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Effect of organic and in organic fertilisers on yield and quality of amaranth in sub-Saharan Africa

C.M. ONYANGO[1], J. HARBINSON[2], S.I. SHIBAIRO[1] & J.K. IMUNGI[3]
[1]Plant Science and Crop protection department, Faculty of Agriculture, University of Nairobi, P. O. Box 29053,00625, Nairobi, Kenya
[2]Horticultural Supply Chains Group, Department of Plant Sciences - Wageningen University, Droevendaalsesteeg 1, P. O. Box 630, 6700 AP, Wageningen, The Netherlands
[3]Food Science, Nutrition and Technology Department, Faculty of Agriculture, University of Nairobi, P. O. Box 29053,00625, Nairobi, Kenya
Corresponding author: cecilia.onyango@uonbi.ac.ke

Abstract This study was designed to evaluate the influence of manure and mineral fertilization on yield, Kjeldahl nitrogen (as protein equivalents), Vitamin C and nitrate accumulation in *Amaranthus hypochondriacus.* The vegetables were produced in field trials, set up in the University of Nairobi, Kenya, during the long rains period between March and May in 2007 and 2008 using diammonium phosphate (DAP; 20, 40 and 60 kg N ha^{-1}) and manure; 40 kg N kg ha^{-1},) and the yield, Kjeldahl nitrogen (K-N; a proxy for protein content), vitamin C and nitrate levels were measured. The manure treatment produced little increase in productivity though it increased K-N levels. The DAP treatments produced increases in yield, but these saturated at 40 kg N ha^{-1}. The K-N content of the leaves was high, ranging from 2.5 % to just over 4 %, and, broadly, increased with fertiliser application though the variation between the treatments was lower than that for yields. Nitrate levels, however, also increased with DAP fertilisation, resulting in an increase in the nitrate:K-N at high DAP applications, especially in younger plants. There were no clear correlations between ascorbic acid content and K-N. Nonetheless, the plants grown under different fertilisation regimes can be placed into three broad groups; those characterised with low to moderate K-N contents and low to high ascorbic acid contents, moderate to high K-N and low to high ascorbic acid content, and lastly high K-N and low ascorbic contents. Therefore, the growth environment of amaranth, can have a major impact not only on the productivity of the crop but its nutritional value. In this respect the mineral nutrient supply to the crop is of particular importance.

Key words: Ascorbic acid, diamonium, phosphate, vitamin C

Introduction

The success in cultivation of amaranth (*A. hybridus, A. hypochondriacus, A. rividis and A. cruentus*) can be attributed to its genetic and physiological flexibility, which allows for adaptation to a wide range of environments, while individual genotypes tolerate a broad range of climatic conditions. In addition, their competitive ability permits cultivation with the minimum of management (Campbell & Abbot, 1982). Like other traditional African leafy vegetables, amaranths grow quickly and thus can be harvested relatively soon after sowing (6 - 8 weeks) (Chweya & Eyzaguirre, 1999). They are one of the most important traditional vegetables in many developing countries, grown mainly for home consumption and to a lesser extent for retail sale (Chweya & Eyzaguirre, 1999; Rajabu *et al.,* 2000). Unlike other traditional vegetables, both the leaves and grains of amaranths are consumed.

Though an adequate and balanced diet is essential for health, for the majority of people in developing and underdeveloped countries, obtaining a balanced diet is hampered by poverty and the insufficient supply of nutritious foods (Negi & Roy, 2003). This problem can be partly solved by increasing the consumption of locally available foods because these are inexpensive and can be highly nutritious. Leafy vegetables are an excellent source of protein, vitamins and minerals, and dietary fibre (Orech *et al.,* 2005) and being familiar and inexpensive, these can be used by large segments of the population to meet essential dietary requirements. These vegetables are one of the most cost-effective and sustainable solutions to counter micronutrient deficiencies, which affect far more people than hunger alone and are widespread in most of sub-Saharan Africa (Prabhu & Barrett, 2009).

Vegetable yields can be increased with proper fertilsation with nitrogen and other mineral nutrients (Turan & Sevimli, 2005) and several studies have investigated the influence of fertilisation with nitrogen on yield and some quality aspects of vegetables (Sorensen *et al.,* 1995; Elia *et al.,* 1998; Æustiæ *et al.,* 2000). The nitrogen status of leaves is an important determinant of their photosynthetic capacity and, therefore, their productivity; nitrogen is a basic component of proteins, and the protein and rubisco content of leaves is strongly correlated with the maximum photosynthetic capacity of the leaves (Evans, 1996). Consequently, it is expected that a well fertilised leafy vegetable crop will be both of high productivity and be more nutritious because of the increased leaf protein content (Suresh *et al.,* 1996). This simple model is complicated, however, by the finite capacity of leaves to convert inorganic N to protein and plants supplied with an excess of nitrate-N accumulate nitrate in their vacuoles (Martinoia *et al.*, 1981). So, though increasing soil N leads to increased yields and the total N content (as protein) of the crop (Evans, 1996), it comes with the penalty of the risk of unacceptable nitrate concentrations.

