"THE EFFECTS OF NITROGEN RATES ON YIELD, QUALITY AND NODULATION, AND STORAGE TEMPERATURES ON WATER AND ASCORBIC ACID CHANGES IN COWPEA (*Vigna unguiculata* (L) Walp) LEAF VEGETABLE"

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DEPARTMENT OF CROP SCIENCE

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DEDICATION

To my family

Lois Kemunto
Rachel Ondicho

Collins Areba
Elvis Ondicho
and

Yucabeth Nyabeta

DECLARATION

I, Daniel Omwoyo Ondicho declare that this is my own work and has not been presented for a degree in any other University.

14/3/1991

Daniel Omwoyo Ondicho Date

This thesis has been submitted for examination with our approval as University supervisors.

5/7/91

Dr. J.A. Chweya Date

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ABSTRACT

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Two experiments were carried out between October and December 1988, and repeated between January and May 1989 at the Field Station, Faculty of Agriculture, Kabete campus, University of Nairobi, Kenya, to investigate the effect of four nitrogen rates (0, 60, 80 and 100 kg N ha^{-1}) on cowpea leaf yield, dry matter, nutrient composition, nitrate accumulation and root nodulation using two varieties (Vita-3 and K-80). Leaves from Vita-3 were also used for in storage experiments in which water and ascorbic acid changes were determined at room ($21\pm7^{o}C$) and refrigeration ($4\pm1^{o}C$) temperatures.

Results showed that application of N had no significant effect on fresh leaf yield, leaf dry matter, crude fibre, beta carotene, ascorbic acid, minerals and nitrate accumulation. The two varieties too, did not show significant differences in leaf nutrient composition.

Nitrogen application reduced root nodule weight per plant significantly but had no effect on nodule numbers per plant. Both varieties differed significantly in nodule numbers and weights per plant.

Leaf protein tended to increase with increased N-rates and was significant in one experiment in which it was 28.6% for cowpea that had received 100 kg N ha^{-1}. Both leaf ether extract and ash increased significantly with applied N. A few minerals such as K^{+} and Ca^{++} tended to increase with applied nitrogen though not significantly.

Laboratory experiments showed that cowpea leaves lo fresh weight, significantly at room and refrigeration storag Losses were,however,less drastic in refrigerated storage relati to room storage. Significant losses in ascorbic acid occured f cowpea leaves stored for three days at room temperature. Wh refrigerated, the vitamin, however,was not significantly altere

CHAPTER ONE

1. INTRODUCTION

Cowpea (<u>Vigna unguiculata</u> (L) Walp) production in Kenya ranks only second in importance as grain legumes to field beans. This crop is drought tolerant and adapted to diverse soils in different agro-ecological zones. The major producing districts include Machakos, Kitui, Meru, Embu, Kilifi, Taita / Taveta and Kwale (Anon.1986). Subsistence production of this crop, however, appears in many other districts of Kenya, mainly for supply of vegetable leaf.

In Kenya, cowpea is commonly grown as an intercrop with either maize, sorghum or millet. Rarely is cowpea grown as a monocrop except in roadside and backyard gardening where it is grown in pure stands for commercial seed and/or for leaf production. Because of the low water requirement of cowpea at some stages a year-round production of this vegetable is possible. Although the can be grown in the long and/or short rains, there is no particular fixed season whenit must be grown.

Cowpea is produced mainly for its protein-rich grains, popularly consumed with cereal foods. In Africa, however, grain yields are extremely low and range from 400 to 750 kg per hectare (Tindall, 1986). Exceptionally high yields of 2500-2900 ha^{-1} have however, been reported when all cultural practices are optimized (IITA, 1973).

