THE EFFECT OF NITROGEN AND PHOSPHOROUS APPLICATION ON GROWTH, YIELD AND NUTRITIONAL QUALITY OF VEGETABLE AMARANTH (AMARANTHUS HYBRIDUS)

BY

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DECLARATION:

I declare that this thesis is my original work and it has not been presented for a degree in any other university.

.....Aluga..... 18TH FEB 2004

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This thesis has been submitted with our approval as the university supervisors.

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This work of thesis is dedicated to my family members; Paul Godfrey Meyo. Noah Hezron Otieno, Ram Justus Ayal, Florence Magdalene Anyango, Joannes Bildad Nyitong and Loise Odindo.

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ABBREVIATIONS

ANOVA	-	Analysis of variance
A.O.A.C.	-	Analytical methods of analysis association of official analytical chemistry
Ca	-	Calcium
CaCl2	-	Calcium chloride
CAN	-	Calcium ammonium nitrate
CEC	-	Cation exchange capacity
CF	-	Crude fibre
CP	-	Crude protein
Fe	-	Iron
Hcl	-	Hydrochloric acid
HPO4-2	-	Diphosphate
H2SO4	-	Sulphuric acid
K	-	Potassium
KARI	-	Kenya Agricultural Research Institute
KMN04	-	Potassium permanganate
N	-	Nitrogen
NaOH	-	Sodium hydroxide
NH+	-	Ammonium ion
NH4OH	-	Ammonium hydroxide
N.R.C.	-	National Research Centre
P	-	Phosphorous
UNESCO	-	United Nations Environmental Soil Conservation
Wk	-	Week

ABSTRACT

Two field studies and laboratory experiments were carried out at Kabete Campus, University of Nairobi, between November 1994 and October 1995. The aim of the study was to find out the effect of nitrogen and phosphorous applications on growth, leaf yield and nutritional quality of vegetable amaranth. There were four levels of each treatments and it was conducted on a split plot design with three replications.

Growth parameters evaluated included plant height, number of leaves, leaf area index and stem diameter. Fresh weight and dry weight were taken to determine yields. Nutritional quality of leaves was evaluated by analysing for crude fibre, crude protein, dry matter, beta carotene, calcium, iron, phosphorous as well as oxalates and nitrate contents.

It was observed that applying seventy five kilogrammes of nitrogen in one hectare gave a maximum height of thirty three centimetres. A height of thirty two point six centimetres was achieved with the application of sixty kilogrammes of phosphorous n one hectare.

Nitrogen application gave a significant effect on stem diameter. Phosphorous application gave a significant effect on leaf area index.

Both fresh weights and dry weight were significantly increased with increased application of nitrogen and phosphorous.

The nutritional quality of leaves were modified by the application of nitrogen and phosphorous. Crude protein and beta carotene contents increased with the application of nitrogen. The mineral contents of calcium, iron and phosphorous tended to increase with the application of nitrogen and phosphorous. However, the levels of crude fibre and oxalates were significantly reduced with the application of nitrogen and phosphorous.

1.0 INTRODUCTION

Amaranth is grown either for leafy vegetable or grain (Sauer,1977; Crubben and van Sloten, 1981). The cultivated species include *Amaranthus tricolor, A. dubius, A. hybridus A. flavus, A. cruentus,* and A. *hypochondriacus* (Kauffman, 1981). Among these, *A. hybridus,* is one of the species that is grown as leafy vegetable. It is superior in succulence and leafiness to *A. dubius, A. hypochodriacus and* A. *flavus,* and can remain in vegetative phase for a relatively long period close to six months (Norman and Sichone, 1903). Some species, like A. *hypochondriacus,* are prolific in flowering and are grown for their grains. There are some species with brilliantly coloured stems, leaves and flowers which are good ornamental crops. One such species is *A. caudatus* which has blazing-red-inflorescence.

Amaranth are popular leafy vegetables in the tropics (Tindall, 1986; Grubben, 1976; Norman and Sichone,,1993). The tender shoots and leaves constitute the edible portion. These are cooked either alone or in mixtures with other leafy vegetables (*Cleone gynandra or Solanum nigrum*) or mixed with other ingredients for making soup (F.A.0. 1990). The vegetable provides a number of nutrients including protein, vitamins A and C and minerals like calcium and iron. The vegetative part also provides limited supplies of amino acids in diets such as lysine and methionine. The plant is capable of producing upto 1.6 g of protein per square meter in a day (Messiaen, 1992). Amaranth greens have nutritional quality similar to other leafy greens such as Swisschard and, in addition, their dry matter, crude protein, iron and calcium p contents are often higher. For that matter, amaranths are good vegetable supplements for the diets of both rural and urban communities. Grubben (1976) noted that amaranth leaves have high β-carotene and if infants and children are fed on 50 to 100 g of the vegetable, the problem of blindness that is common in many tropical areas can be alleviated.

Amaranth utilizes the C4 photosynthetic pathway (Laetsch, 1968; Lawlor 1990), making it an efficient crop plant for the hot tropical conditions. The grain yield potential is estimated at 3 to 5 tonnes per hectare (Mwakia and Nyaga, 1975). In Nigeria, where *A. tricolor* is most popular, leaf yield of about 20t/ha was obtained in 25 to 38 days after germination (Deutsch, 1977). As a leafy vegetable, amaranth grows fast and has a short growing period. It can be harvested several times during the wet season, depending on the harvesting method (Mnzava and Massam, 1985; Chweya, 1993). Most species may be harvested 30 to 50 days from sowing when they are 15 to 20 cm high (Tindall, 1985). Either the whole plant may be uprooted or established plant cut back to 15 cm of the base to encourage lateral growths. Harvesting entire plant can give yields of 20 to 25 t/ha while 30 to 60 t/ha may be realised when shoots are cut back and followed by subsequent harvests (Tindall, 1986). The bundles of shoots sold in the local markets are sometimes collected from the abandoned farms, bushes or from plants left to grow as intercrops.

Amaranth grows well in many agroecological zones of Kenya because of its wide ecological adaptability (Kokwaro, 1976; Michieka, 1987). However, the plant requires fertile soils with adequate supply of nitrogen, phosphorus and calcium (Gudu, 1985) for optimum growth and development. Amaranth is popularly used as leafy vegetable in many parts of Kenya; and it is one of the indigenous vegetables already identified as having appreciable agricultural potential (Chweya, 1985), which is in line with the Sessional paper No. 4 of 1980 on National Food Policy, that advocates for self sufficiency in food production for all Kenyans.

Leafy vegetables play an important role in providing household food security and they form a good portion of major daily meals. Sydenham (1985) pointed out that a regular cultivation of African and Indian spinach (Amaranth) and Chinese cabbage in all vegetable plots would ensure a constant supply of green vegetables to the grower. Among the vegetables of the tropics, amaranths are, easy to grow, and under favourable

conditions they can reseed themselves and continue to produce a useful crop of edible shoots or leaves weekly for upto 6 months.

Currently in Kenya, the crop is not receiving sufficient a agricultural attention to enable realization of its leaf yields and nutrient potentials. There is very little information on its agronomy, especially on fertilizer requirements that can give high yields of leaves of good quality. The demand for this vegetable is growing as the tender shoots are commonly mixed with other vegetables to soften and impart 'nice' taste to them, thereby making delicious dishes.

The study carried out, therefore, had the following two objectives:-

(i) To determine the effect of N and P fertilizer applications on plant growth, leaf yield and
quality of vegetable amaranth.

(ii) To determine the effect of age of the plants on oxalate and nitrate contents of amaranth
leaves.

2.0: LITERATURE REVIEW

2.1: Botany

The genus *Amaranthus* belong to *Amaranthacea* family and it is related to *Celosia.* About 60 species of amaranthus are currently widely distributed throughout the world (Kauffman, 1980). These species have variable chromosome numbers, with *A. caudatus and A. cruentus* having 2n=32 whereas *A. tricolor and A. hybridus* have 2n=34 (Grubben and Sloten, 1981). Amaranth is a dual purpose crop, being grown both for its leaves and grain.

Grain amaranths are native to Guatemala and Mexico (NRC., 1984). *A. hypochondriacus,* and *A. cruentus* were cultivated in valleys of Mexico, Central and South America. The vegetable types *(A. tricolor* and *A. dubius)* have been grown in tropical regions of India, China and South East Asia for over 2,000 years.

The rooting system of vegetable types is well developed whereas the species and varieties cultivated for grain lack the strong taproot (NRC, 1984). The growth habits of amaranth plant vary from branched to unbranched, with grain types having a dominant seed head and fewer side branches (Grubben and Slotten, 1981). The colour of leaves and stems vary from red to green.

The plant head consists of large inflorescence made up of florets, each consisting of 3 to 6 flowers with one staminate flower surrounded by several pistillates (Kauffman, 1980). The flowers are very small, occurring in clusters, they mature to give tiny seeds which are either black (vegetable types) or light (grain types) in colour (NRC, 1984)

Amaranthus hybridus is one of the most common vegetable types. It originated in tropical America and is now widespread in other tropical regions (NRC, 1984). It can grow to a height of 1.5 m. It is a short-lived annual herb that bears simple alternate leaves with long petioles on a thick succulent erect stem. Leaf blades are broad and triangular in shape with obtuse tips.

2.2: Ecological Requirements:

Adequate soil moisture enables amaranth seeds to germinate and seedlings to establish good root system (NRC, 1984). After germination grain amaranths continue to grow well under warm conditions with little water supply whereas the vegetable types require adequate moisture throughout the growing season. Having specialised C4 photosynthetic pathway, amaranths perform well, in terms of converting raw materials of nutrients, water and sunlight into plant tissues, under unfavorable environmental conditions (Kauffman, 1980, 1981) such as high temperatures (16-35°C), high light intensity and dry soil conditions. It is noted that amaranth plants can grow in soils of varying nutrient levels (NRC, 1984, Tindall, 1986). However, vegetable types grow better in soils that have high fertility with adequate supply of potassium and nitrogen.