Differences in nutritional value of vegetables and fruits grown with differing levels of nitrogen fertilisation have been found (Weston & Barth, 1997) Not in reference. Lisiewska & Kmiecik (1996) reported that increasing the amount of nitrogen fertiliser from 8 to 120 kg N ha^{-1} decreased the vitamin C content in cauliflower curd. Similarly, Tedone (2005) working with potato, found that the use of nitrogen fertiliser resulted in a reduction of ascorbic acid levels in the tubers. In addition to its effects on ascorbic acid, nitrogen fertilisation increases in the beta carotene content of vegetables (Flores *et al.*, 2004; Mozafar, 1993). In spite of the importance of nitrogenous fertilisers in increasing the productivity of leafy vegetables and the importance of leafy vegetable as sources of both ascorbic acid and beta carotene, the impact of nitrogenous fertilisers on the levels of ascorbic acid and protein as well as the nitrate content of vegetable amaranth is not well known. The objective of this study was to evaluate the effect of organic and in organic fertiliser N on the yield and quality of Amaranth.

Materials and Methods

Amaranth seeds were obtained from Asian Vegetables Research and Development Centre (AVRDC) in Tanzania. The vegetables were produced in field trials, set up in the University of Nairobi Field Station at the Upper Kabete Campus, during the long rains period between March and May in 2007 and 2008. Kabete is situated about 15 Km to the West of Nairobi city and lies at 1^o 152 S and 36^o 442 E, and at altitude 1930 m above sea level (Sombroek *et al.*, 1982). Not in reference. It has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December (Taylor & Lawes, 1971). The mean annual temperature is 18 ^oC. The soil in Kabete is characterised as a deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996). The land used for the plots had not received fertilisers during the previous year, but pigeon pea (*Cajanus cajan*), followed by chickpea (*Cicer arietinum*) and then grass for hay had been grown on the plots.

The seeds were sown in a seedbed fertilised with either manure or diammonium phosphate (DAP; 18:46:0). The trials were laid out in a complete randomised block design, with five fertilisation treatments as follows: one plot with cattle manure at 40 kg N ha^{-1} (calculated using the content of N found in the manure; approximately 2 tonnes of manure per hectare, three plots with DAP (18:46:0, NPK) at 20, 40, and 60 kg N ha^{-1} and unfertilised control plot. The treatments were replicated three folds. The plots measured 2 m x 2 m with a spacing of 0.15 m x 0.1 m. Both the DAP fertiliser and manure were applied at planting (seeding) by hand mixing with the soil in the planting hole and the seeds embedded in this soil mixture. Four seeds were planted in each hole, but two weeks after germination, the seedlings were thinned to one per hole. Irrigation was used whenever rainfall was insufficient to keep the soil moist. The vegetables were harvested at three maturity stages; 6, 7 and 8 weeks after planting by cutting-off the tender edible stems and leaves.

The percentage reduced nitrogen content was determined by the micro-Kjeldahl method (AOAC, 1999). This method of analysis discriminated between reduced N and nitrate N (NO_3^-)**.** Nitrate levels were determined on weighed leaf samples that were dried in a thermostatically controlled air-oven at 60^{o}C and weighed before being ground to pass through 600 ìm mesh sieve. For determination of nitrates, about 0.1g of the vegetable powder was weighed and suspended in 10 ml distilled water. The suspension was incubated at 45^o C for 1 hour to allow complete leaching of the nitrate, and then filtered through Whatman No. 41 filter paper. The concentration of the nitrate-N in the filtrate was determined by the method of Cataldo *et al.* (1975).