Although the supply of cowpea leaf vegetable is secondary to grain production, the vegetable is becoming important in both urban and rural areas as an alternative to the common leafy vegetables such as cabbage, kale and spinach. In some marginal rainfall areas of Kenya, it is the only vegetable available during dry seasons. The leaves are picked from the plants during early growth so that they dont interfere with seed development after onset of flowers. It has, however, been suggested that defoliation should be light during pod development, to avoid reduction in grain yield (Enyi,1975). Young cowpea plants at three to six weeks of growth,also regularly appear in vegetable markets almost year round in many urban areas of Kenya, perhaps, due to their relatively rapid growth rates. Cowpea varieties in Kenya are either grown for grain or green leaf production. Where grain and leaf are desired dual purpose varieties of cowpea for supply these are becoming popular with Kenyan farmers (Anon., 1978). The important nutrients in cowpea leaf vegetable are mainly vitamins and minerals. Although the leaf protein content of cowpea is lower than grain protein its contribution to dietary protein in almost purely vegeterian diets is not negligible. Studies on cowpea leaf have indicated that vitamin C and provitamin A (carotenes) are abundant in this vegetable (Imungi and Potter, 1983). The vegetable is also reported to have high levels of minerals such as potassium, calcium, iron and magnesium (Imungi and Potter, 1983).

9ks

90ks

Charity

No information is available on the effect of nitrogen on nutrient composition of cowpea leaf vegetable for varieties grown in Kenya. This may perhaps be due to over emphasis in its grain legume research. Because cowpea is increasingly becoming a popular vegetable, research on the effect of fertilizer application on leaf nutrients may be of value.

Cowpea is a legume that obtains most of its nitrogen through symbiotic fixation. Studies on the effect of supplementary nitrogen application on legumes have been carried out yielding both positive and negative responses. Negative responses to applied nitrogen have been indicated in cowpea nodulation. High levels of nitrogen have been reported to reduce nodule size and numbers (Ham, et al.1967; Haque,et al.1980 and Miller, et al.1982). Scanty information is available on the effect of high rates of nitrogen on nodulation of cowpeas in Kenya.

Leafy vegetables have a very short post harvest shelf life because they wilt fast after detachment if/ when kept at high temperatures. The wilting is paralled by changes in nutrients such as vitamin C. In the tropics, one way perhaps of prolonging shelf life of leafy vegetables is by refrigeration. The effect of storage temperature is unknown and has received little attention in Kenya.

With this in mind the objectives of this study, were:

(i) To study the effect of nitrogen rates on cowpea leaf yield.

(ii) To study the effect of nitrogen rates on root nodule sizes and numbers.

(iii) To study the effect of nitrogen rates on nutritive value and nitrate accumulation in cowpea leaf vegetable.

(iv) To study the effect of storage temperature on changes in water and ascorbic acid changes in cowpea leaf vegetable.

CHAPTER TWO

2. LITERATURE REVIEW

2.1.1 The Cowpea

Cowpea (Vigna unguiculata (L) Walp) belongs to the family, Leguminosae, which is one of the largest families in plant kingdom (Janick, 1979). The crop belongs to the genus Vigna in the tribe Phaseoleae, and has about 160 species (Wien and Summerfield,1984). According to Verdcourt (1970), the species unguiculata can be subdivided into five subspecies: sesquipedalis, unguiculata and cylindrica which are domesticated; and, dekindtiana and menensis which are wild forms. Ng and Marechal (1985), however, suggest that the above subspecies are actually hybrids due to the ease with which they outcross.

The growth habit of cowpeas ranges from erect, determinate non-branching types, to prostrate or climbing, indeterminate profusely branching forms (Rachie and Rawal, 1976). In Kenya, cultivars grown mostly for leafy vegetables have prostrate and indeterminate growth, whereas those for grain are erect (Anon, 1978). The roots of cowpea consist of a well developed taproot, with laterals near the soil surface. The laterals usually have large nodules often in groups for symbiotic nitrogen fixation (Kay, 1979). Initiation of root nodules is reported to start as early as seven days after emergence (Pawar and Ghulghule,1980). The cowpea stems are cylindrical but slightly ribbed, twisting, sometimes hollow; and glaborous with scattered minute spinelets.

Pigmentation varies from none or localized purple at the nodes, to solid purple. Axillary buds may develop into branches or flower bearing peduncles (Rachie and Rawal, 1976). The leaves are alternate, trifoliate with one symmetrical terminal leaflet, and two asymmetrical leaflets. The leaf petioles vary from 3 to 25 cm each, and have a swollen pulvinus at the base of their petiolules. The inflorescence is an unbranched axillary raceme, bearing several flowers at the terminal end of the peduncles. The pods are vertically attached to the raceme, axis, and are either linear, curved or coiled in shape. Immature fruits may be green or purple, but upon maturity, they turn straw, deep purple or brown. Grains vary in shape, from square to kidney shape, and frequently, laterally compressed (Rachie and Rawal, 1976).