2.3: Nitrogen and Amaranths

Nitrogen is an essential plant nutrient that promotes vegetative growth (Matoh *et al.,* 1986) and forms integral component of plant proteins and pigments such as chlorophyll (Taeser, 1986). Plants take up N ions in the soil solution (Tisdale et al, 1986). The most available form is NO-2 (Teser, 1986). The nitrate form is reduced to the nitrite form (NO-2) which is rapidly converted into NH+4 and is incorporated into amino acids which are translated into proteins (Salisbury and Ross, 1991; Lawlor, 1990). The form in which nitrogen is translocated depends on the source, uptake and metabolism (Mengel and.

Kirkby, 1979). Nearly all the NH+4 forms are assimilated in the root tissue and translocated as amino acids whereas the N0-2 from the N0-3 are further reduced to amide (Lawlor, 1990) from which branching of metabolic networks occurs leading to formation of amino acids.

2.3.1 Effects on plant growth and yield

A vigorous growing plant like amaranth requires adequate supply of nutrients for its normal growth and development (Hewilt and Smith, 1975), that can result in good leaf yield and quality, and normally leafy vegetables respond well in terms of leaf yields to supply of nutrients that promote vegetative growth. Matoh *et al.* (1986), working in Japan found that the leaf number and stem diameter of amaranths were not affected by N application, but plant height, leaf area and fresh weight increased with increase in N application. Adequate supply of N tends to improve growth and development of leafy vegetables. In kale plants, application of N increased the mean fresh weight and therefore, total leaf yields (Chweya, 1984). Leaf production of kale and collards increased with N supply (Kanampiu, 1987). Fritz and Habben (1973) reported that fresh weight of lettuce improved with increased N application. Sorensen (1984) observed that applying 600 kg N/ha had a significant effect on growth and development of cabbage heads and yields increased from 13.2 to 70.8 t/ha. Knight and Mitchell (1983) noted that supplying N in the form of ammonium nitrate gave good yields in lettuce.

Leaf yields l of vegetable-amaranths are influenced by a number of factors (Tindall, 1985) and fertilizer application is one of the major factors that has a large effect on the yields (NRC, 1984). With adequate nutrients supply, regrowths can provide several harvests in a year resulting in higher total yields (Grubeen, 1977; Mnzava and Masam 1985; Tindall, 1985). Adipala and Mugerwa (1993), working in Uganda; reported

significant increases in leaf yield (about 10%) *in Amaranthus hybridus* subspecies *hybridus* with N and P application.

Nitrogen induces leaf production and expansion in plants (Hewitt and Smith, 1975), and leaf size is determined by cell expansion which is also influenced by nutrient supply. Therefore, under insufficient supply of N these plants are stunted with fewer leaves produced. Hewitt and Smith (1975) noted that chloroplast production is reduced in cauliflowers (*Brassica oleracea* var *botrytis* L.) when nitrogen is deficient, resulting in general chlorosis of plant leaves and premature cell vacuolation and early senescence occur.

Most soils are commonly deficient in nitrogen than any other element, partly because soil parent materials usually contain little or no nitrogen and because this element is easily lost by leaching of nitrate ions or by conversion to volatile Nitrogen gas by microorganisms.

2.3.2: Effect on leaf quality

In leafy vegetables, abundant supply of dark green leaves that are succulent, crispy and savoyed are more preferred (Fritz and Habben, 1973). Adequate supply of N is associated with dark green colour (Tisdale et al. 1985). Nitrogen supply also influences carbohydrate utilization in that when N is limiting carbohydrates are deposited in the vegetative, cells making them thick and less succulent (Hornicks, 1992). Protein:carbohydrate ratio in leaves is influenced by levels of N (Mingel, 1979) such that at lower levels, there is more carbohydrates synthesis than protein, resulting in low crude protein content (Fritz and Habben, 1973). As the amount of N in the growth medium is improved, total N and crude protein contents in the leaves are increased but crude fibre (cellulose, hemicellulose, lignin and pentosans) content is reduced (Mengel, 1979).

Table I: Nutrient of some selected raw vegetable leaves (100g edible portion)

Component	Amaranth (*Amaranthus spp.*)	Spinach *(Spinacia oleracea)*	Basella *(Basella alba)*	Swisschard *(Beta vulgaris)*
Dry matter (g)	13.4	9.3	6.9	8.9
Food energy (Cal)	36.0	26.0	19.0	25.0
Protein (g)	3.5	2.2	1.8	2.4
Fat (g)	0.5	0.3	0.3	0.3
Carbohydrate				
Total (g)	6.5	4.3	3.4	4.6
Fibre (g)	1.3	0.6	0.7	0
Ash (g)	2.6	1.5	1.4	1.6
Calcium (mg)	126.0	93.0	109.0	88.0
Phosphorous (mg)	67.0	51.0	52.0	39.0
Iron (mg)	3.9	3.1	1.2	3.2
Potassium (mg)	411.0	470.0	-	550.0
Vitamin A (iu)	6100.0	8100.0	8000.0	6500.0
Thiamin (mg)	0.08	0.10	0.05	0.06
Riboflavin (mg)	0.16	0.20	-	0.17
Niacin (mg)	0.4	0.6	0.5	0.5
Vitamin C (mg)	80.0	51.0	102.0	32.0

Source: Saunders and Becker, 1983 in NRC (1984)

Since N promotes vegetative growth and enhances deep green colour which is due to the chlorophyll pigment synthesized in the chloroplast (Lawlor, 1992), its supply influences the amount of chloroplasts are rich in proteins and also contain the β-carotenes (Mingel, 1979). Increasing levels of N resulted in increased vitamin A content in spinach (Fritz and Habben, 1973). However, the same authors noted that heavy or excess application of N can result in leaves that are excessively succulent and very susceptible to pests and disease. In cabbages the dry matter content was reduced significantly with increased N supply (Sorensen, 1984). Too much N can diminish taste and flavour and reduce the biological value of protein (Hornick, 1992).

Leafy vegetables are important source of a number of minerals Mingel, 1979; Chweya 1985). Vegetable amaranths have appreciable amounts of calcium, iron and phosphorus (NCR, 1984; Adipala and Mugerwa 1993). Plant species have influence in various mineral content in plant tissues (Hewitt and Smith 1975). Plant growing condition may or may not modify the amounts accumulated in the plant tissue, depending on the particular mineral element and treatments applied. Milton (1994) reported no significant effect on various mineral contents in vegetable parts.

Plants grown with excessive amounts of nitrogen usually have dark green leaves and show abundance of foliage, usually with a poorly developed root system resulting in a high-shoot-to-root ratio, and flowering and formation of seeds of several crops are retarded.

2.4 Phosphorus and Amaranths

Phosphorus is associated with high energy bond molecules like adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) which are responsible for energy release in plant cells. The high energy phosphate compounds are essential in photosynthesis, glycolysis, amino acid metabolism, translocation of photosynthates in phloem and mineral uptake and other life processes of plant growth (Tisdale *et al.,* 1985; Taesar, 1986).

Phosphorus is absorbed by plant roots in the form orthophosphate ions (H2 P04- or $HP0_4^{-2}$) (Tisdale *et al.* 1985). Low soil pH favours absorption of $HP04^{-2}$ ions, and normally P is available to plants at pH range of 5.5 to 6.5.

Soils that are high in 1:1 Kaolinitic clays fix and retain large quantities of P than those that contain 2:1 montimorilonite clays (Tisdale *et al.,* 1985; Hinga, 1973). This is because kaolinitic soils contain high quantities of anhydrous oxides of Fe and Al. Kaolinitic clays are predominant in Kenyan soils (Nyandati and Michieka, 1970) making P one of the most unavailable element in these soils. Addition of P to such soils results in remarkable improvement in crop growth (Tisdale *et al* 1981). Adequate supply of P is required in early stages of plant growth for the development of root system and in later stages for development of fruits and seeds. Normally deficiency of P in plants results in retarded root development and reduced shoot growth and delayed maturity. Even though P occurs in smaller quantities in plant tissues than N and K, it is referred to as a major plant nutrient. However, there is very little information on how this important mineral element affects the growth and development of leafy vegetables on Kenyan soils.

2.4.1: Effect on plant growth and yield

Amaranths have high growth rate and produce large quantities of organic materials and hence have a high demand for stages of growth and development (Mengel and Kirkby, 1979). Follet *et al.*, 1981 observed that the development of seedlings were restricted in many forage plants where P was deficient. The development of early secondary branches may be limited when P supply is inadequate. Plants are stunted when P is lacking (Tisdale *et al.* 1985).

2.4.2: Effect of leaf quality

Phosphorus, as already noted, forms an important structural component of plant cells and it is associated with numerous biochemical processes in plants; including amino acids and fat metabolism, photosynthesis and glycolysis (Lawlor, 1990). Therefore, a good supply

of nutrient P improves the quality of vegetables, fruits and grain crops as well (Tisdale *et al.,* 1985). P is a constituent of nucleic acid, phytin and phospholipids (fatty acids) (Tisdale et al. 1985).

Insufficient supply of P in plants results in a decreased rate of respiration while photosynthesis is still, progressing (Fillet et al., 1981). This results in accumulation of sugars while at the same time anthocyanin pigments are formed giving leaves and stems purple colourations, it was also noted that giving additional P to soils that are deficient in this nutrient can significantly increase the ability of the crop to produce more protein. It is essential in strengthening the cereal straws, and disease resistance is also associated with adequate supply of P (Tisdale *et al.,* 1985). It is readily mobilized in plant tissues such that in deficient conditions it moves from older tissues to the active meristematic regions.

2.5: Accumulation of Oxalates and Nitrates in Amaranths

Oxalates and nitrates are„some of the biochemical products considered anti-nutrients because they have negative contributions in human food (Ferrando, 1981).

2.5.1: Oxalate

Oxalate is derived from oxalic acid which is dibasic, an end product of metabolic process (Carlson, 1983; Lawlor, 1992). It is found in large quantities in rhubarb, sorrel, beetroot, spinach, sesame seeds and amaranths (Ferrando 1981). This acid forms soluble salts with sodium and potassium ions while calcium and magnesium forms are insoluble (Mugerwa and Stafford, 1976). An intake of more than 2 g per kilogramme of body weight is harmful as it can cause kidney lesions and may affect the heart also. High levels of the

acid in the diet can cause calcium deficiency in both man and non-ruminant animals. The effect, however, depends on the amount of calcium ions in the diet available for absorption after a portion of it has been chalated by acid as 1 mg of Ca++ makes 2:25 mg oxalic acid insoluble (Ferrando 1981; Williams, 1993).