For the determination of vitamin C (ascorbic acid), The vegetables were freeze-dried, then ground to pass through a 600 µm sieve. The powder was stored in tightly closed plastic vials in a freezer (-20^{o}C) prior to their analysis for ascorbic acid. All the analyses were done in triplicate samples. Determination of ascorbic acid was by the method of Helsper *et al.* (2003). A weighed sample of approximately 15 mg of freeze dried, powdered leaf was placed into a 2.2 ml eppendorf tube to which 2 ml of 5 % metaphosphoric acid (HPO_3) (prepared each day) was added. The tubes were closed, vortexed then placed in an ultrasonic bath for 15 minutes at ambient temperature. They were vortexed again, and then centrifuged at 8,000 rpm for 5 minutes at 4 ^{o}C. One millimetre of the supernatant was then taken and analyzed for RAA using HPLC (Column: LiChrosper 100 RP-18 (5 ìm), Detection: UV 260 nm, Flow: 0.5 ml min^{-1}, column temperature: 30 ^{o}C). The concentration of ascorbic acid in samples was determined from a standard curve prepared from known concentrations of pure ascorbic acid (Sigma-Aldrich, USA) in aqueous solution.

Data were subjected to the general analysis of variance (ANOVA), using GenStat statistical software (Payne *et al.*, 2006). Not in Refs. Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P \leq 0.05$).

Results

Leaf yield. The use of manure at a rate equivalent to 40 kg N ha^{-1} did not result in a significant increase in yield (fresh weight basis). In contrast to manure, the use of the inorganic fertiliser increases in yield per hectare (Fig. 1). The yields increased steadily from week 6 to week 8, with the highest yield being recorded 8 weeks after planting in plots that received 40 kg N ha^{-1} of DAP. Notably, the effect of the fertilisation on growth was saturated by application of 20 kg N ha^{-1} of DAP at the first harvest. At the second harvest (week 7) yield increased for all DAP dosages, but the differences between the dosages were not significant. It was only by the last harvest (week 8) that differences arose between the DAP treatments and higher though similar yields of the 40 and 60 kg N ha^{-1} treatments on the one hand and the lower yield 20 kg N ha^{-1} treatment on the other.

The Kjeldahl nitrogen content. The Kjeldahl nitrogen (amino-nitrogen) content of the leaves was influenced by the time of harvest and the fertiliser treatment. With increasing time to harvests all treatments showed a trend of decreasing leaf Kjeldahl-N (hereafter K-N) contents, expressed on a dry weight basis. (Fig. 2).

Ascorbic acid content (Vitamin C). Ascorbic acid contents were overall very variable among treatments. The relationship between ascorbic acid content and K-N were not significant. Nonetheless, the plants grown under different fertilisation regimes can be placed into three broad groups; group A (control and manured plants) is characterised with low to moderate K-N contents and low to high ascorbic acid contents, group B (20 and 40 kg N ha^{-1} DAP fertilised plants) contains moderate to high K-N and low to high ascorbic acid content, while group C (60 kg N ha^{-1} DAP fertilised plants) are characterised by high K-N and low ascorbic contents (Fig. 3).

Nitrate content. There was an interaction between fertiliser application, time of harvesting and nitrate content. Fertiliser treatment and time of harvesting had a significant ($P<0.05$) effect on the nitrate content of the vegetables (expressed on a dry weight basis). The nitrate levels were highest for each treatment at week 6, and decreased

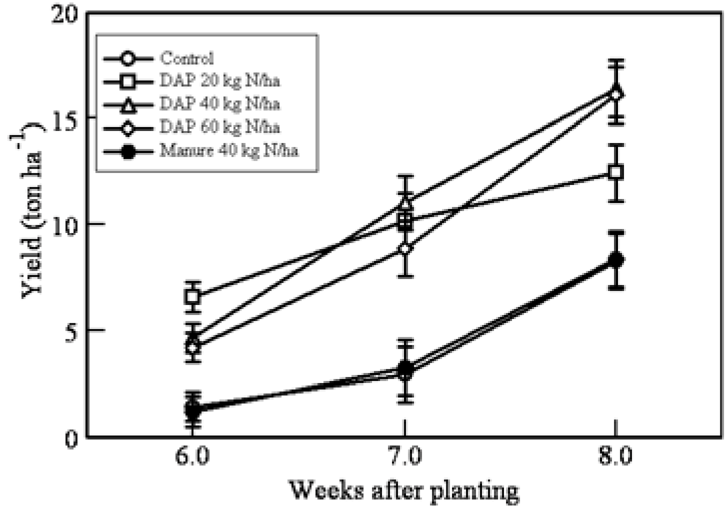

Figure 1. Effect of time of harvesting, chemical fertiliser DAP and cattle manure on leaf yield on fresh weight basis of vegetable amaranth (*A. hypochondriacus*).