2.1.2 Nitrogen nutrition in cowpea plants

Nitrogen is an element required by plants for synthesis of amino acids, the building blocks of proteins (Reisenauer, 1978). Some leafy vegetable crops have a high demand for nitrogen, with yield increases recorded for additional amounts of the nutrient (Dimitrov et al.1971; Mija et al.1971; Edmond et al.1971). Plants absorb nitrogen from the soil as ammonium (NH^{4+}) or nitrate (NO_3^+) ions (Barber,1974). Nitrate nitrogen, however, is preferred over the ammonium nitrogen (Hewit, 1975).

Factors that have been reported to favour uptake of nitrate ion include high nitrate-ammonium ratio in soil solution (Fried et al.1965; Minotti et al.1969); and soil pH lower than 5 (Fried et al.1965). When nitrate nitrogen is absorbed by plant roots, it is reduced to nitrite and further to ammonia by nitrate and nitrite reductase enzymes, respectively (Huffaker and Rains, 1978). Ammonia derived from reduced nitrates or absorbed ammonium ions are then used in synthesis of amino acids (Webster, 1985). The activity of nitrate-nitrite reductases is not uniformly distributed within plant tissues or species. Cereals carry out most nitrate reductions in leaves whereas legumes have their high reductase activity in roots (Pate, 1978).

2.1.3 Nitrogen and legumes:

Most legumes, including cowpeas, are able to fix atmospheric nitrogen through root-rhizobium symbiosis (Chui, 1985). It has been estimated that effectively nodulated cowpeas get 80 - 90% of their total nitrogen requirement through symbiotic fixation (Eaglesham et al. 1977). Quantities of symbiotically fixed nitrogen in cowpea are reported to range between 150 to 354kg ha^{-1} (Agboola and Fayemi,1972; Nutman,1976; Eaglesham et al., 1977). Effective nodulation, however, depends on availability of the correct legume strain (Keya et al., 1982), presence of high soil organic matter (Agboola, 1978), and suitable soil temperatures (Day et al., 1978).

The response of legumes to applied nitrogen in terms of yield, has been reported to be supplementary and inhibitory. Agboola (1978) reported significant cowpea grain yield increases by application of 20 kg of N ha^{-1} in soils with 1% organic matter. Keya et al. (1982) reported that application of nitrogen to cowpea increased dry matter yields. The negative response of cowpea to applied nitrogen pertains to low yields, and reduced nodulation.

Application of more than 45 kg N ha^{-1} has been reported to inhibit nodule formation and subsequent nitrogen fixation for cowpea (Dart and Mercer, 1964; Ham et al., 1967; Haque et al., 1980; Miller, et al. 1982). Root nodule weight has a high correlation with the level of rhizobium nitrogenase activity, the enzyme that reduces fixed nitrogen to ammonium compounds (Ham et al.,1967). According to Lawn and Brun (1974),a significant reduction in soybean fresh root nodules per plant was reported when nitrogen (224 and 448 Kg ha^{-1} of Ammonium Nitrate) was applied. In the same study N-application reduced total and specific nitrogenase activity of root nodules. A study by Chui (1985) reported that application of 40 kg N ha^{-1} reduced root nodule number and weights for cowpea plants.

2.1.4 Nitrogen and leafy vegetable yield:

Extensive literature on the effect of nitrogen on yield of leafy vegetables exist, but most of it is targeted on none leguminous crops. Response to applied nitrogen can be measured directly as fresh yield or as dry matter yield. Most chemical analyses of plant vegetables, however, are expressed on the basis of dry matter (Epstein, 1971). A Germany study showed that application of 240 kg N ha^{-1} increased fresh spinach leaf yield by 500% (Habben and Fritz, 1971). In other studies in Poland, significant fresh leaf yields of cabbage and cauliflower over controls occured when each vegetable was supplied with 340 kg N ha^{-1}. Studies by Chweya (1982) with kale (Brassica oleracea var.acephala D.C) showed that application of 135 kg N ha^{-1} gave a significant leaf yields than controls. Nygaard (1984) reported a significant yield increase in cabbage heads that received 600 kg of N ha^{-1} over controls.