The synthesis and accumulation of oxalic acid is influenced by genetic and environmental factors (Fritz and Habben, 1973). Amaranth is one of the leafy vegetables reported as having relative high levels of oxalic acid in plant tissues especially in the leaves (Carlsson, 1983; Vityakon and Standal, 1988). Fritz and Habben (1973) noted that the content of this acid tended to decrease with increased supply of P. Nitrogen also tends to influence oxalate content in plants (Carlsson, 1983). However, there is no adequate literature on this.

As, the plant matures, total oxalate content tends to increase (Adipala and Mugerwa, 1993). Mugerwa and Stafford (1986) observed significant increases in soluble oxalates in vegetable amaranth with advancing crop maturity. Low temperatures and short day lengths are favourable for enhanced production of oxalic acid and accumulation of total oxalates in plants (Abd-EL-Hadi 1985).

2.5.2: Nitrates

Amaranth requires and absorbs large amounts of nitrates because of its fast growth (Mato *et al.*,1986). However, accumulation of nitrates in the vegetative parts occur if the rate of translocation from roots to shoots is faster than the assimilation in the shoots (Mengel and Kirkby, 1979). Implying that there is little time given for the nitrates to be reduced into nitrates and converted into amino acids in roots (Marynard and Barker, 1979).

Nitrate is harmful to humans when substantial amounts are reduced to nitrites. When the nitrites are absorbed into blood stream, they oxidise the ferrous ions of the haemoglobin to the ferric form, thereby reducing the oxygen carrying capacity of blood. As a result methaemoglobin is formed (Aworh et al., 1980). This results in a condition referred to as methaemoglobinemia (Cantiliffe, 1972) which is a serious problem in ruminants and infants.

The genetic make up of a plant and environment influence nitrate accumulation in plants (Carlsson, 1983). If the plant growing medium is rich in N03-N, and the soil moisture is inadequate, while the relative humidity is high, then there is a tendency of plants to accumulate nitrate in their vegetative parts (Aworh et al. 1980). Light intensity influences NO3 accumulation such that when it is less there is more accumulation of N03-N in the leaves (Cantiliffe, 1972). The same author noted that, as the temperature of the environment was increased, there was less concentration of nitrates in spinach leaves.

Age of the plant part affect nitrate accumulation (Aworh et al., 1980), such that as the plant advances in age there is less N03-N. Kanampiu (1987) noted that petioles had higher N03-N accumulation than laminae and leaves, and that laminae had the lowest contents of NO3-N. When nitrate form of fertilizer is compared to ammoniacal form, more nitrates are accumulated in plant when the former is applied (Pew *et al.,* 1983).

2.5.3: Interactions between N and P

An interaction occurs when the response of one or series of factors is modified by the effect (Tisdale et al. 1985, Steel and Torrie, 1980). When the response to two of the other factors or more inputs used together is greater than the sum of their individual responses, then the interaction is said to be positive, as there is an added effect on the individual factors used.

There are many types of interactions including those between two or more nutrients (Tisdale et al. 1985), which offer a significant effect to progress towards maximum yields. It is also reported that a common interaction is between potassium and nitrogen. The information lacking. However, from the foregoing review on the two nutrient elements, possible interactions may occur; for example, it is noted that excess nitrogen can delay maturity whereas abundant supply of phosphorus accelerates maturity. Again, adequate supply of P stimulates root growth enabling the seedlings get access to other nutrients in soil solution, and in contrast N promotes development of succulent shoots.

2.6: Plant Age and Vegetable Yields

Yields of vegetable amaranths have been reported to vary between 20 to 60 tonnes per hectare. This normally depends on the stage of growth among others) which the initial harvest is made and the harvesting practices that follow. Harvesting entire plant after about fourty (40) days of plant growth can give yields of 20 to 25 t/ha (Tindall, 1986). When shoots are cut back to 15 or 20 centimeters, after 30 to 50 days from sowing and then followed by subsequent harvests of side branches yields of 30 to 60 tonnes hactare may be achieved.

2.7. Plant Age and Leaf Quality.

As the vegetable plants advance in growth, the edible parts (leaves and tender shoots) become less succulent. This is because the synthesized carbohydrates (a by-product of photosynthesis) are deposited in the vegetative cells making them thick and fibrous (Hornicks, 1992).

Total oxalates tend to increase with increase in age amaranth (Adipala and Mugerwa, 1993).

On the other hand, less nitrate N-concentrations have been reported in mature plants of Kales and Collards (Kanampiu, 1987).

3.0: MATERIALS AND METHODS

Two field trials and laboratory work were carried out at Kabete campus, University of Nairobi. The first trial was carried out between November 1994 and March 1995, while the second one was done between May and July 1995. The laboratory work was started in June and continued into October 1995.

3.1: Experimental Site

Field studies were carried out at Kabete Field Station of the Faculty of Agriculture. It is situated at an altitude of 1940 m above the sea level. It is characterised by a bimodal rainfall pattern with peaks in April and November. The average rainfall is about 1000 mm per year. The weather data recorded during the experimental seasons are presented in Appendix I. Minimum temperature was 13.20c and maximum 22.30c. Solar radiation was lowest in July (8151.7) and highest in January (14963.4). A lot of rainfall was received in November, April and May (more than 200mm). January was dry and hot while July was dry but cool.

The soils at the site are Kikuyu friable clay which were derived from tertiary trachytic lava (Siderius, 1970). According to FAO/UNESCO (1974) classification the soils are nitosols characterised by dark, brown colour with a thick, acid top soil. They are deep, well-drained and are resistant to erosion.

Soil samples were taken randomly from the experimental plots at depths of 0 to 15 cm and 15 to 30 cm just before sowing. The samples were air dried and ground to pass through a 2 mm sieve after which they were analysed for total nitrogen, cation exchange capacity (CEC), phosphorus and pH by the methods described in Tisdale et al. (1985). The results are given in appendix II. The pH of the soil samples ranged between 5.90 and 6.0, with CEC of not less than 18.00.

Percent nitrogen was between 0.15 and 0.3. In every 100g of soil P_2O_5, was less than 10mg.

3.2: Treatments and Experimental Design

Two variables (Nitrogen and Phosphorus) were considered in this experiment. Four levels of N; (0, 25, 50, 75kg N/ha) and four levels of P (0, 20, 40, 60kg P/ha). The levels were combined factorially to give 16 treatments which were laid down in a split plot design with three replicates. Nitrogen rates were the main plots while P rates were the subplots. The size of each sub plot was 6 m^2 (5 x 1.2m). *Amaranthus hybridus* seeds used were obtained from grain amaranth project, University of Nairobi.The seeds are tiny, black and glossy/shiny.

3.3: Cultural Practices

3.3.1 Field and seed bed preparations.

The field was ploughed and disc harrowed to give a fine tilth suitable for small seeds of amaranths. The soils were levelled and subplots marked. These operations were carried out during the dry period. Clods and stumps were removed from seedbed.

3.3.2: Sowing and phosphorus application

Seed furrows were made 40 cm apart in each experimental plot. Just before sowing, phosphorus in the form of TSP was applied into the furrows at the various rates and thoroughly mixed with the soil. Amaranth seeds were sown directly by hand drilling into furrows. The seeds were mixed with some soil to enable even distribution. These operations were carried out when the soil was moist.

3.3.3: Weeding and thinning

Weeds were carefully removed by hand rakes. Plants were thinned to leave a space of 20 cm within rows, two weeks after seedling emergence to give plant population of 13 plants/m^2.

3.3.4: Nitrogen application

This was applied once at the rate of 0, 25, 50, 75 KgN/ha 18 days after the seedling emergence. It was placed along the side of each row and watering was done immediately.

3.3.5: Irrigation/watering

This was done wherever there was a dry spell to ensure that there was no water stress in plants. Both watering cans and overhead sprinklers were used.

3.3.6: Pests and disease management

Pale green larvae *(Spodoptera littoralis-Lepidotera)* with white and grey bands on their sides with a brown capsule head, were observed feeding on leaf lamina. The plants were sprayed

once with Dimethoate at a rate of 15 ml/litre of water to control insects. There was no any disease observed during the experimentation.

3.4: Sampling and Data Collection

There were four rows of plants in each subplot. Plants in the middle two rows were used for data collection. Data collected include plant height, leaf number, leaf area, leaf area index, stem diameter and leaf yield. The quality parameters included dry matter, Ca, Fe, P, vitamin A, oxalates and nitrates.

3.4.1 Growth parameters

First sampling began 4 weeks after seedling emergence. Six plants were randomly marked and used to determine growth parameters. Plant height, leaf number, leaf area, leaf area index and stem diameter were taken. Height measurements and leaf numbers were taken at an interval of 7 days for four weeks.

3.4.2 Determination of yields

Shoot samples for fresh and dry weights determination were taken starting six weeks after seedling emergence, and continued for another 6 weeks at an interval of 2 weeks. Plants from an area of 1.2 m were cut at a uniform height of 15 cm above the ground level. Shoots were put in craft paper bags and their fresh weights taken. They were then dried in an air oven at 70°C for 36 hours, after which shoots dry weights were also recorded. In the subsequent harvests, the re-growths were cut at about 3 cm from the main stem.

3.5.3: Determination of specific minerals

A complete wet oxidation of samples was done followed by spectrometric analysis as described in a working manual by KARI (1993).

3.5.3.1: Preparation of the digestion mixtures: A digestion mixture was prepared by dissolving a mixture of 0.42 g selenium powder, 14 g lithium sulphate and 350 ml 30% hydrogen peroxide in 420 ml concentrated sulphuric acid. The acid was slowly added to the mixture and allowed to cool. The mixture was then stored at 2°C.

3.5.3.2: Block digestion: Thirty milligrammes of dried samples were placed in a digestion tube. 44 ml of digestion mixture were added to both the sample and blank tubes. The mixtures were then digested at 360°C for 2 hours. The colourless digests were allowed to cool and 25 ml distilled water added. These were mixed well and transferred to 50 ml volumetric flasks. They were made to mark with distilled water and sediments allowed to settle overnight for the sediments to settle.

