Propositions (Stellingen)

1. If amaranths are given excess easily available N, they would accumulate more nitrates in the leaves rather than use the excess N for photosynthesis (*This thesis*).
2. Even if largely unlisted as an important crop, amaranth can be used to alleviate major problems of underdeveloped and developing countries i.e. hunger, poverty and disease (*This thesis*).
3. When you empower one small woman farmer in Sub-Saharan Africa you have empowered a community.
4. Solutions to problems facing developing countries lie within their geographical boundaries.
5. Exotic can be attractive, but for the health and well being of the people indigenous might be the key.
6. He who marries a woman only for her beauty has ignored a major part of what makes a woman.

Propositions accompanying the PhD thesis **‘Preharvest and postharvest factors affecting yield and nutrient contents of vegetable amaranth (var. *Amaranthus hypochondriacus*)’**

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Wageningen, 29th April 2010

Preharvest and Postharvest Factors Affecting Yield and Nutrient Contents of Vegetable Amaranth (Var. *Amaranthus hypochondriacus*)

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Preharvest and Postharvest Factors Affecting Yield and Nutrient Contents of Vegetable Amaranth (Var. *Amaranthus hypochondriacus*)

Cecilia Moraa Onyango

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Introduction

Recurring economic and political crises, serious environmental problems and widespread insecurity about the future in various parts of the world have increased the gap between the rich and poor between and also within countries (WHO, 2004). Approximately 1.2 billion rural people live on less than U.S.$ 1 per day (the universally accepted poverty line). Expenditure on food accounts for a much larger proportion of family income in developing countries than in developed countries (Caballero, 2005). Globalization of food markets has resulted in the introduction of mass-produced, low cost foods to the domestic food supply of many developing countries. A developing country is one that is poor and whose citizens are mostly agricultural workers but that wants to become more advanced socially and economically (Thesaurus). The World Bank considers all countries with Gross National Income per capita below US$11,905 as developing (World Bank, 2009). Many of these low cost foods are energy-dense and in most cases nutrient poor (Gardner and Halweil, 2000) leading to malnutrition problems in these countries. In addition, agriculture in developing countries faces a number of pressing challenges, including prolonged droughts, population growth and widespread poverty. World population is projected to grow from 6.1 billion today to 7.9 billion in 2025 (U.N., 2000) and most of this population growth will take place in cities of developing countries, whose population is expected to double to 3.4 billion by 2025. Widespread poverty persists, contributing to severe malnutrition and food insecurity. Also alarming is that an 18% rise in the number of malnourished children is projected in Africa by 2020 (IFPRI, 2001). Both poverty and food insecurity are major development problems for economic development in Kenya and most other developing countries. About 56% of Kenyans live below the poverty line and about 50% of the population lacks access to adequate food (GoK, 2004). Moreover, the little food that is available is of poor nutritional value and quality (GoK, 2004). The country persistently suffers from transitory and chronic food insecurity mainly due to drought and floods, which lead to crop failures and over-reliance on one staple food, maize. Diets that are heavily based on cereal grains lack sufficient amounts of essential nutrients such as proteins, minerals (e.g. iron and zinc) and vitamins (Sands et al, 2009). Thus, the overwhelming reliance on cereal grains intensifies malnutrition which is estimated to cause 50% of child deaths in developing countries (Smith et al, 2000). Such malnutrition prevents much of the world’s population from reaching their full potential as human beings; mentally, physically and financially (U.N., 2000). It also contributes to higher rates of deaths caused by heart disease, stroke and cancer (Khaw, 2001).

It is estimated that 20% of people in developing countries suffer from chronic undernourishment (FAO, 1996a) and over 2 billion persons suffer from deficiencies of micronutrients (Essential dietary elements that are needed only in very small quantities e.g copper, zinc, selenium, iodine, magnesium and iron) in their diets (Gardner and Halweil, 2000). About 250 million preschool children suffer from vitamin A deficiency. Vitamin A deficiency is the leading cause of preventable blindness in children and deficiency increases the risk of disease and death from severe infections (Sommer, 2008). This essential vitamin is especially deficient in Africa and Southeast Asia. The dietary sources of vitamin A are preformed vitamin A (commonly found in meats and dairy products) and provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) found in yellow and orange fleshed fruits and vegetables, and in dark-green leafy vegetables (Jaarsveld et al, 2005, cited by Sands et al, 2009). The preformed vitamin A sources are not affordable to the majority of people living in developing countries.

The poverty, food insecurity and malnutrition problems facing most developing countries can be remedied in great measure by increasing the production and consumption of traditional leafy vegetables (TLVs). Commercial production of TLVs can serve as a useful tool for poverty reduction in Sub-Saharan Africa and Southeast Asia (IPGRI, 2003) and the money generated from the sale of these vegetables can be used to attain food security. These vegetables are the most affordable and sustainable dietary sources of vitamins, minerals and other bioactive compounds. Traditional vegetables might not be indigenous to a country, but they can be associated with traditional production systems, local knowledge and usually have a long history of local selection and usage (Keller et al, 2004; IPGRI, 2005). The TLVs have been reported to be particularly rich in provitamin A (β-carotene) and vitamin C, crude proteins, fibre and the minerals potassium, phosphorus, calcium, iron and zinc (Akindahunsi and Salawu, 2005; Orech et al, 2005). These vegetables also contain phytochemicals such as phenolic compounds (Imungi, 2002) (including flavonoids) which possess strong antioxidant properties, and isothiocyanates (group of glucosinolates)(Wattenberg 1977; Cao, et al, 1997; Kähkönen et al, 1999) and have been implicated in the prevention and suppression of diseases such as cancer, arteriosclerosis, aging (Hertog et al, 1992) and currently the management of human immuno-deficiency virus (HIV/AIDS) (Robbins, 2003; Nair et al, 2009). Studies have shown that the best antioxidant effect from ascorbic acid, β-carotene and the phenolics accrues from eating fruits and vegetables and not from the use of dietary supplements (Holick et al, 2002).

Communities in developing countries have in the past depended greatly on the large diversity of traditional crops for food and as a source of nutrition. These foods were, however, edged out of many diets, especially of the urban populations by the introduction of exotic substitutes. However, due to spirited campaigns by governmental, non-governmental organizations and private individuals on the superiority of traditional foods in terms of nutrition, health and taste, they are slowly creeping back into diets (Mwangi and Kimathi, 2006). Of the traditional foods, leafy vegetables such as *Amaranthus spp., Solanum nigrum*, *Gynandropsis gynandra*, *Vigna unguiculata* and *Corchorus olitorius* have successfully been re-introduced into the diets of most of the people in developing countries of Sub-Saharan Africa and Asia (IPGRI, 2003). In the past, traditional vegetables were available for sale only in the open air markets and were purchased largely by members of lower socio-economic groups. More recently, however, these vegetables have started to appear in the modern supermarkets and green-grocer stores where families from the higher socio-economic classes do their shopping, as has been shown in Kenya (Onyango and Imungi, 2007). This is a sure sign that these vegetables are forming part of the diets of those higher socio-economic classes and can no longer be regarded as being only for the poor. In the modern supermarkets, the vegetables sell competitively along side their exotic counterparts like cabbage and spinach (Onyango et al, 2008). As a consequence, traditional vegetables have started to play an increasingly important role in income generation, especially among small women farmers (Ngugi et al, 2006; Onyango and Imungi, 2007). The cash earned contributes significantly to household food security, access to family health care and enables women to attain a degree of financial independence within the family budget (IITA, 2003; Lewis, 1997; IPGRI, 2003). A developmental organization – Farm Concern International - has been assisting small scale farmers in East Africa transform themselves from subsistence farmers into Business Support Units that can produce the vegetables on sustainable basis and ensure consistency in supply to the markets (Ngugi et al, 2006). However, very little research has been done to authenticate the advice on how to profitably produce TLVs.

Although traditional leafy vegetables (TLVs) have been an integral part of agricultural systems in Africa, most countries have not given them priority in crop development. They have not featured significantly on the research agenda of either local or international agricultural organizations, which have often tended to focus on research on highly commercialized crops. However, producing more of these commercialized food crops is not a panacea for the malnutrition and food problems of Africa (Ngugi et al, 2006) and Asia as their nutrient content is low (Gardner and Halweil, 2000). Traditional vegetables on the other hand offer higher levels of nutrients, are easier and cheaper to grow (Watt and Merril, 1975) and possess more familiar tastes to the communities than their exotic counterparts (Akindahunsi and Salawu, 2005; Orech et al, 2005). Consequently, improvement of traditional crops such as amaranths through research and development could produce an easy and cost-effective way of eliminating malnutrition and promoting the people’s health as well as attaining food security. Opportunities exist in Kenya as well as other developing countries to use traditional leafy vegetables (TLVs) to expand the local food base, improve the health of the population, enhance food security and generate income.

In addition to their high nutritional value, however, many traditional African leafy vegetables also accumulate high levels of anti-nutritional factors such as oxalates and nitrates. Many plant species have a tendency to accumulate nitrate-nitrogen in their vegetative parts. This is so especially with the members of the Brassicaceae (Cruciferae) and Chenopodiceae families (Mengel and Kirkby, 1979; Kunsch et al, 1983; Salisbury and Ross, 1992). Accumulation of nitrates occurs if the rate of nitrate translocation from root to shoot is faster than the assimilation into the shoot. While, nitrate itself has not been shown to produce a carcinogenic effect in humans, nitrates can be converted to nitrites by bacteria in human gastro-intestinal tract. Nitrites chemically react with secondary amino compounds present in the food to produce compounds called nitrosamines which have been found to elicit carcinogenesis.

Oxalates also occur in plant materials at relatively high levels, mainly as soluble sodium and potassium salts or as insoluble calcium oxalate. Oxalate contents are high in some leafy vegetables such as *Basella alba* and Amaranths (Schmidt et al, 1971; Dhan and Pal, 1991; Gupta et al, 2005). Oxalic acid contents of <1.5% in *Brassica oleracea* var. *acephala,* 2.8% in *Ipomea aquatica* Forsk, 2.9% in *Amaranthus cruentus* and 10.6% in *Basella alba* on a dry weight basis have been reported by Schmidt et al (1971). Working with *Amaranthus spp,* Ricardo (1993) reported oxalic acid contents ranging from 1.1 % to 7.9% dry weight and 1.8mg/100g fresh weight basis were reported by Imungi (1990). Higher levels of between 8% and 10% dry weight basis have been reported by Radek and Savage (2008) working with *Amaranthus cruentus* and *Amaranthus viridis* respectively. When ingested in the soluble form, oxalic acid is known to reduce absorption of dietary calcium by precipitating calcium as insoluble calcium oxalate. Its presence in large quantities can, therefore result in calcium deficiency even when the diet is otherwise sufficient in calcium. This situation is more critical in infants and children who are experiencing active formation of bones and teeth, and in older persons who can suffer calcium resorption from bones leading to osteoporosis (Harper et al, 1979).

The levels of nutrients as well as antinutrients in fruits and vegetables is known to be influenced by factors such as genotype, environmental growing conditions, growth stage and postharvest handling and storage (Gad et al, 1982; Goldman et al, 1999). Horticultural practices and harvesting crops at the optimal stage of maturity are obvious strategies for optimizing the nutritional quality of plants (Goldman et al, 1999). In addition, postharvest processing of plant tissues has been used to enhance nutritional quality by either the release of nutrients that may otherwise be bound by plant tissues or by elimination of toxins and anti-nutrients (Sands et al, 2009).

Preharvest factors affecting yield and quality of vegetables

It has been reported that both plant and environmental factors affect the yield and nutritional quality of vegetables. Among the plant factors, cultivar and stage of maturity of the plant play a crucial role. Vegetable quality and size can vary between individuals in a population and among cultivars while the timing of harvest can have a great impact on the quantity and quality of produce. For example, Bergquist et al (2005) reported high vitamin C content in ‘baby’ spinach harvested at an earlier stage but a lower yield at harvest. Similarly, small cabbage heads have been shown to be higher in ascorbic acid concentration than large heads (Weston and Barth, 1997). Water availability, and the mineral and organic nutrients of the soil have been shown to have marked effects on the phenolic contents of plants (Barberan-Tomas and Espin, 2001). Phenolic compounds are ubiquitous in plants and are biologically important as a diverse group of secondary chemical metabolites (Lattanzio et al, 2008). In plants, phenolic compounds function as physiologically active compounds, as stress protecting agents (Feucht et al, 1997), as pollination attractants or as feeding deterrents and in general by their significant role in imparting plant resistance to pests (Treutter, 2006). As antioxidants, phenolic compounds act as free radical scavengers and act to prevent diseases which follow free radical mechanism in humans (Wargovich, 2000; Scalbert et al, 2005; Janisch et al, 2006; Stevenson and Hurst, 2007). The beneficial role that phenolics play in both plants and people who consume them has stimulated interest in the regulation of their biosynthesis in plants, especially the role of the plant's environment in this regulation. The environmental factors that impact on accumulation of nutrients/anti-nutrients in plants, thereby influencing nutritional quality of the vegetables are altitude, soil quality, soil pH and salinity, production practices, insect injury and plant diseases. These environmental factors influence the water and nutrient supply to the plant (Goldman et al, 1999) which in turn influence the yield and quality of the material produced. These factors also affect the composition and sensory quality (appearance, texture, taste and aroma) of harvested plant parts (Goldman et al, 1999). For instance intensive cropping, which involves use of high levels of chemical fertilizers, while achieving high levels of production, may not yield the most nutritious food, mainly due to the accumulation of anti-nutrients such as nitrates and oxalates (Goldman et al, 1999).

Of the many factors that affect yield and nutrient quality of vegetables, nitrogen (N) nutrition and maturity at harvest have been found to have the greatest influence on yield and the contents of most nutrients in leafy vegetables (Khader, 2002).

Nitrogen nutrition in plants

Nitrogen (N) has a high influence on plant growth and development (Yoldas et al, 2008) and is an essential component of amino acids, proteins, nucleic acids and many enzymes. It is the key nutrient which promotes vegetative growth of a plant (Huffaker and Rains, 1978). Adequate levels of soil N can result in improved quality of vegetables, generally by allowing for the development of sufficient photosynthetic surface area in higher plants (Weston and Barth, 1997). Plants grown under limited N levels have diminished photosynthetic capacity and if the deficiency is severe enough it leads to depleted leaf chlorophyll levels resulting in stunted growth and ultimately leaf chlorosis (Marschner, 1995). Increased additions of N usually result in an increased yield of vegetables (Greenwood et al, 1980; Szwonek, 1986; Hochmuth et al, 1999). However, toxicity from excessively high N concentrations is possible (Lefsrud et al, 2007). As an example, severe yield depression was reported when cabbage (*Brassica oleracea* L. var. *Capitata*) was grown at elevated N rates (602 mg N L^{-1}; Huett, 1989). In addition, the concentration of nitrate-nitrogen in lettuce and spinach leaves has been shown to be dependent on the dose of applied nitrogen fertilizer. For example, a considerable increase of nitrates in lettuce and spinach was observed when N fertilizer application was increased from 260 to 280 kg Nha^{-1} (Szwonek, 1986). N fertilizers are also known to increase the concentration of nitrates (NO_3) in leaves of most plants (Khader, 2002).

N fertilizer has been shown to influence the nutrient types and levels in vegetables and fruits grown at various levels of N. For instance increasing the amount of N fertilizer from 8 to 120kg ha^{-1}

decreased the vitamin C content in cauliflower (Lisiewska and Kmiecik, 1996). Similarly, Weston and Barth (1997) reported that N fertilization increases β-carotene in vegetables. Based on these reports, it is important to determine the effect of source of N on the yield, nutrients/anti-nutrients and phytochemicals in TLVs.

The two main sources of N used in the production of leafy vegetables are the inorganic chemical fertilizers and organic fertilizers or manure. Proponents of organic agriculture often claim that organically produced plant foods promote health of humans more than products from conventional production systems (Brandt and Molgaard, 2001). However, these claims have not yet been sufficiently validated. A major difference between organic and conventional farming is in the use of fertilizers. Conventional farming allows the use of synthetic fertilizers as well as manure, compost, sewerage sludge and other soil amendments (Bordeleau et al, 2002). Most certified organic farming only allows the use of manure and compost and other soil additives such as bone and blood meal.

Maturity at harvest

Maturity is one of the major factors that determine the compositional quality of fruits and vegetables. Nutrient contents of vegetables differ not only between species or cultivars but are also affected by stage of harvesting (Olsson et al, 2004). In addition, leaf contents vary during plant growth (Yoo et al, 2003). Bergquist et al, (2007) working with ‘baby’ spinach found that the content of vitamin C decreased significantly from 2½ weeks to 3½ weeks after planting while the total carotenoid content significantly increased 3½ weeks after planting. In another study, Giri et al (1984) reported increase in the contents of phosphorus, calcium, magnesium, sodium, iron, copper and manganese and decrease in potassium with the age of the *Chekurmeni* plant (*Chekkurmenis*). A number of TLVs are consumed at different stages of maturity (Khader and Rama, 2003) but limited information is available on their nutrient content at these stages. The socio-economic conditions of households determine the growth stages at which traditional leafy vegetables are harvested, with low socio-economic groups harvesting or consuming the vegetables at more immature stages than the high socio-economic groups. This is because the poor socio-economic groups depend entirely on them as a vegetable source and have very few alternatives if any. The effect of age of plants on the concentration of various nutrients and phytochemical in TLVS consumed in Africa has not been adequately investigated.

Postharvest factors affecting quality of fresh vegetables

As with the pre-harvest factors, the quality of products after harvest is determined by both plant and environmental factors. Maturity at harvest influences the vegetable quality and the development of physical injury arising from handling during harvest and subsequent sorting, cleaning, packaging, transportation and storage. Similarly, environmental factors such as soil type, temperature and weather at harvest can have adverse effects on storage life and quality. Management practices can also affect postharvest quality. For instance, produce that has been stressed by too much or too little watering, high rates of N or has been physically damaged is particularly susceptible to postharvest diseases (Andersen, 1998).

Generally, quality cannot be improved after harvest, but can only be maintained. Delays between harvest and consumption or processing of vegetables can result in direct losses due to water loss, respiration and decay, and indirect losses such as those of flavor and nutritional quality (Kays, 1991). These losses result in a short shelf-life for vegetables whose market value is mainly determined by their physical appearance. Shelf life is the recommended time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected (or specified) conditions of distribution, storage and display (Gyesley, 1991). For leafy vegetables, shelf life is charged by physical appearance, and wilting, discoloration and decay all reduce the shelf life of leafy vegetables. Catabolic processes such as respiration characteristically happen more quickly at higher temperatures. Chemical reactions double their rate for each temperature increase of 10^{o}C because activation energy barriers become more easily surmounted at higher temperatures (Kays, 1991). This is the reason shelf life for leafy vegetables is generally extended by temperature control, for example by refrigeration, insulated shipping containers and a controlled cold chain.

Processing and preparation methods further reduce the composition, including the nutritional value of vegetables. For instance, water soluble vitamins such as the B and C vitamins leach into the cooking water at high rates during cooking and are therefore lost if the cooking water is discarded as is the case with TLVs cooking method. Fat-soluble compounds such as carotenoids may be stabilized or made more available by cooking (Parker-Pope, 2008). The manner in which the quality of TLVs is influenced postharvest by these factors has not yet been adequately documented.

The effects of temperature

Temperature is usually the most important environmental factor limiting shelf life of fresh fruits, vegetables and herbs (Watada and Qi, 1999). Deterioration of fresh commodities can result from physiological breakdown due to natural ripening processes, water loss, temperature injury, physical damage, or invasion by microorganisms. All of these factors can interact, and all are influenced by temperature. Water loss for example from warm products is particularly serious under windy conditions or during transport in an open vehicle (Bartsch and Kline, 1992).

Fresh fruits, vegetables, and flowers are highly perishable because they are alive. They are very vulnerable to moisture loss which may detract from their appearance, saleable weight, and nutritional quality (Kader, 1992; Wills et al, 1998). Stored plant reserves (e.g. starch) are lost via respiration, which means less nutritional value and loss of flavor (Kader, 1992). The respiration rates of commodities are directly related to product temperature; the higher the temperature, the higher the respiration rate.

The respiration rate of a product strongly determines its postharvest life. The higher the storage temperature the higher will be the respiration and transpiration rates and the greater the transit losses (rotting and wilting) leading to a shorter postharvest life of the vegetables. Zepplin and Elvehjein (1944, cited by Lee and Khader, 2000) found that leafy vegetables held at 6°C lost 10% of their ascorbic acid content in 6 days, while those held at room temperature lost 20% in only 2 days. Losses in vitamin C in kale were accelerated at higher temperatures (Lee and Khader, 2000). Therefore temperature management (especially cool storage) seems to be the most important means to extend shelf life and maintain quality of fresh fruits and vegetables that are not chilling sensitive.

Vegetable amaranth

Amaranth is the collective name for the domesticated species of the genus *Amaranthus* (family *Amaranthaceae*). It is one of the oldest food crops in the world with evidence of cultivation dating back to over 6000 years in Puebla, Mexico (Itúrbide and Gispert, 1994). Although virtually unlisted in agricultural statistics, it may be the most widely grown crop in the tropics (Daloz, 1980). This is probably due to the ability of the genus to grow under a wide range of climatic conditions coupled with its competitive ability which permits cultivation with minimum management (Shukla and Singh, 2000; Erika, 1995; Campbell and Abott, 1982). In addition, amaranth grows quickly, requires little inputs and can be harvested within a short time (4-6 weeks after planting).

Physiological, genetic, and nutritional studies have revealed their potential economic value. With respect to its use in agriculture, its importance arises from its high rate of productivity (Ehleringer, 1983) as a rapidly growing summer crop, the large amounts of protein in both seed and leaf with high lysine, the high overall nutritional value, and the water use efficiency for the C_4 photosynthetic pathway. Amaranths are important in the culture, diet, and agricultural economy of the people of Mexico, Central and South America, Africa, and northern India. (McGraw-Hill Encyclopedia, 2005). Genetic, ethnobotanical, and agronomic research needs to be undertaken to develop amaranths as an important food plant in modern agriculture.

Botanical Description of the Amaranths

Amaranthus, collectively known as amaranth or pigweed, is an annual plant (seldom perennial) distributed worldwide in warm, humid regions. Approximately 60 species are recognized (National Research Council, 1984) with inflorescences and foliage ranging from purple and red to gold. Although several species are often considered weeds, people around the world value amaranths as leaf vegetables, cereals, and ornamentals (Toll and von Sloten, 1982). Amaranths are botanically distinguished by their small chaffy flowers, arranged in dense, green or red, monoecious or dioecious inflorescences, with zero to five perianth segments and two or three styles and stigmata, and by their dry membranous, indehiscent, one-seeded fruit (Schippers, 2002).

While grain and vegetable types can be differentiated, often both the grain and leaves are utilized from any type (Saunders and Becker, 1984; Tucker, 1986). The two principal species grown for grain include *Amaranthus cruentus* and *A. caudatus*, while the species grown for vegetable are represented mainly by *A. tricolor*, *A.dubius*, *A. lividus* and *A. hybridus*. *Amaranthus hypochondriacus* is considered to be a dual purpose type i.e. can be used for both grain and vegetable purposes.

The vegetable types are generally smooth leafed, with an indeterminate growth habit, which produces succulent axillary growth. The floral buds arise directly in the leafy axils. Grain types have a main stem axis that terminates in an apical large branched inflorescence. While most amaranth species are used as food crops *Amaranthus retroflexus* is considered one of the world's worst weeds (National Research Council, 1984).

Nutritional Quality of Vegetable Amaranth

Amaranthus spp. are utilized for food in diverse geographical areas. The consumption of vegetable amaranth helps balance vitamin and mineral intake (FAO, 1988; IPGRI, 2003; Shukla et al, 2005) and serves as an inexpensive rich source of protein and dietary fiber (Shukla et al, 2005). In Kenya, vegetable amaranth is cooked in unspecified amount of water for 7 – 9 minutes followed by discarding the cooking water and then frying the vegetables in a small quantity of oil and is eaten as a side dish with *ugali* (a paste made from maize meal) (Imungi and Potter, 1983). The vegetable is also sometimes cooked in admixture with beans, maize and bananas and/or potato and mashed to form a heavy mixture referred to as *kienyeji* (Swahili). The cooked leaves (not mixed with other foods) have been reported to contain about 8% protein, 4% carbohydrates and are rich in calcium, iron and vitamins A and C (Maundu *et al,* 1999). For example, 8mg/100gdw of β-carotene have been reported in *Amaranthus gangeticus* (Rahman et al, 1990), 26.8mg/100gdw of total iron and 629mg/100gdw of ascorbic acid in *Amaranthus tricolor* (Singh et al, 2001). In addition to being a significant source of vitamins and minerals, amaranth also contributes to the intakes of other phytochemicals such as phenolic compounds (Ricardo, 1993; Felician and Harold, 1998; Mposi, 1999; Imungi, 2002). Earlier studies have established the abundance of antioxidants in *Amaranthus* leaves (Sokkanha and Tiratanakul, 2006; Khandaker et al, 2008) and that there was a general trend towards increased antioxidant activity with increased total phenolic content in *Amaranthus tricolor* L (Khandaker et al, 2008). However how the nutritional quality of amaranth is affected by both preharvest and postharvest factors is well documented.

Marketing of vegetable amaranth

Over several years the populations in developing countries have become more urban and there has been a striking separation of food production from food consumption. Transportation of food away from its source of production has amplified the roles of marketing, intermediary distribution and processing, and shelf-life considerations have influenced the choice of varieties grown, thereby leading to reduced importance of traditional foods (Sands, 2009). In Kenya, the volume of production of, and the trade in, vegetable amaranth have increased over the last few years in response to the growing urban vegetable demand (Onyango and Imungi, 2007). The need for a wholesale market arises naturally as the population of a town increases and because of its distant location from producing farms (Mnzava, 1997).

Today, vegetable amaranth is found in many supermarkets and green grocers' stores in the urban centres of Kenya. More than 90% of the supply of the vegetables to these outlets is normally from farms that are within the environs of the urban centre (Onyango et al, 2008). TLVs as a whole have an image problem that does not apply to farm-produced exotic vegetables as there have been concerns that these vegetables are produced using sewage water for irrigation (a practice observed in urban agriculture; Neven and Reardon, 2004). In order to assess the quality of vegetable amaranth sold in the supermarkets there was need to analyze the nutritional quality of the vegetables supplied to these markets and to understand both the pre- and post-harvest factors that affect its yield and nutritional quality. This is important if the vegetable is to make a significant contribution to the nutrition of the people in developing countries and if it is to contribute significantly to food security and poverty reduction.

A survey carried out on the marketing of amaranth showed that the main constraint to marketing of vegetable amaranth is that it is highly perishable and therefore has low storage capacity in the fresh form (Maundu et al, 1999; Onyango and Imungi, 2007). It can not withstand high temperatures during transportation and marketing. Therefore, farmers are forced to sell soon after harvest. Special postharvest treatments are needed to slow deterioration and maintain freshness. Besides refrigerated storage, freshness of produce can also be extended by storage under modified atmospheres, correct humidity and good sanitation (Kays, 1991). Low temperature handling and storage is the most important physical method of postharvest wastage control, and the other methods can be considered as supplemental (Wills et al, 1981; Kays, 1991). The same problem of quality deterioration and loss of freshness is faced by the consumers who buy more than enough for one day's consumption and have to store the surplus. The options that exist for these consumers are that of storing in a refrigerator (available to only a few consumers) or storing at ambient temperatures. Very little information exists on the effect of low temperature storage on the shelf life and the nutritional quality of vegetable amaranth.

Educating small-scale farmers on the effects of both preharvest and postharvest factors on yield and quality of vegetable amaranth will lead to more production and use of these vegetables. With this knowledge, the farmers will learn the benefit of sustainable production practices. They will take advantage of value-added markets for their products. They will produce a year-round supply of safe food that will be more affordable to poor families. This increased availability of food, in turn, will lead to improved health and educational opportunities of families living in the rural sectors. The empowered families can contribute to the development of related industries in the rural sector, thus creating more employment opportunities. All these efforts will contribute to the overall goal of poverty reduction.

Objectives of the study

The main aim of this research is to broaden agricultural research to encompass human health. Vegetable amaranths have been more thoroughly investigated than the grain amaranths. Nevertheless, limited work has been done on the changes in nutritional quality of vegetable amaranth during growth and postharvest. In addition, it is not well known how N fertilization from chemical fertilizer and manure affect yield and nutritional quality of vegetable amaranth. The potential benefits of using vegetable amaranth will not be realized from increased production alone, but from its combination with proper storage to curb the rapid postharvest deterioration.

In the search for solutions to the global problems of poverty, hunger and malnutrition, amaranth is now playing an increasingly important role. The results from this study will be used in increasing production and consumption of this vegetable in Kenya and other sub-Saharan African countries for improved nutrition, food security and income generation.

Thesis layout

Chapter 1: This chapter covers the description of the problems facing developing countries and how African traditional leafy vegetables such as amaranth can play a role in alleviating some of them. It also describes both the botany and nutritional quality of vegetable amaranth. Both the preharvest and postharvest factors affecting quality of leafy vegetables are also discussed. The role of traditional leafy vegetables in the marketing chains of Kenya are also evaluated. **Chapter 2**: Assesses the types and quantities of vegetable amaranths sold in supermarkets and green grocers in the city of Nairobi (Kenya) as well as the manner in which they are sold including prices per unit quantity. It compares amaranth quantities sold with those of other traditional leafy vegetables. Both the sources of these vegetables and the distance between the markets and the production sites are reported. The problems experienced by both producers and traders of these vegetables are also described. **Chapter 3**: In this chapter, the yield response of vegetable amaranth (var. *Amaranthus hypochondriacus*) to diammonium phosphate (DAP) fertilizer and cattle manure as sources of nitrogen (N) is presented. The effect of different levels of N on Kjeldahl nitrogen and nitrate contents is evaluated. This chapter tries to assess the relationship between quantity (yield) and quality (Kjeldahl nitrogen and nitrates) of *A. hypochondriacus* as affected by use of N from chemical fertilizer DAP and manure. Use of N when supplied by DAP increases the yield and Kjeldahl nitrogen content when compared to manure. Effect of maturity at harvest on both quantity and quality of the vegetables is also evaluated. **Chapter 4**: In this chapter, dry matter content, ascorbic acid (vitamin C) and β-carotene (pro-vitamin A) as affected by both N supply and maturity at harvest of *A. hypochondriacus* have been evaluated and discussed. The changes in moisture content, ascorbic acid and β-carotene of the vegetables in storage, in both ambient temperatures and refrigerated storage have also been evaluated. **Chapter 5**: This chapter looks at total phenolics, quercetin and oxalate contents of *A. hypochondriacus* during growth and postharvest. Phenolics have for a long time been considered as anti-nutrients but in the recent past, the flavonoids group of phenolics in particular has been recognized as a major source of antioxidants. For examples, quercetin is suspected to play a role in the prevention of cancer, artisclerosis and heart diseases. **Chapter 6**: This chapter analyses the profitability of producing vegetable amaranth (*Amaranthus hypochondriacus*) as a commercial enterprise. It takes into account the small-scale farmers'economic model of what they consider as "free" resources. It outlines the different gross margins depending on what the farmers consider as "free" resources. **Chapter 7**: This chapter discusses the main results that were found in all the other chapters.

CHAPTER 2

THE PHYSICO-CHEMICAL CHARACTERISTICS AND SOME NUTRITIONAL VALUES OF VEGETABLE AMARANTH SOLD IN NAIROBI-KENYA

Abstract

Twenty one major supermarkets and ten independent green grocers in the city of Nairobi were surveyed for types of vegetable amaranths sold and their post harvest handling. The nutrient composition of the vegetables was also analyzed. In addition, information on three other traditional leafy vegetables (TLVs) namely, *Cleome gynandra*, *Solanum nigrum* and *Vigna unguiculata* was obtained. All the vegetables were sold in bundles of average weight 0.45kg. The edible fraction per bundle averaged 38.9%. Chemical analyses showed that vegetable amaranth had a moisture content of 85.5%, therefore a dry matter content of 14.5%. Expressed on dry matter basis, the mean total ash content was 19.2%, crude protein content 26.1% and the crude fiber content 14.7%. The mean ascorbic acid content was 627mg/100g, zinc content 5.5mg/100g and iron content 18mg/100g. The mean nitrate content was 732.5mg/100g, total oxalates 5830mg/100g and soluble oxalates 3650mg/100g, while the lead content averaged 1.03mg/100g. The study concludes that vegetable amaranth has potential as popular vegetable in the diets of Kenyans to significantly contribute to provision of micronutrients, particularly iron and zinc.

KEYWORDS: Traditional vegetables, marketing, postharvest handling, nutrition, Nairobi-Kenya.

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Introduction

In Kenya, about 200 indigenous plant species are used as leafy vegetables (Maundu et al, 1999). Of these, only a few have been fully domesticated, while more are semi-domesticated and the majority are collected from the wild. The most commonly consumed traditional leafy vegetables (TLVs) in Kenya include the *Amaranthus* spp. (Pig weed), *Vigna unguiculata* (Cowpea leaves), *Solanum nigrum* (Black nightshade*)*, *Cleome gynandra* (Cat's whiskers), *Cucurbita* spp. (Pumpkin leaves) and *Corchorus* spp. (Jute). These vegetables are either purchased or home-grown for personal consumption. The vegetables have been reported to be particularly rich in vitamin A and iron, two nutrients that are currently believed to be deficient in the diet of people in many countries. The vegetables are also rich sources of vitamin C, proteins, fibre and the minerals potassium, phosphorus, calcium and zinc (Akindahunsi and Salawu, 2005; Orech et al, 2005). In addition to antioxidant vitamins, the vegetables also contain high contents of phytochemicals such as phenolic compounds (including flavonoids), which also posses strong antioxidant properties (the phenolics) and which have been implicated in the prevention of aging related diseases such as cancer, arteriosclerosis and diabetes (Hertog et al, 1992), and in the management of HIV/AIDS.

The marketing and consumption of traditional leafy vegetables (TLVs) in Kenya has steadily changed over the past five or so years. The vegetables used to be sold mainly in informal open air markets found in most urban centres and were therefore presumed to be consumed mainly by the lower socio-economic groups. Recently, however, the vegetables have appeared for sale in increasing quantities in supermarkets, where the middle and higher socio-economic classes do their shopping. In the supermarkets, the vegetables are sold alongside their exotic counterparts like cabbage and spinach, with which they must compete. This increase in the demand for TLVs has stimulated many people, especially women, to get involved in the small-scale growing and selling of these vegetables to improve their economic status. There exists, therefore, the opportunity to use traditional leafy vegetables to fulfill several goals including: expansion of the local food base, improvement of community health and nutrition, enhancement of food security and the generation of income and therefore the reduction of poverty.

Amaranth is one of the vegetables for which consumption has greatly increased in the city of Nairobi in the recent past (Mwangi and Kimathi, 2006). Like other TLVs, this vegetable used to be sold only in informal markets, but is now sold in supermarkets and green grocers. Nevertheless, the vegetable has an image problem as it is sometimes cultivated on the banks of drains carrying sewage and irrigated with water from these drains. This deters some consumers from purchasing these vegetables. Another problem is that there are many cultivars and races of amaranth in cultivation, and the growers, because of the small scale of their production, do not know which of these has the highest consumer acceptability. Lastly, once harvested the vegetable has a very short shelf-life.

The study of shelf-life is complicated because the vegetable is normally harvested in three forms i.e leaves, tender young shoots or whole plants. It is likely that these three forms of the vegetable will have different shelf-lives and different postharvest handling requirements. Finally, limited information exists on the nutritional quality and safety of any of the traditional vegetables commonly sold and consumed in Kenya. The safety of the vegetables raises problems not commonly encountered in agriculture because these vegetables are often grown in an urban setting. Lack of land for agriculture close to and within urban areas coupled with desire of many urban poor to generate income has resulted in some vegetables being grown on the banks of drains. This results not only in some consumers having a negative attitude to the vegetables, but raises the real risk of contamination by heavy metals and pathogens. For purposes of health and nutritional planning therefore, it is important to gain knowledge on the quality and safety of these commonly consumed foods.

It was the objective of this study to determine the percentage of amaranth, and its physico-chemical and nutritional characteristics in relation to the main types of TLVs sold in the supermarkets and green grocers in Nairobi.

Materials and Methods

Using a structured, previously tested questionnaire, a survey was carried out in 21 supermarkets (10 of the ‘Nakumatt’ chain and 11 of the ‘Uchumi’ chain) and 10 independent green grocers’ stores in Nairobi, Kenya. The survey determined the types of TLVs sold, the manner in which they were sold, their shelf life in the store, the unit cost per type, the distance from supplier, and the problems encountered in marketing the vegetables, then the types of amaranth in the market and the most popular type.

In addition, samples consisting of six bundles of each vegetable on sale at 10 of the stores (three of the ‘Nakumatt’ chain, four of the ‘Uchumi’ chain and three independent green grocers) were taken. Samples from each supermarket or green grocer were combined to form a batch. The samples were then placed in cool-boxes (at approximately 4-6 ^{o}C) and transported to the laboratories of the Department of Food Science, Nutrition and Technology, University of Nairobi within two hours and prepared for analysis. The vegetables were analyzed for their edible proportion and the nutritional quality of this fraction.

Preparation of the vegetables for analysis

The edible fraction of each batch was quantitatively separated from the hard stalks and other inedible parts. Each fraction was thoroughly mixed, and then samples were taken for analysis.

Analytical methods

Moisture content was determined by drying to constant weight at 60^{o}C in a thermostatically controlled air-oven.

Crude protein was determined as total nitrogen by the semi-microKjeldahl method. The percent nitrogen was multiplied by an empirical factor of 6.25 to convert it to percent protein (AOAC, 1999). Crude fibre and total ash contents were determined by AOAC methods (AOAC, 1999).

