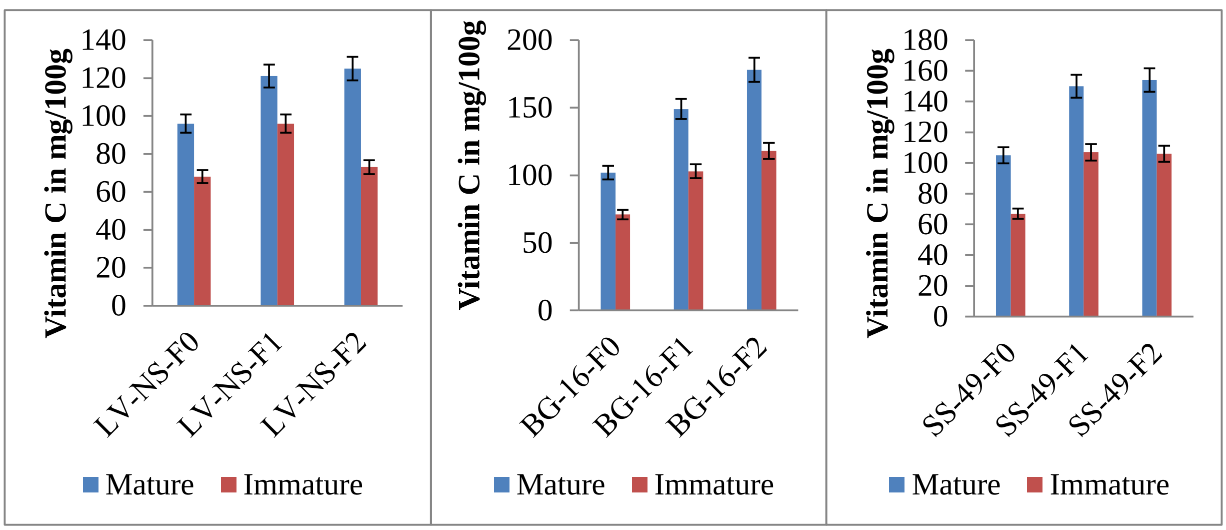

Figure 5. Vitamin C concentration in mature and immature leaves of the three night shade varieties in fields grown at the University of Eldoret Research Farm, Eldoret, western Kenya. F0 = refers to where no fertilizer/manure was used; F1 = mavuno fertilizer; F3 = chicken manure; LV-NS = local variety of nightshade; BG-16 = refers to improved varieties of nightshade; SS-49 = refers to improved varieties of nightshade.

plant parts that are growing rapidly. Ascorbic acid oxidase (AAO) oxidizes vitamin C to dehydroascorbic acid. This is also a possible reason why there was more vitamin C in the mature than immature leaves. Franceschi and Tarlyn (2002) also reported that mature leaves of a plant have a higher capability to synthesize ascorbic acid than flowers and shoot tips. They estimated that mature leaves could easily make vitamin C from its GAL-L precursor than immature ones. These findings are in agreements with that of Rosli et al. (2013) where he

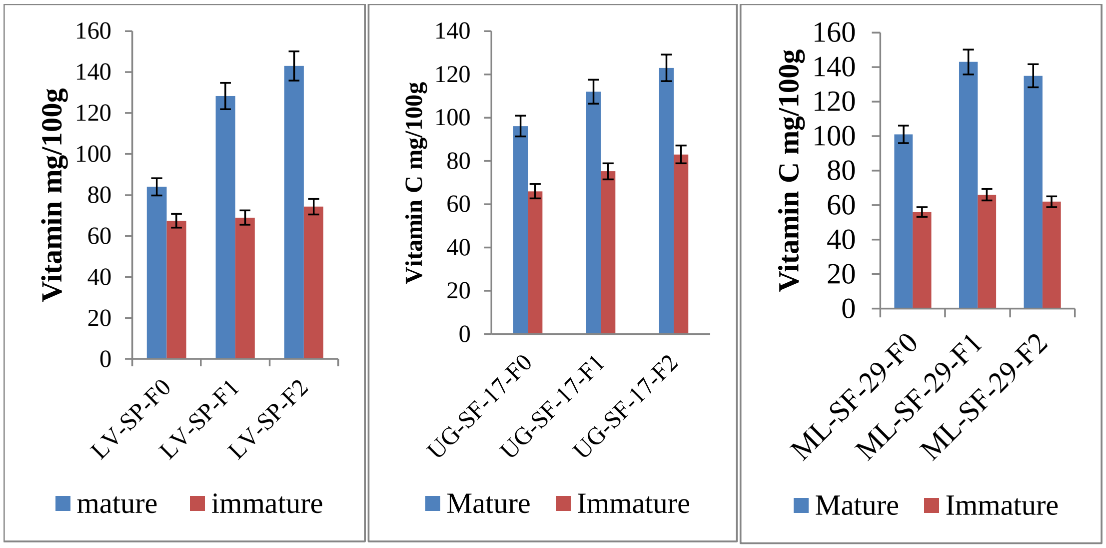

Figure 6. Vitamin C concentration in mature and immature leaves of three spider plant varieties in fields grown at the University of Eldoret, Eldoret, western Kenya. LV-SP= refers to local variety of spider plant; F0 = no fertilizer/manure application; F1 = mavuno fertilizer; F2 = chicken manure; ML-SF-29 = refers to improved spider plant variety; UG-SF-17 = refers to improved variety of spider plant.

observed that mature leaves had more vitamin C than immature ones. However, young leaves have better acceptability and eating quality. In order to optimize vitamin C intake, consumers should thus be sensitized to consume mature leaves so as to benefit more from the leaves. This information is relevant for capacity building purposes for both the producers and consumers so that they are both aware of critical harvesting stages, and the various parts targeted for harvesting in order to maximize nutrient intake from the vegetables. This can help prevent the common harvesting practice of uprooting young plants, particularly spider plant just a few weeks after emergence for consumption even before they form branches.

There was no difference in vitamin C in immature leaves attributed to either inorganic or organic fertilizers. This may be due to similarity in physiological functions of immature leaves across the varieties. Kipkosgei et al. (2003) demonstrated that regardless of the type fertilizer used to increase yield, vitamin C content does not vary in immature leaves.

Conclusions and recommendations

Black nightshade and spider plant are important components of diets of most households in Kenya. Therefore, it is good to increase nutrient composition of these vegetables as an attempt to expand intake of nutrient from the vegetables. The major findings of this study can therefore be summarized as: (1) the common AIVs, namely nightshade and spider plant can contribute to almost the entire recommended daily amounts of vitamin C to all age groups, including pregnant and lactating women, as long as other factors such as postharvest handling, storage and cooking methods are carefully selected to minimize losses; (2) improved varieties of nightshade used in this study are more superior than their local counterparts in terms of vitamin C content; (3) improving soil fertility is key to enhancing vitamin C content of these vegetables. As much as spider plant and black nightshade are good sources of ascorbic acid, vitamin C amounts depend on vegetable maturity, fertilizer application and post-harvest handling procedures. Therefore care needs to be taken during and after harvest, and during preparation to ensure maximum retention of vitamin C is attained. Further studies need to be done on how fertilizer application influences other vitamins and antioxidants in plant tissues. Farmers should also be sensitized on appropriate harvesting practices that target plant parts with higher vitamin C amounts.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The research was funded by Horticulture Innovation Lab led by the University of California – Davis with funding from USAID to Purdue University, Rutgers University and University of Eldoret in support of African Indigenous Vegetables.

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