3.5.3.3: Determination of Cacium, Iron and Phosphorus: Ten millilitres of the above digested sample solutions were placed in a 50 ml volumetric flask. Ten millilitres of 0.15% lanthanum chloride was added, then made to the mark with distilled water. The samples solutions were sprayed into atomic absorption spetrophotometer unicam SP 500 series 2 equiped with an air-acetylene flame and a hollow cathode lamp. The machine was operated under standard conditions using wavelengths and sit widths specified for each element. The quantities of the mineral elements were obtained from standard curves prepared from standard solutions whose absorptions were determined along with the samples. The results were expressed as milligrams per 100 g of dry matter.

3.5.4: Determination of Crude Fibre:

This was determined by A.O.A.C methods (A.O.A.C, 1984). Fifty milligrammes of ground samples were placed in 600 ml beaker and 25 ml of 2.04 N sulfuric acid and small amount of boiling distilled water added. The volume was made to 200 ml and the beaker placed on a heating rak-and condenser fitted under reflux. It was allowed to boil for 30 minutes, with frequent rotation. The contents were then filtered through asbestos ashing dishes packed with glasswool. The residues were rinsed with three portions of 75 ml boiling distilled water. The residue and glasswool were transfered quantitatively back into the beaker and boiled with 25 ml of 1.78N potassium hydroxide, and the volume made to 200 ml with boiling distilled water. It was connected to a reflux condenser and boiled for 30 minutes. The samples were filtered washed with three portions of acetone. The samples and glass wool were dried in an oven at 105°C overnight. They were cooled in a desicator and weights taken. They were then ignited at 550°C in a muffle furnace for 2 hours. They were allowed to cool in a desicator and weighed again. The crude fibre was expressed as percent weight loss of sample taken.

3.5.5 Determination of crude protein

Micro Kijeldahl technique following analytical methods of A.O.A.C. (1984). Fifty milligrammes of ground leave samples and mixed catalyst were added into Kjeldahl digestion tubes and 10 ml of conc. H_2S0_4 added. The mixture was put on pre set burners and blowers turned on. The samples were digested at 60°C until they were clear. The digest was cooled and neutralized with 40 ml distilled water, and 40 ml, of 50% NaOH. Twenty-five millilitres of boric acid solution in 500 ml erlenmeyer flask were placed under the delivery tube from condenser and ammonia was distilled into the solution. The receiving flask was removed when 250ml distillate was collected then filtrated with $0.104NH_250_4$. Nitrogen was determined by formula of

$$\underline{N = (T\text{-}B) \times N \times 1400}$$

Weight of sample (mg)

where

T is filtration (ml)ample

B is the blank filtration (ml)

N is the normality of H_2S0_4

To get % CP of N was multiplied by 6.25 factor

3.5.6: Determination of β-carotene

This was estimated using acetone-hexane solvent mixture method described in A.O.A.C., (1970). One gramme of dried vegetable leaf sample was weighed into a dry test-tube, 15 ml of acetone-hexane mixture (1+9) was added to a flask and left to stand overnight in the dark. The mixture was filtered into 50 ml volumentric flask and made to mark with the solvent. Two ml, of the extract was passed through a chromatographic column prepared from silica gel with, 1 cm layer of anlydrous sodium sulphate on top. The eluate was collected in 25 ml flask and made to mark with the solvent. Absorbance was read at 436 nm, while instrument was set at 100% transmittance with 9% acetone. The content of β-carotene was obtained from standard curve prepared from 0, to 3.0 ug 1 ml and the amount given as mg β-carotene per 100g of dry sample.

3.5.7: Determination of Oxalates

Total and soluble oxalates were determined in dry leaf samples using procedures described by Marshall *et al* (1967). Hundred milligrams were extracted with 30 ml of 0.5 ml HCL. This was placed in a water bath at 100°C for 30 minutes. After cooling and thorough shaking, the mixture was filtered through Whatman No. I filter paper. The pH was adjusted to 8 by adding 8M NH_4OH. The pH of the filtrate was again adjusted to 5.0 with 6N CH_3COOH. Duplicate samples of 10 ml were precipitated with 0.4 ml of 5% $CaCI_2$. It was allowed to settle overnight. The samples were centrifuged at 3000 r.p.m for 15 minutes. The supernatant was discarded. The sample was rinsed twice with 2 ml of 0.35 M of NH_4 OH. The cake was drip dried and dissolved in 10 ml of 0.5 M H_25O_4. This was tiltrated with 0.1M k2 mnO4 at 60°C to a faint violet colour that persisted for 15 seconds.

For soluble oxalates, 30 ml distilled was used for extraction instead of HCL. Similar procedures as for total oxalates were then followed. The results obtained were expressed as milligrams per hundred grams of the dry leaves.

3.5.8: Determination of Nitrate-N

Nitrates-N was determined calorimetrically using Cataldo *et al* (1975) method with slight modification (Chweya, 1985). Dried ground samples were oven dried at 70°C and 100 mg weighed and put into test tubes. Ten millilitre of deonised water were added to the samples and incubated at 45°C. The contents were mixed thoroughly and filtered through Whatman no 41 filter paper and 0.2 ml of extract put in 50 ml flasks. The extract was mixed thoroughly with 0.8 ml 5% salicyclic acid in conc. H_2S0_4 and 19 ml of 2N. NaOH slowly added to the mixture to raise the pH to over 12. The samples were allowed to cool to room temperature, after which optical density was determined at 410 nm against common blank of

0.8 ml of 5% salicyclic acid. Conc. H_2S0_4 and 19 ml 2N NaOH. The machine was set to 100% transmittance with pure solvent.

Nitrates-N standards containing 0 to 60 ug NO3 in 0.2ml aliquots were used with each set of samples.

3.6: Statistical analyses

Data generated from the above methods were subjected to analysis of variance (ANOVA) and means separated using Duncan's Multiple Range Test at 5% level probability (Steel and Torrie, 1980).

4.0 RESULTS

4.1: Effects of nitrogen and phosphorous on growth of Amaranths

The results 'of the effect of nitrogen and phosphorus fertilizer applications on growth parameters are shown in appendix 3 and tables 2 to 9. Applying 50 Kg N/ha resulted in a significant increase in plant height, which was not different from that obtained by 75 Kg N/ha application although plants that were treated with 50 and 75 kgN/ha were noticeably taller than those receiving 0 and 25 kg N/ha.

Number of leaves significantly increased when 50 and 75 kg N/ha were applied to amaranths plants. Stem diameter increased significantly when nitrogen level was increased from 0 to 25 kg/ha. There was no significant increase in stem diameter when nitrogen supply was further increased (Table 4). Leaf area tended to improve as N supply was increased. When the level of N was raised from 25 to 50 kg, there was a significant increase in leaf area. Further increase in N supply did not result in an increase in leaf area. This was observed in both first and second seasons. In both seasons, the least leaf area index (1.0 and 1.1) was obtained where there was no N application.

Records on plant height and number of leaves at various stages of growth are presented in tables 2 and 3. Increasing N level from 25 to 50 kg/ha caused significant increase in plant height at seventh week of plant growth. It was observed that plants that were treated with 50 and 75 kg of N attained heights that were significantly higher than those recorded with 0 and 25 kg N application.

Table 2: Effect of nitrogen on growth of Vegetable amaranth plant at seventh week of plant growth (Season 1)

Treatment	Plant height (cm)	Number of leaves	Stem diameter (mm)	Leaf area (M^2)	Leaf area index
Nitrogen (Kg/ha)					
0	26.0^b	12^b	4.7^b	7.1^b	1.1^c
25	28.0^b	12^b	5.1^a	7.4^b	1.25^b
50	31.5^a	13^a	5.2^a	8.3^a	1.40^a
75	33.0^a	14^a	5.3^a	8.2^a	1.37^a

Means followed with the same superscripts (a, b, c, d) within the columns are not significantly different at 5% probability, according to Duncan's Multiple Range Test (DMRT)

Table 3. Effect of nitrogen on growth of vegetable Amaranth plants after Seventh weeks of growth (season 2

Treatment	Plant height (cm)	Number of leaves	Stem diameter (mm)	Leaf area (M^2)	Leaf area index
Nitrogen (Kg/ha)					
0	26.0^b	10^d	4.5^b	6.0^b	1.0^d
25	28.0^b	11^c	5.0^a	6.8^{ab}	1.2^c
50	32.0^a	13^b	5.1^a	7.5^a	1.3^b
75	32.6^a	14^a	5.2^a	7.9^a	1.4^a

Means followed with the same superscripts (a,b,c,d) within the columns are not significantly different at 5% probability, according to DMRT

20 and from 20 to 40 kg/ha, the resultant heights were significantly increased. A height of 32.6 cm was recorded in the first season with application of 60 kg P/ha. This height was not different, statistically, from that of those plants that received 40 kg P/ha. Significant increases in plant height were observed at various stages of growth with increased level of P (tables 6 & 7). In the fourth week of plant growth, the heights were different at all levels of P. As plants continued in growth in the first season, increases in heights were not

significantly different at 40 and 60 kg P/ha. In the second season, increasing level of P resulted in tall amaranth plants whose heights were significantly different at sixth week of growth.

Number of leaves tended to increase with increased level of P (Tables 6 and 7). Plants treated with 40 and 60 Kg P/ha had more leaves than those receiving 0 and 20 kg P/ha. Number of leaves recorded at 0 and 20 P/ha were the same in fourth and fifth week of growth. Increasing the level of P from 20 to 40 kg P/ha resulted in a significant increase in number of leaves at fifth week of growth. After this more supply of P did not result in production of more leaves. Stem diameter increased with addition of P. Supply of P at all the four levels of P, there was a significant effect such that the higher the level of P the thicker the stems. This was observed in both seasons. (3.la and 3.1b).

Leaf area improved slightly when 20kg P/ha was applied. Supplying 40kg P/ha caused a significant increase in leaf area. Further addition of P did not give a significant change in leaf area. Leaf area index at 0 and 20 kg P/ha were the same. With addition of 40 kg P/ha, there was a significant improvement in leaf area index. No more changes in the index were observed with more supply of P.

Table 6: Effect of phosphorous on vegetable Amaranth plant height and number of leaves from fourth to seventh week of growth.