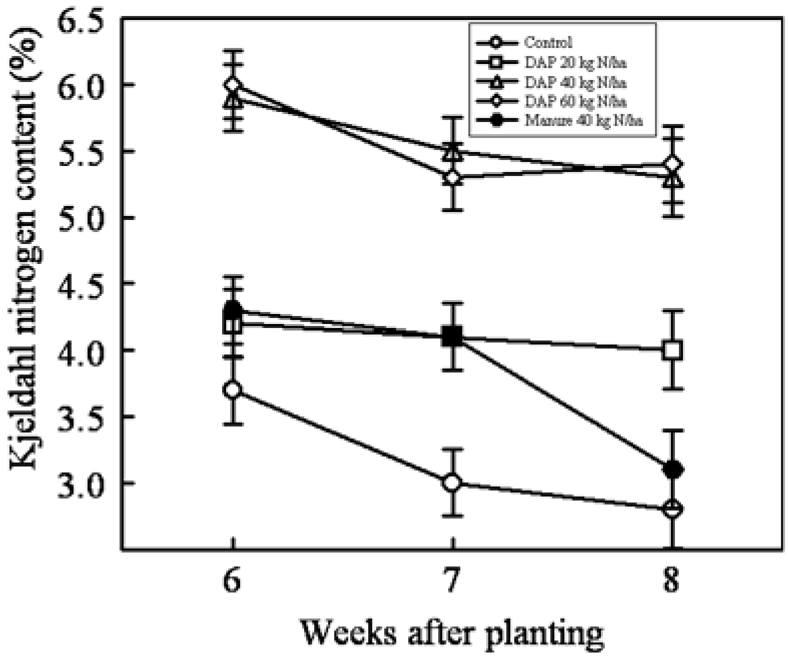

Figure 2. Effect of time of harvesting, chemical fertiliser DAP and cattle manure on percent nitrogen content on dry matter basis of vegetable amaranth (*A. hypochondriacus*).

thereafter. The levels were lowest for the control and manured plots. With DAP fertilisation, levels were higher and increased with increasing application rate of DAP.

Discussion

The growth and yield of amaranth was strongly influenced by soil fertility when an inorganic fertiliser, but not by manure. In common with many plants, nitrogen supply is known to have a considerable effect on the yield of amaranth (Makus, 1986). The results obtained in this study show that, though yields responded to supply of mineral N, the response was not proportional to the supply of N, with the response depending on N supply and harvest date. Working with grain amaranth and using 0, 15, 30, 45 and 60 kg N ha^{-1}, Olaniyi *et al.* (2008) found that fresh and dry shoot yields and grain yield, were significantly influenced by N rates. Although the highest fresh shoot yield increased up to the maximum rate of 60 kg N ha^{-1}, there was no significant difference between the values obtained at 45 and 60 kg N ha^{-1}, which is similar to our experience with vegetable amaranth. The lack of any yield increase in the manured plots was not unprecedented. Adediran *et al.* (2004) working with maize (*Zea mays*) and cow pea (*Vigna unguiculata*), found out that use of manure did not result in significant yield gains.

The nitrogen content of the leaves is important in two major respects; the reduced (amino) K-N content is nutritionally important as this is largely made up of protein, whereas the oxidised (nitrate) nitrogen content is important because of its toxicity. The increase in foliar K-N or protein with increasing N fertilisation under high irradiance conditions is consistent with numerous published reports (Evans & Terashima 1988; Terashima & Evans, 1988; Evans, 1996; Makino *et al.*, 1997).

Though the K-N contents of the control and manured plants were lower than that of the DAP fertilised plants, the decrease was proportionately much less than that of

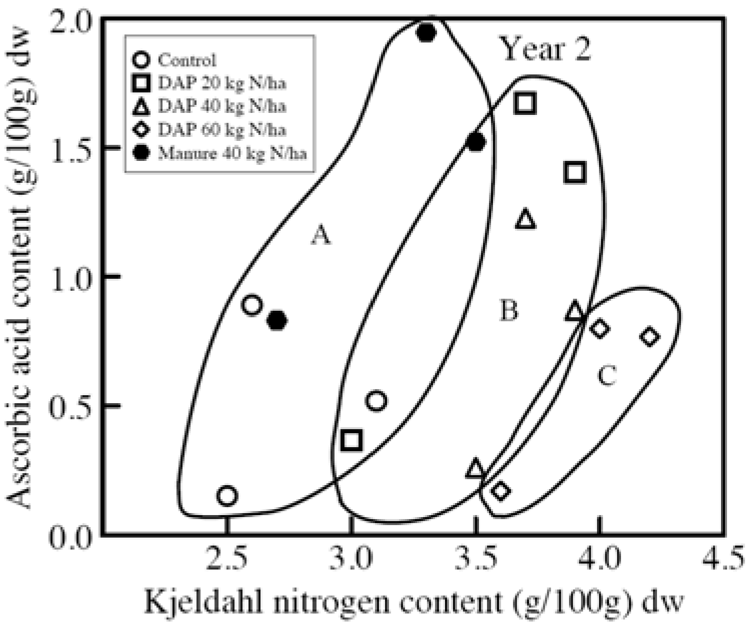

Figure 3. Effect of DAP, manure and time of harvesting on both ascorbic acid and Kjeldahl nitrogen content of vegetable amaranth (*A. hypochondriacus*).