Information exists on the response of cowpea grain yield to applied nitrogen but not on leaf (Malik et al., 1973; Osiname, 1978; Rhodes, 1978; Agboola,1978; Viswanatham et al., 1978; Haque et al., 1980). Oomen and Grubben (1977) reported leaf yield of up to 2 tonnes ha^{-1} besides grains. Cowpeas that was topdressed with 40 kg N ha^{-1} gave a yield of 12 leaves per plant at 50 days after planting , but this was not significantly different from treatments without applied nitrogen (Chui, 1985). Varying reports on the effect of applied N on cowpea dry matter yields exist. For example Swami and pal (1974) in India reported a dry matter decrease in cowpea foliage due to application of an unspecified amount of nitrogen. Application of 90 kg and 60 kg N ha^{-1} on cowpeas, is reported to have given a significant and highly significant dry matter yields, respectively, over controls (Haque et al., 1980). Cowpeas fertilized with 40 kg N ha^{-1} in one Kenyan study had higher but non-significant dry matter yields over control (Chui, 1985).

Increase in dry matter due to applied N may perhaps, be attributed to increased deposition of assimilates, synthesized from the absorbed nitrogen.

2.2 Effect of nitrogen on nutritive quality of leafy vegetables:

2.2.1 Protein:

The protein contents of leaf vegetables has of recent attracted considerable research, interest in the light of the more expensive animal protein alternative (Pirie,1971; Graham and Telek, 1983). Work done by Nagy et al.(1978) singles out cowpea as having a high potential for extraction of leaf protein concentrate in the tropics where protein malnutrition is prevalent. On dry matter basis, cowpea leaf protein has been reported to be 28% (Pirie,1971); 28.5% (Imbamba, 1973); 19.5% (Graham and Telek,1983); and 33% (Imungi and Potter, 1983). Cowpea grains have an average protein content of 24 - 25%, on dry matter basis (Bressani, 1985). The contribution of cowpea leaf to dietary protein, therefore, can be significant.

In a number of leafy vegetables, nitrogen application appears to increase the level of proteins. Increase in leaf total protein, however, may not indicate a corresponding increase in quality, because the applied nitrogen can exist in several non protein nitrogen forms in plant leaves (Graham and Telek, 1983).

Leafy vegetables whose total protein change with nitrogen application, include, spinach (*Spinacea oleracea* (L) Walp), Cauliflower (*Brassica oleracea* var.*italica*), and cowpea (*Vigna unguiculata*) (Sasseville and Mills 1979; Habben and Fritz,1971; Pimpini,et al.1971; and Peavy and Greig,1972). Greenhouse experiments in which cowpea plants received 75 ppm and 100 ppm nitrogen reported leaf protein levels of 12.8 and 20.46% respectively, on dry matter basis (Sasseville and Mills, 1979). A higher leaf protein content of 33% (on dry matter basis) has been reported for cowpeas that received 536 ppm nitrogen (Imungi and Potter, 1983). Field experiments in which cowpea received 0 and 40 kg N ha^{-1} in Kenya, gave no significant difference in foliage protein content (Chui, 1985). Treatments supplied with nitrogen, however, had higher total protein than the control.

2.2.2 Crude fibre

Fibre in plants consists of cellulose, hemicelluloses pectins and lignins (Potter, 1986). Although lacking in food value, fibre promotes the health ofgastro-intestinal tract through prevention of constipation and disease attacks (Anon, 1973; Eastwood, 1974; Wolever and Jenkins,1982; Potter, 1986). Very high fibre contents in vegetables are undesirable, because they reduce digestibility and the quantity consumed (Nagy et al.1978).

According to Mengel (1979), less fibre is produced in vegetables supplied with nitrogen because the application favours synthesis of proteins, rather than carbohydrates. Nygaard (1984) found successive reductions in dietary fibre of cabbage supplied with up to 600 kg N ha^{-1}. Kale fertilized with 135 Kg N ha^{-1} tended to have lower dietary fibre contents compared to ontrols (Chweya, 1984).