		Plant height (cm)				Number of leaves			
Treatment		Week 4	Week 5	Week 6	Week 7	Week 4	Week 5	Week 6	Week 7
Phosphorous Kg/ha	0	5.0^{d}	9.7^{c}	17.6^{c}	24.8^{c}	5^{b}	7^{c}	9^{c}	12^{b}
	20	5.8^{c}	11.0^{b}	20.0^{b}	27.6^{b}	5^{b}	7^{bc}	10^{b}	13^{a}
	40	7.3^{b}	13.0^{a}	23.6^{a}	32.1	6^{a}	8^{a}	11^{a}	13^{a}
	60	8.1^{a}	13.4^{a}	24.5^{a}	32.5^{a}	6^{a}	8^{a}	11^{a}	13^{a}

Means with the same superscripts within the column are not significantly different at 5% probability according to DMRT.

Table 7: Effect of phosphorus on vegetable Amaranth plant height and number of leaves from fourth to seventh week of growth (season 2)

Nitrogen levels (kg/ha) Treatment		Plant height (cm) at:				Number of leaves at:			
		Week 4	Week 5	Week 6	Week 7	Week 4	Week 5	Week 6	Week 7
Phosphorous Kg/ha	0	5.0^{c}	8.6^{b}	16.5^{d}	23.3^{b}	4^{b}	6^{c}	8^{c}	11^{c}
	20	5.9^{b}	9.8^{a}	18.9^{c}	26.2^{b}	4^{b}	7^{b}	9b	12^{c}
	40	6.1^{b}	11.0^{a}	22.1^{b}	31.8^{a}	5^{a}	8^{a}	10^{a}	13^{a}
	60	7.6^{a}	12.2^{a}	23.7^{a}	32.1^{a}	5^{a}	8^{a}	10^{a}	13^{a}

Means followed with the same superscripts (a, b, c) within columns are not significantly different (pc 0.05) - Duncans Multiple Range Test.

Table 8: Effects of Phosphorus on growth of vegetable amaranth plants at seventh week of plant growth (First season)

Treatment		Plant height (cm)	Number of leaves	Stem diameter (mm)	Leaf area m^2	Leaf area index
Phosphorous (Kg/ha)	0	26.0^{c}	12^{b}	2.0^{d}	7.2^{b}	1.2^{c}
	20	28.0^{b}	13^{a}	4.7^{c}	7.3^{b}	1.26^{c}
	40	32.0^{a}	13^{a}	5.8^{b}	8.1^{a}	1.34^{a}
	60	32.6^{a}	13^{a}	7.5^{a}	8.3^{a}	1.38^{a}

Means followed with the same superscripts within columns are not significantly different ($P<0.05$) according to DMRT.

Table 9: Effect of phosphorus on growth of vegetable amaranth, plants at seventh week (second season)

Phosphorous level (Kg P/ha)		Plant height (cm)	Number of leaves	Stem diameter (mm)	Leaf area m^2	Leaf area index
Phosphorous (Kg/ha)	0	25.9^{c}	11^{c}	2.0^{d}	6.8^{b}	1.1^{d}
	20	27.9^{b}	12^{b}	5.0^{c}	7.0^{b}	1.1^{b}
	40	31.5^{a}	13^{a}	6.0^{a}	7.9^{a}	1.3^{a}
	60	31.8^{a}	13^{a}	7.0^{a}	8.1^{a}	1.3^{a}

Means followed with the same superscripts within columns are not significantly different at 5% probability level according to DMRT.

Table 10: Interactions between nitrogen and phosphorus for leaf area (m^2) of vegetable Amaranth plants at tenth week of growth.

Treatments	Phosphorous (Kg/ha			
	0	20	40	60
Nitrogen (Kg/ha) 0	6.1^{h}	7.1^{efg}	7.4^{cefg}	7.9^{bcdef}
25	6.4^{gh}	7.3^{defg}	7.6^{abcdef}	8.2^{abcd}
50	6.9^{gh}	8.1^{defg}	8.9^{a}	8.8^{ab}
75	7.8^{bcdef}	8.3^{ab}	8.2^{abcd}	8.2^{abcd}

LSD 0.45. Means followed with the same letters within columns and across the rows are not significantly different at 5% probability.

4.2. Effect of nitrogen and phosphorus on leaf yields.

The main effects of N and P on fresh weights were very significant (Appendix 4). Fresh weights taken for three harvests are presented in tables 11 and 12.

Where there was no application of nitrogen, harvested shoots weighted less. As the level of N was increased to 25 kg/ha, a significant increase in fresh weight was observed in the first season (Table 13). This was after forty four (44) days of plant growth. Adding more nitrogen did not result in significant increase in fresh weights at this stage. However, at fifty eight and seventy two days after sowing, fresh weights increased significantly where nitrogen level had been raised from 25 to 50 kg/ha. These weights were statistically similar to those obtained with 75kg, N/ha application. When yields from first second and third harvests were added together, fresh weight of 47.6 tonnes per hectare was recorded with application of 25 kg N/ha. There were more cumulated yield with the addition of nitrogen (Table 13).

Dry weights of the harvested shoots were as recorded in Table 14. It was observed that nitrogen application had no significant influence on dry weights in the first harvest. More

dry weights were obtained with 50 Kg N/ha in the second and third harvests. Further addition of N did not significantly increase shoot dry weights. In table 15, effects of phosphorus on fresh weights for three harvests are shown. As was observed with nitrogen application, phosphorus also caused significant production of vegetable shoots. Increasing P supply resulted in production of more fresh shoots (Table 15). From harvests made 58 days after sowing, there was a significant improvement in fresh weight when P supply was raised from 20 to 40 kg/ha. With 60 kg P/ha application, fresh weight of 28.2 tonnes/hectare was recorded but there was no significance in the differences in weights obtained from 40 kg/P/ha. Cumulative weight of fresh vegetable shoot was 53t/ha with 20 kg/,ha of phosphorus application, this increased significantly to 72t/ha when P supply was improved to 40 t/ha.

Dry weights recorded in day 44 increased with more addition of P.

Table 13: Effect of nitrogen on fresh weights (tonnes/hectare) of vegetable Amaranth

		Fresh weight			
Sampling		Harvest 1 44 days	Harvest 2 58 days	Harvest 3 72 days	Cumulative (tonnes/ha)
Nitrogen Kg N/ha	0^{d}	9.2^{b}	14.7^{c}	21.5^{c}	45.1^{c}
	25^{c}	11.4^{a}	16.7^{c}	19.5^{c}	47.6^{c}
	50^{b}	12.0^{a}	24.5^{a}	28.4^{a}	64^{b}
	75^{a}	12.4^{a}	29.4^{a}	26.6^{a}	68^{b}

Means followed with the same superscripts (a, b, c, d) within columns are not significantly different at 5% probability according to DMRT

Table 14: Effect of nitrogen on dry weights (tonnes/hectare) of vegetable Amaranth

			Dry weights (t/ha)			
Sampling			Harvest 1 44 days	Harvest 2 58 days	Harvest 3 72 days	Cumulative (tonnes/ha)
Nitrogen Kg N/ha	0^{d}	0	1.6^{a}	4.2^{b}	3.8^{c}	10.0^{b}
	25^{b}	25	2.0^{ab}	4.2^{b}	5.0^{b}	10.8^{b}
	50^{a}	50	2.3^{a}	6.6^{ab}	8.7^{a}	17.6^{a}
	75^{a}	50	2.1_{ab}	9.0^{a}	7.0^{a}	18.1^{a}

Means followed with the same superscripts (a,b,c) within the columns are not significantly different at 5% probability according to DMRT.

Table 15: Effect of phosphorus on fresh weights of vegetable Amaranth leaves

		Fresh weights (tonnes/ha)			
Sampling		Harvest 1 44 days after sowing	Harvest 2 58 days after sowing	Harvest 3 72 days after sowing	Cumulative
Phosphorous kg P/ha	0	2.3^{d}	11.3^{b}	15.8^{c}	2.9
	20	10.0^{c}	19.5^{b}	23.4^{b}	53
	40	14.3^{b}	26.3^{a}	31.6^{a}	72
	60	18.2^{a}	28.2^{a}	25.1^{b}	71.5

Means followed with the same superscripts (a, b, c, d) within the columns are not significantly different, at 5% probability according to DMRT.

Table 16: Effect of phosphorus on dry weights of vegetable Amaranth leaves

		Fresh weights (tonnes/ha)			
Sampling		Harvest 1 44 days after sowing	Harvest 2 58 days after sowing	Harvest 3 72 days after sowing	Cumulative
Phosphorous kg P/ha	0	2.5^{d}	3.8^{b}	4.7^{c}	9.0
	20	1.9^{c}	6.2^{b}	5.7^{c}	13.8
	40	2.5^{b}	7.6^{a}	7.4^{a}	17.5
	60	3.0^{a}	6.3^{a}	6.6^{a}	15.9

Means followed with the same superscripts (a,b,c,d) within columns are not significantly different, at 5% probability according to DMRT.

Table 17: Effect of Nitrogen on cumulative yields of vegetable Amaranth

Nitrogen levels (kg N/ha)	Cumulative fresh weight (kg/ha)	Cumulative dry weight (kg/ha)
0	3867.5[b]	990.8[b]
25	4515.0[b]	1087.5[b]
50	6579.2[a]	1650.8[a]
75	7291.7[a]	1725.8[a]
LSD (0.0.5)	1287.3	349

Means followed with the same superscripts (a,b,c) down the column are not significantly different ($p<0.05$) Duncans Multiple Range Test.

4.3 Effect of nitrogen and phosphorus on leaf quality

Crude fibre, was reduced significantly with the addition of nitrogen.

Where there was no application of N, crude fibre was about 9% both seasons (Tables 18 and 19). At 25 kg N/ha, crude fibre content was at 7.4%. Applying more N, gave a significant difference in fibre content. However, raising the level of N from 50 to 75 kg/ha did not give less fibre in the vegetable leaves.

On the other hand, crude protein was improved with the addition of N (Table 18). Between 0 and 25 Kg N/ha, the increase was not significant, but contents of CP were much higher at 50kg N/ha. The highest contents of CP (32.2%) was recorded with 75 kg /ha N.

Beta carotenes were also improved with the application of N. Contents of β-carotene in the leaves increased with every rise in level of N in the second season (Table 19) in the first season, no significant increase in β-carotene contents was obtained with raising the level of N from 50 to 75 kg /ha.