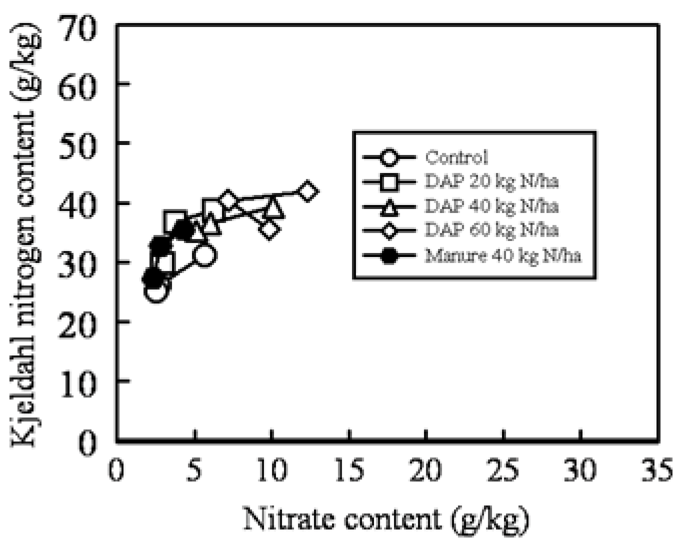

Figure 4. Effect of DAP, manure and time of harvesting on both Kjeldahl nitrogen and nitrate content of vegetable amaranth (*A. hypochondriacus*).

yield. A beneficial effect of the manure treatment, despite its low yield, was the generally higher K-N content of the manured plants compared to the controls. An interesting consequence of these relationships for the consumer is that, even if amaranth is grown on fallow land, the K-N content of its foliage is still appreciable compared to that from fertilised land, so arguably the K-N content of fresh amaranth might always be expected to lie within a limited range of values. The K-N content of leaves of the related *Amaranthus palmeri* grown under natural conditions was observed to decrease from about 4.5 % to just over 2.0% during a prolonged drought stress that ultimately stopped photosynthesis (Ehleringer, 1983). If *A. hypochondriacus* were to behave in a similar way, it would suggest that even drought-stressed plants would retain significant, if diminished, nutritional value.

A consequence of nitrogenous fertilisation is that, if supplied in excess some of the N taken up, will accumulate as nitrate in the vacuoles instead of being converted to amino-nitrogen (Martinoia *et al.,* 1981; Demsar *et al.,* 2004). This accumulated foliar nitrate poses a health risk and is often subject to regulation. Therefore, the benefits of increased yield and increased protein content of leafy vegetables arising from nitrogenous fertilisation need to be balanced against the risk of excessive nitrate contents.

A comparison of the K-N content with the nitrate content of the leaves (Fig. 4) suggests that up to about 35 g kg^{-1} (3.5%) K-N nitrate does not accumulate excessively, and the balance of K-N and nitrate favours K-N. Above 35 g kg^{-1} K-N though further increases of K-N can still occur (they increase to nearly 60 g kg^{-1} K-N), this only occurs with the accumulation of large amounts of nitrate.

Conclusion

The rapidly rising costs of chemical fertilisers often force small scale vegetable farmers to look for alternatives such as manure. The results of this work, however, fail to justify the use of manure over chemical fertiliser due to low yields and low K-N, although the use of manure results in low levels of nitrates and high levels of ascorbic acid (vitamin C). In this study, manure was being used for the first time on the plots and it is possible that its rate of decomposition was insufficient to release N rapidly enough to meet the needs of a fast growing plant such as amaranth. It is possible the repeated use of manure on the same plot as is the case with most small scale farmers would result in improved yields, as might other improvements in land management. Clearly, more research is needed to verify this.

The use of artificial fertiliser results in significant increases in fresh leaf yield and K-N content of the leaves, but the use of these fertilisers can result in the substantial accumulation of nitrate and a significant reduction of ascorbic acid. Improvements in the amount and timing of fertiliser application might help avoid nitrate problems but as with improving the effectiveness of manure, this would require more guidance to the producers.

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