Reduced ascorbic acid was determined by grinding 5 g sample mixed with 5g acid-washed sand in a mortar and pestle with 100ml of 10% trichloroacetic acid until the mixture was homogenous. The homogenate was promptly titrated with a standard solution of 2,6-dichlorophenolindophenol dye solution (Tomohiro, 1990) and a standard solution prepared from pure ascorbic acid and titrated with the same standard dye solution was used to calibrate the essay.The ascorbic acid content was calculated as mg per 100g dry matter.

The contents of iron, zinc and lead were analyzed in all the samples using an atomic absorption spectrophotometer (AAS) (Unicam 939/959, Pye-Unicam, Cambridge, UK) equipped with an air-acetylene flame and a hollow cathode lamp**,** and using lamps specific for each element. The device was operated under standard conditions using wavelengths and slit-widths specified for each element (AOAC, 1999). For analysis, 1g of dried and finely ground sample was accurately weighed and incinerated to constant weight. The ash was extracted with 10ml of HCl:water (1:1) and the extract was quantitatively transferred to 50ml volumetric flask and made up to the mark. Appropriate dilutions were made and the elements analyzed against their standards.

For determination of total oxalate, the sample was extracted with 30ml 1M HCl, while for soluble oxalate the sample was extracted with 30ml distilled water, by shaking in a water bath at 100^{o}C for 30min in each case. This was followed by precipitation with calcium chloride. The suspension was centrifuged at 800g and the supernatant discarded. After washing twice with 2ml 0.35M NH_4OH, the pellet was dissolved in 0.5M H_2SO_4. The solution was titrated with standard solution of 0.1M $KMnO_4$ at 60^{o}C to a faint violet color that was stable for at least 15 seconds (AOAC, 1999).

For determination of nitrates, the samples were ground to pass through a 600µm sieve then re-dried in an air oven at 70^{o}C overnight. Then 0.1g was weighed and suspended in 10ml 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 filtrate was used for analysis of nitrate-N by the method of Cataldo et al (1975)

Data analysis

Descriptive statistics were determined using MS Excel. Using the pivot table, depending on the type of data, means, standard deviations and/or frequencies were computed.

Results and Discussion

Physical characteristics of the vegetables

The survey data are shown in Table 2.1. The distance from the site of cultivation to the shop ranged from 21.9 km for *Amaranthus* spp. to 38.5 km for *Cleome gynandra*. The distance from source is very important in vegetable production and marketing because the longer the transport time the greater will be the degree of product deterioration due to senescence and dehydration. Important factors influencing product deterioration per unit distance traveled are the method of transportation and the condition of the infrastructure.

TABLE 2.1: The types and characteristics of traditional leafy vegetable sold in supermarkets and groceries in Nairobi[1]

	Distance from supply (km)	No.of bundles received per day	Weight of bundle (kg)	Edible portion of bundle (%)	Price per bundle (KSh)	Bundles purchased by customers		Bundles spoilt per day (%)
						1-2	>2	
*Amaranthus spp**	21.9± 8.1	75.2± 58	0.50± 0.1	38.9±21.7	13.9±2.2	11	20	5.5±3.2
Solanum nigrum	31.4±33.5	88.9±88	0.47±0.1	49.3± 5.4	14.4±1.5	5	26	3.5±2.9
Cleome gynandra	38.5±64.4	46.3±33	0.42±0.2	40.5±20.3	14.6± 1.9	9	20	3.1±2.6
Vigna unguiculata	33.9±20.8	71.2±45	0.65± 0.2	41.1±15.2	14.6±1.8	9	20	4.2±3.7

* In the case of Amaranthus, several species are grown and sold (*A. cruentus and A. hybridus*); no distinction is made between these species by growers or retailers. [1]Mean ± SD (N= 31) 1 US $ = KSh. 70.00

The average number of bundles received by each store per day was highest for *Solanum nigrum* at 88.9, followed by *Amaranthus* at 75.2 and lowest for *Cleome gynandra* at 46.3. The weight of bundle was highest for *Vigna unguiculata* at 0.65 kg and lowest for *Cleome gynandra* at 0.42 kg, while that of amaranth was 0.5 kg. The percent edible portion per bundle varied between 38.9% for *Amaranthus* spp to 49.3% for *Solanum nigrum*. The price per bundle averaged KSh. 14.50 (about 0.2 USD) in the supermarkets/green grocers. The supermarkets and green grocers were buying from the farmer/traders at KSh. 10.00 (about 0.14USD) per bundle. This therefore translates to gross earnings of KSh. 752.00 (10.70 USD) per delivery of vegetable amaranth and between KSh. 463.00 (6.6USD) and KSh. 889.00 (12.70USD) per delivery depending on the vegetable type for the three vegetables: *Solanum nigrum*, *Cleome gynandra* and *Vigna unguiculata*. At Ksh. 13.90 per bunch at the supermarkets, *Amaranthus* spp were the cheapest. Finally, the number of bundles that spoiled per day was highest for *Amaranthus* spp. at 5.5% and lowest for *Cleome gynandra* at 3.1%. It appears there was correlation between the number of bundles spoiling and the size of leaves of the vegetables. Amaranthus has the biggest leaves while *Gynandropsis gynandra* has the smallest leaves when compared to the other vegetables used in this study. The number of bundles spoiling increased with increase in leaf size. The harvested products rapidly lose water from their surfaces through transpiration leading to wilting and hence loss of consumer appeal. Moisture loss occurs rapidly in warm, dry environments and is affected by commodity characteristics such as surface area to volume ratio (Eskin and Robinson, 2000). This could have been the case for amaranth leaves, leading to the high losses experienced during marketing.

Amaranthus spp. and *Solanum nigrum* were sold in all the 31 stores studied while *Cleome gynandra* and *Vigna unguiculata* were sold in 94% of the sales outlets. Of all the traditional vegetables sold, consumers indicated a preference for *Amaranthus* spp. with most of them likening it to spinach. The other vegetables were less attractive than the *Amaranthus* spp because consumers indicated that they have a bitter taste. Only two species of amaranth; *Amaranthus hybridus* and *Amaranthus cruentus* were sold. *Amaranthus hybridus* (referred to locally as *kienyeji;* also as *Amaranthus hybridus hypochondriacus* (AVRDC Tanzania) was sold in all the stores, while *Amaranthus cruentus* (which is referred to locally as *agriculture* because it is widely believed to be an agriculturally improved variety) was sold in 19 of the stores. The taxanomic status of *A. hypochondriacus* and *A. hybridus* is unclear as *A. hypochondriacus* is sometimes classified as a sub species of *A. hybridus* (i.e *A. hybridus hypochondriacus*) (Asian Vegetable Research and Development Centre (AVRDC)Tanzania – Personal communication). There was a definite preference by the customers for *Amaranthus hybridus* compared to *Amaranthus cruentus*, which probably explains why the type was the most popular with the stores. The major reason for the preference given by almost all the supermarkets and green grocers surveyed was that because its broad leaves gave it a succulent appearance, *Amaranthus cruentus* was perceived by the customers to be grown using drain water heavily contaminated with sewage and hence was not fit for human consumption. If consumer education to dispel this misconception is carried out, consumption of the vegetable should increase.

The results indicate that in 20 out of the 31 outlets, customers purchased two or more bundles of amaranth (Table 2.1). Those who purchased one bundle of one type of vegetable indicated that they mixed the amaranth with other vegetables in cooking to reduce the pungency of vegetables like *Cleome gynandra* and *Solanum nigrum*. Two bundles of amaranth represent 0.4 kg of edible fraction.

The vegetables were supplied directly by the growers to 28 outlets with some of the green grocers also growing their own vegetables, which they supplemented with those of other growers. The remaining outlets were supplied with the vegetables by middlemen. The main mode of transportation of the vegetables by the suppliers to the stores was either by a lorry or pickup truck. For transportation, the vegetables were either packaged in crates or just stacked in the trucks and covered. The vegetables were received at all the sales outlets between 8h00 -10h00. On arrival, the vegetables were preserved by either holding them in a cold room (6-8 ^{o}C) or by holding them at ambient temperatures but with periodic sprinkling with cold tap water. It was reported by the stores that the vegetables could remain on sale for 2 days, although most of the supermarkets sold the vegetables only on the day of delivery. Of the 31 stores, only 5 reported selling all the vegetables they received. Others sold proportions ranging between 94% and 97% of the vegetables received based on the number of bundles reported as spoilt (Table 2.1).

For vending, the vegetables within each type were tied in bundles of similar average size. No formal packaging was used. The bundled vegetables were displayed by placing them in a trough which was nearly always at room temperature - between 19 ^{o}C and 24 ^{o}C at the time of survey. Only 1 out of the 31 stores surveyed displayed the vegetables at 6– 8 ^{o}C. Wilting was the major cause of quality loss of the vegetables. Substantial losses also occurred through spillage of material onto the ground. It has been reported that leafy vegetables are easily preserved by storing them at temperatures close to 0 °C in packages which are impermeable to the diffusion of water vapor (e.g. polypropylene film) and which create a low O_2/high CO_2 atmosphere (Favell, 1998). Packaging also helps to cut down on losses through spillage.

The main constraint to increased production of TLVs is the absence of any appropriate postharvest management to maintain quality. In the absence of this, they are highly perishable (as are most leafy vegetables in these circumstances) with a postharvest life of 1-2 days, which forces farmers to sell the product in local markets soon after harvest (Maundu et al, 1999). This requirement coupled with a poor road network limits the distance from the point of sale within which the vegetables can be grown. All the supermarkets covered in this study strove to sell all the vegetables within the day of delivery as whatever remained at the end of that day had to be discarded because it had already lost consumer appeal; a loss of outward appearance may also be paralleled by a loss of nutritional quality, especially if yellowing has begun. Some groceries, however, especially those with refrigeration, could keep their TLVs for up to two days before they needed to be discarded due to quality loss. Also unlike the supermarkets, most of the groceries are not much worried if their vegetables look somewhat wilted because of the type of clientele that they target. Their main target customers are the medium to low income groups, who are not very particular about freshness of the vegetables. So, at best the farmers need to deliver fresh harvests early each morning to the supermarkets and every other day to the green grocers. This is both expensive for the small scale farmers and limits the distance over which the vegetables can be marketed.

The farmers as well as the traders require simple postharvest treatments to help slow deterioration and maintain freshness. These technologies will help reduce the cost of delivery to the outlets for the farmers and allow the outlets to sell for longer periods of time. They will also benefit the consumers who buy more than their needs for one day and do not have refrigeration to store the surplus. In addition, good sanitation in post harvest handling is important to prevent microbial contamination, which can accelerate quality deterioration of vegetables, especially in the humid tropical climates where the conditions for growth of spoilage organisms are always favorable. Low temperature handling and storage is, however, the most effective physical method of slowing postharvest deterioration of vegetables and other methods can be considered as supplemental (Wills et al, 1981; Kays, 1991).

Chemical composition

The chemical composition of the raw vegetable amaranth at the point of sale is shown in Table 2.2, the results are expressed on dry weight basis. The results show that the vegetables had a moisture content of 85%, representing a dry matter content of 15%. The protein content ranged between 24.7% and 27.3%, the crude fiber content varied from 13.9% to 15.5% and the total ash content ranged between 18.6% and 20.9%. The results also indicate vitamin C with levels of up to 627 mg/100 g, iron contents of 18 mg/100 g and zinc contents of 5.5 mg/100 g. These values are within the ranges reported previously (Dhan and Pal, 1991; Mwajumwa et al, 1991; Ricardo, 1993; Mathooko and Imungi, 1994; Murage et al, 1996) for raw amaranth leaves. Vegetable amaranth can therefore contribute substantially to protein, the (specific) minerals, vitamin C and crude fiber intake from diets.

The levels of protein, ascorbic acid, iron and zinc are reduced in the cooked, drained vegetables (the form in which they are eaten) due to leaching of the nutrients into the cooking water. For example, Mathooko and Imungi (1994) have reported a loss of 80% ascorbic acid in cooked drained amaranth while Imungi and Potter (1983) reported losses of about 12% of the protein, an apparent increase of about 13% for iron, a loss of less than 1% for zinc and a loss of about 30% for lead in cooked and drained cowpea leaves. The dietary intakes of these nutrients will therefore be lower by similar proportions in the cooked vegetables compared to the uncooked form. An adult person in Kenya will consume about 200g of cooked amaranth vegetables per day, which contain about 7.6g protein, 5.2mg iron, 1.6mg zinc and 182mg vitamin C, assuming similar changes in the nutrient contents during cooking to those shown by cowpea.

It has been reported that Kenya's main nutritional problems include protein deficiency affecting a large proportion of the poor urban and rural populations. Iron deficiency induced anemia has also been reported to be prevalent among the general population, especially among children under five years of age and pregnant women (WHO, 2000).

TABLE 2.2: **Proximate chemical composition, ascorbic acid, iron and zinc contents of raw vegetable amaranth leaves**[1]

Source	Moisture content (%)	Dry matter (%)	Crude protein (Nx6.25)	Crude fibre (%)	Total ash (%)	Ascorbic acid (mg/100)	Iron (mg/100g)	Zinc (mg/100g)
Uchumi	85.2± 1.4	14.8±1.4	27.3± 0.7	15.5± 0.6	18.6±1	444.9±64	16± 3	5.6± 0.8
Nakumat	86.7± 0.9	13.3±0.9	24.7± 1.1	13.9± 0.5	19.1±1	500±119	16± 3	4.9± 0.34
Others	83.5±0.9	16.5±0.9	26.4± 2.6	15± 2.8	20.9±3	627.6±126	24± 2	6.3± 0.6
Mean	85.5± 1.6	14.5±1.6	26.1± 1.7	14.7± 1.3	19.2±2	503.5±118	18± 8	5.5± 0.8

[1] Mean ± SD (N= 6) All values are expressed on dry matter basis

Considering even just these two headline, nutritionally related problems, the consumption of vegetable amaranth in diets would be expected to contribute to partly alleviating these problems. If similar changes in the nutrient contents of the amaranth vegetables as reported for other leafy vegetables are assumed, consumption of equivalent of 200 g fresh weight of vegetable daily will contribute 14% and 17% of the Recommended Dietary Allowances (RDA) of protein for male and female adults respectively, 32.8% RDA of iron for both male and female adults. These vegetables will contribute 8% RDA for protein and 9.8% RDA for iron in the case of lactating and pregnant mothers. The vegetables would also contribute 11% RDA for zinc in adult males and females, which will be 8% RDA for pregnant women and 6.4% RDA for lactating mothers.

Though amaranth is a valuable source of nutrients, the consumption of this vegetable (along with other TLVs) may pose some health risks due to the accumulation of anti-nutritional factors such as nitrates and oxalates, and possible contamination with toxic materials such as lead as a result of poor cultivation practices and/or pollution. The levels of nitrates, oxalates and lead in the vegetable amaranth samples are presented in Table 2.3. Values are expressed on dry matter basis.

As Table 2.3 shows, the nitrate contents ranged between 505 mg/100 g and 1056 mg/100g; this translates to about 73.2 mg to 153.1 mg/100 g fresh weight. These levels are much lower than those reported by (Kariuki, 1998) for *Amaranthus hypochondriacus.* Considering consumption of about 200 g per day of vegetable by adults, therefore, the daily intake of nitrates will be less than 146 – 306 mg. The maximum safe daily intake of nitrate recommended by WHO for adults is 220 mg which is equivalent to consumption of 208 g of fresh vegetable.

TABLE 2.3: Levels of Nitrates, Oxalates and lead in fresh vegetable amaranth leaves[1]

Source	Nitrates (mg)	Total oxalates (mg)	Soluble oxalates (mg)	Pb (mg)
Uchumi	1056± 105	4860± 0.5	2840± 0.5	0.98±0.3
Nakumatt	505± 190	6450± 0.4	3960± 0.5	0.85±0.5
Others	540± 145	6550± 0.5	4200± 0.5	1.50±0.6
General mean	733± 307	5830± 0.9	3560± 0.8	1.03±0.5

[1] Mean ± SD (N= 6) All values are expressed on dry weight basis

Nitrates are water soluble and therefore some loss though leaching is possible in cooked vegetables if the cooking water is discarded (Ricardo, 1993). Abo Bakr et al (1986) reported that 16% of the nitrate was lost from peas, 34% from beans, 51% from carrots and 34% from spinach (most comparable to amaranth) when these vegetables were boiled for unspecified times in water (vegetable: water = 1:2). This method of preparation leads to loss of most kinds of water soluble nutrients to varying extents during cooking (Imungi and Potter, 1983). The cooking method described is comparable to the traditional method used in many Kenyan. If about 34% of the nitrate is lost during cooking (assuming amaranth behaves like spinach) then the safe limit for amaranth consumption increases to 314g fresh weight equivalent of cooked vegetable for a person of 60 kg body weight (Mohri, 1993), which is much higher than the common daily consumption.

Nitrate levels normally found in vegetable amaranths do not present a serious health problem to reasonably healthy individuals if consumption does not exceed 100g of leaf per day (FAO, 1988), 50% of the typical daily intake of amaranth. Our calculations indicate that this limit may be excessively conservative. Most researchers have concluded that there is no danger of the daily nitrate intake exceeding the recommended daily allowance as a result of consuming amaranth considering the nitrate levels found in the vegetable and the daily consumption of the cooked vegetables.

The total oxalate content of amaranth was found to range between 4900 mg/100 g and 6600 mg/100 g. The soluble oxalate values were between 2800 mg/100 g and 4200 mg/100 g (Table 2.3). These levels are similar to those obtained by (Mziray, 1999) working with *Amaranthus hybridus* in Dar-es-Salaam, Tanzania, but are much lower than those reported by (Kariuki, 1998) in *Amaranthus hypochondriacus*. Oxalic acid is a major anti-nutritional factor which is widely distributed in plant foods (Gupta et al, 2005). It is known to interfere with calcium absorption by forming insoluble salts of calcium. Unacceptable levels to humans have been suggested to be 2 to 5 g of oxalic acid per day for populations consuming low levels of calcium (Ricardo, 1993). The levels of free oxalates found in the vegetables do not constitute a serious health hazard for reasonably healthy individuals if consumption does not exceed a maximum of 200g of fresh leaf per day, equivalent to 29g dry weight (FAO, 1988); which is below the amount required to cause health problems. Further, it is expected that if the method of cooking involves discarding of cooking water, the levels of free oxalates and therefore the levels of total oxalates in the vegetables as consumed will be substantially reduced.

As Table 2.3 shows, the levels of lead in the vegetables ranged between 0.98mg and 1.5mg per 100 g dry wt. This translates to consumption of between 0.15mg – 0.23mg for every 100g of fresh leaf. Accumulation of heavy metals differ according to plant species (Hooda et al, 1997), the age of the plant, the environmental conditions in which the plant is grown and the part of the plant analyzed (Isabel and Concepcion, 1997), and the soil mobility of the particular metal ions involved. As lead is relatively immobile in the soil (http://www.atsdr.cdc.gov/toxprofiles/tp13-c6.pdf) leafy crops are most susceptible to contamination from atmospheric deposition of lead from industrial and automotive sources. The US Food and Drug Administration Advisory Panel suggest that no more than 1mg of lead per day be consumed from food (Gordon and Wayne, 1993). It has been assumed that under normal circumstances, the amounts of cooked vegetables consumed by individuals rarely exceed 100g of fresh weight per day (FAO, 1988). In Kenya a daily consumption of 200g is more common (personal observation). This will mean that a person will consume between 0.3mg and 0.5mg of lead for every 200g of vegetable amaranth consumed. This is below the lead limit of 1 mg that has been (Gordon and Wayne, 1993). Moreover, if the cooking water is discarded, this amount is lowered by leaching into the cooking water. Imungi and

Potter (1983) have reported loss of up to 30% lead during cooking of cowpea leaves by boiling in water. Assuming this type of loss in cooking, 200g of the vegetables would then contain between 0.2 – 0.35mg of lead. Therefore, the levels taken in with the cooked vegetables would not be high enough to warrant public health concern. Lead is a naturally occurring element found in the earth’s crust and normally found at only low levels in plant-based food stuffs unless they are grown in sewerage or industrial effluents (David and Craig, 1994). The majority of dietary lead results from environmental pollution due to exhaust gases from automobiles (especially if vegetables are grown near roads) and from processing equipment and storage containers fabricated using lead containing materials such as solder (Pyke, 1981; David and Craig, 1994).

In conclusion, the study showed that production, trade and consumption of vegetable amaranth in Nairobi is on the increase. Fresh cut amaranth vegetables are currently selling competitively with their exotic counterparts in the modern supermarkets and groceries. The most popular type of amaranth among consumers in Nairobi is *Amaranthus hybridus.* The study also showed that the vegetable amaranth has high levels of protein, vitamin C and the minerals iron and zinc that could help in overcoming micronutrient malnutrition at a negligible cost for developing countries. They also had high fiber content and hence would serve as a natural source of fiber. The levels of nitrates, oxalates and lead in the vegetables are not high enough to cause public health concern. Trade in fresh cut amaranth vegetable is, however, limited by its short shelf life. It is therefore recommended that simple and affordable post harvest handling practices that extend shelf life even for limited periods are developed.

CHAPTER 3

THE INFLUENCE OF ORGANIC AND MINERAL FERTILIZATION ON LEAF NITROGEN, NITRATE ACCUMULATION AND YIELD OF VEGETABLE AMARANTH (*Amaranthus hypochondriacus*)

Abstract

The influence of manure and diammonium phosphate (DAP) mineral fertilizer on germination, leaf nitrogen content, nitrate accumulation and yield of vegetable amaranth (*Amaranthus hypochondriacus*) was investigated. Field trials were set up at the University of Nairobi Field Station at the Upper Kabete Campus, Kenya during the long rains of March – May in 2007 and 2008. Trials were laid out as complete randomized block design with four fertilization treatments: 20, 40, and 60kg N ha^{-1} supplied by DAP (18:46:0), 40kg N ha^{-1} supplied by cattle manure and an unfertilized control variant. The vegetables were harvested at three maturity stages; 6, 7 and 8 weeks after planting. Results indicated that there were significant differences between treatments in germination percentage, leaf nitrogen content, nitrate accumulation and vegetable yield. Plants that received manure had a higher germination percentage than those that received the same amount of N supplied by the chemical fertilizer DAP. The yields generally increased from week 6 to week 8. The highest yield was recorded in plots receiving 40kg N ha^{-1} from DAP at 8 weeks after planting. Plots that were supplied with manure recorded the lowest yield when compared to the fertilizer treated plots at all rates. Leaf nitrogen content increased with increasing rate of N but only when N was supplied by DAP fertilizer. The leaf nitrogen content decreased with increasing age of the plants. The leaf nitrate content increased with increase in DAP application rate but decreased with increase in maturity. Results indicate that manure application produced quality vegetables in terms of low nitrate levels, but leaf nitrogen and vegetable yields were low. DAP application effected higher yields, but the vegetables had high though acceptable nitrate levels.

KEYWORDS*: *Amaranth, Manure, diammonium phosphate, yield, nitrogen, Nitrate

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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 and Abbot, 1982). Like other traditional African leafy vegetables, amaranths grow quickly and thus can be harvested relatively soon after sowing (6 - 8 weeks) (Chweya and 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 and Eyzaguirre, 1999; Rajabu et al, 2000). Unlike other traditional vegetables, both the leaves and grains of amaranths are consumed.

The nutritional quality of amaranth combined with its environmental tolerance and flexibility makes it a suitable crop for improving food security and reducing micronutrient malnutrition in developing countries. Together with some other indigenous vegetables it is therefore important in the strategies for improving livelihoods of many people in the rural and peri-urban areas of developing countries. In Kenya, for example, where these vegetables are widely grown for subsistence, they also offer a significant opportunity for poor households to generate income through commercial production now that urban dwellers of all cadres increasingly appreciate the value of traditional food crops in their diet (Onyango et al, 2008). However it is clear that the growth environment of amaranth, as with other leafy vegetable crops, 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.

Vegetable yields can be increased with proper fertilization with nitrogen (N) and other mineral nutrients (Turan and Sevimli, 2005) and several studies have investigated the influence of fertilization with nitrogen on yield and some quality aspects of vegetables (Sorensen et al, 1995; Elia et al, 1998; Ćustić, 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 and dry matter content may also decrease (Guttormsen, 1996), possibly being due to an increase in succulence (Singh and Whitehead, 1996).

If consumed, nitrate can have toxic effects by two main routes. First, nitrate is reduced to nitrite in the saliva and the gastrointestinal tract and this can then be re-oxidized to nitrate by oxyhemoglobin in the bloodstream with the formation of methemoglobin (in which the central iron of the haem group is Fe^{3+}) (Kosaka et al 1979). Unlike hemoglobin, methemoglobin has no ability to bind oxygen (Santamaria, 2006) so the capacity of the blood to deliver oxygen to the body tissues is impaired (Hill, 1999). This condition is referred to as methemoglobinemia and is more serious with infants than with grown children or adults. Second, nitrites react with secondary amino compounds commonly present in diets to form nitrosamines, which have been implicated in carcinogenesis (Hanssen and Marsden, 1987; Wogan, 1976). The nitrate content of vegetables is therefore a determinant of their quality and there are recommended limits for the nitrate content of vegetables (Food Standards Agency, 2001). So though amaranth can constitute a valuable source of micronutrients in diets, its excessive consumption may pose some health risk due to excessive intake of nitrates, especially where nitrogen fertilization is poorly controlled. It is possible however that as they are fast growing, suggesting a high photosynthetic activity, amaranths will be less vulnerable to excessive nitrate accumulation than other plants. However there have been few studies reported on the effects of organic and mineral fertilizers on the yield and quality of fast growing leafy vegetables such as amaranth (Gontcharenko, 1994; Ćustić, et al, 2003).

Studies of the impact of diverse fertiliser regimes are especially relevant to agriculture practiced by the poor in developing countries where fertilization of crops is likely to be less organized and more improvised in terms of the timing of applications and the type of fertilizer used. Small scale farmers in developing countries can ill afford chemical fertilizers so they often either use manure or no fertilization. Manure is cheap because it is a by-product of the shed rearing of cattle, especially in Kenya where zero grazing of dairy cattle is widely practiced. Sometimes the vegetables are planted in old and abandoned cattle sheds. Few studies, however, have been carried out to simulate these methods of production. Also information on the effect of mineral fertilizer and cattle manure on the yield and quality of vegetable amaranth is very scanty. Turan and Sevimli (2005) working with cabbage found that fertilizer type and dose significantly influenced the weight and mineral content of the cabbage heads. Increasing the level of nitrate nutrition stimulated dry matter production. When 400, 200, 100 kg N ha^{-1} of chemical fertilizers and farmyard manure were used, plant yields were 66%, 61%, 49% and 44% higher than without fertilization. The nitrate contents of plants also increased with the increasing N application, but they found that the foliar nitrate content was lower when the plants were fertilized with manure than with mineral fertilizer. A possible problem with the use of manure as a fertilizer for fast growing plants such as amaranth is that during the short growth period insufficient mineralization of manure might take place to noticeably improve growth.

This study was therefore designed to evaluate the influence of manure and mineral fertilization on germination, yield, Kjeldahl nitrogen and nitrate accumulation in *Amaranthus hypocondriacus.* The taxonomy of *A. hypochondriacus* is not fully resolved; it is often classed as a subspecies of *A. hybridus* (i.e *A. hybridus hypochondriacus*) but here we will use the name *A. hypochondriacus* for the sake of brevity.

Materials and Methods

Amaranth seeds were obtained from Asian Vegetables Research and Development Centre (AVRDC) 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 Latitude 1^{o} 15′S and Longitude 36^{o} 44′E, and at altitude 1930 m above sea level (Sombroek et al, 1982). 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 and Lawes, 1971). The mean annual temperature is 18^{o}C. The soil in Kabete is characterized as a deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996). The land used for the plots had not received any fertilizer 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 fertilized with either manure or diammonium phosphate (DAP; 18:46:0) fertilizer. In 2007, planting was done on the 11th April while in 2008, planting was done on the 6th of March. The differences in planting days were due to the different times the long rains season started in the two years. The trials were laid out in a complete randomized block design with five fertilization treatments as follows: one plot with cattle manure at 40kg N ha^{-1} (calculated using the content of N found in the manure; approximately 2 tonnes of manure per hectare, Table 3.1), three plots with DAP (18:46:0, NPK) at 20, 40, and 60kg N ha^{-1} and unfertilized control plot. While we will focus on the N content of these fertilizers, it is important to note that the manure used is a source of a diverse range of macro- and micro-nutrients (Table 3.1) and DAP is a phosphorus (P) as well as an N source; for the manure, the P added was 40 g and 2.6 kg P ha^{-1} in year 1 and 2 respectively. For the DAP 20, 40 and 60 kg N ha^{-1} the P added was 50, 100, and 150 kg P ha^{-1} respectively. Phosphorus (P) (as phosphate) is an essential nutrient for plant growth, development, and reproduction that forms part of key molecules such as nucleic acids, phospholipids, ATP, and other biologically active compounds. After nitrogen, P is considered to be the second most important nutrient limiting agricultural production (López-Bucio et al, 2000). However, the phosphate ion is precipitated by many cations and is easily available for plant uptake at a narrow range of neutral soil pH values. In acid soils, P forms low-solubility precipitates with aluminum (Al) and iron (Fe), whereas in alkaline soils, it combines efficiently with calcium (Ca) and magnesium (Mg) to form sparingly soluble phosphate compounds (Bar-Yosef, 1991) Therefore, although the total amount of phosphorus in the soil may be high, in most cases it is unavailable for plant uptake. It is likely that under the acid conditions of the soil used in our study, the phosphate in either the manure or the DAP was not available for plant uptake.

The treatments were replicated three fold. The plots measured 2 m x 2 m with a spacing of 0.15 m x 0.1 m (Palada and Chang, 2003). Care was taken in this trial to use a manure treatment and, where possible, cultivation practices that were comparable to those employed by local small-scale growers. Both the DAP fertilizer 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 in 2008 to keep the soil moist. The plants were grown in 2007 (year 1) and 2008 (year 2), but the measurements made on the year 1 plants were not as comprehensive as those made in year 2 so not all results are available for both years. The vegetables were harvested at three maturity stages; 6, 7 and 8 weeks after planting.

Physico-chemical analysis of the soil and manure

Soil samples were collected from the uncultivated land meant for the plots at depths of 0 – 15 cm and 15 – 30 cm using a soil auger. The soil samples were air-dried and ground to pass through a 2 mm sieve. The samples were analyzed for organic carbon, pH, total nitrogen, the exchangeable bases Ca, Mg, K and Na, and for phosphorus. Organic carbon was analyzed using the Walkley-Black wet oxidation method (Okalebo et al, 1993). The pH was determined using a glass electrode (Metrohm 605, Pye Unicam, Cambridge, UK) on the supernatants after suspending the sample in either water or 0.01 M $CaCl_2$ solution and allowing the mixture to settle. Total nitrogen was determined using micro-Kjedahl method (AOAC, 1999). Exchangeable bases (Ca, Mg, K and Na) were determined by displacing them from a soil sample using 0.1N ammonium acetate at pH 7.0**.** Calcium and magnesium were determined using an atomic absorption spectrophotometer, while sodium and potassium were determined using a flame-photometer (Okalebo et al, 2002). Phosphorous content was determined by first extracting a 5g soil sample using the double-acid method (0.05N HCl in 0.025 N H_2SO_4, Okalebo et al, 2002) and then measuring the concentration of phosphorous in the extract using an atomic absorption spectrophotometer (AAS) (Unicam 939/959, Pye-Unicam, Cambridge, UK).

For analysis of manure, samples were obtained from old cattle shed on the Kabete Campus Veterinary Farm. The shed had been abandoned for approximately one year. This manure resembled that commonly used by Kenyan farmers, who normally remove fresh wet manure (containing dung, urine and uneaten straw) from the cattle shed and allow it to partially decompose in an uncovered heap outside the shed for at least two rain seasons (about 1 year old). The manure samples were air-dried and ground to pass through a 4mm sieve. The samples were then analyzed for organic carbon, total nitrogen and exchangeable cations using the same methods as used for soil analysis. Phosphorous content was determined by first extracting a 5g manure sample using the double-acid method (0.05N HCl in 0.025 N H_2SO_4, Okalebo et al, 2002) and then measuring the concentration of phosphorous in the extract using an atomic absorption spectrophotometer (AAS) (Unicam 939/959, Pye-Unicam, Cambridge, UK).

The contents of iron, zinc, copper and manganese were analyzed using AAS (Unicam 939/959, Pye-Unicam, Cambridge, UK) equipped with an air-acetylene flame and a hollow cathode lamp, and using lamps specific for each element. The device was operated under standard conditions of wavelengths and slit-widths specified for each element (Okalebo et al, 2002). The chemical composition of the soil and manure are shown in Table 3.1.

Determination of percentage germination, number of leaves and harvest yields

Germination was determined two weeks after planting while the number of leaves per plant from different treatments was determined 3 weeks after planting. The yields were determined at three different stages of maturity *viz.* 6, 7 and 8 weeks after planting by cutting-off the tender edible stems and leaves. The harvest from each plot was weighed to determine the yield per plot. This was then scaled up to yield per hectare.

Determination of leaf area and leaf mass (fresh weight basis) per unit area

Determination of the leaf area and leaf weight was done by using a cork borer of known diameter. Leaf area was calculated by taking 10 leaf discs and calculating an average area of disc as: area of leaf disc (circle) = πr^2 where $\pi = 3.14$ and r = radius of the cork borer. Leaf weight was done by taking the weight in grams of the 10 leaf discs and getting an average. Leaf mass per unit area was given by weight of leaf disc/area of leaf disc.

Determination of nitrogen and nitrate content

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°C and weighed before being ground to pass through 600 µm mesh sieve. These dry-weight results were also used to calculate the dry matter content of the samples. For determination of nitrates, about 0.1g of the vegetable powder was weighed and suspended in 10ml distilled water. The suspension was incubated at 45° 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).

Climate records

The daily rainfall (mm), temperature (°C), sunshine hours (h) and solar irrandiance ($MJm^{-2}day^{-1}$) were obtained from a mini-meteorological station located on the research farm in Kabete campus of the University of Nairobi. The readings of rainfall were used to calculate the amount of rainfall received per week during the cropping season. For the temperature, sunshine hours and solar irrandiance, the averages of each week were calculated for the cropping season. The solar irrandiance measurements were done by using a pyranometer CM6B sensor (Kipp and Zonnen Co., Delft, Netherlands).

Data analysis

Data was subjected to the general analysis of variance using Genstat statistical software (Payne et al, 2006). Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P<0.05$).

Results

Soil and manure analyses and climate during the experiments

There were no major differences between year 1 and year 2 soil and manure nutrient contents used in the study except for the phosphorus and magnesium contents of manure. The posphorus content was more in manure used in year 2 compared to that used in year 1 while the magnesium content was more in manure used in year 1. The soil and manure analyses are shown in Table 3.1 for year 2007(year 1) and 2008 (year 2) growing periods respectively. During the first 3 weeks after sowing in year 1 the mean temperature was 19.4^{o}C and during the 2nd 3 week period it was 18.8 ^{o}C, in the same period, the total number of sunshine hours was 13.2h and 18.5h, the solar irradiance intergrated for the period was 428.4MJ m^{-2} and 350.7 MJ m^{-2} and the total amount of rainfall was 770mm and 22mm respectively. In year 2 during the first 3 weeks after sowing the mean temperature was 20.1 and during the 2nd 3 week period it was 18.8^{o}C, in the same period, the total number of sunshine hours was 20.5h and 21.3h, the solar irrandiance intergrated for the period was 453.6 MJ m^{-2} and 468.3 MJ m^{-2} and the total amount of rainfall was 175mm and 85.1mm respectively. The crop was irrigated twice a week whenever rainfall was insufficient keeping the soil moist at all times to avoid moisture stress. The weather conditions are shown in Tables 3.2a and 3.2b. Conversion of the incident solar irradiance to potential dry matter accumulation was achieved by multiplying by 0.8 to estimate the percentage radiation absorption (values for intercepted solar irradiance reach to over 0.9 for a fully developed canopy (Piedade et al 1991, Kamnalrut et al 1992, Qiu et al 1992), and then by 2 gMJ-1 (the standard conversion factor for intercepted solar irradiance in energy terms to dry matter for C4 plants (derived from Monteith 1978).

Germination, growth and yield

The manure and control treatments resulted in higher germination percentage when compared to DAP fertilizer treatments (data only for year 2). Each of the three levels of DAP had significantly ($P<0.05$) lowered rates of germination, with the lowest occurring at the highest rate of N. These results are shown in Table 3.3.

The growth of the plants after germination was faster in plots that received DAP compared to the manure and control plots. Three weeks after planting (data only from year 2) the plants on the plot with the lowest DAP application (20 kg N ha^{-1}) had almost three times the number of leaves compared to the plants on the manure and the control plots (Table 3.3). Increasing the DAP application further resulted in a small increase in leaf number, and there was no significant increase in leaf number at applications of over 40 kg N ha^{-1}. In neither year 1 nor year 2 (Fig 3.1) did the use of manure at an application rate equivalent to 40 kg N ha^{-1} result in a significant (physically or statistically) increase in yield (fresh weight basis) per hectare compared to the control.