Influence of phosphorus on the contents of crude fibre, crude protein and β-carotene was less compared to that realised with N application (Appendix 6 and Table 20). Phosphorus application did not result in any significant improvement of either CP or β-carotene contents.

Table 18: Effect of Nitrogen on crude fibre, crude protein and carotene contents of Amaranth leaves determined at eighth week of growth

Treatment Nitrogen (kg/ha)	Crude fibre (%dm)	Crude protein (% dm)	β-carotene (mg/100g)
0	9.0[a]	29.6[b]	0.75
25	7.9[b]	30.0[b]	0.87[b]
50	7.4[c]	31.0[a]	0.82[a]
75	7.5[c]	32.2[a]	0.95[a]

Means followed with the same superscripts (a,b,c) within the columns are not significantly different at 5% probability according to DMRT

Table 19: Effect of nitrogen on crude fibre, crude protein and β-carotene contents of Amaranth leaves at eighth week of growth

Nitrogen levels (Kg P/ha)	Crude fibre (%dm)	Crude protein (% dm)	β-carotene (mg/100g)
0	9.1[a]	29.6[a]	0.51[c]
25	8.0[b]	29.7[a]	0.52[b]
50	7.5[c]	30.0[a]	0.86[b]
75	7.3[c]	30.2[a]	0.95[a]

Means followed with the same superscripts (a,b,c) within columns are not significantly different ($P<0.05$) according to DMRT

Table 20: Effect of phosphorus on crude fibre, crude protein and β-carotene contents of Amaranth leaves determined at eighth week of plant growth.

Treatment (kg/ha)	Phosphorous	Crude fibre (%dm)	Crude protein (% dm)	β-carotene (mg/100g x 10^{-2})
	0	8.9^{a}	29.6^{b}	7.9^{a}
	20	8.0^{b}	29.6^{a}	7.9^{a}
	40	7.8^{b}	30.2^{a}	7.8^{a}
	7560	8.2^{b}	30.6^{a}	7.7^{a}

Means with same superscripts (a,b) down the columns are not significantly different at 50% probability according to DMRT

Table 21: Effect of phosphorous on crude fibre, crude protein and β-carotene contents of Amaranth leaves determined at eight week of growth in season two

Phosphorous (Kg/ha)	Crude fibre (%dm)	Crude protein (% dm)	β-carotene (mg/100g)
0	7.8^{b}	29.6^{a}	0.77^{a}
20	7.8^{b}	29.6^{a}	0.78^{a}
40	7.9^{b}	30.2^{a}	0.79^{a}
60	8.2^{a}	30.0^{a}	0.79^{a}

Means followed with the same superscripts (a,b) down the column are not significantly different (P<0.0.5) according to DMRT.

Table 22. Interaction between N and p for crude fibre contents in amaranth leaves at eight week of plant growth.

Treatment	Phosphorous (Kg/ha)			
	0	20	40	60
Nitrogen (Kg/ha)				
0	8.8^{b}	9.1^{b}	8.6^{c}	9.7^{a}
25	7.9^{de}	7.8^{de}	8.2^{cd}	8.2^{cd}
50	7.6^{efg}	7.5^{efgh}	7.2^{efgh}	6.9^{fh}
75	7.5^{gh}	7.7^{gh}	7.4^{efgh}	7.9^{de}

LSD (0.05) = 0.58 Means followed with the same letters within columns and across the rows are not significantly different.

4.4. Effects of nitrogen and phosphorus on minerals

Total minerals determined as total ash, improved with the addition of N (Table 22). However, the effect was only significant when 50kg/ha was applied. Supplying more N did not result in significant increase in mineral content.

The content of calcium ions (Ca^{++}) increased with every increased supply of N. Where there was no application of nitrogen, 1.4 mg/10g of calcium was recorded. This increased to 1.7mg/100g when 25kgN/ha were added, giving a significant difference in calcium contents at two levels similar effects were observed when N level was raised further to 50 and 75kg/ha. The mineral contents of iron and phosphorus did not change much with addition of nitrogen.

The application of phosphorus to vegetable amaranths gave insignificant changes in total minerals. However, the contents of calcium and iron tended to improve with the addition of phosphorus (Table 24).

Table 23:1 Effect of Nitrogen on mineral contents of amaranth at eighth week of plant growth in season 1

Nitrogen levels (Kg N/ha)	Total minerals (% dm)	Calcium (Mg/100g)	Iron (Mg/100g)	Phosphorous (PPM)
0	19.6[a]	1.5[b]	20.4[a]	0.38[b]
25	19.7[a]	1.7[a]	20.7[a]	0.39[a]
50	18.0[a]	1.8[a]	20.8[a]	0.41[a]
75	18.1[a]	1.8[a]	20.9[a]	0.41[a]

Means followed with the same superscripts within the columns are not significantly different ($P<0.05$) - according to DMRT

Table 23.2: Effect of nitrogen on mineral contents of Amaranth leaves at eighth week of plant growth in season two

Nitrogen levels (Kg N/ha)	Total minerals (% dm)	Calcium (Mg/100g)	Iron (Mg/100g)	Phosphorous (PPM)
0	19.4[a]	1.4[d]	20.4[a]	0.21[a]
25	19.6[a]	1.7[c]	20.7[a]	0.20[a]
50	17.8[a]	1.8[a]	20.8[a]	0.21[a]
75	17.9[b]	1.9[a]	20.9[a]	0.20[a]

Means followed with the same superscripts (a,b,c,d) down the column are significantly different (P<0.0.5) - Duncan's Multiple Range Test.

Table 24: Effect of phosphorus on mineral contents of Amaranth plants at eighth week of plant growth (First season)

Phosphorous levels (Kg N/ha)	Total minerals (% dm)	Calcium (Mg/100g)	Iron (Mg/100g)	Phosphorous (PPM)
0	19.6[a]	1.5[c]	18.4[c]	0.38[c]
20	18.4[a]	1.7[b]	20.1[b]	0.39[c]
40	18.5[a]	1.8[a]	21.0[b]	0.43[b]
60	18.7[a]	1.9[a]	22.5[a]	0.46[a]

Means with the same superscripts within the columns are not significantly different at < 5% according to DMRT.

Table 25: Effect of phosphorus on minerals contents of Amaranth plants at eighth week of growth (Season 2).

Phosphorous levels (Kg P/ha)	Total minerals (% dm)	Calcium (Mg/100g)	Iron (Mg/100g)	Phosphorous (PPM)
0	19.5[a]	1.4[c]	20.4[a]	18.4[c]
20	18.6[a]	1.6[b]	20.7[a]	20.1[b]
40	18.3[a]	1.8[a]	20.8[a]	21.0[b]
60	18.5[a]	1.8[a]	20.9[a]	22.5[a]

Means followed with the same superscripts (a,b,c) down the column are not significantly different (P<0.05) Duncans Multiple Range Test.

4.5 Effects of nitrogen and phosphorus on oxalate and nitrate contents.

Three determinations for oxalates and nitrate N-contents in the leaves were carried out from samples obtained from shoot harvests. Only one determination for soluble oxalates was done from samples of second harvest.

Applying nitrogen resulted in less contents of total oxalates (Table 26). After fifty eight days of growth, about 4.6 mg/100g oxalates could be found in amaranth leaves which had not received nitrogen. This went down to 4.0 mg/100g oxalates when 25 kgN/ha were applied, giving a significant difference in total oxalates at the two levels. Similarly, raising the level of N caused further reduction in total oxalates at 72 and 86 days of growth. It was noted that as plant progressed in growth, more oxalates accumulated in the leaves (Table 26). Nitrogen application had no effect on soluble oxalates.

As was observed with nitrogen application, supply of phosphorous also tended to lower the contents of oxalates (Table 27). There was a significant difference in total oxalate contents in the four levels of P observed at 58 and 72 days of growth. There were more concentrations of oxalates in the plants that had not received P treatment. This was significantly reduced with the application of P. There was no observable influence of p on soluble oxalates.

Table 26: Effect of Nitrogen on oxalate and nitrate - N contents determined at sixth eighth and tenth week of plant growth.

	Total oxalate contents (mg/100g) in three harvests/samples				Nitrate contents (PPM) in three harvests		
Nitrogen levels	1	2	3	Soluble oxalates	1	2	3
Nitrogen (kg/ha)							
0	4.6[a]	4.8[a]	5.0[a]	1.7[a]	112.2[c]	102.2[c]	99.8[c]
25	4.6[a]	4.7[a]	4.9[ab]	1.7[a]	131.6[b]	141.2[b]	138.8[b]
50	4.3[a]	4.4[b]	4.6[c]	1.7[a]	139.2[b]	148.6[ab]	142.8[b]
75	4.5[a]	4.5[b]	4.7[c]	1.8[a]	149.9[a]	161.3[b]	148.8[a]

Means followed with the same superscripts within columns are not significantly different - using Duncan's Multiple Range Test at 5% level.

Table 27: Effect of nitrogen on nitrate contents of Amaranth leaves determined at sixth, eighth and tenth week of plant growth (season 2)

Nitrogen level (kg N/Ha)	Nitrate-N contents (ppm)		
	Week 6 (58 days)	Week 8 (72 days)	Week 10 (86 days)
0	98.8[d]	98.3[d]	96.8[d]
25	119.3[c]	138.5[c]	131.2[c]
50	121.1[b]	142.3[b]	140.0[b]
75	147.5[a]	155.7[a]	150.2[a]

Means followed with the same superscripts letters (a,b,c,d) down the column are not significantly different ($p< 0.05$) Duncans Multiple Range Test.

Table 28:Effect of nitrogen on total and soluble oxalates in Amaranth leaves (season 2)

Nitrogen level (kg/N/ha)	Total oxalates (mg/100g)			Soluble oxalates
	58 days	72 days	86 days	72 days
0	4.6[a]	4.6[a]	5.1[a]	1.7[b]
25	4.0[b]	4.7[a]	4.9[ab]	1.7[b]
50	3.9b[c]	4.1[b]	4.5[bc]	1.8[ab]
75	3.8[c]	4.0[b]	4.7[c]	1.8[a]

Means followed with the same superscripts (a,b,c) down the column are not significantly different ($P<0.05$) Duncans Multiple Range Test.

Table 29: Effect of phosphorus on nitrate contents of Amaranth leaves determined at sixth, eighth and tenth weeks of growth (first season)

Phosphorous level (kg P/ha)	Nitrate - N contents (ppm)		
	Week 6 58 days	Week 8 72 days	Week 10 86 days
0	131.7[a]	137.2[a]	128.3[d]
20	133.2[a]	136.5[c]	130.8[c]
40	132.8[a]	140.0[a]	133.1[b]
60	134.3[a]	140.5[a]	136.2[a]
LSD (0.05)	3.5	4.0	2.0

Means followed with the same superscripts (a,b,c,d) down the column are not significantly different (P0.05) Duncans Multiple Range Test

Table 30: Effect of phosphorus on total and soluble oxalates in Amaranth leaves determined at 58, 72 and 86 days of growth.

Phosphorous level 9 kg P/ha	Total oxalates (mg/100g)			Soluble oxalates
	58 days	72 days	86 days	
0	4.7[a]	5.2[a]	5.0[a]	1.80[a]
20	4.4[b]	4.6[b]	4.9[ab]	1.7[ab]
40	3.7[c]	4.1[c]	4.8[b]	1.72[b]
60	3.5[d]	3.8[d]	4.6[c]	1.70[b]
LSD (0.05)	0.2	0.2	0.1	0.06

Means followed with the same superscripts (a,b,c,d) down the column are not significantly different (P< 0.05) Duncan's Multiple Range Test.

Table 31: Effect of phosphorus on oxalate and nitrate - N contents of Amaranth leaves determined at sixth, eighth and tenth weeks of plant growth (second season)

	Total oxalate contents (mg/100g) in the three harvests/samples				Nitrate contents (PPM) in three harvests		
Phosphorous levels (Kg P/ha)	1	2	3	Soluble oxalates	1	2	3
Phosphorous (kg/ha)							
0	4.9[a]	4.7[a]	5.0[a]	1.7[ab]	132.3[a]	128.9[a]	130.1[b]
20	4.5[b]	4.6[a]	4.8[b]	1.8[a]	132.1[a]	136.5[a]	130.9[b]
40	4.4[b]	4.6[a]	4.9[ab]	1.7[b]	132.8[a]	139.3[a]	134.2[a]
60	4.2[c]	4.4[b]	4.6[c]	1.7[ab]	135.6[a]	140.3[a]	135.1[a]

Means followed with the same superscripts (a,b,c) within columns are not significantly different - Duncan's Multiple Range Test (P= 0.05)

Table 32: Interactions between N and P for the contents of total oxalates in Amaranth leaves in the second harvest.

	Phosphorous (Kg P/ha)			
Nitrogen (kg N/ha)	0	20	40	60
0	5.70[a]	4.93[cd]	4.80[d]	4.70[de]
25	5.50[ab]	4.50[d]	4.60[de]	4.40f
50	5.07[bc]	4.47[e]	4.60[de]	4.60[de]
75	4.60[d]	4.50[e]	4.40[f]	4.16[f]

Means followed with the same superscripts within the columns and across rows are not significantly different (LSD)

Nitrate contents in amaranth leaves increased with supply of N. Raising the level of N from 0 to 25 kg/ha resulted in a high increase in NO_3-contents in the leaves. More supply of N further increased NO_3, contents, giving significant difference in contents in all the four levels of N application. This was observed in the three stages of growth. On the other hand, applying phosphorus did not change the contents of nitrate-N in the leaves.

Nitrate contents in amaranth leaves increased with supply of N. Raising the level of N from 0 to 25 kg/ha resulted in a high increase in $N0_3$-contents in the leaves. More supply of N further increased $N0_3$ contents, giving significant difference in contents in all the four levels of N application. This was observed in the three stages of growth. On the other hand, applying phosphorus did not change the contents of nitrate -N in the leaves.

5.0 DISCUSSION

5.1 Effect of Nitrogen and Phosphorous Application on Growth and Yields of Leafy Vegetable Amaranth.

Results obtained from this study indicate that both nitrogen and phosphorous had significant effects on growth and subsequent yields of vegetable amaranths. Plants that were treated with N and P showed more vigorous growth than the untreated ones. This is because, under normal conditions, amaranths like any other leafy vegetable require adequate supply of nutrients for normal growth and development. Thus increasing N and P levels resulted in significant increases in plant height, leaf numbers, leaf area and leaf area index. Motoh *et al.,* (1986) reported increases in plant height, leaf area and fresh weight, of vegetable amaranths with increased supply of N. Nitrogen induce expansion in plants (Hewith and Smith, 1975).

Normally leaf size is determined by cell expansion which is also influenced by nutrient Supply. Thus plants receiving sufficient supply of N tend to have more leaves produced. In kale plants, application of N increased the mean fresh weights and total leaf yields (Chweya (1984).

Leaf production of kales and collards increased with N supply (Kanampiu, 1987). Adipala and Mugerwa also reported significant increases in leaf yield in *Amaranthus hybridus* subspecies *hybridus* with N and P application.

Phosphorous gave a high significant increase in plant height and stem diameter. This is because amaranths have high growth rate and have a high demand for P in the early stages of growth and development (Mengel and Kirkby, 1979). Ample supply of P results in plants good root developments and shoot growth. Follet *et al.* (1981) noted that the development of seedlings were limited in many forage plants where P was deficient. Phosphorous forms high energy bond molecules (AMP, ADP and ATP) responsible for energy release in cells Tisdale *et al.* (1985). These compounds are responsible for photosynthesis, glycolysis, translocation of photosynthates and mineral uptake which are all important life processes for plant growth. Leaf area index, which is an important parameter since it shows photosynthetic capacity of the plant was significantly increased. Where the level of P was increased from 20 to 40 Kg/ha. As a result there were significant increases in both fresh and dry weights with P supply.

The interactions between N and P gave positive significant increases in yields indicating that the two nutrient elements are dependant on one another for yield. This is probably because P stimulates root growth, enabling plants get access to other nutrients in soil solution. Nitrogen on the other hand promotes development of shoots resulting in good yields.

Effect of Nitrogen and Phosphorous Application on quality of Amaranth leaves.

Nitrogen gave a significant reduction in crude fibre. This is because high levels of N results in the synthesis of carbohydrates and more proteins, making the leaves more succulent with low crude fibre. Fritz and Habben (1973) reported low crude protein contents at low levels of N. It is also reported that Protein: Carbohydrates ratios in the leaves is influenced by

levels of N (Mingel, 1979). The results indicate that increased N supply gave significant increases in crude protein. Mingel (1979) reported that as the amount of N in growth medium is improved, total N and crude protein contents in the leaves are increased but crude fibre content is reduced.

The influence of N on carotene was significant as it gave significant increase in the content of β-carotene. This is because N promotes vegetative growth and also enhances deep green colour formation. This deep green colour is due to the chlorophyll pigment synthesized in chloroplast (Lawlor, 1992). Therefore N supply influences the amount of chloroplasts that are rich in protein and plant pigments such as β-carotene. Fritz and Habben (1973) (1973) reported increased vitamin A content with increased N levels. This positive quality attribute was less prominent with P application as there was, no significant statistical evidence realized for β-carotene.

As already noted in the results, both N and P had a negative effect on the contents of total oxalates as the nutrients elements caused significant reduction in oxalate content. Applying N and P facilitates rapid plant growth and more protein synthesis is favoured and less oxalates are synthesized. Fritz and Habben (1973) noted that the content of oxalic acid tended to decrease with increased P supply. Carlson (1983) also reported some influence of N on oxalate content in plants though no specific qualities were given. Mugerwa and Stafford (1986) observed significant increase in soluble oxalates in vegetable amaranth with advancing crop maturity.

High concentrations of nitrate-N is a negative quality attribute. More $N0_3$-N was accumulated when the level of N was increased. At higher rates of N, the rate of translocation of nitrates from roots to shoots is faster than their assimilation in the shoots (Mingel and Kirkby, 1979). This implies that there is less time for conversion of nitrates to nitrates and into amino acids, thus the accumulation of nitrates in the leaves/shoots. Results

also indicated that as plants advanced in age, there was less NO_3-N accumulated. As plants become mature, the plant system can sufficiently convert NO_3-N into the nitrates and further incorporate them into amino acids (Maynard and Barker, 1979). Aworh *et al.* (1980) reported that there is a tendency of plants to accumulate nitrates if growing medium is rich in $N0_3$-N, and as the plant advances in age there is less concentration of $N0_3$-N in the leaves.

On mineral contents, phosphorous gave significant increases in P, Ca and Fe contents, while nitrogen played insignificant role. Phosphorous application normally facilitates root growth, thereby establishing good root structure. This enables absorption of other nutrient elements from the soil solution. These nutrient elements are necessary for the growth and development of plants. Follet et al (1981) observed that the development of seedlings were restricted in fora where P was deficient.

6.0 CONCLUSIONS AND RECOMMENDATIONS

From the results obtained from this study, conclusions may be drawn that applying 50Kg N/ha and 40kg P/ha were the optimum levels that affect growth and yield significantly. These levels are more economical and they give sufficient nutrients needed for optimum vegetable growth. It would be advisable to farmers apply N and P to amaranth vegetables so that better yields may be realized.

Moderate application of N can improve vegetables quality in terms β-carotene content, crude protein without causing a lot of NO_3-N accumulation in the leaves. Applying more than 50kg N/ha can result in accumulation of NO_3-N. Phosphorous has a significant role in influencing the levels of iron, phosphorus and calcium contents in vegetable leaves, it plays little role in the β-carotene content and crude fibre contents. However, further investigations on the interactions between N and P for yields and crude fibre contents may shade more light on the effects of N and P on these parameters.