Table 3.1: Chemical properties of soil and manure used in the study

PROPERTY	**Year 2007**			**Year 2008**		
	Soil		**Manure**	**Soil**		**Manure**
	0-15cm	**15-30cm**		**0-15cm**	**15-30cm**	
pH (H_2O)	6.5	6.4	9.3	6.7	6.7	9.0
pH (0.01M $CaCl_2$)	5.9	6.0	9.0	5.7	5.7	8.8
Total nitrogen (g/kg)	3	2.8	17.9	2.5	2.6	19
Organic carbon (%)	2.5	2.6	41.64	2.5	2.4	33.6
C:N ratio	-	-	23:1	-	-	18:1
P (g/kg)	0.013	0.012	0.018	0.024	0.023	1.25
K (mmol/kg)	22.0	19.2	505	15.2	18.7	600
Na (mmol/kg)	8.0	8.4	15.7	10.8	11.3	21.0
Ca (mmol/kg)	100.8	99.5	116.0	112.3	101.3	125.5
Mg (mmol/kg)	25.0	23.6	81.7	25.2	23.0	49.2
Fe (g/kg)	-	-	0.002	-	-	0.002
Cu (g/kg)	-	-	Trace	-	-	0.004
Zn (g/kg)	-	-	Trace	-	-	0.002
Mn (g/kg)	-	-	Trace	-	-	0.06

Table 3.2a : Weather summary during the growing period of vegetable amaranth 2007

Month	***Week of the year***	***Total Rainfall per week (mm)***	***Mean daily temperature (^{o}C)***	***Mean daily Sunshine hours (h)***	***Mean daily Solar irradiance (MJ m $^{-2}$day^{-1})***	***Expected dry matter production (kg ha^{-1})***
April	13	3.8	20	5.2	23.7	379.2
	14	124.1	19.7	2.1	18.6	297.6
	15 (3rd day) Planting	441.7	19.5	4.4	20.3	324.8
	16	204.1	18.9	6.7	19.5	312.0
	17	5.4	19.5	8.4	23.8	380.8
May	18	1.0	18.8	6.4	17.9	286.4
	19	15.2	18	3.7	14.6	233.6
	20	64.5	18.8	5.8	19.2	307.2
	21	17.0	18.7	7.3	16.3	260.8
	22 (1st day) First harvest	85.6	18.6	6.1	17.4	278.4
	23 (1st day) Second harvest	5.5	19.1	-	18.0	288.0
	24 (1st day) Third harvest	6.0	17.6	-	17.0	272.0

Source: Kenya Meteorological Department (Kabete sub-station)

Table 3.2b : Weather summary during the growing period of vegetable amaranth 2008

Month	*Week of the year*	*Total Rainfall per week (mm)*	*Mean daily temperature (^{o}C)*	*Mean daily Sunshine hours (h)*	*Mean daily Solar irradiance (MJ m $^{-2}$day^{-1})*	*Expected dry matter production (kg ha^{-1})*
March	9	3.1	20.2	9.0	26.2	419.2
	10 (4th day) Planting-	1.7	19.9	8.8	26.8	428.8
	11	35.5	21.0	8.0	26.6	425.6
	12	137.6	19.5	3.7	17.0	272.0
	13	0.2	18.7	6.3	21.2	339.2
April	14	0	19.0	9.1	24.5	392.0
	15	84.9	18.7	5.9	20.2	323.2
	16	16.7	19.1	8.3	22.2	355.2
	17 (1st day) First harvest	102.6	18.1	6.6	17.8	284.8
	18 (1st day) Second harvest	0	19.3	7.4	19.7	315.2
May	19 (1st day) Third harvest	0	18.2	8.2	21.5	344.0

Source: Kenya Meteorological Department (Kabete sub-station)

Table 3.3: Effect of levels of nitrogen and manure on germination and growth of vegetable amaranth (year 2)

Source	**Percent germination**	**No. of leaves 3 weeks after planting**
Control	60.0c	5a
DAP 20kgN/ha	30.6b	14b
DAP 40kgN/ha	21.1ab	16c
DAP 60kgN/ha	15.6a	17c
Manure 40kgN/ha	62.2c	5a
l.s.d	10.0	1.4

DAP = Diammonium phosphate fertiliser, Figures along a column followed by the same letter are not significantly different ($P \leq 0.05$).

In contrast to manure, the use of the inorganic fertilizer did produce increases in yield per hectare (Fig 3.1). 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 (Fig 3.1). Notably the effect of the fertilization on growth is largely saturated by the application of 20 kg N ha^{-1} of DAP at the first harvest in both years. At the second harvest (week 7) of year 1 yield increased for all DAP dosages, but the differences between the dosages was not significant. It was only by the last harvest (week 8) in year 1 that any 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.

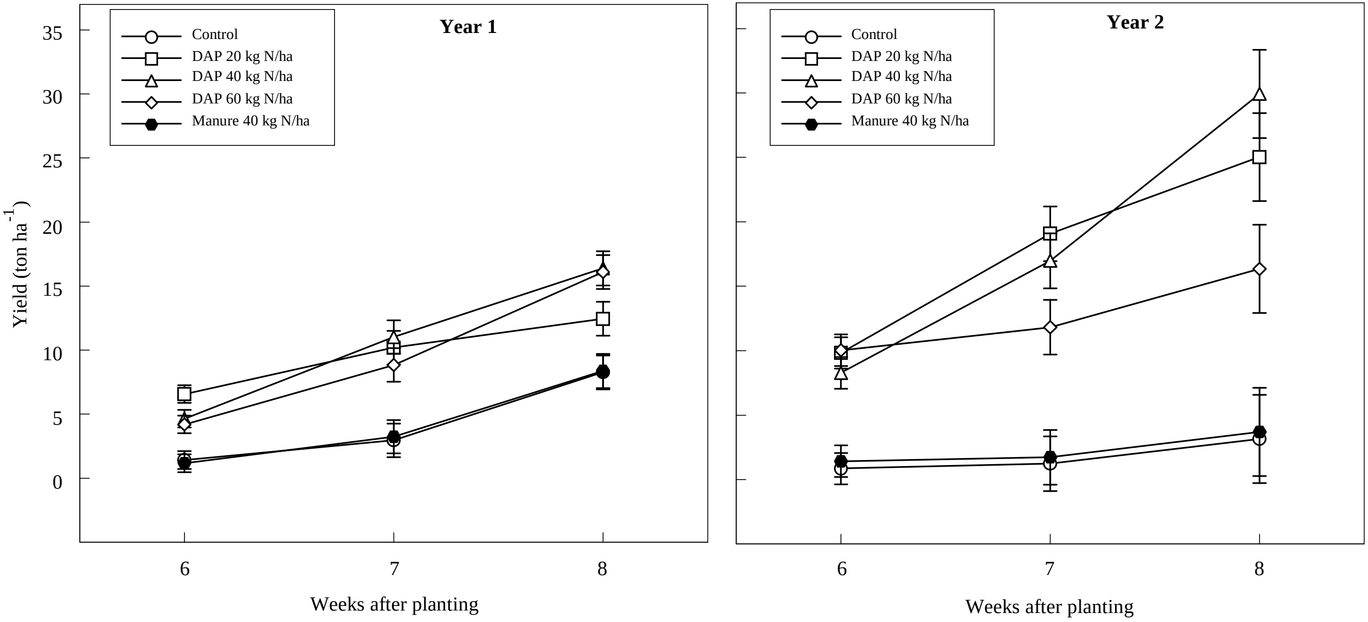

Figure 3.1: Effect of time of harvesting, chemical fertiliser DAP and cattle manure on above ground biomass of vegetable amaranth (A. hypochondriacus)

During year 2 the yields of DAP treated plots also increased with time, but in this case the increase of the 60 kg N ha^{-1} performed less well than either the 20 and 40 kg N ha^{-1}, both of which had insignificant yield differences. The poor performance of the 60 kg N ha^{-1} plot in year 2 can be attributed to poor seed germination and therefore to a low plant number in those plots. Yields, overall, in year 2 were about twice those in year 1.

On per plant basis (data only available for year 2) the plots that received 40 and 60 kg N ha^{-1} recorded the highest yield (Fig 3.2), though at the first harvest the difference between the 20, 40 and 60 kg N ha^{-1} was not significant. At weeks 7 and 8 the per plant yield from the 20 kg N ha^{-1} plot was less than that of the 40 and 60 kg N ha^{-1} plots, though still conspicuously higher than the per plant yields from the control and manured plots which were both very low.

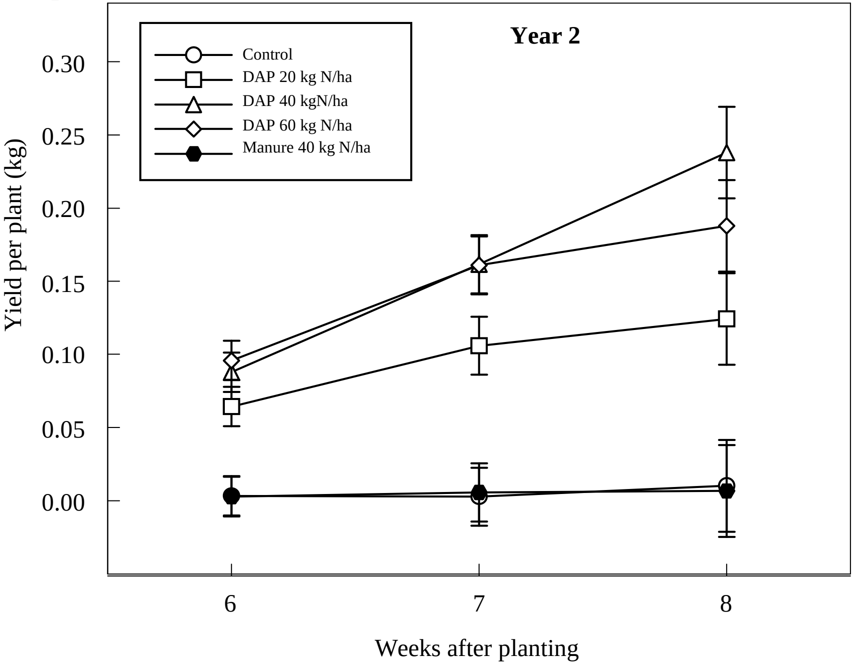

Figure 3.2: Effect of maturity at harvest, chemical fertiliser DAP and cattle manure on fresh leaf yield per plant (kg) of vegetable amaranth

The fresh leaf mass per unit area (LMA) was highest in plants that received 60 kg N ha^{-1}. There were no significant differences in the LMA of the plants that received 20 kg N ha^{-1} and those that received 40 kg N ha^{-1} although these were significantly higher than those that received manure and the control (Fig 3.3, year 2 data only). The leaf weight per unit area of the manured plants was higher than that of the controls, so though manuring had no effect on overall growth there was a morphological effect.

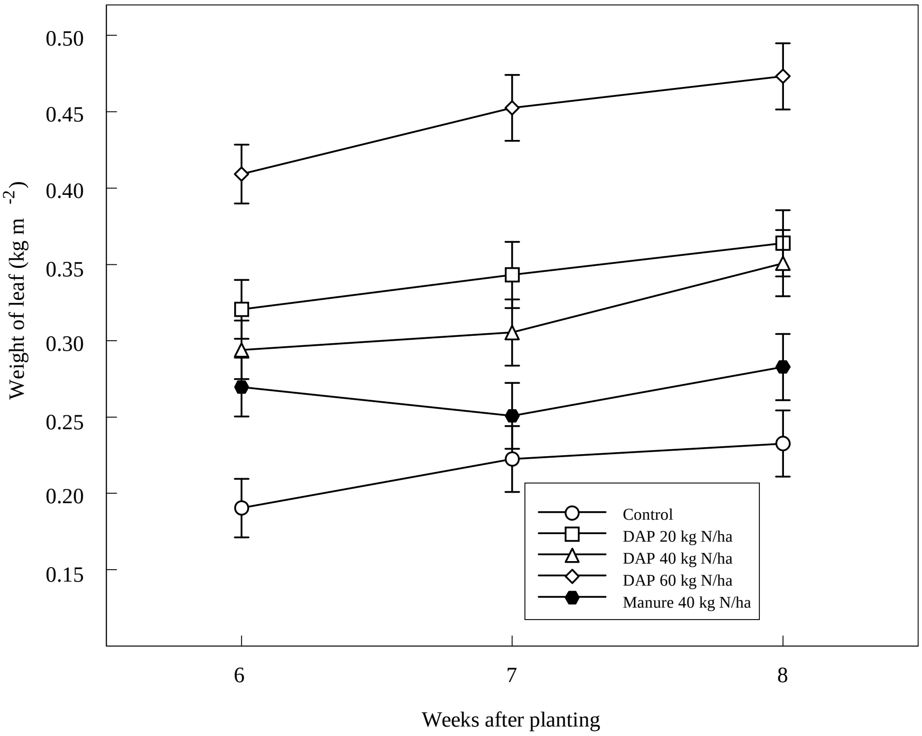

Figure 3.3 : Effect of chemical fertiliser DAP and cattle manure on leaf weight per unit area (kg m^{-2}) of vegetable amaranth

Kjeldahl-nitrogen content

The Kjeldahl nitrogen (amino-nitrogen) content of the leaves was influenced by the time of harvest and the fertilizer treatment (Fig 3.4). With increasing time to harvest all treatments showed a trend of decreasing leaf Kjeldahl-N (hereafter K-N) contents, expressed on a dry weight basis. In years 1 and 2 the K-N content of the control plants for all harvests was lower than that of all other treatments in those years. The K-N content of the leaves from the other treatments were closer to that of the control plants in year 2 than in year 1. In year 1, the K-N content of the manured plants was similar to that of 20 kg N ha^{-1} DAP fertilized plants on weeks 6 and 7, though by week 8 the K-N content of the manured plants had fallen to that of the control plants. Application of 40 and 60 kg N ha^{-1} DAP increased the K-N content to between 5% and 6%. In year 2 there was a trend to increasing K-N with increasing DAP application, but the differences between the treatments was not statistically significant ($P<0.05$).

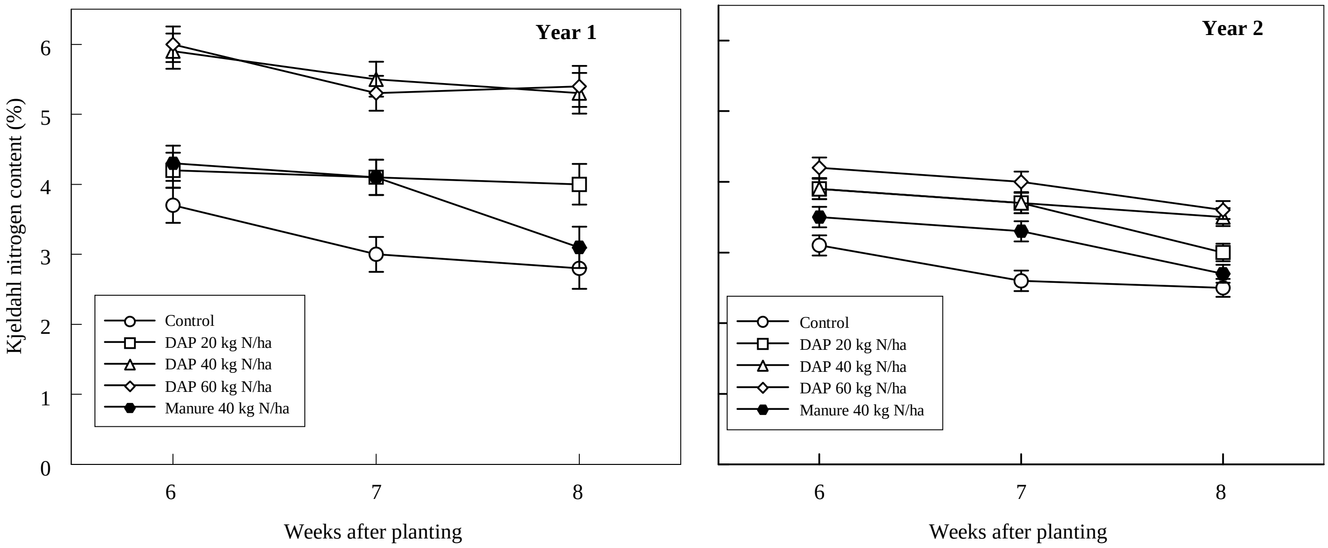

Figure 3.4: Effect of time of harvesting, chemical fertilizer DAP and cattle manure on percent Kjeldahl nitrogen content on a dry matter basis of vegetable amaranth

The K-N content per unit area (Fig 3.5, year 2 only) showed similar trends to those shown by the K-N content per unit weight. However, the content per unit leaf area was relatively independent of time of harvest as a result of decreasing K-N content per unit weight being more or less balanced by an increasing specific leaf weight. The other major difference was the conspicuously greater K-N content per unit area of the 60 kg N ha^{-1} plants compared to those of the 20 and 40 kg N ha^{-1} plants, a consequence of the higher specific leaf weight of the 60 kg N ha^{-1} plants. There were no significant differences in nitrogen content per unit area between plot that received 20kg N ha^{-1} and those that received 40 kg N ha^{-1} (Fig 3.5).

The yield of K-N per hectare (Fig 3.6) gives, a different, more practical perspective on the production of human nutrition to that offered by the per plant values. The K-N production per hectare parallels the weight of leaf in kg per meter square (Fig 3.3, year 2). The addition of DAP leads to significant increases in the K-N yield per hectare though the difference between the 20 and 40 kg N ha^{-1} is small at all harvests. The 60 kg N ha^{-1} dose is the treatment with the highest K-N yield per hectare although it did not give the highest total biomass yield (Fig 3.1, year 2). The total yield in both year 1 and 2 contained both the tender stems and leaves but the nitrogen content analysed was on the leaves therefore only the leaf nitrogen produced per hectare is given.

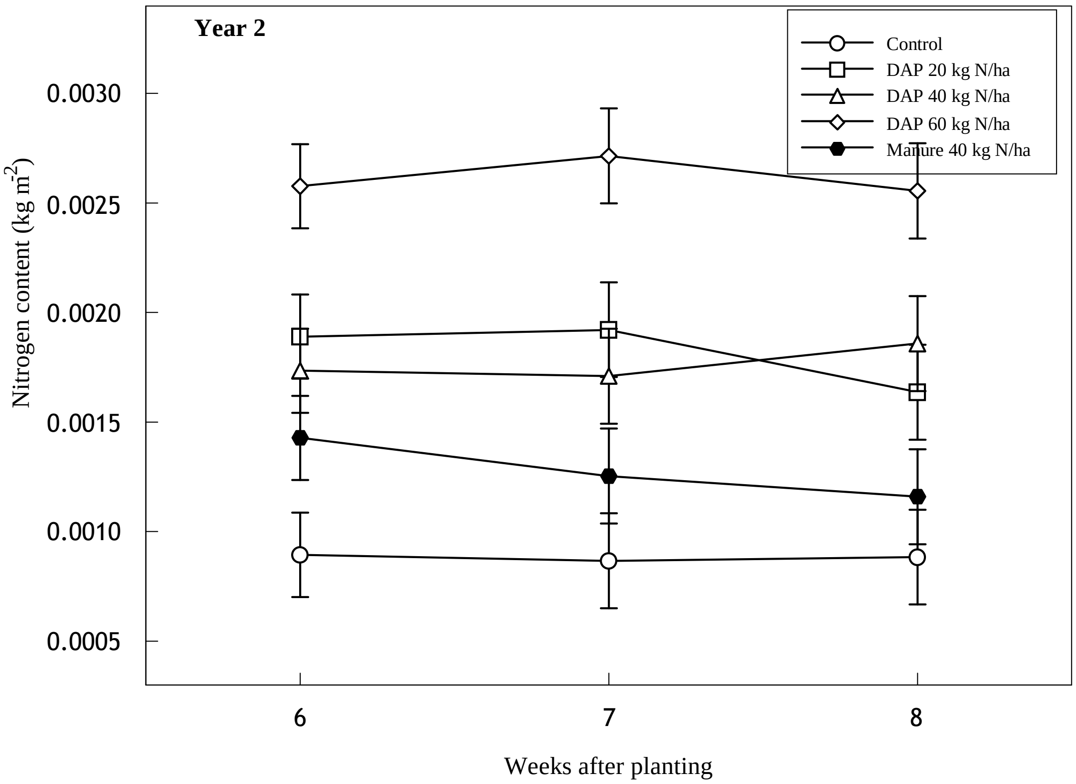

Figure 3.5 : Effect of maturity at harvest, chemical fertiliser DAP and cattle manure on Kjeldahl nitrogen content per unit leaf area of vegetable amaranth (fresh weight basis

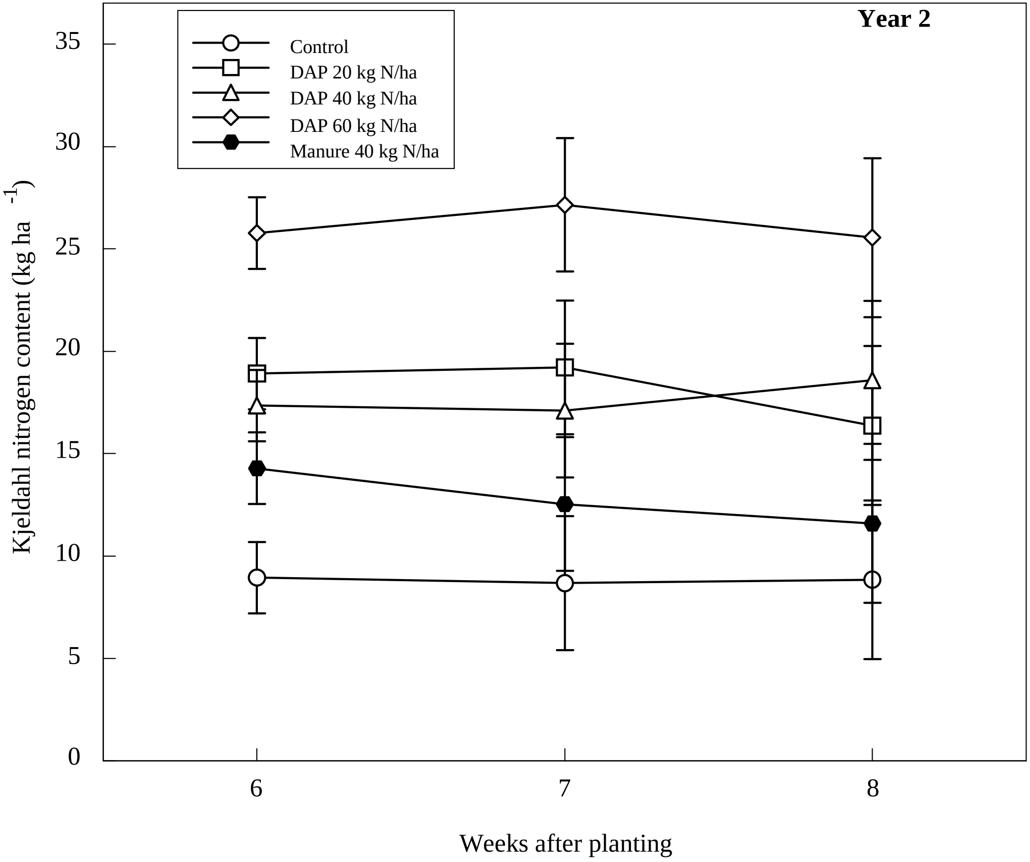

Figure 3.6: Effects of maturity at harvest, chemical fertilizer DAP and cattle manure on Kjeldahl nitrogen content per hectare of vegetable amaranth (fresh weight basis)

The K-N yield per hectare was lowest for the control plots and the addition of manure and DAP increased the yield of K-N per hectare.

Nitrate content

There was an interaction between fertilizer application, time of harvesting and nitrate content; fertilizer 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) as shown in figure 3.7. In year 1 the nitrate levels were, overall, higher than for year 2, and in both years nitrate levels were highest for each treatment at week 6 and decreased thereafter. In year 1 the nitrate levels were lowest for the control and manured plots. With DAP fertilization, levels were higher and increased with increasing application rate of DAP. In year 2 the ordering of the nitrate levels was similar, but as the nitrate levels in this year were only 50% of those in year 1 the differences between the manured, control plots and the plot fertilized with 20 kg N ha^{-1} were insignificant. Higher applications of DAP resulted in higher nitrate levels, but the differences between the 40 and 60 kg N ha^{-1} plants was only significant at week 8, though the 60 kg N ha^{-1} material had a trend to higher nitrate levels at the earlier harvests.

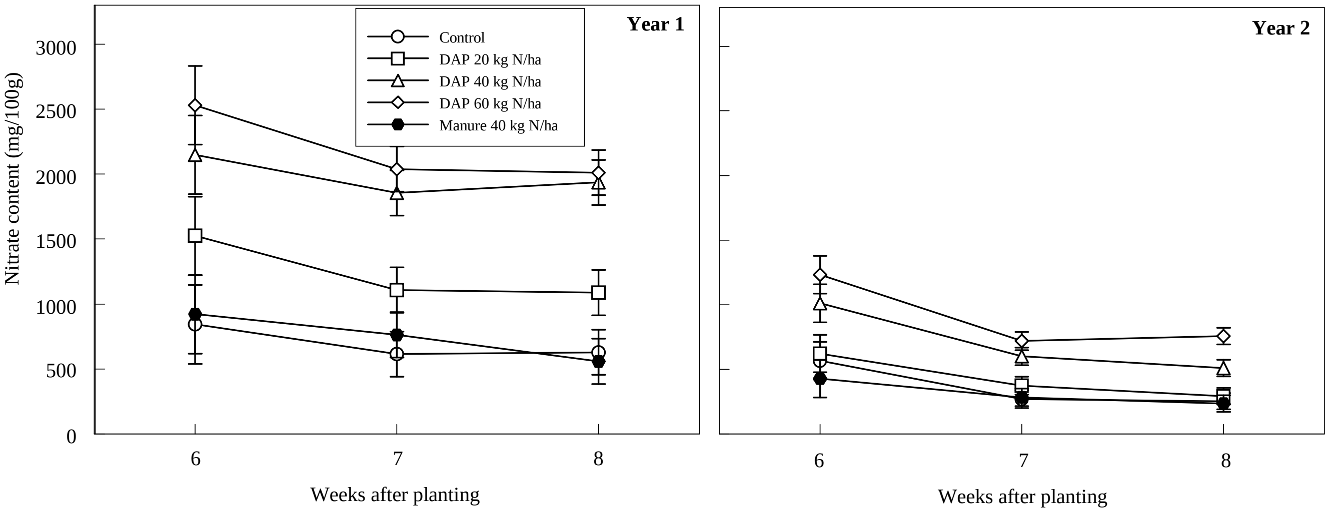

Figure 3.7: Effect of chemical fertiliser DAP and cattle manure on nitrate content (dry weight) of vegetable amaranth

Discussion

Germination

Amaranth seeds have been reported to have tough seed coats which do not rupture easily. While the hard seed coat does not block water uptake, it retards germination (Bond et al, 2007) since the plumule and radicle cannot penetrate it. Manure has also been shown to enhance decomposition of the endocarp resulting to enhanced permeability of moisture to the seed (Mwaburi et al, 2005). Our results, however, indicate that manure, rather than stimulating germination, simply lacks the apparent inhibitory effects of the artificial fertilizers. Even the lowest application of DAP inhibits germination relative to the control (Table 3.3), and with increasing amounts of DAP germination decreases further. Kumar et al (2008) working with *Amaranthus powellii* found out that its emergence declined with increasing N fertilization via an osmotic effect. Higher concentrations of ammonium nitrate fertilizer have also been shown to significantly inhibit germination of redroot pigweed (*Amaranthus retroflexus*) (Sardi and Beres, 1996). Overall, therefore, the application of artificial fertilizer of the kind used in this study prior to germination has a significant negative effect on germination.

Growth and yield

The growth and yield of amaranth is strongly influenced by soil fertility when this has been enhanced by an inorganic fertilizer, 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 is not proportional to the supply of N, with the response depending on N supply and harvest date. The typical growth duration of amaranth in Kenya is 8-10 weeks from planting to flowering, with the main harvesting of leaves occurring between week 6 and 8. Under this regime yield saturated at an application of DAP of no more than 40 kg N ha^{-1} when this was applied as a single dose.

Working with grain amaranth and using 0, 15, 30, 45 and 60kg N ha^{-1}, Olaniyi et al (2008) found that fresh and dry shoot yields and grain yield per hectare were significantly influenced by applied 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 kg and 60 kg N ha^{-1} which is similar to our experience with vegetable amaranth. The grain yield per hectare was increased as the N rates increased from 0 up to 45 kg N ha^{-1} and declined at 60 kg N ha^{-1}. Since there was no increase in yield when 60 kg N ha^{-1} were used, it could be that higher rates of N result in luxury N uptake leading to nitrate accumulation or loss of N through leaching (Maurao and Brito, 2001). Higher rates (45, 90 and 135 kg N ha^{-1}) have been successfully used to increase the production of vegetable amaranth but in this case the N amount was applied as a split application, half at germination and the other half two weeks later (Singh and Whitehead, 1996). In this case leaf area increased with N-fertilization until 90 kg N ha^{-1} then decreased at 135 kg N ha^{-1}.

The lack of any yield increase in the manured plots was disappointing but 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 when compared to the control. Recent work by Sousa et al (2008) demonstrated an overall trend of higher total phenolics concentrations in organically grown tronchuda cabbage, accompanied by lower plant fresh weight, as compared with conventionally fertilized samples. They suggested that the lack of nutrients, particularly insufficient nitrogen supply as a result of a low mineralization rate under organic production, could have boosted synthesis of phenolic compounds while limiting rapid growth of new leaves. Other studies have shown that manure is not as effective as artificial fertilizer (Van Lauw et al, 2001, Turan and Sevimli, 2005). Compared to mineral fertilizers, manure contains relatively smaller amounts of nutrients readily available for growth of plants (Edmeades, 2003). It is possible that after emergence, the supply of these essential nutrients was insufficient to promote continued rapid growth.

Care was taken in this trial to use a manure treatment that was comparable to that employed by local small-scale growers, so the differences in the growth responses obtained between the manured and DAP-fertilized plots are both significant and relevant. Manure is an important source of nutrients for many smallscale farmers in East Africa who cannot afford chemical fertilizers (Onduru et al, 2008).The manure used in this study was 1 year old and from an uncovered old cattle shed. It has been reported that old manures tend to be lower in nutrients due to the long periods of decomposition and mineralization (Harby et al, 2001). In addition, in an uncovered or loosely covered manure heap, free nitrogen is likely to be lost through volatilization during hot periods or through runoff or leaching during the rain season thereby reducing availability to plants. The variation in nitrogen (N) contents of cattle manures from Africa is large depending on ration composition and utilization by animals, and collection and storage of the manures (Snijders et al, 2009). Lekasi et al, 2003 reported total N contents of between 1.12 % and 1.4 % in Central Kenya. Our values of 1.79 % in year and 1.9 % in year 2 are slightly above these values. However, not all the nutrients in manures are readily available for uptake by plant roots. Much of the N and P in manures is combined with organic substances and only becomes available to plants when these substances decay. In Cattle manure, only about 20 % of the total N and 40 % of the total P becomes available in the year of application (http://agriculture.kzntl.gov.za/portal/agricpublications/327/). N content in manures from temperate countries are often higher, probably due to higher protein contents in feed rations and more favourable collection and storage conditions, including lower temperatures (Snijders et al, 2009). In the Netherlands, for example, average N contents in cattle manure of 2.5 % have been reported (Anonymus, 1997 In Snijders et al, 2009).

It is evident that yields in year 1 (especially at the rate of DAP application) are lower than in year 2. A comparison of the harvests in the two years suggests that the plants in year 1 were about one week behind those of year 2. The composition of the soil of the two plots was comparable in the two years (Table 3.1) and though there are differences in the weather of the two years (Table 3.2a and 3.2b), we cannot reliably attribute any particular cause to the yield differences observed. The results suggest, however, that amaranth is more sensitive to environmental factors than is commonly supposed. Vegetable amaranth has been reported to achieve optimum growth when air temperatures are above 25^{o}C (Singh and Whitehead, 1996). During the growing period in our study, the air temperature ranged between 18 ^{o}C and 19.7^{o}C in year 1 and between 18.7 ^{o}C and 21^{o}C in year 2. The crop received a total of 43hours in year 1 and 59 hours in year 2 of solar energy and especially early in the growing season (the first and second 3 weeks periods). The number of sunshine hours in year 1 was much less than that in year 2. It is possible that the extra hours of solar energy received in year 2 may have contributed to the higher yields realized.

The greatest increase in above ground fresh weight, 13 tons ha^{-1}, occurred between weeks 7 and 8 for the 40 kg N ha^{-1} treatment of the year 2 experiment. This equates to 1.95 tons ha^{-1} dry matter using a 15 % dry matter content (see chapter 4), or about 279 kg ha^{-1} day^{-1} dry matter. Expected plant production can be estimated from the intercepted total solar irradiance and estimates of conversion of absorbed irradiance to dry matter, (Monteith, 1978) which for C_4 plants is conservatively estimated to be 2 g MJ^{-1} (*Echinochloa polystachya*, for example, growing in the Amazon basin has an energy conversion efficiency 2.3 g MJ^{-1} (Piedade et al, 1991). Assuming that the absorbed irradiance is only 80 % of the incident, then the highest actual productivity is comparable to that expected from the total solar irradiance measured near the field site (Table 3.2) (315 kg ha^{-1} expected productivity versus 279 kg ha^{-1} actual productivity).

Foliar nitrogen contents

The nitrogen content of the leaves is important in two major respects; the reduced (amino) nitrogen content (in our case measured by the micro-Kjeldahl method) is nutritionally important as this is largely made up of protein, whereas the oxidized (nitrate) nitrogen content is important because of its toxicity. The increase in foliar K-N or protein with increasing N fertilization under high irradiance conditions is consistent with numerous published reports (e.g. Terashima and Evans, 1988; Evans and Terashima 1988 , Makino, et al, 1997; Evans, 1996).

A comparison of the differences between the fertilization treatments with respect to productivity and K-N content is useful. The yield of the DAP fertilized plots was much higher than that of either the control or the manured plots. In terms of the K-N content, the leaves from the DAP fertilized plots generally had the highest content, though in both years the leaves from the 20 kg N ha^{-1} plot were in some harvests no better than those from the manured plots. If this higher K-N content translates directly into available protein then clearly there are nutritional benefits to be gained by using DAP at 40 kg N ha^{-1}, but above that treatment the benefit is minimal. The nutritional value of young leaves from young plants also exceeds that from older plants. In terms of per hectare yield of K-N, the 60 kg N ha^{-1} treatment is more productive though this benefit is outweighed by the lower leaf yields achieved under this treatment (Fig. 3.1) compared to that from the 40 kg N ha^{-1} treatment.

Though the K-N content of the control and manured plants was lower than that of the DAP fertilized plants the decrease was proportionately much less than that of yield. So the K-N content of control plants at week 6 of year 1 (the most extreme year in terms of K-N content) on a weight basis was about 60% of that of the 60 kg N ha^{-1} plants, despite their productivity being approximately 18 % of the 60 kg N ha^{-1} plants. 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. However the increased K-N content of the manured plants needs to be viewed in the context of the low productivity of the manured plots (Fig. 3.1). This results in a low K-N productivity per hectare (Fig 3.6).

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 fertilized 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.

Other African leafy vegetables (*Solanum africanum, Amaranthus hybridus, Amaranthus caudatus, Telfaria occidentalis, Venonia amygdalina* (fertilizer treatments used not known) have been reported to contain a crude protein content of between 31.7% - 34.6% (Aletor et al, 2002). Dividing the crude protein with 6.25 a factor used to convert the percent nitrogen to crude protein, these vegetables contained a K-N content of between 5.1% - 5.5%. These values are within the range of that obtained in *A. hypochondraicus* used in this study which were 6.0% in year 1 and 4.2% in year 2 when produced using

60 kg N ha^{-1} DAP. In the absence of fertilization or when using manure, the K-N is conspicuously lower than the values reported for other comparable vegetables (Fig. 3.8).

Nitrogen is a basic component of protein and the total N, protein or rubisco contents of leaves is strongly correlated with the maximum photosynthetic capacity of the leaves for both C3 and C4 plants, though the nitrogen use efficiency of C3 is lower than that of C4 leaves (Ehleringer, 1983; Field and Mooney, 1986; Evans and Terashima, 1988; Evans, 1996; Makino et al, 1997; Makino et al, 2003). In the case of the North American *A. palmeri* (a C4 amaranth like *A. hypochondriacus*) rates of CO_2 fixation of over 80 μmol m^{-2} s^{-1} at an irrandiance of 2000 μmol m^{-2} s^{-1} are achieved with a K-N content of 4% - 4.5% (Ehleringer, 1983); to the best of our knowledge this is the highest rate of CO_2 fixation ever measured from a leaf in air. This is comparable to the highest K-N content found in *A. hypochondriacus* in year 2 and less than that found in year 1 and suggests that like *A. palmeri*, *A. hypochondriacus* might have exceptional photosynthetic capacity. High leaf protein contents are associated with high leaf levels of other nutritionally valuable components, such as fatty acids (Witkowska et al, 2008), minerals and organic cofactors associated with photosynthesis.

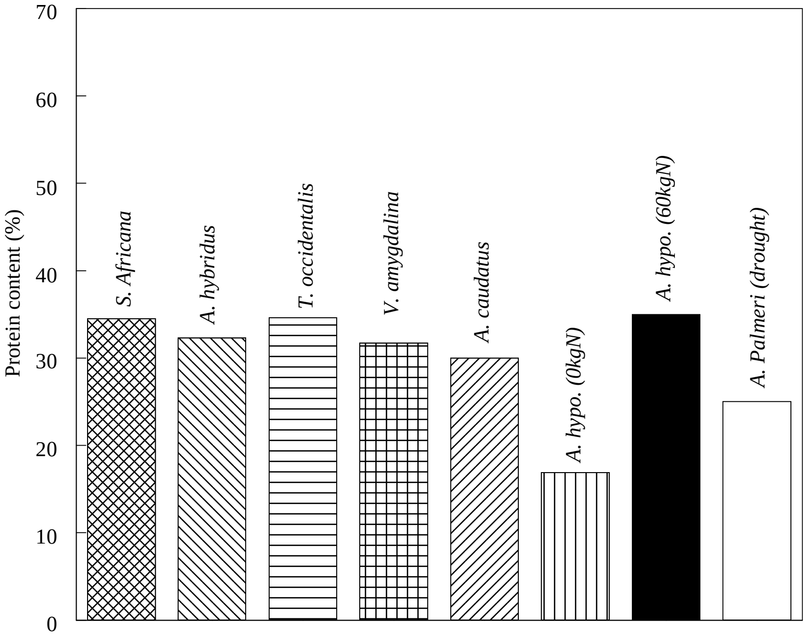

Figure 3.8: Crude protein content of other leafy vegetables compared with that of *Amaranthus hypochondriacus* in year 2. Source: Aletor et al, 2002. Food Chemistry 78: 63 – 68.