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Appendix 1:: Weather data recorded during the experimental season

Month	Temperature (°C)		Relative Humidity at		Radiation	Total rainfall
	Max	Min	9 am	12 noon		
October 1994	24.1	13.8	81.5	48.8	12407.1	87.8
November	22.3	14.0	89.0	64.0	11148.8	301.4
December	20.0	13.3	81.2	59.2	13072.9	64.7
January	-	13.2	70.5	46.8	14963.4	8.6
February	-	13.3	75.2	42.0	1315.5	139.7
March	22.3	14.0	84.9	58.0	13002.4	166.2
April	-	14.9	89.8	60.9	11704.5	259.0
May	-	15.2	88.0	67.8	10820.1	244.5
June	-	12.2	89.3	60.1	105292.2	120.3
July	20.2	11.3	88.7	65.4	8151.7	18.2
August	21.3	11.5	86.4	56.4	10175.6	30.8

Appendix 2: Summary of Soil Analyses

	Experiment I (Season 1)			Experiment II
	0.15 cm	15-30 cm	0.15 cm	15.30 cm
pH in water	5.90	6.40	5.80	6.00
pH in 0.01 m $CaCL_2$	5.13	5.38	5.00	5.40
% Nitrogen	0.13	0.18	0.27	0.15
% Carbon	3.21	2.02	2.81	1.55
Mg (m/100g)	3.73	2.89	3.41	2.87
Ca (me/100g)	11.00	8.63	10.06	7.89
Na (me/100g)	0.43	0.87	0.45	0.79
K (me/100g)	1.81	1.47	1.50	1.44
P (mg P2O5/100g)	9.20	8.00	6.98	7.24
CEC (ME%)	24.00	18.00	22.53	20.34

Appendix 3a: ANOVA for plant height, number of leaves stem diameter, leaf area and leaf area index (First season)

Sour of error	Degree of freedom	Plant height	No. of leaves	Stem diameter	Leaf area	LAI
Replication	2	12.600	0.646	2.616	15206	0.047
Nitrogen (A)	3	124.248	11.833***	0.910**	32913***	0.119*
Error (A)	6	4.955	0.312	0.057	5861	0.019
Phosphorous (B)	3	118.094***	2.333	656.44***	34880***	0.050*
A and B	9	6.585 ns	0.463 ns	0.045 ns	8904**	0.018 ns
Error (B)	24	4.212	0.368	0.810	2842	0.010

Significances: ns (non significant), *(0.05), **(0.01), ***(0.001),

** - Significant at 5% level

Ns - non-significant

Appendix 3b: ANOVA for plant height, number of leaves, stem diameter, leaf area and leaf area index (LAI) (Second season)

Sour of error	Degree of freedom	Plant height	No. of leaves	Stem diameter	Leaf area	LAI
Replication	2	49.103	2.271	4.470	1672727	0.010
Nitrogen (A)	3	202.138***	9.055***	1.112	63350***	0.150***
Error (A)	6	11.192	0.410	0.664	10285	0.023
Phosphorous (B)	3	173.707***	2.500**	49.432***	34205*	0.075**
A and B	9	9.952 ns	0.29 ns	0.209 ns	5566 ns	0.015 ns
Error (B)	24	12.289	0.263	0.463	5309.722	0.011

Significances: ns (non significant, *(0.05), **(0.01), ***(0.001)

Appendix 4: AVOVA for total fresh and dry weights

	Degree of freedom	Fresh weight	Dry weight
Replication	2	0.018	0.003
Nitrogen (A)	3	0.320**	0.018**
Error (A)	6	0.016	0.001
Phosphorus (B)	3	0.393***	0.017***
A and B	9	0.019	0.003**
Error (b)	24	0.006	6.34x10

Significances ns (non - significant), *(0.05), **(0.01), ***(0,001)

Appendix 4b: ANOVA for yield

Source of error	Degree of freedom	Fresh weight
Replication	2	1851402.1
Nitrogen (A)	3	31975627,8***
Error (A)	6	1660571.5
Phosphorus	3	39305100.0***
A and B	9	1935935.2*
Error (B)	24	608734.7

Significances: *(0,05), **(0.01), ***(0.001)

Appendix 5b: ANOVA (analysis of variance) for crude fibre crude protein β-carotene

Source of error	Degree of freedom	Crude fibre	Crude Protein	β-carotene
Replication	2	0.061	7.90	0.006
Nitrogen (A)	3	7.422***	0.833 ns	0.453**
Error (A)	6	0.134	3.5130.004	
Phosphorus (B)	3	0.298*	0.780 ns	0.001 ns
A and B	9	0.386**	2.620 ns	0.005 ns

Error (B)	24	0.118	2.095	0.002

Significances: ns (non significant), *(0.05), **(0.01) **(0.001)

Appendix 6b: Analysis of variance for minerals

Source of error	Degree of freedom	Total minerals	Calcium	Iron	Phosphorus
Replication	2	0.309	0.005	13.121	0.0000
Nitrogen (A)	3	12.167*	0.2005**	1.963	0.0011 ns
Error (A)	6	12.117	0.012	2.634	0.0006
Phosphorus (B)	3	3.556 ns	0.386***	34.387**	0.0574***
A and B	9	3.213 ns	0.021	1.961	0.0002 ns
Error (B)	24	2.363	0.005	3.098	0.0003

Significances: ns (non significant), *(0.05), **(0.01) ***(0.00)

Appendix 7b: ANOVA table for total oxalates, soluble oxalates and nitrates

Source of error	Degree of freedom	Total oxalates	Soluble oxalates	Nitrate -N
Replication	2	0.026	0.012	21.141
Nitrogen (A)	3	0.770***	0.004ns	7786.4***
Error (A)	6	0.211	0.003	36.48
Phosphorus (B)	3	1.358***	0.005ns	37.242ns
A and B	9	0.114*	0.003ns	17.46ns
Error (B)	24	0.039	0.003	23.140

Significances: ns (non significant), *(0.05), **(0.01), ***(0.001)

Appendix 5: ANOVA for total minerals, calcium, iron and phosphorus

Source of error	Degree of freedom	Total minerals	Calcium	Iron	Phosphorus
Replication	2	0.436	0.005	13.156	3.33x10
Nitrogen (A)	3	10.624*	0.201**	1.965ns	$11.7x\ 10^{-4}$
Error (A)	6	1.351	0.012	2.626	6.3×16^{-4}
Phosphorus(B)	3	3.443ns	0.387***	34.457***	$57.5x10^{-3}$***
Error (B)	24	2.303	0.005	3.094	$3.67x10^{-4}$ns

A and B	g				

Significances: ns (non significant), *(0.05), **(0.01), ***(0,001)

Appendix 6: ANOVA for crude fibre, crude protein and β - carotene

Source of error	Degrees of freedom	Crude fibre	Crude protein	β-carotene
Replication	2	0.026	0.354	0.006
Nitrogen (A)	3	6.837***	0.827**	0.455**
Error (A)	6	0.153	0.084	0.004
Phosphorus(B)	3	0.282 ns	1.592	0.001 ns
A and B	9	0.627	0.257ns	0.005ns
Error (B)	24	0.111	0.152	0.002

Significant: ns (non-significance), *(0.05), **(0.01), ***(0.001)

Appendix 7. ANOVA table for oxalates and nitrates

Source of error	Degree of freedom	Total oxalates	Soluble oxalates	Nitrate - N
Replication	2	0.154	0.003	0.984
Nitrogen (4)	3	0.534**	0.015ns	5921.494***
Error (A)	6	0.053	0.006	18.482
Phosphorus	3	0.378***	0.018ns	69.809***
A and B	9	0.029ns	0.006ns	10.355 ns
Error (B)	24	0.025	0.006	7.689

Significances: ns (non significance), *(0.05), **(0.01), ***(0.001)

Appendix 6b: Analysis of variance for minerals

Source of error	Degree of freedom	Total minerals	Calcium	Iron	Phosphorus
Replication	2	0.309	0.005	13.121	0.0000
Nitrogen (A)	3	12.167*	0.2005**	1.963	0.0011ns
Error (A)	6	12.117	0.012	2.634	0.0006
Phosphorus(B)	3	3.556ns	0.386***	34.387**	0.0574
A and B	9	3.213ns	0.021	1.961	0.0002ns

Error	24	2.363	0.005	3.098	0.0003

Significances: ns (non significant), *(0.05), **(0.01), ***(0.00)

Appendix 7b: ANOVA table for total oxalates, soluble oxalates and nitrates

Source of error	Degree of freedom	Total oxalates	Soluble oxalates	Nitrate - N
Replication	2	0.026	0.012	21.141
Nitrogen (A)	3	0.770***	0.004ns	7786.4***
Error (A)	6	0.211	0.003	36.48
Phosphorus (B)	3	1.358***	0.005ns	37.242ns
A and B	9	0.114*	0.003ns	17.46ns
Error (B)	24	0.039	0.003	23.140

Significances: ns (non significant), *(0.05), **(0.01), ***(0.001)

Appendix 5: ANOVA for total minerals, calcium, iron and phosphorus

Source of error	Degree of freedom	Total minerals	Calcium	Iron	Phosphorus
Replication	2	0.436	0.005	13.156	$3.33x10^{-5}$
Nitrogen (A)	3	10.624*	0.201**	1.965ns	$11.7x10^{-4}$
Error (A)	6	1.351	0.012	2.626	$6.3x16^{-4}$
Phosphorus (B)	3	3.443ns	0.387***	34,457***	$57.5x10^{-3}$***
Error (B)	24	2.363	0.005	3.094	3.67×10^{-4}ns
A and B	g				

Significances: ns (non significant), *(0.05), **(0.01), ***(0.001)

Appendix 6: ANOVA for crude fibre, crude protein and β-carotene

Source of error	Degree of freedom	Crude fibre	Crude protein	β-Carotene
Replication	2	0.026	0.354	0.006
Nitrogen (A)	3	6.837***	0.827**	0.455**
Error (A)	6	0.153	0.084	0.004
Phosphorus(B)	3	0.282 ns	1.592	0.001ns
A and B	9	0.627***	0.257ns	0.005ns

Error (B)	24	0.111	0.152	0.002

Significant: ns (non significant), * (0.05), **(0.01), ***(0.001)

Appendix 7. ANOVA table for oxalates and nitrates

Source of error	Degree of freedom	Total oxalates	Soluble oxalates	Nitrate-N
Replication	2	0.154	0.003	0.984
Nitrogen (4)	3	0.534**	0.015ns	5921.494***
Error (A)	6	0.053	0.006	18.482
Phosphorus	3	0.378***	0.018ns	69.809***
A and B	9	0.029ns	0.006ns	10.355ns
Error (B)	24	0.025	0.006	7.689

Significances: ns (non significance), *(0.05), **(0.01), ***(0.001)