Foliar nitrate levels and toxicity

A consequence of nitrogenous fertilization 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; Santamaria et al, 1999a; Santamaria et al, 2001; 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 fertilization 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 in years 1 and 2 (Fig 3.9) 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, a response that is particularly conspicuous in year 1. Overall, the leaves from the first harvest (week 6) have relatively the greatest amounts of nitrate compared to K-N, and in year 1 the decrease in nitrate as the crop matures is paralleled by insignificant decreases in K-N. Early in the growing season the levels of leaf K-N and nitrate are both high and though the levels of both decrease with increasing time to harvest this effect is relatively greater for nitrate content than for K-N content.

Both foliar nitrate and K-N are coupled to yield; in year 1 when leaf yields were overall lower than for year 2 both K-N and nitrate levels were conspicuously higher at the higher levels of DAP application, but the increase in nitrate was relatively greater than that for K-N. The week 6 leaves from the 20kg N ha^{-1} showed a similar response. So though the lower yield of the crops in year 1 was compensated for by higher K-N contents, the relatively larger increase in nitrate content raises the risk of nitrate toxicity from the more heavily fertilized (with DAP) crops from year 1. In contrast to the DAP fertilized plots, the leaves from the control and manured plots while possessing reasonable K-N contents (about 30 g/kg) had relatively low nitrate levels (about 5 g/kg). This favourable situation in relation to nitrate, however, is largely outweighed by the overall low yields obtained from these treatments. The problem with DAP fertilization, where yields and K-N levels are high, is balancing the benefits of these increases against the risk of excess nitrate, especially when nitrate levels increase strongly if yield is decreased.

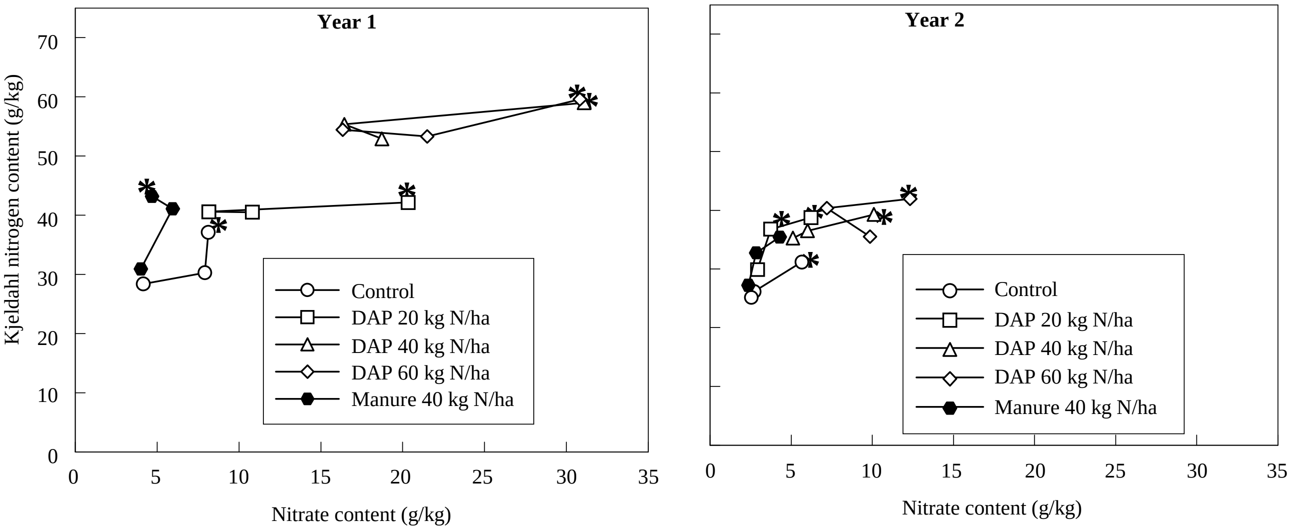

Figure 3.9: Effect of maturity at harvest, chemical fertilizer DAP and cattle manure on the nitrate-nitrogen balance in vegetable amaranth

Increased nitrate accumulation often follows high rate application of nitrogen fertilizers. Nitrogen fertilization and weather conditions have also been found to be of equal importance as factors influencing nitrate accumulation in spinach, maize biomass and potato tubers (Schmidt et al, 1971; Bassioni et al, 1980; Amalin et al, 2002) in that the accumulation of nitrates by the same plants differed significantly in different years. The differences were shown to be due to different levels of illumination or different lengths of sunny periods, fluctuations in temperature and humidity. In these studies, increased nitrate content was found to be due to even an insignificant deviation from the optimal value of the crop. Accumulation of nitrates in the plants decreased with the the increase in the number of sunny days during the vegetative growth (Amalin et al, 2002) which is similar to what was found in this study in which nitrate amounts recorded in year 2 were lower than those of year 1 that received less sunshine hours.

DAP at the rate of 40kg N ha^{-1} gave the highest yields with nitrate levels of 2150mg/100gdw (year 1, week 6 harvest). This is equivalent to 320mg/100g fresh weight. These levels are slightly higher than the levels of between 250mg – 300mg permitted in 100g of edible lettuce (Santamaria, 2006; Food standards Agency, 2001). Unlike lettuce, however, in Kenya and most developing countries amaranth leaves are normally cooked by boiling in large quantities of water, draining and discarding the water and then frying the vegetables in a small quantity of oil. Nitrates are water soluble and therefore some loss through leaching is expected, especially if the cooking water is discarded. Such losses have been reported by Ricardo (1993) and Imungi and Potter (1983). Abo Bakr et al (1986) reported losses of 16% of the nitrate from peas, 34% from beans, 51% from carrots and 34% from spinach (a leafy vegetable comparable to amaranth) through boiling with water (vegetable: water = 1:2) for unspecified periods of time. Using the release of nitrate from cooked spinach as a guide, cooked amaranth would contain about 210mg/100g. According to the FAO (1988), nitrate levels normally found in vegetable amaranths do not pose a serious health problem to healthy individuals if consumption does not exceed 100g of leaf per day and our results are consistent with this. Notably, amaranth is often cooked with other leafy vegetables and therefore it is unlikely that more than 100g would be consumed in a single meal. Even if amaranth was prepared alone for consumption, conventionally very few families will consume more than 100g of the vegetable per day per person.

The rapidly rising costs of chemical fertilizers 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 fertilizer due to low yields and low K-N (a measure of protein content), although the use of manure results in low levels of nitrates. 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 fertilizers can result in the substantial accumulation of nitrate, especially when growth is poor. Improvements in the amount and timing of fertilizer application might help avoid nitrate problems but as with improving the effectiveness of manure, this would require more guidance to the producers.

CHAPTER 4

INFLUENCE OF MATURITY AT HARVEST, N FERTILIZER AND POSTHARVEST STORAGE ON DRY MATTER, ASCORBIC ACID AND β-CAROTENE CONTENTS OF VEGETABLE AMARANTH (*Amaranthus hypochondriacus*)

Abstract

Vegetable amaranth is a leafy vegetable traditionally grown in sub-Saharan Africa and Asia where it is the most consumed traditional vegetable in these countries. It is considered to have high nutritional quality, containing relatively large amounts of vitamins A and C. We have assessed the influence of the maturity of the vegetable and soil nutrition and on the visual and internal quality of amaranth. We found out that leaf ascorbic acid content is strongly influenced by both maturity and soil nutrition, with leaves of 7 week old manured plants having the highest content. β-carotene increases with increasing amount of soil nitrogen and with increasing plant age. The loss of both visual and internal quality during storage was influenced more by maturity at harvest and the temperature of storage than the soil nutrition.

KEYWORDS: *Amaranthus hypochondriacus*, growth stage, N fertilizer, ascorbic acid, β-carotene, moisture loss

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Submitted as: Cecilia M. Onyango, Jeremy Harbinson, Jasper K. Imungi and Olaf van Kooten. Influence of maturity at harvest, N fertilizer and postharvest storage on dry matter, ascorbic acid and β-carotene contents of vegetable amaranth (*Amaranthus hypochondriacus*)

Chapter 4

Introduction

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 and 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 and Barrett, 2009).

A high intake of fruits and vegetables is correlated with a low incidence of a number of chronic diseases, including cancer and cardiovascular diseases (Hertog et al, 1992). It is suggested that antioxidants, such as the ascorbic acid (vitamin C), carotenoids and flavonoids commonly found in high concentrations in fruits and vegetables, are involved in the protection against these diseases (Carr and Frei, 1999). Studies have also shown that to maximise the benefits from ascorbic acid and β-carotene it may be better to eat fruits and vegetables containing these antioxidants than to rely on supplements for their supply (Holick et al, 2002).

The antioxidant content of fruits and vegetables is known to vary with factors such as genotype, environmental growing conditions, growth stage at harvest, and postharvest handling, storage (Goldman et al, 1999) and processing (Verker and Dekker, 2004). The ascorbic acid and β-carotene contents of leafy vegetables such as spinach have been found to vary extensively during plant growth (Bergquist et al, 2005) and even during diurnal cycle (Bartoli et al, 2006). While variation in ascorbic acid content during plant growth has been observed in spinach there appears to be no consistent pattern to this variation. Bergquist et al (2006) working with immature spinach found a higher ascorbic acid content in younger leaves (stage I) than in older leaves (stage II and III) while Stino et al (1973) reported that ascorbic acid concentration increased with plant age. In other leafy vegetables, ascorbic acid and carotenoid contents have been found to change during growth, but with no consistent trend to increase or decrease (Guil-Guerrero et al, 2003; Sørensen et al, 1994; Weston and Berth, 1997).

Differences in nutritional value of vegetables and fruits grown with differing levels of nitrogen fertilization have been found (Weston and Barth, 1997). Lisiewska and Kmiecik (1996) reported that increasing the amount of nitrogen fertilizer from 8 to 120kg N ha^{-1} decreased the vitamin C content in cauliflower curd. Similarly, Tedone (2005) working with potato, found that the use of nitrogen fertilizer resulted in a reduction of ascorbic acid levels in the tubers. In addition to effects on ascorbic acid, nitrogen fertilization produces increases in the β-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 β-carotene, the impact of nitrogenous fertilisers on the levels of ascorbic acid and β-carotene of these vegetables is not well known. However, as both are involved in photosynthesis and nitrogen nutrition is an important determinant of photosynthetic capacity it would be expected that the nitrogen status of the crop will affect their content.

With regards the nutritional value of a vegetable the influence of post-harvest factors on nutrient content are as important as those of the production phase. Leafy vegetables are highly perishable with relatively low storage potential in terms of visual quality, as well as other quality variables such as wilting and nutritional content. This loss of quality is proportional to temperature (Kays and Paull, 2004). Several studies have been carried out on the effect of different storage conditions on ascorbic acid and carotenoids (Lee and Kader, 2000; Negi and Roy, 2003; Prabhu and Barrett, 2009) but we have found very few comparing changes during storage in leaves of different ages (Bergquist et al, 2005) grown using both organic and inorganic fertilizers. Since metabolic rates are usually higher in younger leaves, they could be expected to loose antioxidants more quickly than older leaves. The ascorbic acid contents of most leafy vegetables declines quickly during storage, especially at ambient temperature, mainly through oxidation while the carotenoids are relatively more stable compared to ascorbic acid. Vegetable amaranth is usually displayed at ambient temperatures for vending (Onyango et al, 2008). The high temperatures cause rapid deterioration through loss of water, resulting in wilting and loss of consumer appeal.

Based upon results obtained from other vegetables (Stino et al, 1973; Sørensen et al, 1994; Weston and Berth, 1997; Guil-Guerrero et al, 2003; Flores et al, 2004; Bergquist et al, 2005; Bergquist et al, 2006; Bartoli et al, 2006), it is expected that the ascorbic acid and β-carotene contents in vegetable amaranth will be modified by the cultivation practices used in the production of the vegetables. Nitrogen nutrition is important in increasing the productivity of vegetable amaranth, and both manure and artificial fertilisers are commonly used as nitrogen sources, with weathered manure from cattle sheds being more commonly used by small-scale farmers in Kenya than artificial fertilisers (Personal observation). As it is likely that fertilisation will affect the ascorbic acid and β-carotene contents at the time of harvest, the potential interaction with crop productivity is important in assessing the overall outcome of the production process in terms of the production of nutrition.

During the postharvest phase the retention of the ascorbic acid and β-carotene produced during the production phase is expected to be influenced by the storage conditions, and possibly by the production conditions, of the vegetables. So far, however, very little is known about how either the ascorbic acid or β-carotene content of vegetable amaranth is influenced by either pre or post-harvest conditions. The aim of this study therefore, was to investigate the influences of N from chemical fertilizer diammonium phosphate (DAP) and cattle manure (fertilizers commonly used to increase amaranth productivity), and maturity at harvest on dry matter, ascorbic acid and β-carotene contents of *Amaranthus hypochondriacus.*

Likewise it is not known how postharvest storage affects moisture, ascorbic acid and β-carotene contents of this vegetable.

Materials and Methods

Amaranth seeds were obtained from Asian Vegetables Research and Development Centre (AVRDC) 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 Latitude 1° 15′S and Longitude 36° 44′E, and at altitude 1930 m above sea level (Sombroek et al, 1982). 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 and Lawes, 1971). The mean annual temperature is 18°C. The soil in Kabete is characterized as a deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996). The land used for the plots had not received any fertilizer 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 fertilized with either manure or diammonium phosphate (DAP; 18:46:0, N:P:K) fertilizer; manure is potentially a source of not only nitrogen (N) but other plant macro- and micro-nutrients while the DAP will also provide phosphorus as well as N: In 2007, planting was done on the 11th April while in 2008, planting was done on the 6th of March. The differences in planting days were due to the different times the long rains season started in the two years. The trials were laid out in a complete randomized block design with five fertilization treatments as follows: one plot with cattle manure at 40kg N ha^{-1} (calculated using the content of N found in the manure, approximately 2 tonnes of manure per hectare, Table 3.1), three plots with DAP (18:46:0) at 20, 40, and 60kg N ha^{-1} and unfertilized control plot. While we will focus on the N content of these fertilizers, it is important to note that the manure used is a source of a diverse range of macro- and micro-nutrients (Onyango et al, in preparation) and DAP is a phosphorus (P) as well as an N source; for the manure, the P added was 40 g and 2.6 kg P ha^{-1} in year 1 and 2 respectively. For the DAP 20, 40, and 60kg N ha^{-1} the p added was 50, 100, and 150 kg P ha^{-1}. Phosphorus (P) (as phosphate) is an essential nutrient for plant growth, development, and reproduction that forms part of key molecules such as nucleic acids, phospholipids, ATP, and other biologically active compounds. After nitrogen, P is considered to be the second most important nutrient limiting agricultural production (López-Bucio et al, 2000). However, the phosphate ion is precipitated by many cations and is easily available for plant uptake at a narrow range of neutral soil pH values. In acid soils, P forms low-solubility precipitates with aluminum (Al) and iron (Fe), whereas in alkaline soils, it combines efficiently with calcium (Ca) and magnesium (Mg) to form sparingly soluble phosphate compounds (Bar-Yosef, 1991) Therefore, although the total amount of phosphorus in the soil may be high, in most cases it is unavailable for plant uptake. It is likely that under the acid conditions (pH 5) of the soil used in our study, the phosphate in either the manure or the DAP was not available for plant uptake.

The treatments were replicated three fold. The plots measured 2 m x 2 m with a spacing of 0.15 m x 0.1 m between and within rows in each plot (Palada and Chang, 2003). Care was taken in this trial to use a manure treatment and, where possible, cultivation practices that were comparable to those employed by local small-scale growers. 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 in 2008 to keep the soil moist. The plants were grown in 2007 (year 1) and 2008 year 2, but the measurements made on the year 1 plants were not as comprehensive as those made in year 2 so not all results are available for both years. The vegetables were harvested at three maturity stages; 6, 7 and 8 weeks after planting by cutting-off the tender edible stems and leaves.

Storage trials

The harvested vegetables were then stored in a domestic refrigerator ($4\underline{+}1^oC$) and at ambient temperature ($20\underline{+}3^oC$) for a period of 4 days, the typical time period that elapses between harvesting and consumption in Kenya (Onyango and Imungi, 2007). The edible portion of the fresh and stored vegetables were analyzed for dry matter/moisture, β-carotene and reduced ascorbic acid, the stored vegetables were analyzed after every two days of storage.

Preparation of the vegetables for analysis

The vegetables were freeze-dried, then ground to pass through a 600µm sieve. The powder was stored in tightly closed plastic vials in a freezer (-20^oC) prior to their analysis for ascorbic acid and β-carotene. All the analyses were done in triplicate samples.

Determination of moisture and dry matter content

Moisture and dry matter were determined as a percentage by drying 5g of fresh vegetables in a thermostatically controlled air-oven at 60^oC to constant weight (AOAC, 1999).

Determination of β-carotene

β-carotene was determined by the method of García-Plazaola and Becerril (1999) with slight modifications. About 20 mg of freeze-dried, powdered leaf was accurately weighed and placed into a plastic centrifuge tube. About 5mg magnesium hydroxide carbonate and 2.5 ml tetrahydrofuran:methanol (50:50) mixture were added and vortexed. The tube was placed in an ultrasonic bath for 15 minutes in the dark, then 2.5 ml methanol was added and vortexed. The mixture was centrifuged at 4,500 rpm for 5 minutes at 4^oC. One-and-half millilitres of the supernatant were then placed into an eppendorf tube and centrifuged at 8,000 rpm for 5 minutes at 4^oC. From the eppendorf tube, 1ml of supernatant was withdrawn and used for analysis of β-carotene using HPLC (Column: Alltima C18, 3µm + guard column, Detection: UV 470 nm, Flow: 1 ml/min, Column temperature: 30°C). The concentration of β-carotene in the samples was determined from a standard curve prepared from known concentrations of pure β-carotene (Sigma-Aldrich, St. Louis, MO, USA) in methanol.

Determination of Reduced Ascorbic Acid (RAA)

Determination of ascorbic acid was by the method of Helsper et al (2003). A weighed sample of approximately15mg of freeze dried, powdered leaf was placed into a 2.2 ml eppendorf tube to which 2ml 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°C. One millimetre of the supernatant was then taken and analyzed for RAA using HPLC (Column: LiChrosper 100 RP-18 (5µm), Detection: UV 260nm, Flow: 0.5 ml/min, column temperature: 30°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.

Statistical analysis

Data was subjected to the general analysis of variance (ANOVA), using Genstat statistical software (Payne et al, 2006). Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P \leq 0.05$).

Results

Preharvest

Dry matter

The dry matter contents of the harvested vegetables did not differ in Year 1, but differed significantly ($P < 0.05$) among treatments in Year 2 (Fig 4.1) when the highest dry matter contents were recorded in control plots followed by those of the manure treated plots. In no case were there significant differences in dry matter content of the vegetables receiving mineral fertilizer at different rates. There was a general increase with maturity of the vegetables in the dry matter content in all the treatments.

β-carotene content

The β-carotene content increased between 6 and 8 weeks after planting for all plants that received DAP at all N levels in both years. Comparing year 1 with year 2, the β-carotene levels were overall higher (typically by 50%) in year 1 (Fig 4.2). There was no significant increase in β-carotene for those that received manure. There was no interaction between the source of N and the time of harvest on the β-carotene contents of vegetable amaranth grown using DAP at different levels in either year 1 and 2.

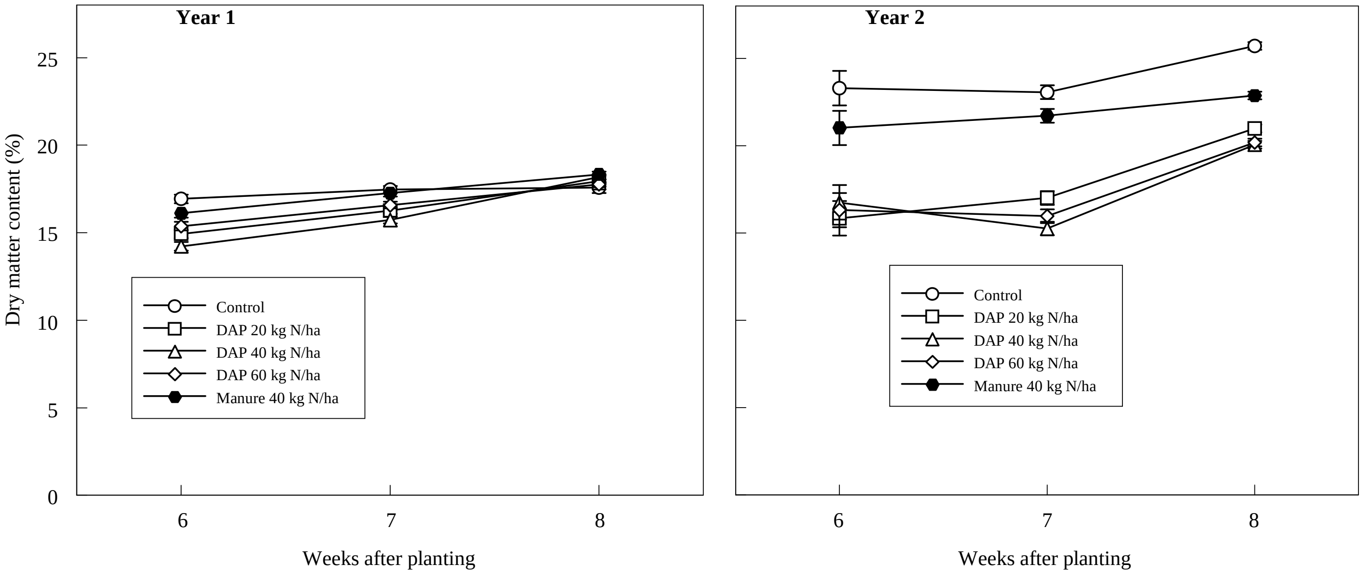

Figure 4.1: Effect of diammonium phosphate fertilizer, manure and maturity at harvest on dry matter content of vegetable amaranth (*A. hypochondriacus*).

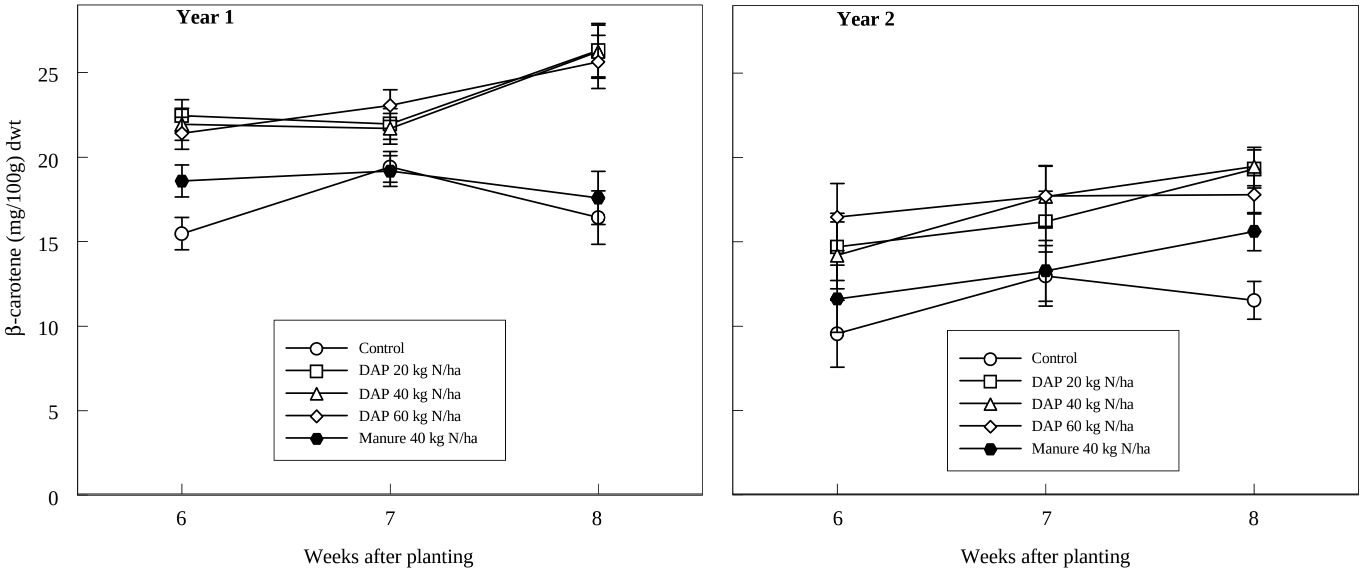

Figure 4.2: Effect of diammonium phosphate fertilizer, manure and maturity at harvest on β-carotene content of vegetable amaranth (*A. hypochondriacus)*

In both years the use of DAP as a source of N resulted in higher β-carotene contents than manure (Fig 4.2). The use of DAP also resulted in higher Kjeldahl nitrogen (K-N) contents than in either the control and manured plots (Fig. 4.3) and comparing K-N content with that of β-carotene reveals a correlation between both; as β-carotene tends to zero K-N tends to about 2.5 g/100 g (Year 2, Fig 4.4) , and with increasing K-N the β-carotene levels increase (Fig 4.4) until β-carotene levels reach a maximum at around 25 mg/100 g (fig 4.4, year 1).

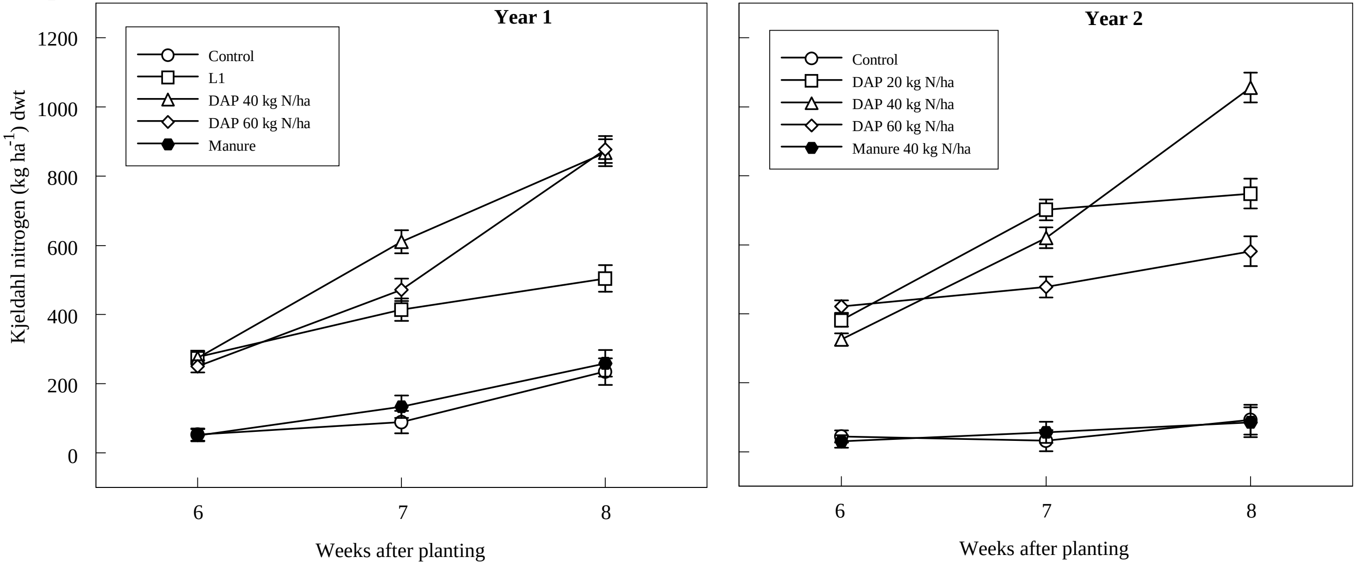

Figure 4.3: Effect of diammonium phosphate fertilizer, manure and maturity at harvest on Kjeldahl nitrogen content of vegetable amaranth (*A. hypochondriacus*).

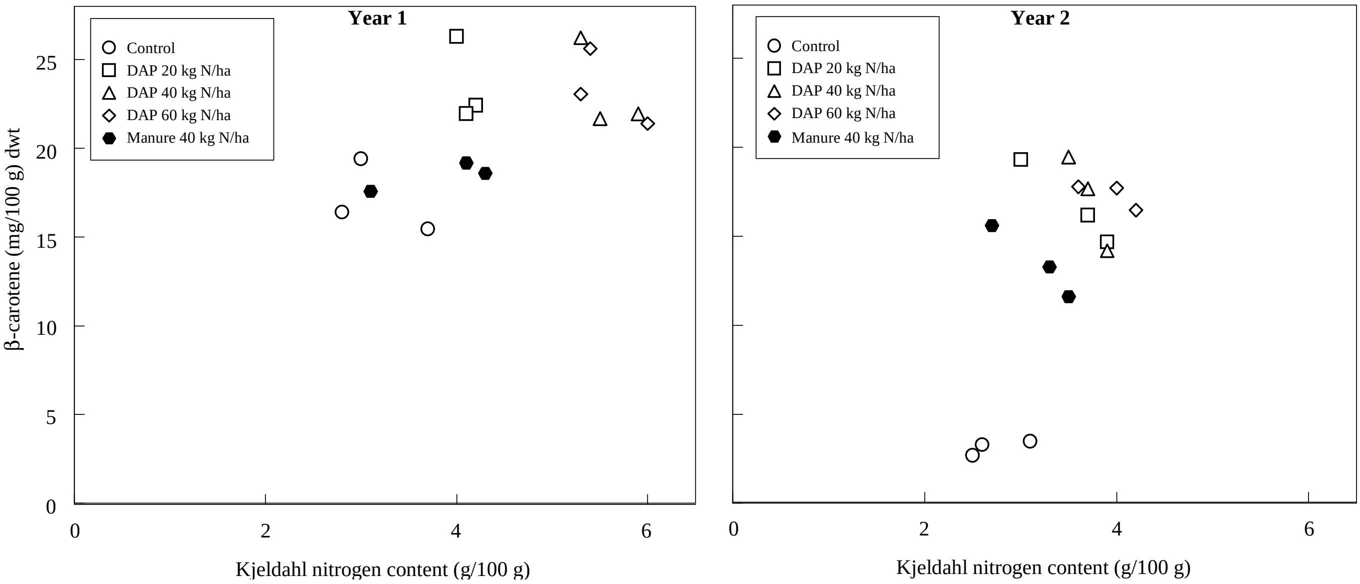

Figure 4.4: Effect of diammonium phosphate fertilizer, manure and time of harvesting on both β-carotene and Kjeldahl nitrogen content of vegetable amaranth (*A. hypochondriacus*).

Ascorbic acid content

Ascorbic acid contents (data only available for Year 2) were overall very variable, ranging from nearly 0.3g to over 3 g/100 g on dry weight basis and differed significantly (P=0.05) among treatments. The highest ascorbic acid contents were recorded in plants receiving manure at 40 kg N ha^{-1}, followed by those receiving DAP at 20kg N ha^{-1} (Fig 4.5). With DAP at higher rates than 20 kg N ha^{-1} there was a decrease in ascorbic acid content. The lowest ascorbic acid content was found in either the control or 60 kg N ha^{-1} plants, depending upon the harvest date. The ascorbic acid contents increased between week 6 and 7, but decreased in week 8 (Fig 4.5). Unlike the relationship between β-carotene and K-N content, that between ascorbic acid content and K-N shows no clear correlations (Fig 4.6). 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.

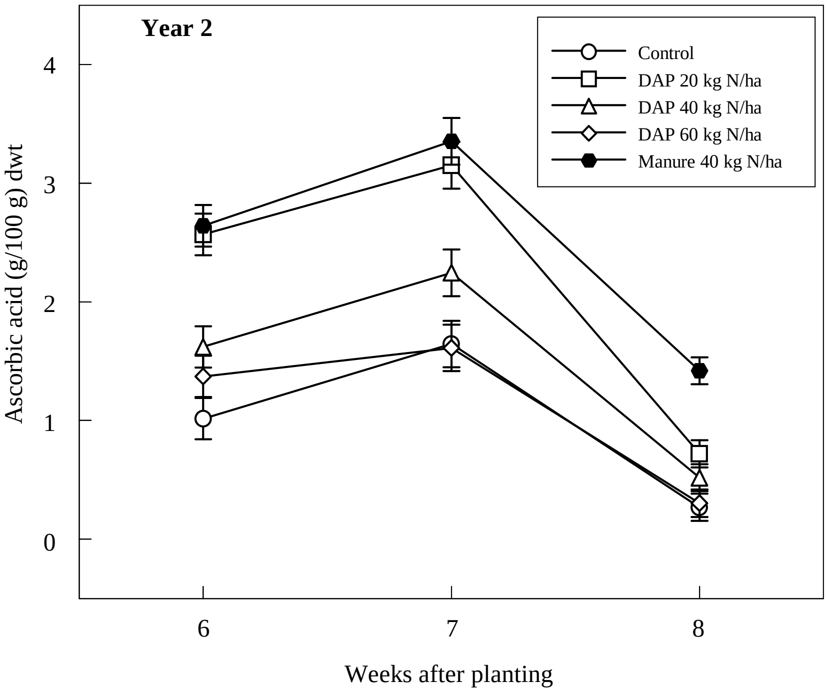

Figure 4.5: Effect of diammonium phosphate fertilizer, manure and maturity at harvest on ascorbic acid content of vegetable amaranth (*A. hypochondriacus*).

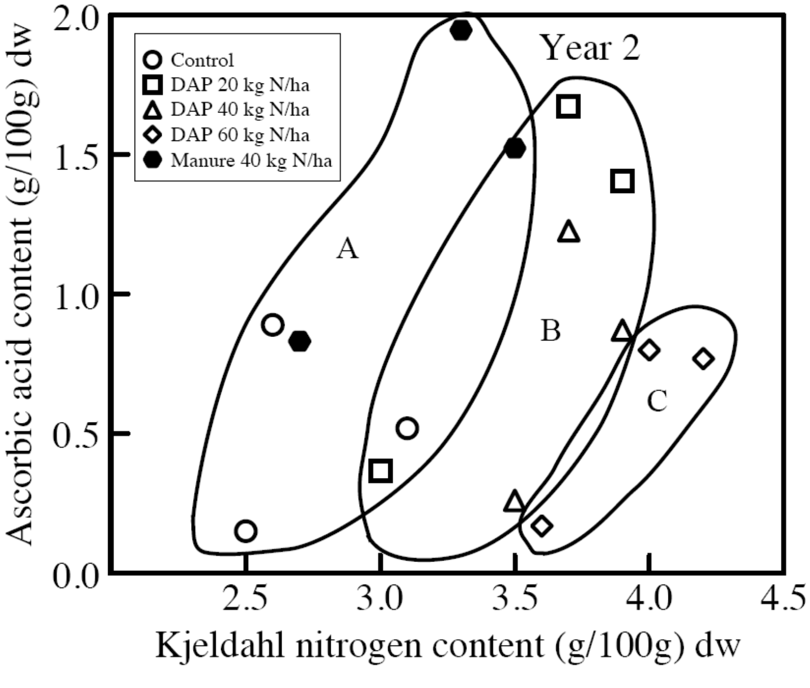

Figure 4.6: Effect of DAP, manure and time of harvesting on both ascorbic acid and Kjeldahl nitrogen content of vegetable amaranth (*A. hypochondriacus*).

Moisture content

The loss in moisture content postharvest did not differ between the cultivation treatments and the different harvest maturities so the results from these have been combined. During storage, higher moisture losses were experienced at ambient (20±3°C) than refrigerated (4±1°C) temperature of storage. These results are shown in Fig 4.7. The loss of moisture after two days of storage under ambient conditions was about twice that under refrigeration. After 4 days in storage, the vegetables held at ambient temperature had lost nearly 10% of their initial moisture content (Fig 4.7). While those kept in a refrigerator (which was without any humidity control) lost about 5 % of their initial moisture content.

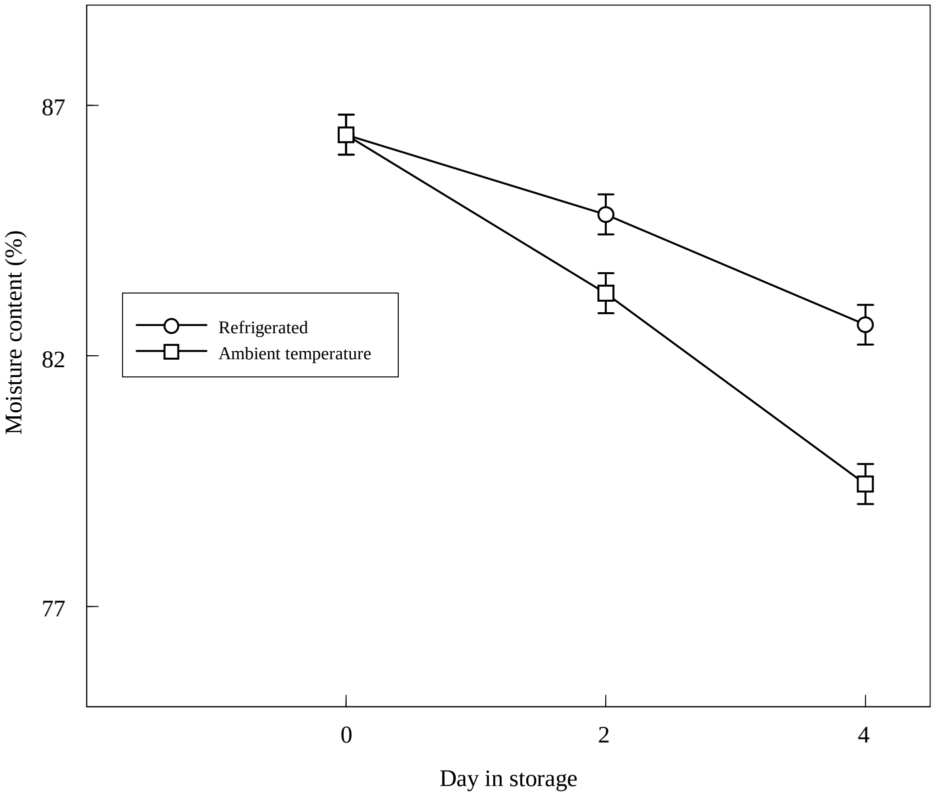

Figure 4.7: Changes in percent moisture content of vegetable amaranth during refrigerated and ambient temperature storage

β-carotene content

The effect of fertilizer application and maturity at harvest on beta carotene during storage was not significant but the effect of storage temperature was significant ($P<0.05$) (4.8). There was no significant change in β-carotene when the vegetables were stored under refrigeration for up to 4 days. However, there was a significant ($P<0.05$) decrease in β-carotene when the vegetables were stored at ambient temperature for a period of 2 or 4 days (Fig 4.8); 10% after 2 days and 25% after 4 days. There were no significant differences between leaves harvested at different maturities and stored either refrigerated or at ambient temperatures.

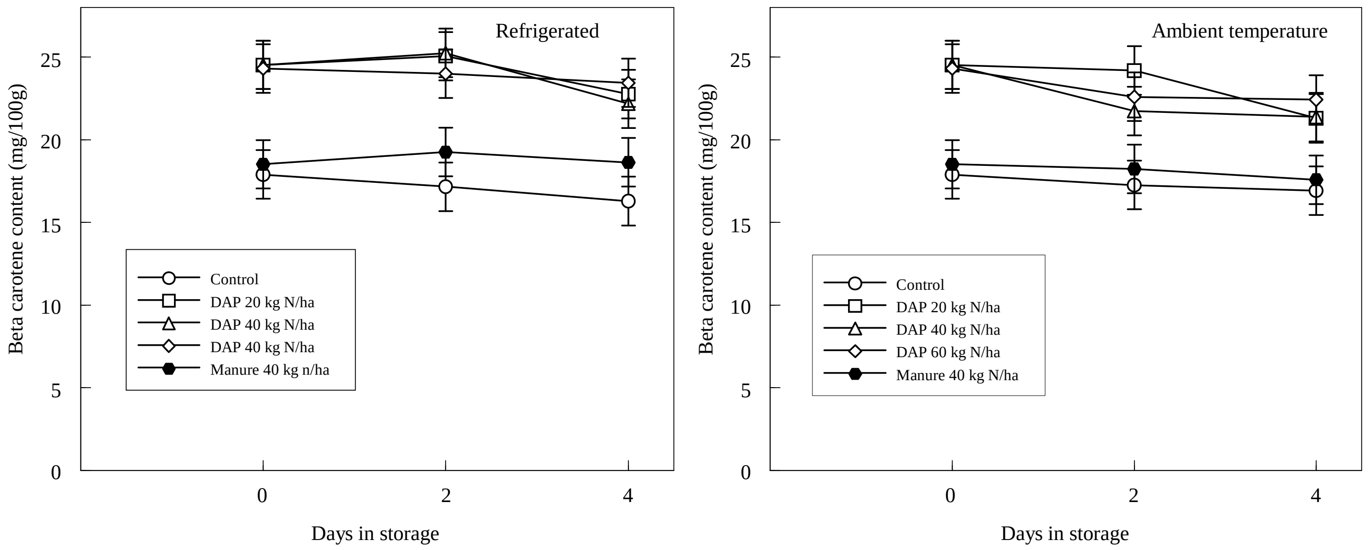

Figure 4.8: Effect of source of nitrogen, temperature and duration of storage on β-carotene content of vegetable amaranth

Ascorbic acid content

The ascorbic acid content of the amaranth leaves decreased significantly whether they were refrigerated or stored at ambient temperatures (Fig 4.9). The rates of decrease were much higher for the leaves stored at ambient temperature compared to those stored in the refrigerator. Figure 4.9 shows ascorbic acid losses with conditions such as temperature and storage period of preservation. The ascorbic acid content slowly decreases with duration of storage when refrigerated while under ambient temperature storage close to 100% of the ascorbic acid is lost within 2 days.

Considering postharvest losses separately for each harvest maturity the effect of a low initial ascorbic acid content (i.e. at time of harvest) can be clearly seen (Fig 4.10). At ambient temperatures the ascorbic acid decrease rapidly to practically zero within 2 days. While under refrigeration the already poor ascorbic acid content of week the week 8 vegtables decreases further. So by day 2 all material, except for that from the manure plots had an ascorbic acid content that was close to zero, comparable to the week 7 material after 2 days of storage at ambient temperatures.

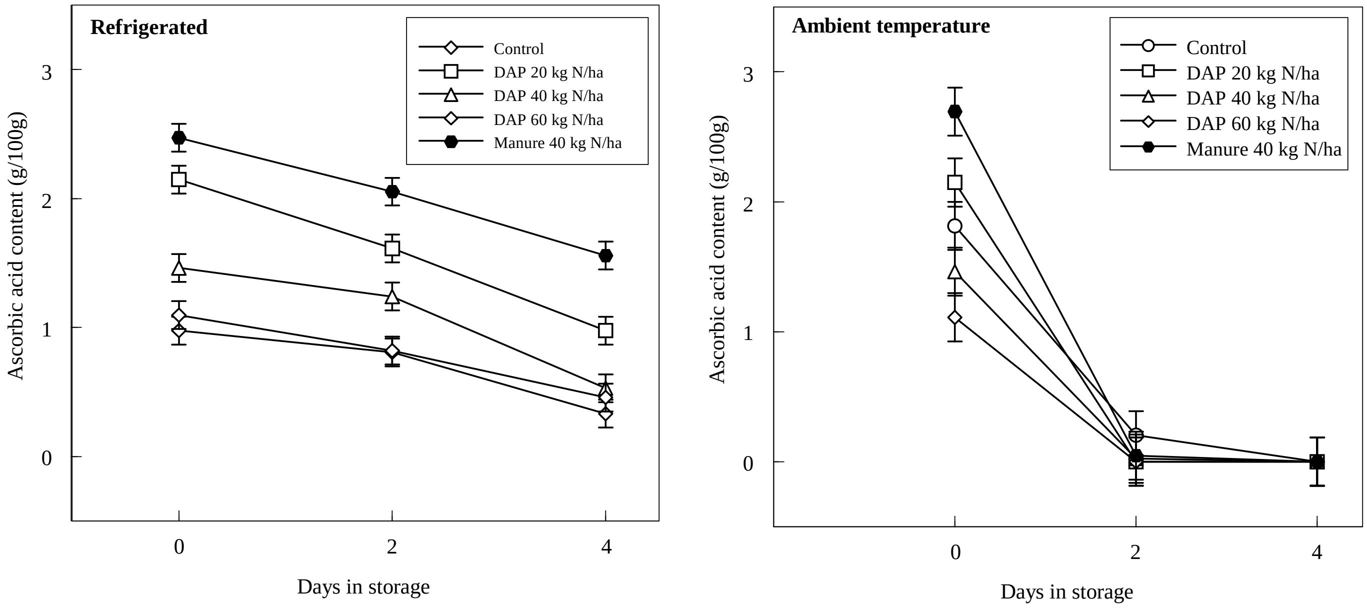

Figure 4.9: Effect of source of nitrogen, temperature and duration of storage on ascorbic acid content of vegetable amaranth

Discussion

Preharvest

The dry matter of plants, including that of their edible parts, is a complex mixture of components some of which, such as proteins and low molecular weight carbohydrates have a clear nutritional value (about 25% of the edible parts of an amaranth are protein, see chapter 3) while other parts are undigestable fibre, which may still be beneficial. Overall, it is not possible to attribute specific benefits to the dry matter content of a vegetable though generally speaking the dry matter component is the nutritionally significant part of the vegetable. The moisture content of a vegetable is also important because it affects the weight of the feed but does not provide nutritive value to the consumer (htt:www.extension.org/dry matter determination).

Vegetable amaranth dry matter content is often high, hence an equivalent amount of fresh amaranth often provides from 2 to 3 times the amount of nutrients found in other vegetables (Ruskin, 1984). The dry matter content in our study ranged between 19% and 23 % by the third harvest (8 weeks after planting). Grubben (2004) reported ranges of between 9 – 22% in different vegetable amaranth types. The dry matter content was higher in plots that received manure than the DAP and was either greater or similar in the plots that received no fertilizer at all. The growth rate in both the control plots and the manure plots was however lower than that of the DAP plots (Personal observation). The fresh yields were higher in plots that received DAP than the manure (Onyango et al, submitted).

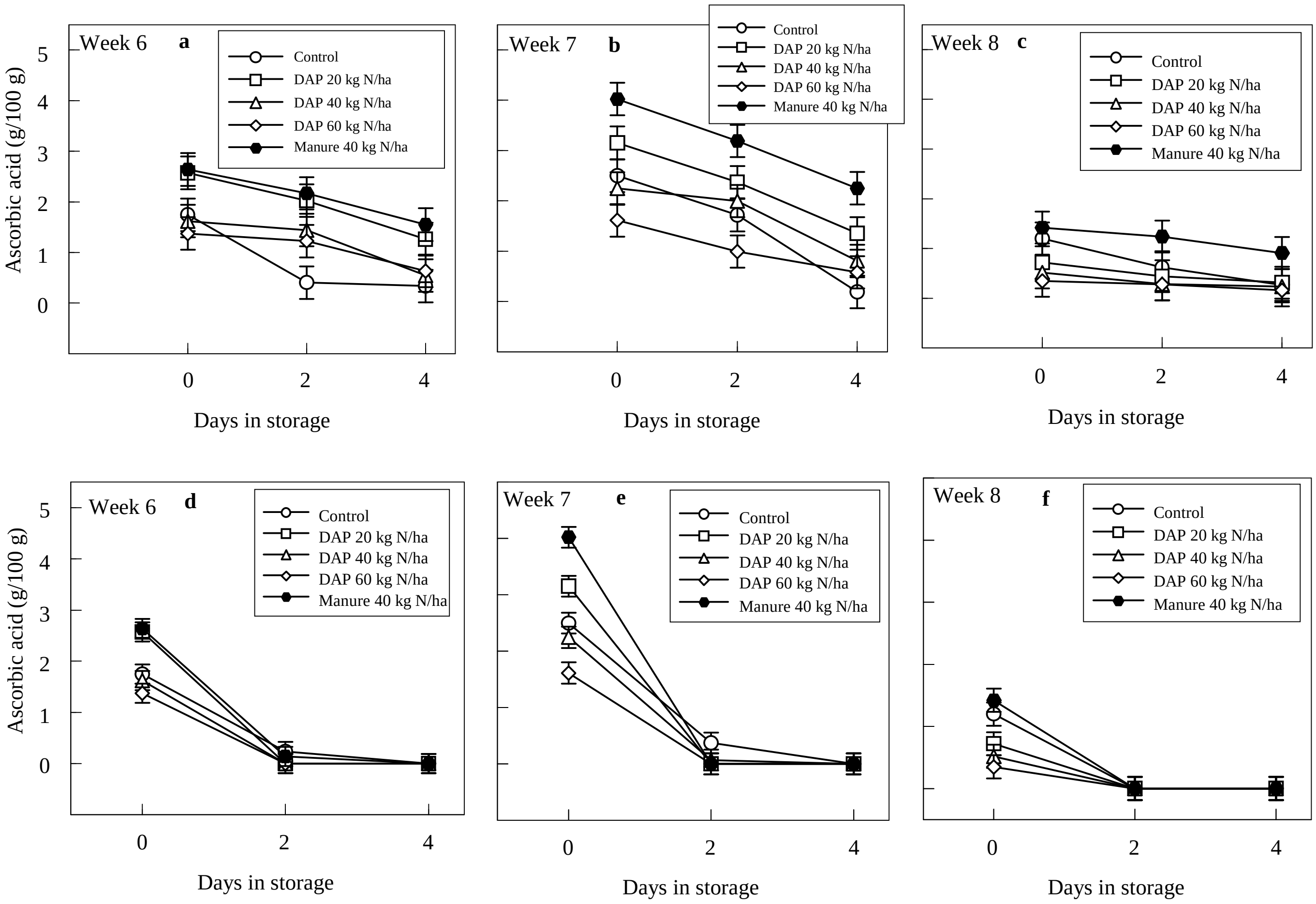

Figure 4.10: Effect of source of N, maturity at harvest and days in refrigerated (a, b, c) and at ambient (d, e, f) temperatures of storage on ascorbic acid content of vegetable amaranth.

The fresh weight of the harvested material cannot be easily compared to the relative dry matter content, but whenever the relative amount of dry matter decreases there is an increase in succulence (Singh and Whitehead, 1996). Lisiewska and Kmiecik (1996) working with broccoli and cauliflower reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg N ha^{-1} significantly increased their commercial yield but led to a decrease in dry matter content, though, this decrease was not significant.

The β-carotene content increased with maturity of the vegetables between week 6 and 8. This is similar to immature spinach harvested at 18, 24 and 30 (Stages I, II and III) days after sowing (Bergquist et al, 2006) in which the major carotenoids, violaxanthin and β-carotene, were significantly higher at stage III than at stages I and II. The higher contents of total carotenoids in mature leaves than in young leaves have also previously been reported in other leafy vegetables (Guil-Guerrero et al, 2003). The effect of DAP on β-carotene content was more significant than that of the manure, with high concentrations giving high levels of β-carotene. This is probably due to the fact that nitrogen from DAP is more readily available to the plants compared to the slow-N-release and slow nitrification properties of manure (Goh and Vityakon, 1986).

Since DAP at 40 kg N ha^{-1} gave the highest yields on a fresh weight basis (Fig 3.1, Onyango et al, in preparation) it is necessary to determine whether the concentration of β-carotene in these vegetables would be sufficient to meet the needs of the consumer. The highest β-carotene content obtained ranged between 19 mg/100g (year 2) and 26 mg/100g (Year 1) dry weight basis. This translates to 2.9mg/100g and 3.9 mg/100g fresh weight. There is no recommended dietary allowance for β-carotene but studies have used doses ranging between 15 and 180 mg/day (Longe, 2004). Therefore a person would have to consume between 480-550g of fresh leaf per day to obtain 15mg of β-carotene per day. Since people in developing countries consume two hot meals per day, it would be necessary to consume between 240-275g of amaranth per meal. Given that amaranth is not eaten in the fresh form but is cooked and the cooking water discarded, there may be losses of β-carotene during cooking. Rahman et al (1990) reported loses of β-carotene of 31% of the initial content in *Amaranthus gangeticus* when cooked in water for 7 – 9 minutes followed by discarding the cooking water and then frying the vegetables in a small quantity of oil for 4-6 minutes. While, Sungpuag et al (1999) working with *Amaranthus viridis* found out that steaming the vegetables at 100^{o}C for 8 minutes resulted in a 15% increase in β-carotene. This increase was attributed to the changes in tissue morphology, which occur, as a result allowing greater penetration of organic solvents into the cells and enhances release of β-carotene. The method of preparation of vegetable amaranth in Kenya and most sub-Saharan countries compares very well with that of Rahman et al (1990). This means then that after cooking, the β-carotene content decreases to between 2mg and 2.7 mg/100g fresh weight. This implies that depending on the initial content of β-carotene in the vegetable, a person would have to consume between 560-750g of vegetable to obtain 15mg of β-carotene per day. It is, therefore, possible to use amaranth to alleviate the vitamin A deficiency in developing countries by either increasing the quantity consumed or changing the preparation methods, for example encouraging people to steam the vegetables rather than boil to reduce losses of β-carotene due to cooking. Nonetheless it is clear that the normal consumption of amaranth (200g per day) in Kenya will not provide the minimum recommended daily intake for β-carotene of 15mg. Increasing the quantity of vegetable consumed will lead to an increase in the intake of nitrates (NO^{-3}) (chapter 3, this thesis). Using the fertilizer level of 40 kg N ha^{-1} and using the release of nitrate from cooked spinach as a guide, cooked amaranth would contain about 210mg/100g. Increasing the consumption to between 560 and 750g will mean a consumption of more than 1g of NO^{-3} from the vegetables. This is 3 times the highest limit intake from lettuce, (300mg) (Food standards Agency, 2001). The only option left therefore, is to change the cooking method, for example by adopting the method used by Sungpuag et al (1999) working with *Amaranthus viridis* who found out that steaming the vegetables at 100^{o}C for 8 minutes resulted in a 15% increase in β-carotene.

Nitrogen fertilizers at high rates tend to decrease ascorbic acid accumulation in many fruits and vegetables (Lee and Kader, 2000). In our study, ascorbic acid content decreased with increasing rate of N from 20 to 60 kg N ha^{-1} when supplied by the chemical fertiliser DAP. Lisiewska and Kmiecik (1996) found that increasing the rate of nitrogen fertilizer from 80 to 120 kg N ha^{-1} resulted in a decrease in the vitamin C content of cauliflower curd by 7%. The ascorbic acid level decreased as N amount increased in the fruit of yellow grape tomato (Simonne et al, 2007). Nitrogen fertilization generally enhances plant growth so that an apparent solute dilution effect is observed in the plant tissues (Lee and Kader, 2000; Pieper and Barrett, 2009). Nitrogen fertilizer is also known to increase plant foliage, which may reduce the light intensity onto the leaves, thereby reducing accumulation of ascorbic acid in the shaded plant parts (Lee and Kader, 2000; Bartoli et al, 2006) and causing accumulation of potentially hazardous concentrations of nitrates, which adversely affect nutritional quality (Maynard, 1984). Since high rates (60 kg N ha^{-1}) of nitrogen fertilizer as DAP also causes an increase in accumulation of nitrates (NO_3) and a simultaneous decrease in ascorbic acid, this is a double negative effect on the quality of vegetable amaranth.

Weston and Barth, (1997) reported that in snap beans and other green vegetables, ascorbic acid tends to increase with maturation and decrease with advanced maturation and senescence. This is in agreement with our results in which ascorbic acid increased between 6 and 7weeks, followed by a decrease at 8 weeks after planting. Biesiada et al (2007) working with leeks found out that plants harvested at later stages of maturity contained higher amounts of dry matter and total and reducing sugars and this was accompanied by a significant decrease in vitamin C.

Postharvest

In storage more moisture was lost when the vegetables were stored at ambient than at refrigerated temperatures. At the end of 4 days of storage the vegetables had lost close to 10% of the initial moisture content when held at 20^{o}C. Generally, a loss of 5 – 10 % moisture renders a wide range of products unmarketable (Kays and Paull, 2004). For leafy vegetables, such as amaranth, transpiration causes wilting, which lowers consumer appeal. The loss of water also leads to loss of saleable weight, implying a loss in monetary returns for vegetables such as amaranth which are sold by weight. Leafy vegetables with large surface area to volume ratios are particularly vulnerable to loss of water during storage which will increase with storage temperature and vapour pressure deficit. Low temperature of storage has been shown to slow down evapo-transpiration (Kays, 1991) and hence ensures a longer shelf life of the vegetables and in this study, the loss of water was slowed down by storing the vegetables under refrigerated temperatures.

The β-carotene content did not change significantly during storage at refrigerated temperatures but losses were observed during ambient temperature storage. Carotenoids have been found to be relatively stable in storage, but are still susceptible to oxidation and isomerisation (Buescher et al, 1999). It has been reported that temperatures of 20^{o}C or more causes some of the carbon-carbon double bonds of the long polyunsaturated carbons chain to undergo inflexion from the trans- to the cis- form, leading to loss of the vitamin activity (Tannenbaum, 1976). With the existing cooking methods of amaranth, it is likely that some of the β-carotene would be lost. However, if the cooking method is changed amaranth would be a good source of the vitamin.

Our results indicate that more ascorbic acid is lost at higher than low temperatures of storage. These results compare well with those of Lee and Kader (2000) working with kale. They reported that losses in ascorbic acid were accelerated by high storage temperatures. Favell (1998) observed greater losses for example only 10% retention in spinach within 3 days of storage at 20°C, and 20% retention when stored under refrigeration (4°C) for 7 days the same temperatures as evaluated in this study. Prabhu and Barrett (2009) working with two African leafy vegetables (*Cassia tora* and *Corchorus tridens*) reported ascorbic acid retention of between 28 and 69% of their initial content when stored at 4°C for 14 days. Yadav and Sehgal (1995) observed 44.8 and 34.1% ascorbic acid retention in *Amaranthus tricolor* leaves stored at 30°C for 24 and 48 h respectively. Our results indicate that ascorbic acid is lost after harvest even under refrigerated storage

The concentration of ascorbic acid declines fairly rapidly in many of the more perishable fruits and vegetables after harvest. Losses are greater with increasing storage temperature and duration (Kays and Paull, 2004, Lee and Kader, 2000, Yadav and Sehgal, 1995). This was true with the current study where more ascorbic acid was lost during storage at ambient rather than at refrigerated temperatures for a period of 4 days. The study also demonstrates that refrigeration of vegetables with a low initial ascorbic acid content, like those found in vegetable amaranth harvest at 8 weeks after planting, does not preserve its concentration during storage. Overall, ascorbic acid levels are labile and fluctuate substantially in short term; ascorbic acid levels decrease during the dark period and still further during the early photoperiod before recoverying the photoperiod (Bartoli et al, 2006). Dark storage which is typical for leafy vegtables in boxes or bunches would be expected to provoke a rapid loss in ascorbic levels, though this loss is delayed, though not prevented, by refrigeration.

The ascorbic acid of plants is known to change extensively before and after harvest, and a complete understanding of these changes is necessary if the full potential of Amaranth as a source of dietary ascorbic is to be realized. (Bergquest et al, 2005; Rahman et al, 2007). From our study, the contribution of amaranth in terms of ascorbic acid can be realised mainly when the leaves are utilized immediately after harvesting. For storage of the vegetables, harvesting should be done 6 and 7 weeks after planting and these should be under refrigeration for a maximum period of 4 days.

For consumption, vegetable amaranth is normally cooked in large amounts of water that is drained and discarded after cooking followed by frying in a small quantity of oil (person observation). Ascorbic acid is known to be heat labile and sensitive to light, oxygen and oxidizing agents (Astier-Dumas, 1975, cited In Yadav and Sehgal, 1995). The extent of the decrease will depend upon the extent and duration of exposure to heat. Cooking involves heat treatment therefore a decrease of ascorbic acid during cooking of vegetable amaranth is expected. Cooking of both *Cassia tora* and *Corchorus tridens* leaves for 20 minutes in 50ml of water for every 2 g of vegetable (2.5L of water/100g of vegetable) followed by draining of the cooking water resulted in a substantial loss of ascorbic acid, with only 1-4% retention of the fresh content (Prabhu and Barrett, 2009). Amaranth leaves are cooked in a similar way followed by frying in a small quantity of oil. Therefore the losses in vegetable amaranth leaves may even be more than those recorded in *Cassia tora* and *Corchorus tridens* because frying causes a further decrease in ascorbic acid. The losses from the cooked vegetable may be due to both leaching into the cooking water and thermal breakdown. This effect of cooking is a major problem for the use of amaranth as an ascorbic acid source as the vegetable can not be eaten uncooked. Vegetables prepred by microwave-steaming and stir fried with oil have been shown to contain significantly higher (1.3-1.8 fold) ascorbic acid rention values than those that were boiled (Masrizal et al, 1997). However, this method of cooking is not possible in Kenya and most of the developing countries. Steaming the vegetables rather than boiling will help reduce the ascorbic acid losses but steaming will cause a small decrease or no change in the nitrate or soluble oxalate content which are the major anti-nutrients associated with increased application of N fertilization.

It can therefore be concluded that with proper fertilizer management, it is possible to obtain both high yields and high quality vegetable amaranth in terms of β-carotene content. DAP as fertilizer up to 40 kg N ha^{-1} enhances the product quality. In addition, maturity at harvest influences the vitamin contents of vegetable amaranth with higher contents of ascorbic acid found in younger leaves while that of β-carotene is found in more mature leaves. After harvest, ascorbic acid and moisture contents decreases with increase in both temperature and duration of storage. A negligible amount of ascorbic acid remains after two days of storage at ambient temperatures. However, substantial amounts of ascorbic acid are retained when the vegetables are refrigerated but if the initial ascorbic acid content is already low, vegetables stored this way may be little better after 2 days of storage than vegetables stored at ambient temperatures beginning with a high ascorbic acid content. As a source of ascorbic acid the temperatures used for display during marketing and the cooking method currently used by most of the people consuming amaranth makes it an inapproprate source. β-carotene is relatively stable in storage especially when the vegetables are held at refrigerated temperatures with substantial amounts being retained after 4 days of storage. A problem that remains, however, is that even if the vegetables are refrigerated prior to sale, the storage life with the consumer will be short as this also depends on roting. Storage at low temperature is advantageous for preventing degradation of ascorbic acid and β-carotene contents and reducing water evapo-transpiration, slowing down these processes considerably. Therefore to maintain the quality in terms of ascorbic acid, β-carotene and general appearance (using percent moisture content as a proxy) of vegetable amaranth after harvest, refrigerated storage is required.

CHAPTER 5

EFFECTS OF SOURCE OF NITROGEN FERTILIZATION, MATURITY AT HARVEST AND STORAGE ON PHENOLICS AND OXALATE CONTENTS OF VEGETABLE AMARANTH (*Amaranthus hypochondriacus*)

Abstract

For long, nutritionists have considered dietary oxalates and phenolics as nutrition antagonists; the oxalates impairing absorption of calcium and some of the phenolics impairing absorption of proteins. However, recently it has come to dawn that classes of phenolics, among them quercetin, fulfill beneficial health functions in the body. This study was therefore designed to assess the effect of chemical fertilizer and manure on the accumulation of total phenolics, quercetin and oxalates during production and storage of amaranth vegetables. Results showed that during growth, the total phenolics content decreased with increasing N, the extent of the decrease depending on the level and source of N. The levels of phenolics and quercetin increased; the oxalate contents decreased with age of plants during both years of the study. Phenolics increased in refrigerated storage, but the increase was not significant. The levels of oxalates did not change appreciably during refrigerated storage. There was a general decrease in phenolics content during storage at ambient temperatures. Storage of the fresh vegetables at 4°C for limited periods improves their quality by increasing total phenolics and quercetin contents.

KEYWORDS: Vegetable amaranth, mineral fertilizer, manure, phenolics, oxalates, storage

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To be submitted as: Cecilia M. Onyango, Jeremy Harbinson, Jasper K. Imungi, Solomon I. Shiabairo and Olaf van Kooten. The influence of organic and mineral fertilization on germination, leaf nitrogen, nitrate accumulation and yield of vegetable amaranth (*Amaranthus hypochondriacus*)

Chapter 5

Introduction

Vegetables have had conferred upon them the status of functional foods (Hasler, 1998) because they seem to be delivering health benefits besides fulfilling physiological nutritional needs. Their consumption is associated with protection against major diseases including cancer and cardiovascular diseases (Wargovich 2000; Kaur and Kapoor 2001; Scalbert et al, 2005). The protective action of the vegetables has been attributed to the presence of antioxidants (Prior and Cao, 2000). Studies have shown that the majority of the antioxidant activity may be from secondary metabolites such as polyphenol compounds which include flavonoids, isoflavones, anthocyanin and catechin rather than from vitamins C and E, and β-carotene alone (Cao et al, 1997; Kähkönen et al, 1999). Moreover, a positive correlation between total phenolics and antioxidant activity in some vegetables and fruits has been reported (Gil et al, 2000; Pyo et al, 2004; Khandaker et al, 2008).

Vegetable amaranth, members of the genus Amaranthus, are widely grown in the tropics. They are a group of fast growing C4 herbs that yield a harvestable product within 4 – 6 weeks after planting, which makes them attractive to resource-poor farmers. In some countries such as Kenya, they are largely grown by women thus providing this gender with a degree of financial independence (IPGRI, 2003). The vegetable type selected for this study represents an important source of proteins (Carlsson, 1980) and vitamins A and C as well as minerals such as iron, calcium and magnesium (National Research Council, 1984) for a majority of people living in sub-Saharan Africa. In Kenya, for example, the diet of lower socio economic groups consists largely of maize and various traditional leafy vegetables, such as amaranth.

Earlier studies have established the abundance of antioxidants in *Amaranthus* leaves (Sokkanha and Tiratanakul, 2006; Khandaker et al, 2008) and that there was a general trend towards increased antioxidant activity with increased total phenolics content in *Amaranthus tricolor* L. The beneficial effects of phenolics makes it important to understand which circumstances they are synthesized and accumulate in these plants. Among the group of flavonoids, quercetin has been reported to be a strong antioxidant (Hollman, 1997). It has been found to chelate metals, scavenge oxygen free radical and prevent oxidation of low density lipoprotein in *in vitro* studies (Bors et al, 1990; de Whalley et al, 1990; Kandaswami and middleton, 1994).

Unfortunately, in addition to their high nutritional value, amaranths also accumulate high levels of anti-nutritional factors such as oxalates (Vityakon and Standal, 1989; Gupta et al, 2005). A growing body of evidence indicates that oxalate plays various functional roles in plants, including calcium regulation, plant protection and detoxification of certain metals (Franceschi and Nakata, 2005). For instance, several plants such as buckwheat, taro and rice exude and/or accumulate oxalate in vivo to cope with aluminum and lead toxicity (Ma et al, 1997; Ma and Miyasaka 1998; Yang et al, 2000). More interestingly, oxalate can serve as an antioxidant to quench the oxidative burst during plant response to pathogen attacks

(Cessna et al, 2000; Weir et al, 2006). A molecular genetic analysis confirmed a long-held hypothesis that calcium oxalate crystals act as an effective defense against chewing insects (Korth et al, 2006).

Despite these suggested functional roles in plants, excess levels of oxalate in plants have long been a concern to human nutrition and health (Franceschi and Nakata, 2005). The high levels of oxalate in any edible parts of vegetables and food crops will significantly lower their nutritional quality and lead to health concerns. Soluble oxalate decreases calcium bioavailability by forming calcium oxalate crystals (Radek and Savage, 2008) which can also lead to the formation of kidney stones (Libert and Franceschi 1987; Massey, 2003). As oxalate can have dual and contrasting roles in plants, depending on its concentrations and/or tissue localization, a proper manipulation of its concentration in vegetative parts for its natural functions and in edible parts for better human food quality, therefore, becomes increasingly important.

Studies have shown that availability of plant nutrients can be an important factor in determining the activity of secondary metabolism within plants (Gad et al, 1982; Kopsell et al, 2003; Aires et al, 2006). Nitrogenous fertilizer is one of the most important factors controlling the yield and quality of vegetables (Juan et al, 2008). In developing countries, such as Kenya, nitrogenous fertilizer is often supplied as manure because of the cost of synthetic fertilizers. However information on the effect of manure or inorganic fertilizers on the polyphenols and oxalic acid contents of vegetables is still scanty. Only a few studies (Stout et al, 1998; Juan et al, 2008) have investigated the effect of different levels of nitrogen on the total phenolics and oxalate contents of vegetables.

Secondary metabolites are also known to vary in amount and content depending on the age of the plant (Dumas et al, 2003). Although it has been reported that there exists a large variability in the levels of phenolic compounds at various stages of maturation (Ellnain-Wojtaszek et al, 2001) of some leafy vegetables, the changes that occur in the content of these chemicals at different growth stages of traditional leafy vegetables found in Sub-Sahan Africa is poorly understood.

Information on the fate of the harvested amaranth vegetables during storage is limited and even more so the fate of their constituents. The present study was therefore designed to assess the levels of total phenolics, quercetin, and total and soluble oxalates in vegetable amaranth (*Amaranthus hypochondriacus*) grown using a mineral fertilizer diammonium phosphate (DAP) and cattle manure, at different maturity stages. The changes in the levels of these substances during storage at ambient and refrigerated temperatures were also assessed.

Materials and Methods

Amaranth seeds were obtained from Asian Vegetable Research and Development Centre (AVRDC) Tanzania and grown in the University of Nairobi Field Station at the Kabete Campus during the long rains periods between March and May of 2007 and 2008, by methods already described (Palada and Chang, 2003). Kabete is situated about 15 km to the West of Nairobi city and lies at Latitude 1^{o} 15′S and

Longitude 36° 44′E, and at altitude 1930 m above sea level (Sombroek et al, 1982). Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December (Taylor and Lawes, 1971). The mean annual temperature is 18°C. The soil in Kabete is characterized as a deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996). The land used for the plots had not received any fertilizer 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 fertilized with either manure or diammonium phosphate (DAP; 18:46:0) fertilizer. The trials were laid out in a complete randomized block design with five fertilization treatments as follows: one plot with cattle manure at 40kg Nha^{-1} (calculated using the content of nitrogen (N) in the manure; Onyango et al, in preparation), three plots with DAP at 20, 40, and 60kg Nha^{-1}, and an unfertilized control plot. While we will focus on the N content of these fertilizers, it is important to note that the manure used is a source of a diverse range of macro- and micro-nutrients (Onyango et al, in preparation) and DAP is a phosphorus (P) as well as an N source; for the manure, 20, 40 and 60 kg N ha^{-1} treatments, the P added by the manure was very low in both years while that added by the DAP was 50, 100, and 150 kg P ha^{-1}. Phosphorus (P) (as phosphate) is an essential nutrient for plant growth, development, and reproduction that forms part of key molecules such as nucleic acids, phospholipids, ATP, and other biologically active compounds. After nitrogen, P is considered to be the second most important nutrient limiting agricultural production (López-Bucio et al, 2000). However, the phosphate ion is precipitated by many cations and is easily available for plant uptake at a narrow range of neutral soil pH values. In acid soils, P forms low-solubility precipitates with aluminum (Al) and iron (Fe), whereas in alkaline soils, it combines efficiently with calcium (Ca) and magnesium (Mg) to form sparingly soluble phosphate compounds (Bar-Yosef, 1991) Therefore, although the total amount of phosphorus in the soil may be high, in most cases it is unavailable for plant uptake. It is likely that possible that under the acid conditions (pH 5) of the soil used in our study, the phosphate in either the manure or the DAP was not available for plant uptake.

The treatments were replicated three times. The crop was irrigated twice a week whenever rainfall was insufficient keeping the soil moist at all times to avoid moisture stress. The vegetables were harvested at 6, 7, and 8 weeks from planting by cutting-off the tender edible stems and leaves.

Storage trials on the vegetables

Bundles of average weight 0.45 kg from each fertilizer treatment harvested at 6, 7 and 8 weeks after planting were stored at ambient (20±3°C) and refrigerated (4±1°C) temperatures for 4 days. Initially and at 2 days interval, the vegetables were analyzed for total phenolics, quercetin, total and soluble oxalates.

Preparation of the vegetables for analysis

The edible parts of the vegetables which consisted of leaves and tender stems were separated and freeze-dried. The residue was ground to pass through a 600µm sieve. The powder was stored in tightly closed plastic vials in a freezer (-20°C) to await analyses for total phenolics, quercetin, and total and soluble oxalates.

Determination of total phenolics

For the analysis 25mg of the freeze-dried powdered amaranth was weighed into a 50 ml centrifuge tubes and 20ml of 50% methanol:water added. The mixture was allowed to stand overnight at room temperature. The mixture was then centrifuged at 2400g for 15 minutes, filtered and the filtrate made up to 20 ml each with the methanol:water mixture (1:1). The total phenolic contents of the filtrate were measured with a slightly modified method of Ragazzi and Veronese (1973). To 1.0 ml of filtrate in a 25ml volumetric flask, 1ml Folin's Reagent (1 N) and 2 ml of 20% Na_2CO_3 were added and mixed thoroughly with a cyclomixer. The mixture was made to 25 ml with distilled water then allowed to stand at room temperature for 30 minutes. The absorbance of the mixture was measured at 720nm on a UV-Visible spectrophotometer (UV mini 1240, spectrum bandwidth 5nm, Shimadzu Corporation, Tokyo, Japan). The concentration of total phenolics in the filtrate was determined using a standard curve prepared from known concentrations of pure gallic acid aqueous solutions subjected to the same analytical procedure. The concentration in the filtrates was used to calculate the total phenolic contents of the vegetables as mg gallic acid equivalent /g on a dry weight basis.

Determination of quercetin

Quercetin content was determined using the method of Bovy et al (2002) with slight modifications. Into a 50ml plastic centrifuge tube, 50mg of the freeze-dried powder was weighed and 4ml of 62.5% aqueous methanol containing 2g/L tert-butylhydroquinone (TBHQ) were added, followed by 1 ml 6N HCL and carefully mixed. The centrifuge tubes were closed and placed in a shaking water bath at 90°C for 2 hours. The extract was cooled, and made to 10ml with methanol and placed in an ultrasonic bath for 5 minutes. This was followed by centrifugation at 1360g for 3 minutes. An aliquot of 1.5 ml was then transferred to an Eppendorf tube and centrifuged at 3600g for 10 min. A 1ml sample of the supernatant was then injected and eluted through HPLC (Column:Waters Nova-pak C18, 4µm, 3.9x150mm; Eluent: 25% acetonitrile in 0.1% trifluoracetic acid; Detection: Dionex UVD 340S at 370nm; Flow: 0.9 ml/min; Injection: 20µl; Temperature: 30°C). The concentration of quercetin was determined from a standard curve prepared from known concentrations of pure quercetin in methanol.

Determination of total and soluble oxalates

For determination of the oxalates, 0.1g of powder was weighed and mixed with 30ml of 1M HCl. Each mixture was then shaken in a water bath at 100°C for 30min. To each mixture was added 0.5ml of

5% calcium chloride and thoroughly mixed to precipitate out the calcium oxalate. The suspension was centrifuged at 800g for 15minutes and the supernatant decanted. The pellet was washed twice with 2ml of 0.35M NH_4OH then dissolved in 0.5M H_2SO_4. The solution was titrated with standard solution of 0.1M $KMnO_4$ with the temperature being maintained at 60^oC to a faint violet color that persisted for at least 15 seconds (AOAC, 1999). For soluble oxalates, the same procedure was used except that instead of extracting the oxalates with 30 ml of 1M HCl, the extraction was with 30ml distilled water. The total and soluble oxalate contents were calculated as percent of dry weight.

Statistical analysis

Data was subjected to the general analysis of variance (ANOVA), using Genstat statistical software (Payne et al, 2006) to check for both main effects of treatments and their interactions. Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P<0.05$).

Results and Discussion

Preharvest

Total phenolics and quercetin content

The phenolic contents differed significantly ($P<0.05$) among the sources of N and maturity at harvest. These results are shown in Figures 5.1. The highest content was recorded in the manure and control plots at all maturity stages. The use of DAP led to a decrease in the phenolic contents. The antagonistic effect of DAP on total phenolics content was increased with increasing levels of N.

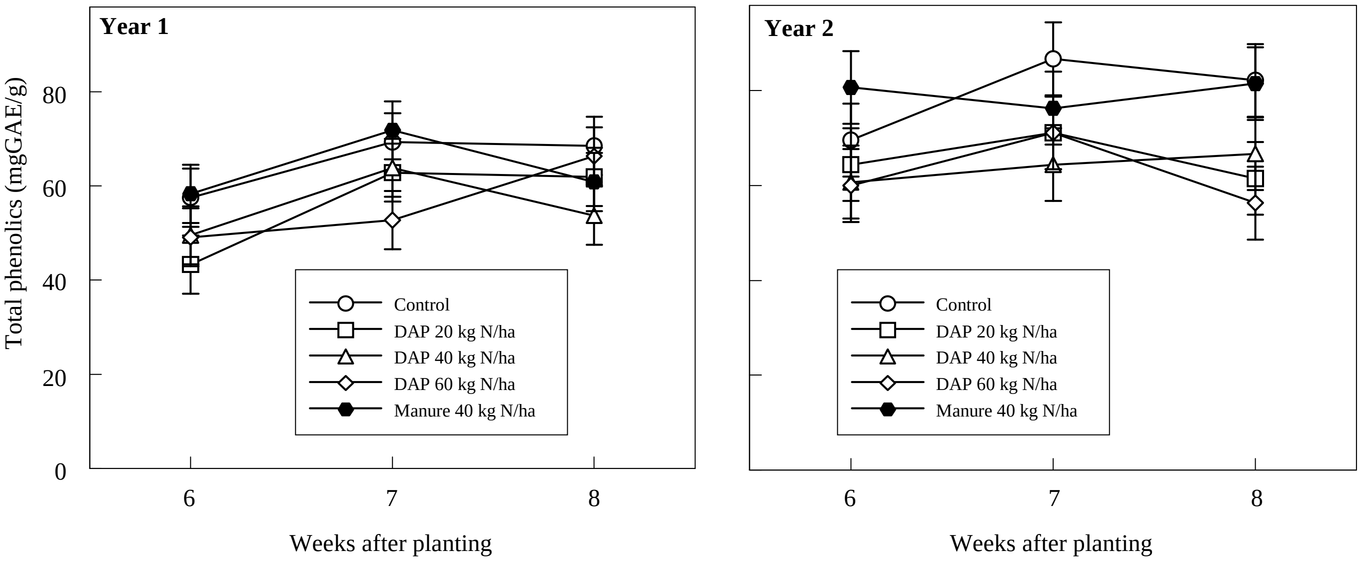

Figure 5.1: Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total phenolic content of vegetable amaranth (*A. hypochondriacus*) (GAE = Gallic acid Equivalent**)**

These results are in agreement with those of Li et al (2008) who found that the total phenolics concentrations of leaf mustard (*Brassica juncea* Coss) were considerably decreased by increasing nitrogen supply. This negative correlation between nitrogen application and total phenolics could be explained by the protein competition model (PCM) (Jones and Hartley, 1999). The PCM hypothesis makes a contingent prediction: when biomass increases in response to elevated nitrogen nutrition, phenolic concentrations will decline because increased protein demand for growth will decrease partitioning of carbon skeletons to phenolics (Jones and Hartley, 1999).

In addition, it has been reported that water availability, and the mineral and organic nutrients of the soil have marked effects on the phenolic contents of plants (Barberan and Espin, 2001). Under the conditions of abundant insolation normally encountered in the tropics, the rate of photosynthesis of carbohydrates may be so high that if the levels of available nitrogen and minerals are low, they get quickly depleted by synthesis of primary metabolites. The excess carbohydrates can then only be shunted to production of nitrogen- and mineral-free molecules such as the phenolic compounds (Waterman et al, 1984). This is probably what happened with the plants in the control and the manure plots. However though within each year there is support for the PCM hypothesis, between the years the picture is not so clear. Compared to year 1, the plants from year 2 had lower leaf N and considerably better growth (Onyango et al, in preparation) (chapter 3 this thesis). The Leaf N content in DAP fertilized plants were lower in year 2 compared to year 1, the opposite to the prediction of the PCM hypothesis. It is however, relatively easy to accommodate this conflict by modifying the PCM model to include not only the use of carbon skeletons for protein synthesis but also for growth

Recent work by Sousa et al (2008) demonstrated an overall trend of higher total phenolics concentration in organically grown tronchuda cabbage, accompanied by lower plant fresh weight, as compared with conventionally fertilized samples. They suggested that the lack of nutrients, particularly insufficient nitrogen supply as a result of a low mineralization rate under organic production, could have boosted synthesis of phenolic compounds while limiting rapid growth of new leaves. In our study the control and manure plots that recorded the highest phenolic content, recorded the lowest leaf yield at all maturity stages (Onyango et al, in preparation).

The levels of total phenolics found in our samples from all treatments ranged between 50 and 70mgGAE/g dry weight. These levels are higher than the 45.5mg/g recorded in leaves of *Larrea tridentata* a desert shrub (Hyder et al, 2002), but are less than 98.6mg/g recorded in leaves of other desert shrubs (Hollechek et al, 1990). The presence of these compounds in green stems and leaves is related to deterring herbivory, with protection of photosynthetic tissues from damage by ultra-violet radiation being a secondary function (Gonzalez-Coloma et al, 1988). It has also been shown that the biosynthesis of phenolic compounds can be induced by sunlight (Harbowy and Balentine, 1997). This could explain the higher levels of phenolics recorded in vegetable amaranth used in this study which was grown under tropical conditions where sunlight is not limiting.

For the flavonoid quercetin, there were very significant differences between the two years of study with very low amounts recorded in year 1. The only difference recorded in the two years was the sunshine hours received by the crop during the growing season and the air temperatures were slightly cooler in year 1 than year 2. This could have caused the differences shown in Fig 5.2. This results are in agreement with those by Goldberg et al (1998) who found out that quercetin concentrations were clearly highest in wines from warmer climates notable for high sunshine while Patil et al (1995) working with onions also recorded higher quercetin contents in onions that were grown under high light intensity than those from low light intensity locations.

There was a significant difference in content of quercetin among fertilizer treatments and time of harvesting in year 2 (Fig 5.2). Quercetin contents increased significantly ($P<0.05$) between weeks 6 and 8 in all treatments except the 60 kg Nha^{-1} DAP in year 2 . The highest increase occurred when 0, 20 kg N ha^{-1} DAP and manure were used. Use of higher levels of N supplied as DAP (60 kg N ha^{-1}) resulted in only slight, further increase in quercetin content. At 60 kg N ha^{-1}, the quercetin content of week 7 harvest was almost equal to that of week 8 harvest. Just like the total phenolics, there was a negative correlation between N levels supplied by DAP and quercetin content.

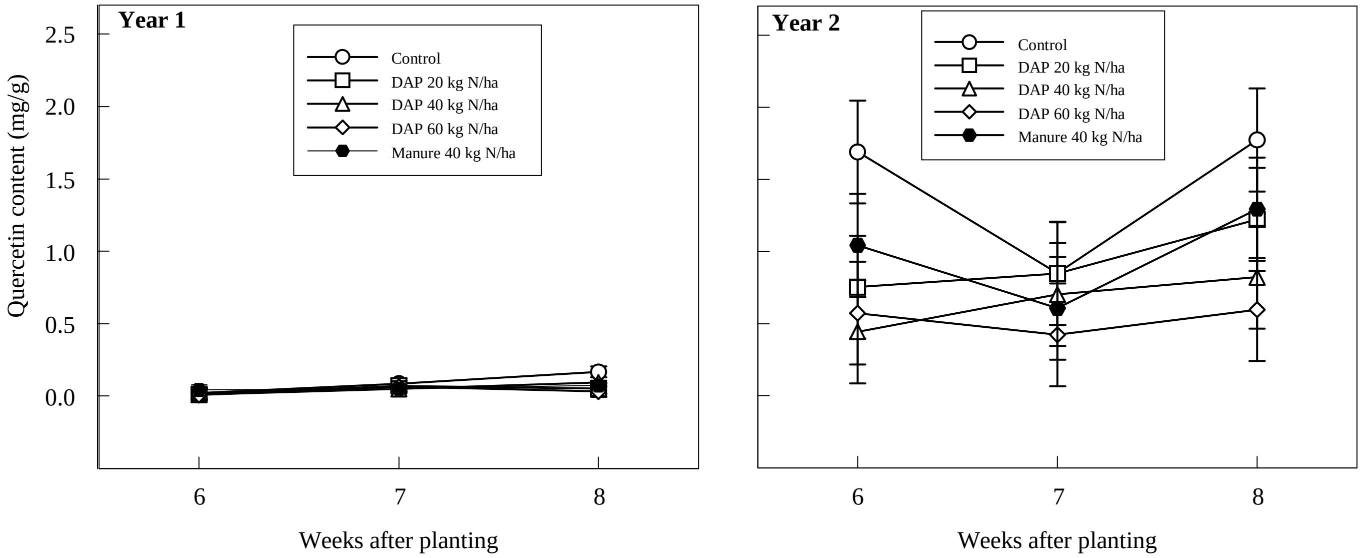

Figure 5.2: Effects of diammonium phosphate fertilizer, manure and maturity at harvest on quercetin content of vegetable amaranth (*A. hypochondriacus*)

Total and soluble oxalates

Measurement of the total and soluble oxalates content in the vegetables was done only in year 2. The results are shown in Fig. 5.3. There were no significant differences between treatments in terms of the total and soluble oxalate contents. The total oxalates decreased with maturity of the vegetables for all treatments but this decrease was not significant. These results are comparable with those of Kitchen and

Burns (2006) working with Dark Green Bloomsdale spinach who found that total oxalate content was maximum at 32 days after planting and decreased subsequently as the plants developed vegetatively. Some weeds have been known to accumulate compounds such as oxalates to act as a defense against chewing insects (Korth et al, 2006). This could be the case in *Amaranthus hypochondriacus*.

There was no significant difference in soluble oxalates among fertilizer treatments as well as the time of harvesting (Fig 5.4). The soluble oxalates remained unchanged throught the growing period. The soluble oxalate contents range between 3% and 4% dry weight in all treatments (Fig 5.4). Schmidt et al (1971) reported 2.9% in *Amaranthus cruentus* L., while Radek and Savage (2008) reported 4.7% soluble oxalates in *Amaranthus viridis* and 4.4% in *Amaranthus cruentus*.

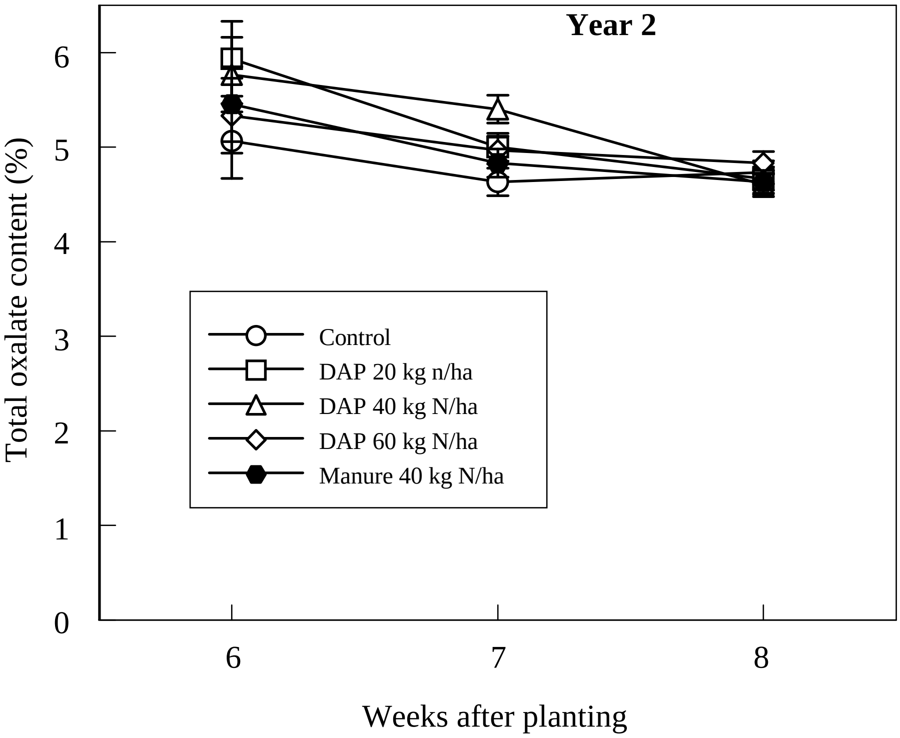

Figure 5.3: Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total oxalate content of vegetable amaranth (*A. hypochondriacus*)

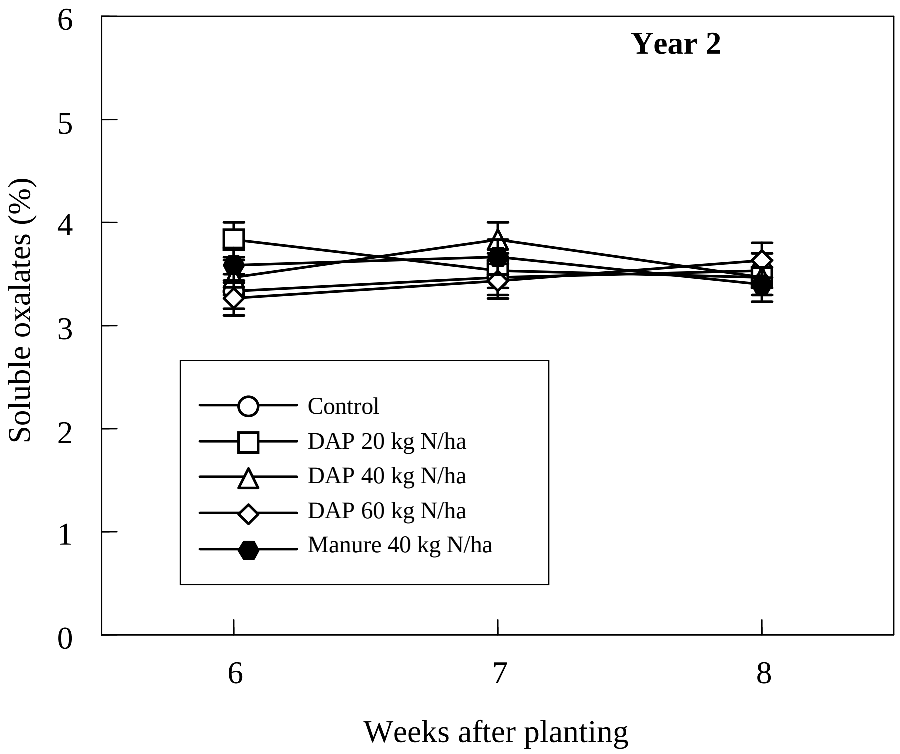

Figure 5.4: Effects of diammonium phosphate fertilizer, manure and maturity at harvest on total oxalate content of vegetable amaranth (*A. hypochondriacus*)

Our results fall between the ranges reported by Schmidt et al (1971) and Radek and Savage (2008). This translates to consumption of between 0.5g and 0.6g of oxalates per 100g of fresh vegetable. Unacceptable levels for humans have been indicated as 2 to 5g of oxalic acid per day for populations consuming low levels of calcium (Ricardo, 1993). The oxalate levels in the vegetables that received manure and DAP are within safe limits as long as consumption does not exceed the equivalent of 800g of fresh leaves per day which is far above the amounts normally consumed, for example, in Kenya which are 200g fresh weight per day (Personal observation). Cooking by boiling has been shown to significantly reduce the amount of soluble oxalates in *Colocasia esculenta* (L) Schott, and *Oxalis tuberose* (Catherwood et al, 2007; Albihn and Savage, 2001b) through leaching into the cooking water. Since vegetable amaranth is cooked before consumption, it is expected that during cooking, which involves boiling in water which is thereafter discarded the levels of soluble oxalates and therefore the levels of total oxalates in the vegetables as consumed will be substantially reduced.

Postharvest

Total phenolics and quercetin content

There were no differences in the trends between year 1 and year 2 during storage, therefore only data from year 2 is presented. The N source and levels did not cause significant changes on total phenolics and quercetin contents during storage. However, maturity at harvest as well as storage temperature resulted in significant ($P<0.05$) changes in both the phenolics and quercetin content. There was a gradual decrease in total phenolics throughout the storage period when the vegetables were stored under refrigeration and this decrease was greater when the material was stored at ambient temperatures (fig 5.5). For quercetin, there was a slow initial increase after 0 – 2 days in storage followed by a gradual decrease from 2 – 4 days in storage under refrigeration (4^{o}C). Storage of vegetable vegetable at ambient temperatures led to an accelerated decrease of the quercetin content (fig 5.6).

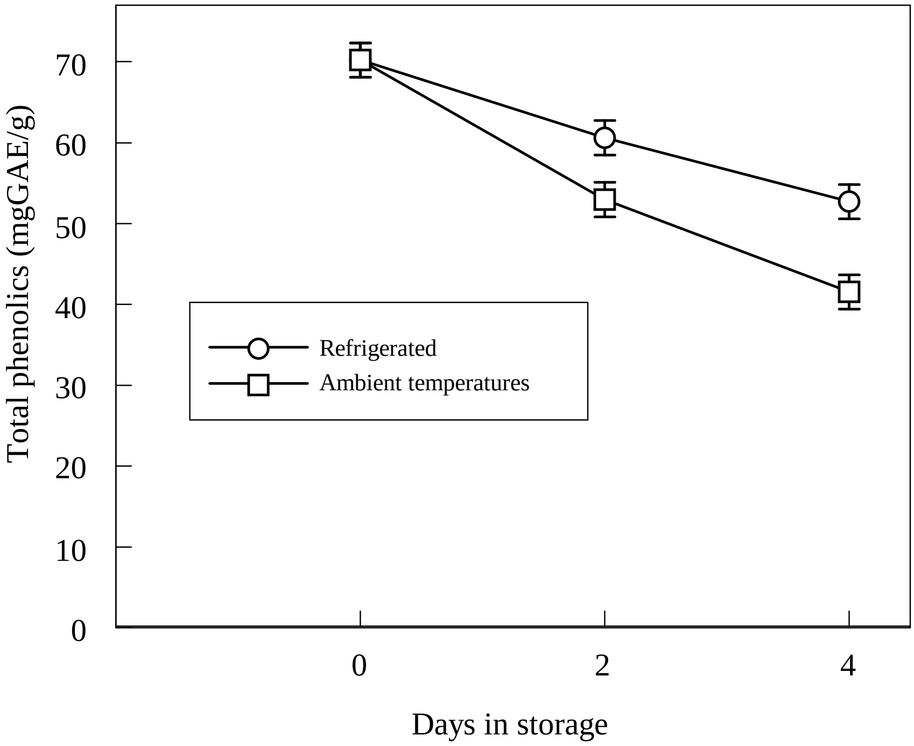

Figure 5.5: Effect of temperature and duration of storage on total phenolic content of vegetable amaranth[*]

*Results from material grown under different fertilizer regimes and harvested at different times have been combined.

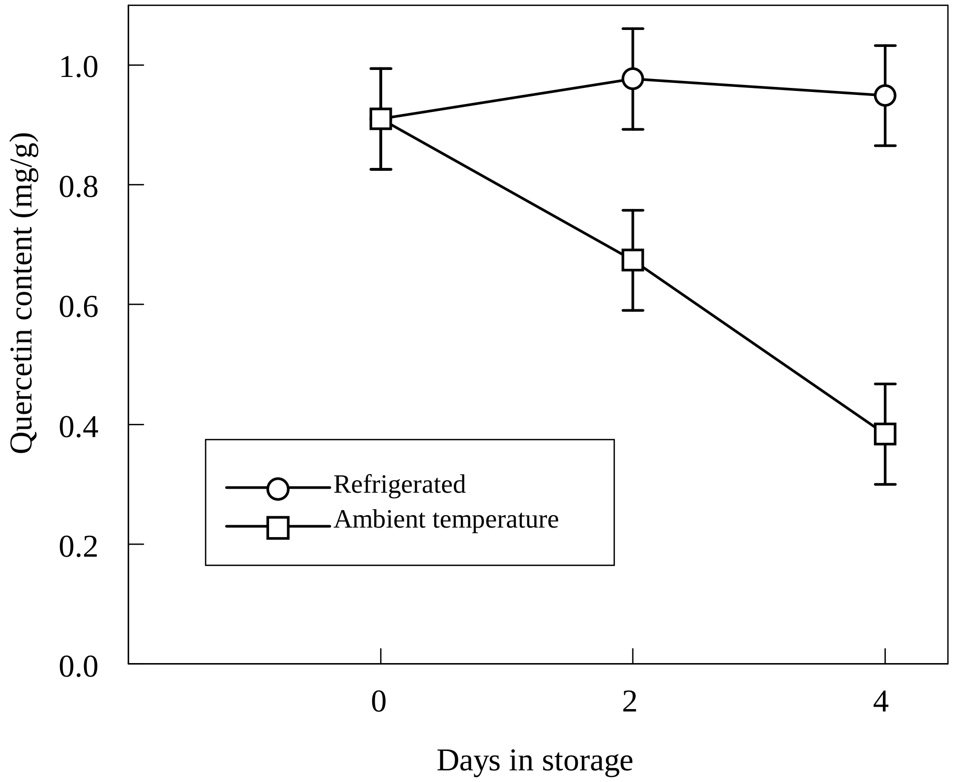

Figure 5.6: Effect of temperature and duration of storage on quercetin content of vegetable amaranth*[*]

*Results from material grown under different fertilizer regimes and harvested at different times have been combined

Changes in both total phenolics and quercetin content depended on the maturity at harvest. Vegetables harvested 6 weeks after planting and stored refrigeratred had their total phenolics content increase after 2 days in storage followed by a gradual decrease (Fig 5.7). For vegetables harvested 8 weeks after planting, the increase in total phenolics was recorded after 4 days in refrigerated storage. Vegetables harvested at 7 weeks after planting had a non-significant increase in total phenolics after 2 days in refrigersted storage followed by a gradual decrease. Unlike the total phenolics, quercetin content of vegetables harvested 7 and 8 weeks after planting increased significantly while that of the week 6 harvest decreased when the vegetables were stored refrigerated (Fig 5.8).

Venere et al (2005) storing escarole (*Cichorium endivia* L. *latifolium* group) and radicchio (*Cichorium intybus* L. (*rubifolium* group) at 4^{o}C found that in escarole, the most abundant phenolic compound increased markedly during storage after the fifth day. In radicchio, the phenolic content showed a oscillated trend during storage: after an initial increase, it showed a gradual decrease, followed by a sharp increase around the ninth day in storage. Our results are comparable with those of Venere et al (2005) although our storage period was only for a period of 4 days after which the amaranth becomes unsaleable.

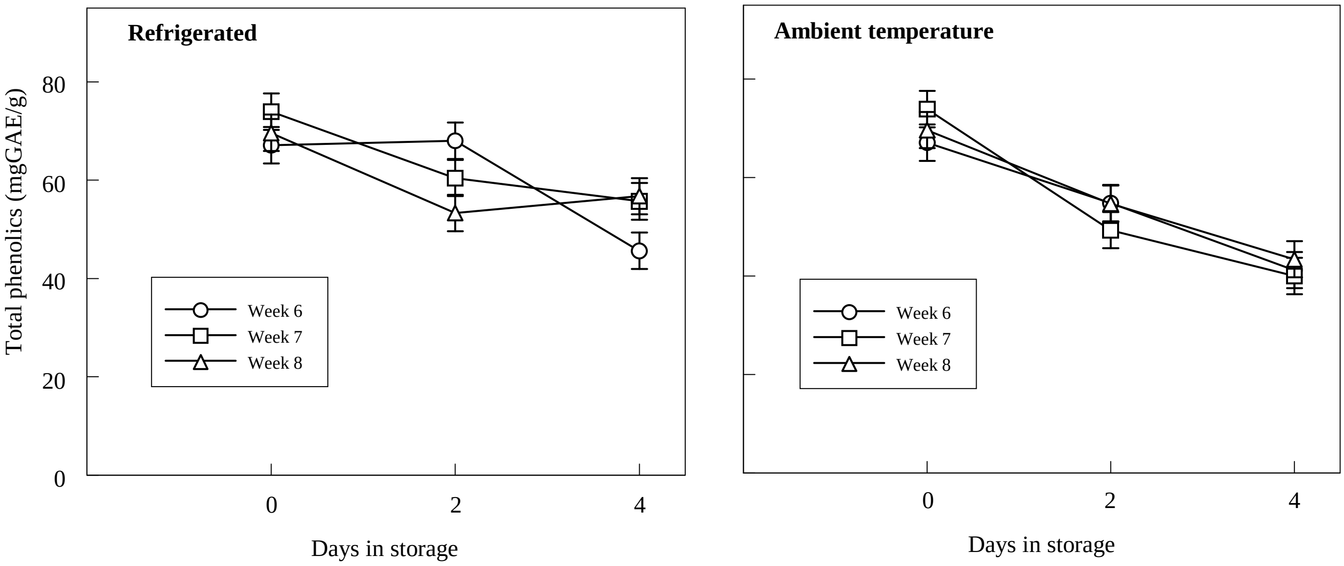

Figure 5.7: Effect of maturity at harvest, temperature and duration of storage on total phenolic content of vegetable amaranth (*A. hypochondriacus*)

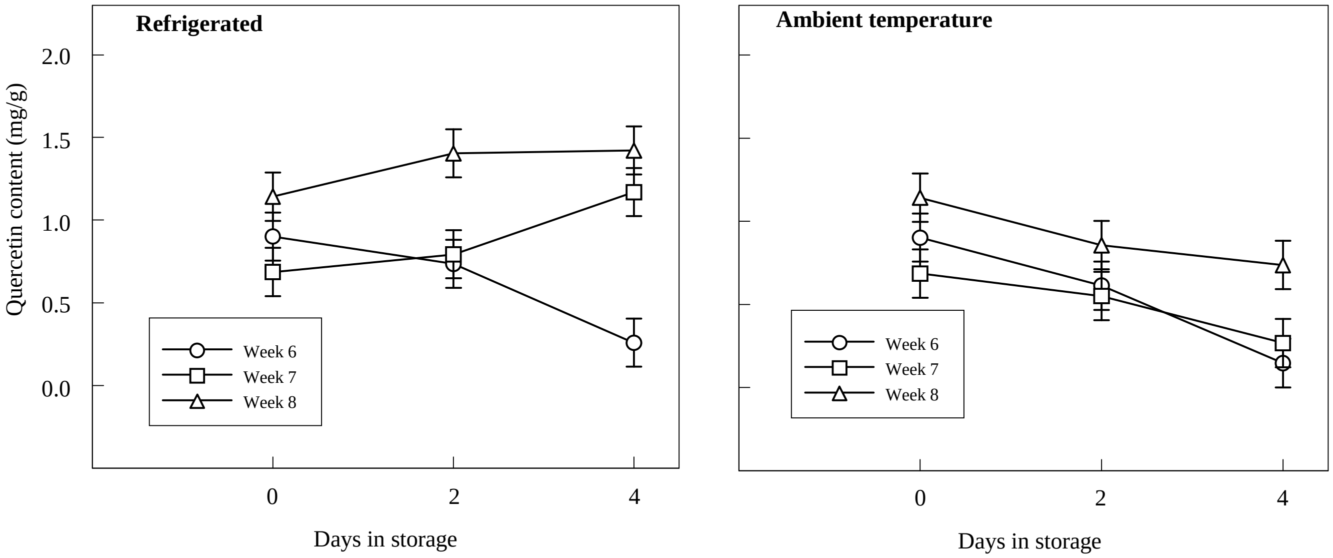

Figure 5.8: Effect of maturity at harvest, temperature and duration of storage on quercetin content of vegetable amaranth (*A. hypochondriacus*)

In storage at low temperature (4±1°C), the total phenolics and quercetin contents increased after 2 or 4 days in storage depending on the stage of maturity at harvest. These observations agree with reports by Souzan and Abd El-aal (2007) and Rivera et al (2006), both groups working with lettuce. They found that the phenolics content increased during low temperature storage reaching a maximum after 2 and 3 days, then decreased marginally until the end of storage of 8 days. The basis for the increase is not easy to explain, but there is a possibility that depending on the amount and type of stress, the synthesis of phenolic compounds may be induced as a result of increased transcription of genes encoding the enzymes in their biosynthetic pathway (Dixon and Paiva, 1995; Ferrante et al, 2009); the synthesis of phenolics is known to be up regulated in exposure to light, cold and wounding (Tavarini et al, 2007). While this model is attractive its relevance to *Amaranthus* which is a tropical genus and therefore probably chilling sensitive would need to be confirmed. In storage at high temperatures, most of the phenolics may be lost in the respiration and oxidation processes which results in the decrease observed**.**

Total and soluble oxalates

There were no significant changes in both total and soluble oxalates during refrigeration as well as ambient temperatures of storage. The fresh samples had an average total oxalate content of 5.1% which after storage for 4 days in the refrigerator was found to be 5% and while that of the material stored at ambient temperatures was 5.2%. The soluble oxalate content of the fresh samples was 3.4% which increased to 3.5% after storage for 4 days in the refrigerator and to 3.7% if stored at ambient temperatures. A small increase in soluble oxalates during storage has been shown to be related to an overall loss in moisture content of other vegetables (Catherwood et al, 2007). This can explain the small increase shown in vegetable amaranth after 2 and 4 days in storage. Our results for soluble oxalates indicate that minimal changes occurred during storage. This is in agreement with those of Kuan (2003) working with soyabeans who found that the levels of soluble oxalates remained constant through 6 months of storage at -20^{o}C, 4^{o}C and 25^{o}C; the temperatures used 4^{o}C and 25^{o}C are similar to those used in this study, although the periods of storage used in this study were considerably shorter.

Conclusion

The study concludes that vegetable amaranth (var. *Amaranthus hypochondriacus*) accumulates higher levels of total phenolic and quercetin when grown with manure than with DAP for similar levels of N. Total phenolics and quercetin levels increase, while that of oxalates decrease, with maturity of the vegetables. The accumulation of oxalates during the periods considered normal for growth are low enough to maintain their levels within safe limits for human consumption. Low temperature storage of *A. hypochondriacus* helps maintain the contents of both phenolics and quercetin. Both total and soluble oxalates do not change significantly during storage of vegetable amaranth (var. *A. hypochondriacus*) for up to 4 days.

Evidence is slowly mounting that the phenols in foods are absorbed by humans (Vinson et al, 1998). For example one Dutch group found that quercetin from onions was still detectable in human plasma 48h after ingestion of 215g of dried onions that provided 64.2mg of quercetin equivalents (Hollman, et al, 1996). Spiking studies (Vinson et al, 1998) indicate that phenols in vegetables, once absorbed, can enrich the low density lipoprotein and prevent them from oxidizing. This effect provides one mechanism for the beneficial effect of vegetables such as amaranth against diseases e.g heart disease. In addition, phenols belong to a reactive group of compounds that can be attacked by the weak electrophile nitrosonium ion (NO^{+}) and nitrosation may occur easily in aromatic rings bearing more activating groups such as –OH and -OR (Mohammed, 2002). Therefore in addition to free radical neutralization, the natural phenolic compounds may play some important role in prevention of nitrosamines (potent carcinogens) formation which are produced due to the reaction of nitrite with amines in vivo (See chapter 3, this thesis)

From this study, the contents of total oxalates remain unchanged during growth and in postharvest under the conditions described. It is possible that these conditions do not influence the content of oxalates found in this vegetables. The two fractions of oxalates that have been found to be dorminant in amaranth are the boiling-water soluble fraction and an insoluble fraction (Vityakon and Standal, 19890). The total oxalates in amaranth is made up of both the soluble and the insoluble fractions. As it also precipitated by Ca^{2+} the insoluble oxalate cannot inactivate further Ca^{2+}, whereas the soluble forms can combine with Ca^{2+} from food and reduce its availability. Therefore for nutrition purposes, the contents of the soluble oxalates in the vegetables are of concern. The quantities of soluble oxalates found in A*maranthus hypochondriacus* used in this study are not high enough to cause public health concerns. However, further research on the effect of other types of fertilizers and different modes of application is recommended.

CHAPTER 6

FEASIBILITY OF COMMERCIAL PRODUCTION OF AMARANTH (*Amaranthus hypochondriacus*) LEAF VEGETABLE BY SMALL-SCALE FARMERS IN KENYA

Abstract

Vegetable amaranth (*Amaranthus hypochondriacus*) was grown under a range of fertilizer treatments (manure and a range of diammonium phosphate (DAP) applications) to determine the potential profitability under commercial production conditions. These trials were carried out at the University of Nairobi Field Station in the Upper Kabete Campus during the long rains of March – May 2008. Trials were laid out as complete randomized block design on plots measuring 2m x 2m, with four fertilization treatments: 20, 40, and 60kg N ha^{-1} of DAP (18:46:0), 40kg N ha^{-1} from cattle manure, and an unfertilized control. The vegetables were harvested at 6, 7 and 8 weeks after planting. Gross margins were calculated based on a range of the possible choices; hired or family labour, rented or owned land, and purchased manure or that produced by cattle on the farm. A sensitivity analysis was performed on the possible effects of changes in fertilizer cost and the price of vegetables on the resulting revenue. The week 8 harvests resulted in the maximum yields for all treatments. The highest yield, which was obtained from the plots receiving 40kg N ha^{-1} DAP, was equivalent to 29,925 kg ha^{-1} . The yield from the manured plots was equivalent to 3,700kg ha^{-1} . Based on use of manure alone and the different options the predicted gross income per hectare ranged between KSh 53,107 with purchased manure, and hired land and labour, to KSh 81,780 with own land, labour and manure. Using DAP, the predicted income ranged from KSh 483,273 with hired land and labour to KSh 498,140 with own land and labour. The change in the gross income to the farmer was more sensitive to the change in the price of the vegetables than to the cost of fertilizer. These results show that small farmers can make money from commercial production of amaranth vegetables. This conclusion is based on the farmer judging the opportunity costs of his own land, family labour and manure from his own farm as zero. The results make it clear that at current market prices the revenue from growing *Amaranthus hypochondriacus* can be increased by using artificial fertilizer rather than manure.

KEYWORDS*: Amaranth, Commercial production, small-scale farmers, Kenya*

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Chapter 6

Introduction

Most of the farmers in the developing world are small-scale. For example, in south Asia, there are 125 million farms with an average size of 1.6 ha, in Bangladesh, 96% of all holdings have a size of less than 0.3ha (AVRDC, 2002) while in most countries in Sub-Saharan Africa small scale farmers operate on an average size of 0.4ha. Though most of these farmers grow traditional leafy vegetables (TLVs) in their kitchen gardens only for home consumption, in Kenya, these vegetables are found in increasing quantities in formal markets where the middle and higher socio-economic classes shop (Ngugi, *et al,* 2006). In terms of quantities supplied to these markets, amaranths rank second after *Vigna unguiculata,* but they are the more preferred by consumers. In these markets, TLVs are sold competitively alongside their exotic counterparts like cabbage and spinach (Onyango and Imungi, 2007). The increase in demand has stimulated many entrepreneurs, especially women, to grow and trade in these vegetables on a small scale. Currently, therefore, opportunities exist in Kenya to use TLVs, such as amaranth, to generate income.

It has been previously demonstrated that commercial production of TLVs by small scale enterprises can be viable (Ngugi, *et al,* 2006; Besong *et al,* 2001). This form of commercial production can serve as a useful tool for poverty reduction for women with little capital, limited access to land and working under labour constraints (Lewis, 1997). The revenue generated contributes significantly to enhancement of household food security, access to family health care and enables women to attain some degree of financial independence within the family budget (IITA, 2003).

Production of TLVs is very simple and is practised with very few inputs - normally just the use of farmyard manure as fertilizer and very rarely is chemical fertilizer used in the production of TLVs. For marketing, the harvested vegetables are packed in bundles of sizes dependent on the method of harvesting. Driven by trade liberalization and new technologies in food processing and retailing, agri-food markets are becoming more concentrated at all levels (wholesale, retail and intermediary traders). Market expansion results in increasing price competition and new large distribution agents are setting stricter standards in terms of product quality, reliability and scale of delivery. Procurement patterns are being adapted to these new requirements and changing the market opportunities for farmers in the process (Vorley et al, 2007). In order to assist farmers to grow TLVs and access markets, a developmental organization – Farm Concern International - has been assisting small scale farmers across Sub-Saharan Africa to transform themselves from subsistence farmers into Business Support Units that can produce the vegetables on a sustainable basis and ensure consistency in supply to the markets (Ngugi et al, 2006).

In developing countries, small-scale agriculture remains a critical contributor to poverty reduction and rural development (through direct linkages to the non-farm economy) (The World Bank, 2008). It has been shown that that there is high demand of TLVs in major cities of developing countries e.g. Kenya (Onyango et al, 2008) and that these vegetables are becoming a major source of income to rural and peri-urban small scale farmers. In order for the TLVs to play a significant role in poverty reduction, farmers involved in their production and marketing require education on profitable production and marketing aspects. The purpose of this study is to present a Gross Margin analysis for producing vegetable amaranth as a commercial enterprise. The Gross Margin of an enterprise is the enterprise total income less its variable costs. It is a simple measure of an enterprise's contribution to the overall cost of running the farm. Thus if all the enterprises that go to make up the farm show a positive Gross Margin, then the farm is likely to make a profit (FAO, 2007). The Gross Margin calculation may be used as a convenient step in preparing a farm budget or investment plan for the future. The costs charged to an enterprise when calculating its Gross Margin are called variable costs because they vary according to the size and nature of the enterprise. Examples are: Seeds (purchased or home-grown), fertilizers, chemicals, casual labour and hired equipment. Fixed costs are not shown in the Gross Margin analysis. Fixed costs are those costs that would occur no matter what quantity is produced. Examples are: Depreciation on buildings and machinery and interest on capital.

The Gross Margin analysis for vegetable amaranth production is likely to be affected by both changes in artificial fertilizer (which is imported) costs due to the fluctuating dollar to the Kenya shilling exchange rate and the changes in vegetable prices in the different seasons (rain and dry seasons). An analysis of the Gross Margins given the changes in both fertilizer cost and vegetable price will determine to which of the two factors the gross margin is most sensitive to.

It is also necessary for the farmers to know the quantity of the vegetables they will need to produce in order to cover all the costs incurred during the production (the break-even point). The break-even yield is the quantity of yield required to just recover the cost spent on producing the commodity at a given price. The higher the expected or actual yields above the break-even yield level the greater the profitability. At the planning stage, if after calculating the break-even yield, it is found to be much lower than the recommended or farmer’s usual yield, and there is no way to push the yield above the break-even point, then the farmer should not produce the crop in question.

The objective of this study was therefore to use A*maranthus hypochondriacus* yields obtained from experimental plots using manure and DAP fertilizer to generate gross margins based on small scale farmers’ economic model.

Materials and Methods

Amaranth seeds were obtained from Asian Vegetables Research and Development Centre (AVRDC), 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 Latitude 1^o 15′S and Longitude 36^o 44′E, and at altitude 1930 m above sea level (Sombroek et al, 1982). 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 and Lawes, 1971). The mean annual temperature is 18^oC. The soil in Kabete is characterized as a deep, well drained, dark reddish-brown to dark brown, friable clay (Mburu, 1996). The land used for the plots had not received any fertilizer 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 fertilized with either manure or diammonium phosphate (DAP; 18:46:0) fertilizer. The trials were laid out in a complete randomized block design with five fertilization treatments as follows: one plot with cattle manure at 40kg N ha^{-1} (calculated using the content of N found in the manure; Table 3.1), three plots with DAP (18:46:0) at 20, 40, and 60kg N ha^{-1}, and unfertilized control plot. The treatments were replicated three fold. The plots measured 2m x 2m with spacing of 15 cm x 10 cm (Palada and Chang, 2003). Care was taken in this trial to use a manure treatment and, where possible, cultivation practices that were comparable to those employed by local small-scale growers. 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 plants were grown in 2007 (year 1) and 2008 (year 2) but only the yields from year 2 are used for gross margin calculation. Year 2 was used to because conditions experienced during this year were considered to be the average encountered by farmers in Kenya. The vegetables were harvested at three maturity stages; 6, 7 and 8 weeks after planting.

The yields were converted to bundles per hectare as they would if being produced for sale by the small-scale farmers and the gross margins per hectare computed for a harvest at 8 weeks after planting (a season), when the vegetable yields are at the maximum. Yields from year two (2008) when using manure at 40 kg N ha^{-1} and DAP at 40 kg N ha $^{-1}$ were used for the Gross Margin calculations. These two levels were used because small scale farmers in Kenya would normally use manure and the recommended rate of N for vegetable amaranth is 40 kg N ha $^{-1}$. The fertilizer costs used in the analysis were those that small scale farmers would have paid when buying fertilizer in small quantities from local suppliers; these prices are somewhat higher for DAP than when the fertilizer is purchased in bulk by large scale farmers. Gross margin is a proxy for profitability and is in turn a reflection of the commercial viability of growing amaranth. All values and calcultions are presented on a growing season basis.

Gross Margin = GI – TVC

Where:

GI = Gross income (Ksh ha^{-1} season^{-1})
= Yield in (kg ha^{-1} season $^{-1}$)x Price (Ksh kg^{-1})

TVC = Total variable cost (cost of land, labour, manure/fertilizer, seed)

Land rates = 4,000 (Ksh season^{-1}ha^{-1})

Labour = 4,400 (Ksh ha^{-1} $season^{-1}$)

Seed = 360 (KSh ha^{-1} $season^{-1}$)

Cost of fertilizer = 166,500 (KSh ha^{-1} $season^{-1}$)

Cost of Manure = 13, 888 (KSh ha^{-1} $season^{-1}$)

Ksh: 1 Kenya shilling is equivalent toUSD 0.014

Note: The rates of the variable costs used in this study were given by Farm Concern International – an organization that assists small scale farmers produce and sell TLV in East and Central Africa.

Amaranth production like most agricultural production, suffers from changes in input and output prices. In order to assess whether amaranth production could be commercially viable, a sensitivity analysis was performed by simulating the effect of realistic vegetable and fertilizer price changes on the Gross Margins obtained above. This was followed by calculation of the break even points (BEP) for yield for different vegetable prices and fertilizer costs.

Gross Margin Sensitivity analysis = Change in Gross Income with a change in vegetable price and cost of fertilizer

Break-Even-Point (BEP) occurs at the point when GI=TVC.

The Break-Even Point for Yield = the quantity of yield required to just recover the cost spent on producing the commodity, at a given price.

Where:

GI = Gross Income
TVC = Total Variable Cost

BEP (Yield)(kg ha^{-1} $season^{-1}$)= TVC (Ksh $season^{-1}ha^{-1}$)/Price (Ksh kg^{-1})

Results and Discussion

The yield data are presented in Table 6.1. Total yield per hectare increased steadily from week 6 to week 8 with the highest yield being recorded in plots that received 40 kg N ha^{-1} of DAP at week 8 harvest. Plots with manure application also recorded the highest yield at week 8 harvest. Evidently, the growth and yield of amaranth is strongly influenced by soil fertility when this has been enhanced by an inorganic fertilizer. 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, this response is not proportional to the supply of N, with the response depending on N supply and harvest date.

Table 6.1: Vegetable amaranth yields (kg ha^{-1}) during a long rains season at different levels of nitrogen application and stages of maturity at harvest; all values sre for one growing season

Source of N	**Yield (kg ha^{-1}) at different stages of maturity (weeks after planting)**		
	6	**7**	**8**
Control	800	1,225	3,150
DAP 20kg N/ha	8,275	16,975	25,025
DAP 40kg N/ha	9,825	19,075	29,925
DAP 60kg N/ha	10,025	11,825	16,350
Cattle manure	1,425	1,725	3,700

DAP = Diammonium phosphate fertilizer

The manure treatment used in these trials was comparable to that employed by local small-scale growers. Therefore, the differences in the growth responses obtained are both significant and relevant.

Conventionally, a majority of the farmers will grow crops on the same plot of land season after season without any soil fertilization. Farmers' crop yields therefore continually dwindle. A possible redeeming factor for manure application (other than the improved germination – Onyango et al, in preparation) is that many farmers may be using a soil that is more exhausted than the soil employed as the control in this study. Application of manure to nutrient depleted plots, such as those typical of small farms, would probably have elicited discernible differences in the yields between the manured and the control plots.

Gross margin analysis at 8 weeks after planting

Farmers in Kenya sell vegetable amaranth in bundles of average weight 0.45 kg per bundle (Onyango et al, 2008). Each bundle is sold for a mean price of KSh 10.00. This translates to a price of KSh 22.2 per kilo. The different gross margins are shown in Table 6.2 and 6.3 for manure and fertilizer respectively.

Case 1: application of manure 40 kg N ha^{-1}

Yield (kg ha^{-1} season^{-1})= 3,700.

Gross Income (Ksh ha^{-1} season^{-1}) = Yield(kg ha^{-1} season^{-1}) x Price (Ksh kg^{-1})
= 3,700 x 22.2
= 82,140.00

Most small-scale farmers use family labour, have their own land and practise zero grazing of dairy animals so manure is readily available. These farmers consider land, labour and manure as ‘free’ resources. Although these resources have opportunity costs, these are normally considered zero. Consequently the only variable cost that should be reflected in the gross margin calculations is that of the seed. Sometimes the seed will be own produced or donated by a neighbour or friend. In this case, the seed can also be placed in the same category of ‘free’ inputs by the farmer, and the opportunity cost becomes zero. These considerations vary the gross margins depending on what the farmers consider as ‘free’ inputs and create the following scenarios; in each case seeds being purchased:

Farmer 1: Purchased manure, hired labour and land
Farmer 2: Hired labour and land, own manure
Farmer 3: Hired land, own manure and labour
Farmer 4: Own land, manure and labour
Farmer 5: Purchased manure, hired labour, own land
Farmer 6: Purchased manure, own land and labour
Farmer 7: Hired labour, own land and manure

The different gross margins based on these scenarios are shown in Table 6.2.

Table 6.2: Gross Margin analysis on season basis for production of vegetable amaranth using cattle manure based on seven scenarios (values are in Ksh)

	Farmer1	***Farmer2***	***Farmer3***	***Farmer4***	***Farmer5***	***Farmer6***	***Farmer7***
Gross Income (GI) (Ksh ha^{-1}season^{-1})	82,140	82,140	82,140	82,140	82,140	82,140	82,140
Total variable costs (TVC) (Ksh ha^{-1} season^{-1})							
Seed cost	360	360	360	360	360	360	360
Manure cost	13,888	0	0	0	13,888	13,888	0
Labour cost	10,866	10,866	0	0	10,866	0	10,866
Land Rent	4,000	4,000	4,000	0	0	0	0
Total cost	**29,114**	**15,226**	**4,360**	**360**	**25,114**	**14,248**	**11,226**

Gross Margin (GI-TVC) (Ksh ha^{-1}season^{-1})	**53,107**	**66,914**	**77,780**	**81,780**	**57,026**	**67,892**	**70,914**

1US Dollar = Ksh 74

Many small-scale farmers in Kenya today practise zero grazing and will therefore use manure from their cattle sheds in the production of these vegetables.

Case 2: application of DAP 40 kg N ha^{-1}

Yield (kg ha^{-1} season^{-1}) = 29,925.

Gross Income (*Ksh ha^{-1}season^{-1}*) = *Yield (kg ha^{-1} season^{-1}) x Price (Ksh kg^{-1})*

= *29,925 x 22.2*

= *664,335.00*

Just like the farmers who produce the vegetables using manure, small-scale farmers using fertiliser will consider the use of family labour and their own land as 'free' resources hence the opportunity cost of these resources is zero. With this in mind, there arises several scenarios in which the seed and fertilizer is purchased:

Farmer 1: Hired land and labour

Farmer 2: Own land, hired labour

Farmers 3: Owns land and labour

Farmers 4: Hired land, own labour

Gross margins for these scenarios are shown in Table 6.3

The prohibitive costs of chemical fertilizer means that it is very rarely used in the production of TLVs such as *Amaranthus hypochondriacus*. Whenever it is used, it is usually in combination with manure, and the fertilizer is usually applied in levels that would be sub-optimal for fertiliser alone.

Table 6.3: Gross Margin analysis on a season basis for production of vegetable amaranth using DAP fertilizer (18:46:0) (values are in Ksh)

	Farmer1	*Farmer2*	*Farmer3*	*Farmer4*
Gross Income (GI) (Ksh ha^{-1}season^{-1})	664,335	664,335	664,335	664,335
Total variable costs (TVC) (Ksh ha^{-1} season^{-1})				
Seed cost	360	360	360	360
Fertilizer cost	166,500	166,500	166,500	166,500
Labour cost	10,866	10,866	0	0
Land rent	4,000	0	0	4,000
Total cost	**181,726**	**177,726**	**166,860**	**170,860**
Gross Margin (GI-TVC)				

(Ksh $ha^{-1}season^{-1}$)	**482,609**	**486,609**	**497,475**	**493,475**

1US Dollar = Ksh 74

From the gross margin analysis, it can be seen that application of the recommended rates of DAP fertilizer results in higher yields of the *Amaranthus hypochondriacus* and therefore better returns to the farmers than from the use of manure. Most of the vegetable amaranth farmers have access to an average of 0.1ha (0.25 acre) of land for production of the vegetables. If the gross incomes estimated for 1 hectare are scaled down to one-quarter acre, a farmer will make a gross income of Ksh 66,434 and Ksh 8,214 when the vegetables are produced using DAP and manure respectively. This shows very clearly that farmers will obtain higher yields and hence gain higher returns by applying DAP fertilizer at the recommended rate of 40 kg N ha^{-1}. Some farmers will use sub-optimal artificial fertilizer levels in admixture with the manure. The yields and returns from this option will fall between that of production using 40 kg N ha^{-1} DAP and 40 kg N ha^{-1} manure.

Sensitivity analysis

The Gross margins shown in Table 6.2 and 6.3 will change depending on the changes in the variable costs and the changes in market prices of the vegetable. The artificial fertilizers form the largest fraction of the total variable cost under this type of production and therefore the changes in its cost will have significant effects on the total variable costs incurred for production of the vegetables. The vegeteable prices determine the gross income obtained from production. It is therefore important to determine which of the two the Gross Margins will be most sensitive.

Vegetables normally sell at Ksh 22.2 per kg (Onyango et al, 2008). However during the rain season the prices drop to Ksh 11.5 per kg and during the dry season the prices rise to Ksh 33.3 per kg. Cost of Fertilizer = USD 1 per kg (The dollar rates to the Kenyan shilling fluctuate between Ksh 75 and Ksh 100).

Our results show that the gross margins are more sensitive to change in vegetable prices than the change in fertiliser prices (Figure 6.1). The price of vegetables will normally rise during the dry season in response to diminished supply in that period.

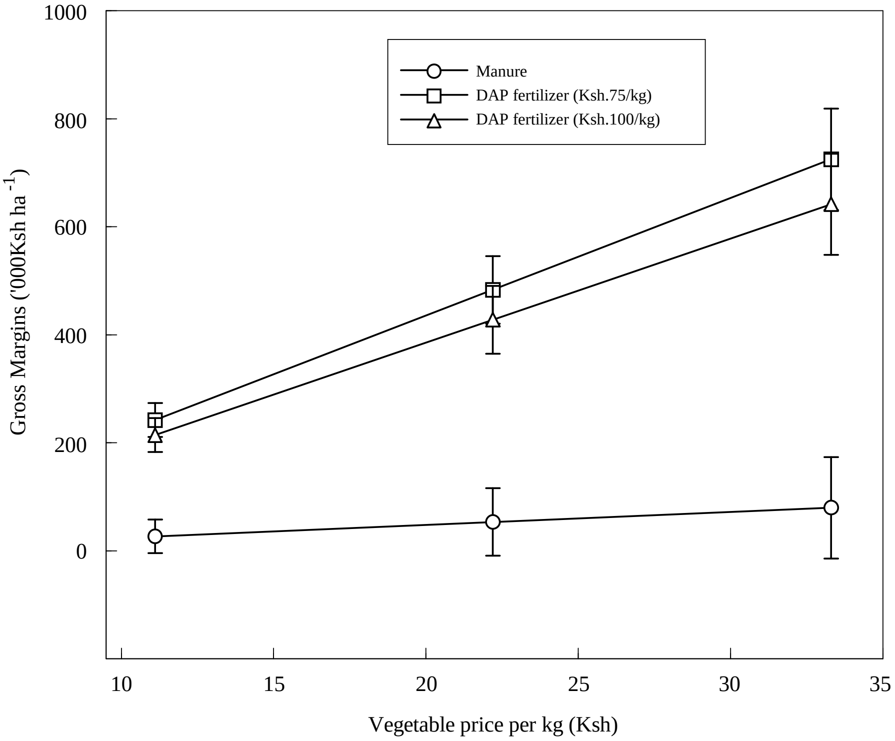

Figure 6.1: Effect of fertilizer (DAP) cost and vegetable price on the Gross margins for vegetable amaranth production per hectare per season (1 US Dollar = Ksh 74)

The changes in fertilizer prices are unpredictable because fertilizers are usually imported and their prices are pegged to the hard currencies whose exchange rates with the Kenya shilling usually fluctuate. In most cases however, these fluctuations are minimal resulting in only small changes in cost of fertilisers and small effects on the Gross Margins. This implies that the returns obtained per unit of land by the farmers depend greatly on the price the vegetable amaranth will attract. The small scale farmers can therefore take advantage of the high prices of vegetables during the dry season. Most of the small scale farmers in Kenya grow TLVs near rivers and hence can carry out irrigation using watering cans to produce these vegetables during the dry season.

Break Even Point (BEP) Analysis

The break-even yield is the quantity of yield required to just recover the cost spent on producing the commodity at a given price of the commodity in this case, price of the vegetable. The break-even point with the use of DAP fertiliser requires a higher productivity of the vegetable than when manure is the fertiliser (Fig 6.2). This arises from the high cost of the chemical fertiliser as compared to manure. However as the price of the vegetable increases, the break-even point for yield when DAP fertiliser is used decreases significantly. The BEP analysis is important to the planning stage because if after calculating the break-even yield it is found to be much lower than the recommended or farmer’s usual yield, and there is no way to push the yield above the break-even yield, then the farmer should not produce the crop in question. This is because producing at a yield lower than the break-even yield would mean producing at a loss.

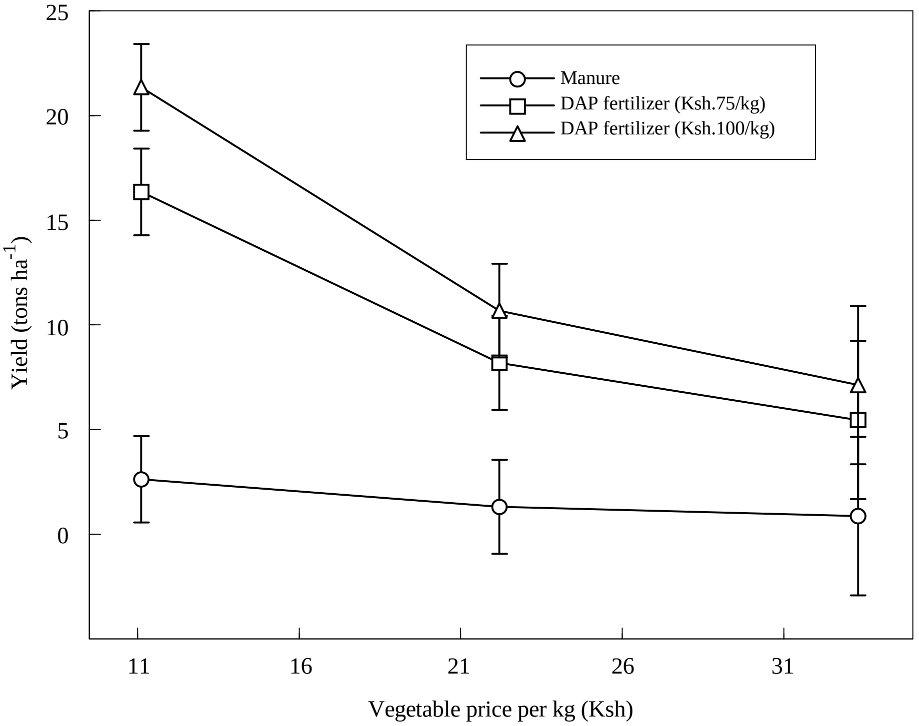

Figure 6.2: Effect of fertilizer (DAP) cost and vegetable price on the break even yield for vegetable amaranth production per hectare per season (1 US Dollar = Ksh 74)

As vegetable productivity is increased by using DAP fertilizer at the recommended rate of 40 kg N ha^{-1} the break-even point can be reached.

Example: If the DAP cost rises to Ksh 100 per kg, using the yield obtained by using DAP at 40 kg N ha^{-1} at 8 weeks maturity and the lowest price for the vegetable (Ksh 11.1Kg^{-1})

$$BEP\ (Yield)(kg\ ha^{-1}\ season^{-1}) = TVC/Price\ kg^{-1}$$

$$= 237226/11.1$$

$$=21371kg$$

$$=21.37Tons$$

The yield of 21.4 tons will be equivalent to the break even point. To the small scale farmer who considers use of family labour and land as zero cost, the cost assigned to these two factors will be perceived by the farmer as accrued income. At the normal price of Ksh 22.2, the farmers will produce yield that are above the break-even point and therefore will make a profit. However, using the same rate of DAP, lower yield have been recorded in vegetable amaranth produced under different conditions (Onyango et al, in preparation, chapter 3 this thesis). Since farmers have no control over the market price of the vegetables, it is clear that at the lower yields and using a price of Ksh 11.1 per kg of vegetable used during the long rains season, the farmers would not achieve the break-even point.

In spite of the benefit of being free, the use of manure results in less income than the use of artificial fertiliser. In this study gross margin calculations have been based on small scale farmers' perception of the costs of required inputs and income from production in order to produce results that are consistent with the farmers' economic model and which will, therefore be more convincing to them, rather than the conventional calculations based upon standard economic principles which, however, often seem unrealistic to small-scale farmers.

Our results indicate that returns to investment for the TLV farmers depend largely on the vegetable prices. Development of proper infrastructure between production sites and markets is required to be able to get the produce to the markets offering high prices. The presence of middlemen in the marketing of the TLVs in Kenya (personal observation) results in small scale farmers getting lower prices for their produce than it would be if they sold directly to the marketing outlets. Formation of farmers groups to take advantage of the economies of scale in accessing the markets directly will result in higher incomes. Considering the whole value chain from farming to the final consumer is vital for furthering sustainable livelihood in amaranth.

Agriculture, in particular pro-poor agricultural growth is back on the international development agenda (U.N. 2000). Use of traditional leafy vegetables such as amaranth to promote the role of agriculture in economic growth and poverty reduction in developing countries is possible. As the global economy becomes knowledge-based, the management and utilization of information will increasingly drive agricultural development. Knowledge on intensive technologies and services (pest management strategies, seed, fertilization strategies, value-added markets), will continue to increase in importance within the agricultural sector. All farmers, including small-scale farmers, will need both ingenuity and competency to remain competitive.

Most African governments have clearly not prioritised agricultural transformation and continue to treat smallholder agriculture as just a way of life for a peasant population, with little to contribute towards economic growth and poverty alleviation. The farmers have also remained poorly organised, and fail to lobby for an adequate share of public resources. Purposeful and sustained engagement of these stakeholders is required to unlock the potential of smallholder agriculture as the best option for transforming the lives of large numbers of poor households in rural communities across Africa (Mutamba and Nyagah, 2009).

CHAPTER 7

GENERAL DISCUSSION

Production of amaranths and other traditional leafy vegetables (TLVs) is increasingly being viewed as an important part of the livelihood strategies of many people in the rural and peri-urban areas of most of the developing countries. Apart from subsistence production, amaranths also offer significant opportunity for poor households to generate revenue through commercial production (Chapter 6), because due to increase in their consumption by people in urban areas, market demand for traditional leafy vegetables has increased.

This study established that the growth and yield of amaranth is more strongly influenced by soil fertility when this has been enhanced by inorganic fertilizer but not by manure. Common with many plants, nitrogen supply is known to have considerable effect on the yield of amaranth (Makus, 1986). There was no significant yield increase when manure was used as a source of N when compared to the control. The lack of yield increase due to manure application was disappointing but not unprecedented. Adediran et al, (2004) working with maize (*Zea mays*) and cowpea (*Vigna unguiculata*) found that use of manure did not result in significant yield gains compared to control plots. Other studies have shown that manure is not as effective as artificial fertilizer (Van Lauw et al, 2001, Turan and Sevimli, 2005). Compared to mineral fertilizers, manure contains relatively smaller amounts of nutrients readily available for growth of plants (Edmeades, 2003). It is possible that after emergence, the supply of these essential nutrients was insufficient to promote continued rapid growth. The results obtained in this study show that although yields responded favourably to supply of mineral N, the response depended on N supply and harvest date. The typical growth duration of amaranth in Kenya is 8-10 weeks from planting to flowering, with the main harvest of leaves occurring between weeks 6 and 8. Under this regime yield saturated at an application of diammonium phosphate (DAP) fertilizer of no more than 40 kg N ha^{-1} when this was applied as a single dose.

Working with grain amaranth and using 0, 15, 30, 45 and 60kg N ha^{-1} (NPK 15-15-15), Olaniyi et al, (2008) found that fresh and dry shoot yields and grain yield per hectare were significantly influenced by applied N rates. Although fresh shoot yield increased with increasing level of fertilizer application between 0-60 kg N ha^{-1}, there was no significant difference between the mean yields obtained at 45 kg and 60 kg N ha^{-1} . These results are similar to our results with vegetable amaranth (*Amaranthus hypochondriacus*). Since there was no increase in yield when 60 kg N ha^{-1} were used, it could be concluded that higher rates of N than optimal result in luxury N uptake, leading to nitrate accumulation or loss of N through leaching (Maurao and Brito, 2001). Higher rates (45, 90 and 135 kg N ha^{-1}) have been shown to cause an increase in the yields of vegetable amaranth (*Amaranthus tricolor*), but in this case a split application of N , half at germination and the other half two weeks later, was used (Singh and Whitehead, 1996). Leaf area increased with N-fertilization until 90 kg N ha^{-1} then decreased at 135kg N ha^{-1}.

There was continuous increase in yield between 6 and 8 weeks after planting. The highest yield was recorded at 8 weeks after planting in all treatments. This is important for the farmers to optimize their income from the vegetables especially if they are to produce it for commercial purposes.

Nutritional quality of vegetable amaranths

The nutritional value of vegetable amaranth has been rated to be equal, or even superior to that of spinach, as it is considerably higher in vitamins A and C, calcium, iron and phosphorus (Watt and Merril, 1975). The vegetable amaranths sampled from the Nairobi markets in this study contained an average protein content of 26%, an average vitamin C content of 627 mg/100 g, an iron content of 18 mg/100 g and a zinc content of 5.5 mg/100 g on dry weight basis. The vegetables were also analyzed for nitrates and oxalates, and possible contamination with the toxic metal lead to assess the safety of cultivation practices and/or pollution. The nitrate contents ranged between 505 - 1056 mg/100g dry weight; this translates to about 75 - 158 mg/100 g fresh weight. This level is below the maximum safe daily intake of 220mg/100g fresh weight recommended by WHO for adults (WHO, 2000). The total oxalate contents were found to range between 4900 - 6600 mg/100g and soluble oxalates between 2800 - 4200 mg/100g dry weight. Levels toxic to humans have been indicated to be 2 to 5 g of oxalic acid per day for those populations consuming low levels of calcium (Ricardo, 1993). The levels of free oxalates found in this study therefore do not constitute a serious health hazard for reasonably healthy individuals if consumption does not exceed 200g of fresh leaf per day (FAO, 1988). Further, it is expected that if the method of cooking involves discarding of cooking water as is often the case with leafy vegetables in Kenya, the levels of total and free oxalates as well as levels of nitrates in the vegetables as consumed will be substantially reduced.

The levels of lead in the vegetables ranged between 0.98 - 1.5mg/100g dry weight, which translates into 0.15 – 0.2 mg /100g fresh weight. The US Food and Drug Administration Advisory Panel suggest that no more than 1mg of lead per day be consumed from food (Gordon and Wayne, 1993). The levels that were found in the vegetables in this study would lead to intakes of less than 0.5mg assuming consumption of 200g of leaf by adult persons which is below the recommended limits.

Lack of land for agriculture in the urban and peri-urban areas coupled with desire by the poor populations in these areas to generate income has resulted in some vegetables being grown on the banks of urban drains and sewers. This results not only in some consumers having a negative attitude to the vegetables, but raises the real risk of contamination by heavy metals and pathogens. For purposes of health and nutritional planning therefore, it was important to gain knowledge on the quality and safety of these vegetables and the possible methods of preparation to reduce potential of intoxication. Findings in chapter 2, indicated that most of the vegetable amaranths together with other TLVs sold in supermarkets in Nairobi are cultivated 20 - 40 km away from the city. Vegetables grown in the banks of drains and sewers will probably be found in some of the multiplicity of open air markets within the city and not in these formal markets.

The Kjeldahl nitrogen contents of edible parts of *Amaranthus hypochondriacus* ranged between 3.7% to 6% in vegetables produced in Year 1; and 3.1% to 4.2% in those produced in year 2. Converting these values to crude protein by multiplying by 6.25, the protein content ranged between 23% and 37.5% in year 1 and 19.4% and 26.3% in year 2 (Chapter 3). The values obtained in year 1 were slightly higher than those already reported in literature for vegetable amaranths (Aletor et al, 2002; Sleugh et al, 2001) and the average values for the vegetable samples collected from the supermarkets and green groceries in Nairobi (Chapter 2). The values in year 2 were comparable to those reported in literature and those that were obtained in samples collected from the markets. The β-carotene contents ranged between 15.5mg/100g and 26.3mg /100g dry weight for harvests in year 1; and between 9.6mg/100g and 19.5 mg/100g dry weight for harvests in year 2. The contents of ascorbic acid (vitamin C) ranged between 126 mg/100g (control) and 1699 mg/100g (plots that received manure) depending on the time they were harvested (Chapter 4). The other vegetable amaranth spp would be expected to give values that are comparable with these results. The protein as well as the β-carotene contents of *A. hypochondriacus* are greatly improved by the use of the chemical fertilizer DAP which provides an easily available form of N (and P). At the rate of 40 kg Nha^{-1}, it is possible to obtain both high yields of vegetables with high protein and beta carotene contents (Chapter 4). However at this high levels the ascorbic acid content was decreased. Use of manure led to higher amounts of ascorbic acid compared to the use of DAP but harvestable yield and protein levels were the lowest.

It has been reported that Kenya's main nutritional problems are the same as those of other developing countries, and include protein and vitamin A deficiencies which affect a large proportion of the poor urban and rural populations. Iron-deficiency induced anemia has also been reported to be prevalent among the general population, especially among children under five years of age and pregnant women (WHO, 2000). Considering just these two headline nutritionally related problems the consumption of vegetable amaranth in diets would be expected to partly contribute to alleviating these problems. The vegetables contribute substantially to the protein, the mineral (iron, calcium, phosphorus and potassium) vitamins A and C and crude fiber intakes from diets. The levels of protein, ascorbic acid, iron and zinc are reduced in the cooked, drained vegetables (the form in which they are eaten) due to leaching of the nutrients into the cooking water. For example Mathooko and Imungi (1994) have reported loss of 80% ascorbic acid in cooked drained *Amaranthus hybridus* while Yadav and Sehgal (1995) reported ascorbic acid losses of 93% and β-carotene losses of 1.3% in *Amaranthus tricolor.* Imungi and Potter (1983) reported losses of about 12% of the protein, an apparent increase of about 13% of the iron and a loss of less than 1% of the zinc in cooked and drained cowpea leaves. With the exception of iron, the dietary intakes of these nutrients from the cooked *A. hypochondraicus* vegetable is therefore expected to be substantially lower than that expected from the levels in fresh vegetables.

The phenolic content including that of quercetin, are enhanced by use of manure, low levels of N as DAP and with advancing maturity of the vegetables at harvest (Chapter 5). Polyphenols, principally the group of flavanols consisting of kaempferol, quercetin and myricetin, have bee implicated in playing an important role in human health. Of special interest is their antioxidant activity and their wide range of pharmacologic properties (Wargovich, 2000). They have been associated with anticarcinogenic and antiarteriosclerotic properties and they have the ability to fight dental carries and diarrhoea (Hertog et al, 1992; Prior and Cao, 2000; Imungi, 2002). The polyphenols are also considered to have potential for the management of HIV/AIDS. For example, caffeic acid derivatives (e.g dicaffeoylquinic and dicaffeoyly-tartaric acids) have been shown to selectively inhibit Human Immunodeficiency Virus type1 (HIV-1) integrase (Robbins, 2003). In addition, *in vitro* studies have shown that quercetin prevents oxidation of low density lipoprotein (Kandaswami and Middleton, 1994), a process which is thought to be an intermediate step in the formation of ateriosclerotic plaques (Hollman, et al, 1997). It is possible that the preference for some of the traditional leafy vegetables shown by some communities arises not only from desire to enrich their diet, but also from their ability to prevent and cure diseases. It is possible to manipulate the contents of various nutrients in these vegetables during production by changing the time of maturity at which the vegetables are harvested and also by the use of various doses of N supplied by either manure or chemical fertilizer.

Though *A. hypochondriacus* is a valuable source of nutrients, the consumption of this vegetable may pose some health risk due to the accumulation of anti-nutritional factors such as nitrates and oxalates. A consequence of nitrogenous fertilization 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; Santamaria et al, 2001; Santamaria et al, 1999a). This accumulated foliar nitrate poses a health risk and is subject to regulation as it is known to interfere with the blood haemoglobin in the oxygen transport. If consumed, nitrate can have toxic effects by two main routes. First, in the saliva and the gastrointestinal tract, nitrate is reduced to nitrite which can then be re-oxidized to nitrate by oxyhemoglobin in the bloodstream with the resultant formation of methemoglobin (in which the central iron of the haem group is Fe^{3+}) (Kosaka et al, 1979). Unlike hemoglobin, methemoglobin has no ability to bind oxygen (Santamaria, 2006) so the capacity of the blood to deliver oxygen to the body tissues is impaired (Hill, 1999). This condition is referred to as methemoglobinemia and is more serious with infants than with grown children or adults. Second, nitrites react with secondary amino compounds commonly present in diets to form nitrosamines, which have been implicated in carcinogenesis (Hanssen and Marsden, 1987; Wogan, 1976). The nitrate content of vegetables is therefore a determinant of their nutritional quality and hence the recommended limits for the nitrate content of vegetables (Food Standards Agency, 2001) need to be strictly followed.

The field experiments in year 1 revealed that the highest levels of nitrates were found at 6 weeks after planting and ranged from 844 to 2530mg/100g on dry weight basis. These contents dropped to between 629 to 2012mg/100g dry weight by 8 weeks after planting. In year 2 the nitrate content was lower and ranged from 567 to1233 mg/100g dry weight at 6 weeks after planting. These values dropped to 255 to 758 mg/100g at 8weeks after planting (Chapter 3). Taking the highest amount of nitrates recorded in year 1 (2530mg/100g dry weight) when 60 kg N ha^{-1} was used in production of the vegetables and harvesting done 6 weeks after planting, it will mean that a person will consume 266mg/100g fresh weight, assuming that there will be a 30% loss due to leaching in the cooking water. Assuming an average consumption of 200g of vegetable per day, this translate to a consumption of 530mg of nitrates per day. This is far above the 220 mg maximum safe daily intake of nitrate recommended by WHO for adults of (WHO, 2000). Therefore the benefits of increased yield, protein and β-carotene contents of leafy vegetables arising from nitrogenous fertilization need to be balanced against the risk of excessive nitrate contents.

The total oxalate content of vegetable amaranths was found to range between 3700 - 6800 mg/100 g while that of soluble oxalate ranged between 2800 - 4200 mg/100 g dry weight, both for samples collected from the markets and those grown in experimental plots. For *A. hypochondriacus,* the oxalate contents decreased with maturity of the vegetables (Chapter 5) the highest levels were recorded in vegetables that were harvested 6 weeks after planting. Our oxalate levels are comparable to those reported by Schmidt et al, (1971) who reported a soluble oxalate content of 2900mg/100g dry weight in *Amaranthus cruentus* L., while Radek and Savage (2008) reported a content of 4700mg/100g dry weight in *Amaranthus viridis* and 4400mg/100g dry weight in *Amaranthus cruentus*. These levels are similar to those obtained by (Mziray, 1999) working with *Amaranthus hybridus* in Dar-es-Salaam, Tanzania., but are much lower than those reported by Kariuki, (1998) for *Amaranthus hypocondriacus*.

Oxalic acid is the major anti-nutritional factor found widely in plant-derived foods (Gupta et al, 2005). It occurs mainly as soluble sodium and potassium salts or as insoluble calcium oxalate and it is the soluble forms that are most damaging as they can precipitate Ca^{2+}. Variation in oxalate contents of plants can occur depending on season, species, variety, age and part of the plant, and soil conditions during growth (Gad et al, 1982). The highest levels are reported in spinach (Wills et al, 1981), but the levels reported in vegetable amaranths are also high (Dhan and Pal, 1991; Lathika et al, 1995). Levels ranging from 1.1% to 7.9% have been reported by Ricardo (1993). Oxalic acid is known to reduce absorption of dietary calcium by precipitating insoluble calcium oxalate. Its presence in large quantities can, therefore cause an apparent manifestation of calcium deficiency even when the diet is sufficient in calcium. This situation is more critical in infants and children who have active formation of bones and teeth, and to older persons who can suffer bone resorption leading to osteoporosis (Harper et al, 1979).

The levels of soluble oxalates found in the vegetables used in this study do not constitute a serious health hazard for reasonably healthy individuals if consumption of the vegetables does not exceed 200g of equivalent fresh leaf per day (FAO, 1988)**.** Further, it is expected that if the method of cooking involves discarding of cooking water (as occurs in the preparation of vegetable amaranths for consumption), the levels of free oxalates, and therefore the levels of total oxalates, in the vegetables will be substantially reduced.

Amaranthus hypochondriacus is an important source of ascorbic acid (vitamin C) and β-carotene (pro-vitamin A) and phenolics (including quercetin). Of these pro-vitamin A (β-carotene) is especially important as its deficiency results in the most common nutritional deficiency in the tropics and leads to blindness in thousands of children each year. The high content of quercetin is also significant as this is currently rated as one of the strongest antioxidants and helps to prevent chronic diseases such as diabetes, cancer and arteriosclerosis (Scalbert et al, 2005, Wargovich, 2000). Quercetin has also been considered as a tool for the management of HIV/AIDS. Nair et al (2009) have reported that quercetin significantly down-regulates HIV-1 p24 antigen production and viral infectivity in a dose dependent manner (5-50 μM) as compared toan HIV infected untreated control.

Amaranths have a high potential to contribute to the reduction in malnutrition, especially among people in rural areas where it constitutes an important part of the diet. In addition, they grow quickly, require little input and can be harvested within a short period of time (6-10 weeks after planting). This makes them useful in nutrition-intervention programmes (Okigbo, 1977). This is especially so when produced using DAP rates of not more than 40kg N ha^{-1}. With proper fertilizer management, it is possible to obtain both high yields and high quality *A. hypochondriacus.* In addition, maturity at harvest influences the vitamin and phytochemical contents.

Commercial production of vegetable amaranths

In order to assist farmers to grow these vegetables and access markets in East and Central Africa, a developmental organization Farm Concern International has been assisting small-scale farmers transform themselves from subsistence farmers into Business Support Units that can produce the vegetables on sustainable basis and ensure consistency in supply to the markets (Ngugi *et al,* 2006). Most small-scale farmers use family labour, have their own land and can use manure generated from the zero-grazing dairy units. These farmers therefore consider land, labour and manure as ‘free’ resources. Although these resources have opportunity costs, these are normally considered zero from the point of view of the farmers. Consequently the only variable cost that should be reflected in the gross margin calculations for amaranth production is that of the seed (Chapter 6). Sometimes the seed will be produced on the farm or donated by a neighbour or friend. In this case, the seed can also be placed in the same category of ‘free’ inputs by the farmer, and the opportunity cost becomes zero. These considerations vary the gross margins depending on what the farmers consider as ‘free’ inputs. Just like the farmers who produce vegetables using manure, small-scale farmers using fertiliser will consider the use of family labour and their own land as ‘free’ resources hence the opportunity cost of these resources is zero.

The prohibitive cost of chemical fertilizer means that it is very rarely used in the production of TLVs such as amaranth. Whenever it is used, it is usually applied in combination with manure, and the fertilizer is usually applied at levels that would be sub-optimal for fertilizer alone. From the yield data and gross margin analysis (Chapters 3 and 6 respectively), it can be concluded that application of the recommended rates of DAP fertilizer results in higher yields of the vegetables and therefore better returns to the farmers than from the use of manure. Most of the traditional leafy vegetable farmers have access to an average of 0.1ha (0.25acre) of land for production of the vegetables. If the gross incomes estimated for 1 hectare are scaled down to one-quarter acre, a farmer will make a gross income of Ksh 66,434 and Ksh 8,214 (US $ 900 and 120) when the vegetables are produced using DAP and manure respectively (Chapter 6). This means that farmers will obtain higher yields and hence gain higher returns by applying DAP fertilizer at the recommended rate of 40 kg N ha^{-1}. Some farmers will use sub-optimal chemical fertilizer levels in admixture with the manure. The yields and returns from these variants will be expected to fall between that of production using 40 kg N ha^{-1} DAP and 40 kg N ha^{-1}manure.

From this study, commercial production of amaranth vegetables (var. *A. hypochondriacus*) by small-scale farmers on land holdings as small as 0.4ha (1 acre) and using purely manure, fertilizer or mixtures of these is viable (Chapter 6). It is expected that the farmers will find the use of manure more attractive even though it gives lower yields than chemical fertilizer (Chapter 3). This is because manure is much cheaper than fertilizer and in most cases will be considered as a ‘free’ by-product of the farmer’s cattle rearing activities. In this latter case, the opportunity cost of the manure, like the costs of family labour and family land, will be perceived by the farmer as zero. In spite of the benefit of being free, however, the use of manure results in less income than that use of either artificial fertiliser or combinations of manure and artificial fertiliser. The gross margin calculations (Chapter 6) have been based on farmers’ perception of the costs of required inputs and income from production in order to produce results that are consistent with the farmers’ economic model. This will therefore, be more convincing to them, rather than the conventional calculations based upon standard economic principles which, unfortunately, often seem unrealistic to small-scale farmers. It is also possible to sensitize the farmers on the use of fertilizer to get more income and use the additional revenue for purchase of fertilizer.

Agriculture, in particular pro-poor agricultural growth, is back on the international development agenda (U.N., 2000). Use of traditional leafy vegetables such as amaranth to promote the role of agriculture in economic growth and poverty reduction in developing countries is possible. As the global economy becomes knowledge-based, the management and utilization of information will increasingly drive agricultural development. Knowledge on intensive technologies and services (pest management strategies, seed, fertilization strategies, value-added markets), will continue to increase in importance within the agricultural sector. All farmers, including small-scale farmers, will need both ingenuity and expertise to remain competitive.

In most poor countries, agriculture is a major employer and a source of national income and export earnings. Growth in agriculture tends to be pro-poor – it harnesses poor people’s key assets of land and labour, and creates a vibrant economy in rural areas where the majority of poor people live (OECD, 2006). Given its dominance in the economy, it will remain a primary source of growth and means of poverty reduction for some time. It remains the backbone of the rural economy, and employs the majority of the world's poor people. The proportion of poor people remains highest in sub-Saharan Africa, where slow economic growth has left millions at the margins of survival (OECD, 2006). Improved productivity of traditional crops such as amaranth through research will go a long way in improving their contribution to poverty reduction. For example a 10% increase in crop yields leads to a reduction of between 6 - 10% of people living on less than 1 USD a day (Irz et al, 2001).

Postharvest handling

Results of this study show that in terms of quantities of TLVs supplied to the markets, amaranths rank second after *Solanum nigrum* but they are the most preferred by the consumers so demand for them is, therefore, higher. However, high losses are incurred for vegetable amaranths and are due to wilting and rotting at the point of sale (Chapter 2).

One of the main constraints to increased production of vegetable amaranths is the absence of any appropriate postharvest management to maintain quality (Onyango and Imungi, 2007). In the absence of this, and because they are highly perishable (as are most leafy vegetables in these circumstances) their postharvest life is limited to 1-2 days, which forces farmers to sell the product in local markets soon after harvest (Chapter 2). This problem coupled with a poor road network limits the distance from the point of sale within which the vegetables can be grown. The distance from supply is very important in vegetable production and marketing because the longer the transport time the greater will be the degree of product deterioration due to wilting and possibly senescence, important factors influencing product deterioration per unit distance traveled.

Leafy vegetables have a relatively low storage potential in terms of visual quality, as well as other quality parameters such as microbial growth and nutritional quality. They are usually stored in polypropylene bags, and the recommended storage temperature is close to 0 °C (Favell, 1998). However, for vending in Kenya, vegetable amaranth is sometimes kept at higher temperatures (>20^{o}C) in display for sale. Compared to more massive vegetables, such root vegetables, increased storage temperatures pose even more risk for leafy vegetables which because of their very high surface area to volume ratios exhibit very high rates of transpiration, and they are metabolically active leading to high rates of respiration. Transpiration results in the product wilting and shriveling while respiration causes rapid heat buildup if the vegetables are tightly packed and results in depletion of nutrients (Kays and Paull, 2004). The high potential benefits from the growth and consumption of amaranth vegetables can not be realized simply by increased and improved production alone, but from their combination with proper storage to curb the rapid postharvest deterioration.

In this study, more moisture, ascorbic acid and β-carotene content were lost when *A.*

hypochondriacus was stored at ambient than at refrigerated temperatures (Chapter 4). At the end of 4 days in storage, the vegetables had lost close to 10% of the initial moisture content when held at 20^{o}C. Generally, a loss of 5 – 10 % moisture renders a wide range of products unmarketable (Kays and Paull, 2004). For leafy vegetables such as amaranth, not only wilting due to water loss but colour changes associated with senescence lower the consumer appeal of the product. The loss of water leads to loss of saleable weight implying a loss in monetary returns from the vegetables such as amaranth which are sold by weight. In addition, conditions favourable to water loss after harvest result in the loss of water soluble vitamins such as ascorbic acid especially in leafy vegetables (Lee and Kader, 2000) leading to a low quality product.

The study also demonstrated that there was a faster loss of ascorbic acid in younger leaves (6 and 7 weeks) than the older leaves (8 weeks) during storage, most probably because metabolic rates are usually higher in younger leaves than older ones; therefore, they loose antioxidants such as ascorbic acid more quickly. However, due to a high content of ascorbic acid found in the younger leaves before storage, refrigerated storage helped to preserve substantial amounts of ascorbic acid. This was not the case with leaves harvested 8 weeks after planting as refrigerated storage did not help preserve the low quantities of ascorbic contained in the leaves. The concentration of ascorbic acid declines fairly rapidly in many of the more perishable fruits and vegetables after harvest. Losses are greater with increasing storage temperature and duration (Kays and Paull, 2004; Lee and Kader, 2000; Yadav and Sehgal, 1995). Conditions favourable to water loss after harvest also result in a rapid loss of ascorbic acid, especially in leafy vegetables (Lee and Kader, 2000). Carotenoids have been found to be relatively stable in storage, but are still susceptible to changes due to senescence (Buescher et al, 1999) both of which are favoured by high temperatures such as the ones used in this study for ambient storage. For the phenols, low temperature reduced the rate at which they were lost in storage and in some cases even it led to slight increases in their content.

Use of DAP as a source of N in vegetable amaranth production results in high fresh leaf yield, high protein, β-carotene, nitrates and oxalate contents. The use of manure as an N source results in very low fresh leaf yields, reasonable amounts of protein and β-carotene contents, high levels of ascorbic acid and phenolics and very low levels of nitrates. For commercial production of vegetable amaranth the use of DAP, and not manure, combined with harvesting at 8 weeks maturity gives high returns on investment for the farmer. However, at this maturity the ascorbic acid content is very low which makes vegetable amaranth, which is otherwise a very rich source of ascorbic acid, a poor source of the vitamin. This could be overcome by harvesting more immature plants (e.g. 6 or 7 weeks after planting) and changing the cooking method from boiling followed by discarding the cooking water to steaming. These options, however, exacerbate problems due to nitrate and oxalic acid toxicity. Nitrate levels are higher in younger plants fertilized by DAP, and boiling the vegetables though it results in a major loss of ascorbic acid, also reduces the nitrate and soluble oxalate content of the vegetables and, especially for nitrate, reduces the toxicity risk. Alternative cooking methods which avoid the use of large quantities of water would result in less, or even no loss of nitrates and oxalates from the vegetables. In addition, the farmers cannot break-even given the low yields obtained at these stages of maturity. Hence there is a conflict of interest between amaranth becoming a source of income for poverty reduction and, a source of major nutrients such as ascorbic acid to solve the nutriton problems. In the short-term it is unlikely that the custom of cooking amaranth by boiling can be changed and given the catastrophic effect cooking has on ascorbic acid levels, it implies that despite being a potentially good source of ascorbic acid, the role of amaranth in this respect should be discounted. In the long-term, better cultivation practices or improved varieties of amaranth (i.e more ascorbic acid at later maturity stages, less accumulation of nitrates) combined with better cooking methods might improve the value of amaranth as an ascorbic acid source. As a β-carotene source vegetable amaranth can make a major contribution to people consuming it expecially when produced using manure and if new cooking methods are used in its preparation for consumption.

Further research on the response of vegetable amaranth to continuous use of manure as well as the different sources of manure is recommended as this is normally the case with small scale farmers in developing countries. A detailed study on the photosynthetic capacity of different varieties of vegetable amaranth under various field conditions is required to document their leaf yield potential if vegetable amaranth is to play a crucial role in food security among developing countries. Breeding programs to come up with varieties of amaranth that accumulate less nitrates will help in solving the nitrate toxicity problem in immature leaves. Traditional preservation methods such as boiling for limited periods of time followed by sundrying should be evaluated to determine the quality of the vegetables since these methods compared to refrigeration are more affordable to majority of the people living in developing countries.

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SUMMARY

Kenyan communities have in the past depended greatly on a large diversity of traditional green leafy vegetables as a source of nutrition and to give variety to the diet. These vegetables were, however, edged out of many diets, especially of the urban populations by the introduction of exotic substitutes such as cabbage and spinach. However, due to spirited campaigns on the superiority of traditional vegetables in terms of nutrition, health and taste, these vegetables have started to make a come-back into the diets of Kenyans of all social classes. Some of these vegetables are therefore currently being sold not only in open-air markets, where low socio-economic populations do their purchases, but even in formal supermarkets and green grocer stores in the urban centres where middle and upper socio-economic classes do their shopping. In these latter markets they are competitively sold alongside their exotic counterparts. This unfolding scenario, therefore, provides an opportunity to use traditional leafy vegetables to expand the local food base, improve health, and enhance individual and household food security and income.

The most commonly consumed traditional leafy vegetables (TLVs) in Kenya include the *Amaranthus spp., Vigna spp., Solanum spp., Cleome gynandra, Cucurbita spp.* and *Corchorus spp.* These vegetables are particularly rich sources of vitamin A, C, B_1 and folacin, crude protein, fibre and minerals such as iron, zinc, sodium, phosphorus, and calcium. These vegetables also contain other phytochemicals such as phenolic compounds including flavonoids) and glucosinolates, which possess strong antioxidant properties and have been implicated in the prevention of diseases such as cancer, arteriosclerosis, aging (Hertog et al, 1992) and currently the HIV/AIDS.

Traditionally, agricultural scientists and nutritionists have worked independently. The agriculturalists endeavored to produce adequate food in quality and quantity, while the nutritionists endeavored to ensure that the food was wholesome and contained the required nutrients in the right amounts for life sustenance. In order to bring the promise of enhanced health due to better diet to fruition, it has been recognized that these two sciences need to work together (FAO, 2004). This will provide a framework for agronomists, plant physiologists, breeders, genetic engineers, food chemists, food and agricultural engineers, and food marketing and distribution experts to work together to ensure that production from these crops reach the family table with the maximum possible nutritional and health values. Limited work has been done on the changes in nutritional quality of traditional green leafy vegetables during growth and postharvest handling. This study was designed to assess the quantity and quality of vegetable amaranth sold in major markets in Nairobi, Kenya. Subsequently, field trials of *Amaranthus* cultivar *Amaranthus hypochondriacus* were carried out to evaluate the yields and nutrient contents as affected by different levels of N, as supplied by the artificial fertilizer diammonium phosphate (DAP) and cattle manure, and the maturity at harvest. Further, the effect of ambient and low storage temperature (4^{o}C) on nutritional quality and shelf life was determined.

In Chapter 2 we set a baseline for the study by assessing the physico-chemical characteristics and

some nutritional values of vegetable amaranth sold in Nairobi, Kenya. Through a survey of major supermarkets and green grocers we identified the types of traditional leafy vegetables sold, the quantities sold, the proportion of the edible portion as sold, and their post harvest handling. In addition, problems encountered by both the producers and the traders were identified. The main TLVs found in the markets were the A*maranthus spp*, , *Cleome gynandra, Solanum nigrum, Cucurbita spp* and *Vigna unguiculata*. Of all the TLVs, only amaranths were sold in all the supermarkets and green grocers. All the vegetables were sold in bundles of an average weight of 0.45kg. Chemical analyses showed that vegetable amaranth had a moisture content of 85.5%, total ash 19.2%, crude protein 26.1% and the crude fiber 14.7%. The ascorbic acid content was 627mg/100g, zinc 5.5mg/100g and iron 18mg/100gdw. The nitrate content was 732.5mg/100g, total oxalates 5830mg/100g and soluble oxalates 3650mg/100g, while the lead content averaged 1.03mg/100g on dry weight basis.

In Chapter 3 we analyzed the quantity and quality of *Amaranthus hypochondriacus* as affected by different levels of N supplied by DAP and cattle manure. This was carried out under field conditions during the long rains season of 2007 and 2008, in the University of Nairobi, Kabete, Kenya. We also combined the fertilizer levels and stage of maturity at harvest to determine optimum leaf yields that contain high levels of Kjeldahl nitrogen (a measure of protein content) and acceptable levels of nitrates. The highest yield was recorded in plots receiving 40kg N ha^{-1} from DAP at 8 weeks after planting. Plots that were supplied with manure recorded the lowest yield when compared to the fertilizer treated plots at all rates. Leaf nitrogen content increased with increasing rate of N but only when N was supplied by DAP fertilizer. The leaf nitrogen content decreased with increasing age of the plants. The leaf nitrate content increased with increase in DAP application rate but decreased with maturity of the vegetables.

In Chapter 4 we compare the influence of soil nutrition and maturity of the vegetable on the visual and internal quality of amaranth. We also assessed the effect of storage temperature on these two qualities. The visual quality was assessed by dry matter/moisture content, while the internal quality was assessed by the ascorbic and β-carotene contents. The study showed that ascorbic acid content is susceptible to plant nutrition and maturity. The ascorbic acid content increased with reduced levels of N and with the use of manure as a source of N and attained a maximum at 7 weeks after planting. β-carotene increased with increasing soil nitrogen and with advanced maturity of the vegetable. Loss of both visual and internal quality during storage were influenced more by maturity at harvest and the temperature of storage than by soil nutrition.

In chapter 5 we examine the effects of source of nitrogen fertilization, stage of harvesting and storage on the total phenolics and oxalate contents of A*maranthus hypochondriacus*. Nutritionists have long considered dietary oxalates and phenolics as nutritional antagonists; the oxalates impairing absorption of calcium and some of the phenolics impairing absorption of proteins. However recently it has become clear that some classes of phenolics, among them quercetin, fulfill beneficial health functions in the body. Results showed that during growth, the phenolics decreased with increasing N levels when supplied by DAP. Use of manure promoted increase of total phenolic contents. The levels of total phenolics and quercetin increased while the oxalate contents decreased with age of plants during the study. The Phenolics increased in refrigerated storage, but the increase was not significant. The levels of oxalates did not change appreciably during refrigerated storage. There was a general decrease in phenolics content during storage at ambient temperatures. Storage of the fresh vegetables at 4^{o}C for limited periods led to insignificant change of both total phenolics and quercetin contents.

In chapter 6 we present an analysis of the feasibility of commercial production of vegetable amaranth (var. *Amaranthus hypochondriacus*) by small-scale farmers in Kenya. We use the vegetable yields obtained in chapter 3 to calculate gross margins based on a range of the possible choices; hired or family labour, rented or owned land, and purchased manure or that produced by cattle on the farm. A sensitivity analysis was performed on the possible effects of changes in fertilizer cost and the price of vegetables on the revenue generated by farmers. By using hired land and labour, the DAP treatment resulted in a gross margin that was about 9 times higher (KSh 483,273 against KSh 53,107) than when using manure. Also use of DAP in combination with own land and labour yields a gross margin that is 6 times higher than when using manure (i.e KSh 498,140 against KSh 81,780). The change in the gross income to the farmer was more sensitive to the change in the price of the vegetables than to the cost of fertilizer. These results show that small farmers can make money from commercial production of amaranth vegetables. This conclusion is based on the farmer considering the opportunity costs of his own land, family labour and manure from his own farm as zero. The results make it clear that at current market prices the revenue from growing *Amaranthus hypochondriacus* can be increased by using artificial fertilizer rather than manure.

In chapter 7 we present a synthesis of the most important findings of the study. The study shows that amaranth yields can be increased by use of chemical fertilizers. The study also shows that *Amaranthus hypochondriacus* is an important source of protein, ascorbic acid (vitamins C) and β-carotene (pro-vitamin A) and both total phenolics and quercetin contents. Important to note is pro vitamin A (β-carotene), the lack of which results in a most serious nutritional deficiency in the tropics and leads to blindness in thousands of children each year. Consequently, amaranths have a high potential to contribute to the reduction in malnutrition, especially among people in rural areas where it constitutes an important part of the diet. An addition advantage is that amaranths grow quickly, require little input and can be harvested within a short period of time (6-10 weeks after planting). However, increases in leaf yields that come with use of high levels of N fertilizers comes with a penalty in terms of poor quality due to accumulation of the antinutrients nitrates and oxalates. Therefore, the benefits of increased yield and increased protein content of leafy vegetables arising from nitrogenous fertilization need to be balanced against the risk of excessive nitrate contents. With proper fertilizer management, it is possible to obtain both high yields and high quality *A. hypochondriacus.* In addition, maturity at harvest influences the nutrients and anti-nutrients content of *A. hypochondriacus.*

This study shows that commercial production of amaranth vegetables (*A. hypochondriacus*) by small-scale farmers on land holdings as small as one acre (0.4ha) and using purely manure, fertilizer or mixtures of these is viable. The farmers will find the use of manure more attractive even though it gives lower yields than chemical fertilizer because manure is cheaper than fertilizer and in most cases will be considered as a 'free' product of the farmer's shed. In this latter case, the opportunity cost of the manure, like the costs of family labour and family land, will be perceived by the farmer as zero. In spite of the benefit of being free, however, the use of manure results in less income than the use of either artificial fertiliser or combinations of manure and artificial fertiliser. The gross margin calculations have been based on farmers' perception of the costs of required inputs and income from production in order to produce results that are consistent with the farmers' economic model. This will therefore, be more convincing to them, rather than the conventional calculations based upon standard economic principles which, however, often seem unrealistic to small-scale farmers.

To minimise the losses incurred during post harvest of vegetable amaranth, use of low temperatures (4^oC) is required. This helps to preserve both the physical appearance as well as the nutritional quality for up to 4 days after harvest.

Further research on the response of vegetable amaranth to continuous use of manure is recommended. The effect of different sources of manure on yield and nutrient contents of vegetable amaranth will be very useful given that most small scale farmers in developing countries have access to diversified sources and quality of manure. Detailed study of the photosynthetic capacity of different varieties of vegetable amaranth under various field conditions is also required. Traditional and cheaper preservation methods such as sun-drying and fermentation should be evaluated and modified to optimize nutrient and quality retention as these methods will be more appealing and affordable to majority of the people living in developing countries as compared to refrigeration.

SAMENVATTING

Een groot deel van de bevolking in Kenia was in het verleden grotendeels afhankelijk van de grote diversiteit aan traditionele groene bladgroenten als bron van voedingsstoffen en voor het bieden van variëteit in de dagelijkse maaltijd. Deze groenten zijn echter grotendeels verdwenen uit de maaltijden, vooral in stedelijke gebieden. Uitheemse groenten hebben hun plaats ingenomen. Momenteel keren deze traditionele groenten weer terug in de maaltijden van Kenianen in alle sociale klassen van de bevolking. Dit dankzij campagnes die de voordelen van traditionele groenten met betrekking tot voedingswaarde, gezondheid en smaak benadrukken. Een aantal van deze groenten wordt tegenwoordig niet alleen verkocht op de markt, waar mensen uit lagere socio-economische klassen hun boodschappen doen, maar ook in supermarkten en groentezaken in het centra van de stad, waar de middel en hogere socio-economische klassen hun boodschappen halen. In deze laatst genoemde zaken worden zowel de traditionele groenten als hun uitheemse tegenhangers, zoals spinazie en kool, verkocht. Dit biedt de mogelijkheid om het gebruik van traditionele bladgroenten te stimuleren. Het biedt mogelijkheden om de diversiteit aan groenten te vergroten, de gezondheid te verbeteren en de voedselzekerheid en inkomen van individuen en huishoudens te vergroten.

De meest gegeten traditionele blad groenten (TBG's) in Kenia zijn *Amaranthus spp., Vigna spp., Solanum spp., Cleome gynandra, Cucurbita spp.* and *Corchorus spp.* Deze groenten zijn vooral rijk aan vitamine A, C, B_1, folacine, ruwe eiwitten, vezels en de mineralen ijzer, zink, natrium, fosfor en calcium. Ze bevatten ook fytochemicalieën zoals fenolen, flavenoiden en glucosinolaten. Deze stoffen hebben sterk anti-oxiderende eigenschappen en spelen een rol bij het voorkomen van ziekten als kanker en arteriosclerosis, veroudering (Hertog et al., 1992) en momenteel ook HIV/AIDS.

Agronomen en voedingsdeskundigen werkten tot voor kort onafhankelijk van elkaar. Agronomen streefden ernaar om genoeg voedsel van de juiste kwaliteit te produceren. Voedingsdeskundigen streefden naar voedzaam eten, wat de juiste nutriënten bevatte voor een goede gezondheid. Echter, om de fytochemicalieën ook daadwerkelijk te laten bijdragen aan een beter gezondheid, moeten agronomen en voedingsdeskundigen gaan samenwerken (FAO, 2004). Dit zal er toe moeten leiden dat agronomen, plantfysiologen, veredelaars, voedingschemici, levensmiddelentechnologen, marketing- en logistieke deskundigen eendrachtig samenwerken om de traditionele gewassen met zo goed mogelijke voedingskundige- en gezondheidseigenschappen bij de consument te brengen. Er is slechts zeer beperkt aandacht besteed aan de veranderingen in voedingskundige eigenschappen van de TBG's, die optreden bij de groei en de naoogst fase. In deze studie is eerst de kwantiteit en kwaliteit van de groente amaranth (*Amaranthus hypochondriacus*) in de voornaamste verkoopplaatsen van Nairobi (Kenia) bepaald. Daarna zijn er proeven gedaan om na te gaan wat de opbrengst en het gehalte aan voedingsstoffen van amaranth cultivar *Amaranthus hypochondriacus* waren bij verschillende hoeveelheden stikstofgift. Deze stikstofgiften werden gedaan als de chemische meststof diammonium fosfaat (DAF) en als koemest. Verder is het effect van bewaren bij omgevingstemperatuur en een lage temperatuur (4°C) op de

voedingswaarde en de houdbaarheid van het product bestudeerd.

In hoofdstuk 2 is de basis van het onderzoek gelegd met het inventariseren van de fysisch-chemische eigenschappen en een aantal voedingseigenschappen van de groente amaranth in Nairobi, Kenia. We hebben bij de belangrijkste supermarkten en groentezaken een inventarisatie gedaan naar de soorten traditionele bladgroenten die werden verkocht, in welke hoeveelheden, welk gedeelte van het verkochte product gegeten kon worden, en hoe er na de oogst mee werd omgegaan. Daarnaast werden knelpunten bij zowel de boeren als de verkopende organisaties geïdentificeerd. De belangrijkste TBG's die op de markt werden verkocht waren A*maranthus spp, Cleome gynandra, Solanum nigrum, cucurbita spp* en *Vigna unguiculata*. Van de TBG's werd alleen amaranth ook in supermarkten en bij groentezaken verkocht. Alle groenten werden in porties van 0.45kg verkocht. Chemische analyses lieten zien dat de groente amaranth had een vochtgehalte van 85.5%, het totale asgehalte was 19.2%, de ruwe eiwitten maakten 26.1% van het gewicht uit en ruwe voedingsvezels 14.7%. Het gehalte aan ascorbine zuur was 627g/100g, het zinkgehalte was 5.5 mg/100g en het ijzergehalte 18 mg/100g. Het nitraatgehalte was 732.5mg/100g, het totale oxalatengehalte was 5830 mg/100g en het gehalte oplosbare oxalaten was 3650 mg/100g. Het loodgehalte was gemiddeld 1.03 g/100g (alle voorgenoemde gehaltes zijn uitgedrukt ten opzicht van het drooggewicht). Uit het berekenen van de bruto inkomen werd duidelijk dat de groente amaranth een belangrijke rol kan spelen in het genereren van inkomen, met name bij kleine vrouwelijke boeren. In voedingskundig opzicht kan het significant bijdragen aan de inname van micronutriënten, met name eiwitten, ijzer en zink.

In hoofdstuk 3 worden productie en kwaliteit van *Amaranthus hypochondriacus* bekeken, zoals die beïnvloed wordt door verschillende stikstofgiften, toegediend als DAF en als koemest. De proeven werden uitgevoerd tijdens het lange regenseizoen van 2007 en 2008 op de universiteit van Nairobi, Kabete, Kenia. De uitkomsten bij combinaties van verschillende bemestingsniveaus en rijpheid bij oogst zijn vergeleken om tot een optimale bladoogst te komen, die hoge concentraties Kjeldahl stikstof bevat (een maat voor het eiwitgehalte) en toelaatbare nitraatgehaltes. De hoogste opbrengst werd behaald in velden die bemest werden met 40kg N ha^{-1}, gegeven als DAF, en 8 weken na het planten werden geoogst. Op alle bemestingsniveaus behaalden de velden die met koemest waren bemest een lagere opbrengst dan de velden die met DAF waren bemest. Het gehalte aan stikstof in het blad nam toe met toenemende stikstofbemesting, maar alleen als de stikstof als DAF was gegeven. Het gehalte aan stikstof in het blad nam af naarmate de planten ouder waren. Het gehalte aan nitraat in het blad nam toe met toenemende DAF bemesting, maar werd minder naarmate de groente rijper was.

In hoofdstuk 4 vergelijken we de invloed van bodembemesting en rijpheid van het gewas op de visuele en interne kwaliteit van amaranth. Ook het effect van bewaartemperatuur op deze twee kwaliteitsaspecten is bekeken. Onder visuele kwaliteit werd verstaan het droge stof gehalte/vochtgehalte. De interne kwaliteit werd bekeken aan de hand van het gehalte ascorbinezuur en β-caroteen. Het ascorbinezuur gehalte liet een optimum zien in de veldproeven. Het ascorbinezuur gehalte nam toe als de stikstofgift afnam en als de stikstof als koemest werd gegeven. Het ascorbinezuur gehalte was maximaal bij 7 weken na het planten. β-caroteengehaltes namen toe met het stikstofgehalte in de bodem en met de rijpheid van het gewas. Afname van visuele en interne kwaliteit tijdens het bewaren werd voornamelijk bepaald door de rijpheid bij oogst en de bewaartemperatuur, en minder door de bodembemesting.

In hoofdstuk 5 bekijken we het effect van de vorm van stikstofbemesting, de rijpheid bij oogst en bewaring op het totale gehalte van fenolen en oxalaten in *Amaranthus hypochondriacus*. Voedingsdeskundigen hebben oxalaten en fenolen lange tijd beschouwd als antagonisten. De oxalaten houden de opname van calcium tegen en bepaalde fenolen remmen de opname van eiwitten. Echter, recentelijk is aan het licht gekomen dat sommige typen fenolen, waaronder quercetine, gunstige gezondheidseigenschappen hebben in het lichaam. De resultaten lieten zien dat de fenolen gedurende de groei afnamen met toenemende N-niveaus toegediend als DAF. Koemest bevorderde de toename van het totale fenolengehalte. De totale fenolen- en quercetinegehalten namen toe, terwijl het oxalaatgehalte afnam met de plantleeftijd gedurende deze proef. De fenolen namen toe in gekoelde bewaring, maar de toename was niet significant. Ook de oxalaat- en quercetinegehalten namen niet noemenswaardig toe gedurende gekoelde bewaring. Er was een algemene trend van afname in het fenolengehalte gedurende bewaring bij kamertemperatuur.

Hoofdstuk 6 bevat een analyse van de haalbaarheid van commerciële amarant (var. *Amaranthus hypochondriacus*) groenteteelt door kleinschalig producerende boeren in Kenia. De groenteoogsten beschreven in hoofdstuk 3 zijn gebruikt om het bruto inkomen te berekenen, gebaseerd op een reeks van mogelijke keuzes: Arbeid door familie of personeel, land in pacht of in eigendom en gekochte koemest of koemest geproduceerd door vee op eigen boerderij. Een gevoeligheidsanalyse is gebruikt voor de mogelijke effecten van veranderingen in de kunstmest kostprijs en de groenteprijs op de door de boeren behaalde inkomen. Bij gepacht land en arbeid door personeel resulteerde de DAF-behandeling in een bruto inkomen dat ongeveer 9 keer hoger was dan het inkomen bij gebruik van koemest (KSh 483.273 tegenover KSh 53.107). Ook het gebruik van DAF in combinatie met land in eigendom en arbeid door familie resulteerde in een bruto inkomen dat 6 keer hoger was dan bij gebruik van koemest (KSh 498.140 tegenover KSh 81.780). De verandering in het bruto inkomen van de boeren was gevoeliger voor de groenteprijs dan voor de kostprijs van de kunstmest. De resultaten tonen aan dat kleinschalig producerende boeren geld kunnen verdienen met commerciële amarant groenteteelt. Deze conclusie is gebaseerd op de aanname dat de boer opportuniteitskosten van zijn eigen land, arbeid door zijn familie en koemest geproduceerd door zijn eigen vee nihil acht. De resultaten laten duidelijk zien dat bij de huidige marktprijzen de inkomsten door het telen van *Amaranthus hypochondriacus* kunnen worden vergroot door kunstmest te verkiezen boven koemest.

In hoofdstuk 7 wordt een samenvoeging van de meest belangrijke bevindingen van dit onderzoek gepresenteerd. Het onderzoek toont aan dat de opbrengst van amarant kan worden vergroot door chemische meststoffen te gebruiken. Het onderzoek toont tevens aan dat *Amaranthus hypochondriacus* een belangrijke bron is voor eiwitten, vitamine C en β-caroteen (pro-vitamine A) en voor zowel totale fenolen- als quercetinegehalten. Het is van belang om op te merken dat pro-vitamine A (β-caroteen)-gebrek veel voorkomt in de tropen. Het gebrek resulteert in zeer ernstige ondervoeding en leidt ieder jaar tot blindheid bij duizenden kinderen. Aldus biedt amarant belangrijke mogelijkheden om bij te kunnen dragen aan de afname van ondervoeding, vooral onder mensen in rurale gebieden, waar het een belangrijk bestanddeel van het dieet vormt. Een bijkomend voordeel is dat amarant snel groeit, weinig input vergt en al na een korte groeiperiode geoogst kan worden (6-10 weken na planten). Echter, de toename in bladoogst door het gebruik van hoge doseringen N houdende kunstmest brengt het nadeel met zich mee dat de kwaliteit slecht is vanwege ophoping van de anti-nutriënten nitraat en oxalaat. Daarom moeten de voordelen van een toegenomen oogst en eiwitgehalte van bladgroenten door stikstofbemesting afgewogen worden tegen het risico van een excessief nitraatgehalte Door een goede bemestingsstrategie is het mogelijk om zowel een grote oogst als een hoge kwaliteit van *A. hypochondriacus* te verkrijgen. Bovendien beïnvloedt het ontwikkelingsstadium van de planten tijdens de oogst het nutriënten- en anti-nutriëntengehalte van *A. hypochondriacus.*

Dit onderzoek toont aan dat commerciële amarant groenteteelt (*A. hypochondriacus*) door kleinschalig producerende boeren op percelen niet groter dan 0.4 Ha en gebruikmakend van alleen koemest, alleen kunstmest of een combinatie hiervan levensvatbaar is. De boeren zullen het gebruik van koemest een aantrekkelijkere optie vinden ondanks de lagere oogst vergeleken met kunstmest, omdat koemest goedkoper is dan kunstmest en meestal beschouwd zal worden als “gratis” product uit de stal. In dit laatste geval worden de opportuniteitskosten van koemest, evenals de kosten van arbeid door familie en land in eigendom, door de boer als nihil beschouwd. Ondanks het voordeel gratis te zijn, echter, resulteert het gebruik van koemest in minder inkomen dan het gebruik van óf kunstmest óf combinaties van koemest en kunstmest. De bruto inkomensberekeningen zijn gebaseerd op de perceptie van de boer op de kosten van de vereiste inputs en inkomen uit productie, om resultaten te verkrijgen die consistent zijn met het economische model van de boer. Deze aanpak zal daarom overtuigender zijn voor hen dan de conventionele berekeningen gebaseerd op algemene economische principes, die vaak onrealistisch lijken in de ogen van kleinschalig producerende boeren. Om de naoogst verliezen bij amarantgroente te minimaliseren is een lage bewaartemperatuur vereist (4^oC). Dit helpt om zowel de visuele kwaliteit als de voedingswaarde te behouden tot aan 4 dagen na de oogst.

Verder onderzoek naar de reactie van amarantgroente op het continue gebruik van mest is aan te bevelen. Het effect van verschillende mestbronnen op oogst en voedingswaarde van amarantgroente is een goed bruikbaar gegeven omdat de meeste kleinschalig producerende boeren in ontwikkelingslanden toegang hebben tot diverse soorten en kwaliteiten mest. Detailonderzoek naar de fotosynthesecapaciteit van de verschillende variëteiten amarantgroente onder verschillende groeicondities is tevens aan te bevelen. Traditionele en goedkope bewaarmethodes zoals zondrogen en fermentatie zullen geëvalueerd moeten worden en aangepast om het behoud van voedingsstoffen en kwaliteit te optimaliseren, omdat deze methoden voor de meerderheid van de mensen in ontwikkelingslanden aantrekkelijker en beter betaalbaar zijn dan koeling.

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Cecilia

CURRICULUM VITAE

Cecilia Moraa Onyango was born on 15th May, 1973 in Kisii, Kenya. After completing her basic education in Nyansakia II primary and Nyabururu Girls' high school, she enrolled at the University of Nairobi and received an honors degree in B.Sc. Agriculture in 1996. From October 1997 to November 2000 she followed a postgraduate program in Horticulture at the Faculty of Agriculture, in the University of Nairobi. In November, 2001 she joined the Department of Plant Science and Crop Production in the Faculty of Agriculture of the University of Nairobi as a Tutorial Fellow. In February, 2006 she was awarded a grant from the Netherlands Fellowship Programme (Nuffic) to study for her PhD degree on a sandwich program between the Horticultural Supply Chains Group of Wageningen University of Research, The Netherlands and Plant Science and Crop Protection Department of the University of Nairobi, Kenya. This thesis is the outcome of the study.

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