2.2.3 Ether extract:

Ether extract consists of a heterogenous group of compounds that are insoluble in water but highly soluble in organic solvents (Berhane and Nganga,1975). These compounds include simple lipids, chlorophyll, cutin and carotenoids (Kramer and Kozlowski, 1979). Only carotenoids and chlorophyll have, however, been reported to increase with applied nitrogen (Mengel, 1979). There is no evidence that associates nitrogen with changes in the other components of ether extract. It is possible, however, that other ether extract components may change independently with stage of growth.

2.2.4 Beta carotene:

Carotenoids refer to a group of closely related plant constituents capable of conversion to vitamin A. These are largely hydrocarbons that exist as red, orange or yellow pigments in plants (Kramer and Kozlowski, 1979). Three types of carotenoids (alpha,beta and lambda) exist in plants, but beta carotenes consist of over 70% of total carotenoids in most plants (Vereecke and Maercke, 1979). Each molecule of beta carotene can be broken down to release two molecules of retinol, whereas alpha and lambda carotenes release a molecule each (Imungi, 1984). The importance of Vitamin A in human health lies in the promotion of vision by the rod and cone light perceptors of the retina of the eye (Wald and Hubbard,1970). The vitamin also prevents xerosis in membranes, and bone deformities (Moore, 1970). Vitamin A deficiency therefore,leads to xeropthalmia due to drying of the cornea; and night blindness due to low sensitivity of both rod and cone vision (Wald and Hubbard, 1970; Pirie, 1981). The recommended dietary allowance of vitamin A for infants and adult human beings ranges from 400 to 1000 micrograms of retinol equivalents per day respectively (Anon.1980). A retinol equivalent is equal to 1 microgram of retinol or 6 micrograms of beta carotene or 12 micrograms of other carotenoids having vitamin A activity (Potter, 1986). Statistics of FAO (1978) indicate that 100,000 to 300,000 people suffer from Vitamin A deficiency, while many more show mild symptoms of the disorder. A majority of these cases are in developing countries, where green vegetables can be easily cultivated (Pirie, 1981). The level of beta carotene in vegetables has been shown to vary with plant species (Nilsson, 1979); growth stage (Abe and Imbamba, 1977); processing and storage (Imungi and Potter, 1985); and nitrogen application (Fritz and Habben,1971; Nilsson, 1979). Nitrogen application increase carotenoids in green plants through the synthesis of more chloroplast organelles (Mengel, 1979). The effect of applied nitrogen on carotenoid levels in vegetables is, however, erratic. Fritz and Habben (1971) observed a 79% increase in the carotenoid contents of spinach leaves fertilized with 200 kg N ha^{-1} over controls without N.

In Sweden, there was some carotenoid increase though not signifcantly different in cabbage that was fertilized with 200 kg N ha^{-1} (Nilsson, 1979). The level of carotenoids for cowpea leaves is reported to be 69 mg (Maeda and Salunkhe, 1981); and 57 mg $100g^{-1}$ of dry weight (Imungi and Potter,1983).

2.2.5 Ascorbic Acid.

Ascorbic acid is the chemical name for vitamin C which is mainly found in green vegetables, fruits and tubers. Deficiency of Vitamin C leads to fragile capillary walls, bleeding of gums, loosening of teeth and bone joints, and abnormal formation of collagen protein (Potter, 1986). Studies done by Bhodal et al., (1969) report that green vegetables are the main suppliers of Vitamin C in the diets of most Kenyans. The recommended dietary allowances for infants and adults range from 35 to 60 mg per day, respectively (Anon. 1980).

Factors that have been reported to influence the level of ascorbic acid in leafy vegetables include the type of species (Werner,1957), growing stage (Abe and Imbamba, 1977), temperature during the growing period (Rosenfeld,1979), light during growth period (Mengel,1979), nitrogen Werner, 1957; Mengel, 1979) and storage condition (Nilsson,1979). Although information on the effect of nitrogen application on ascorbic acid levels in leafy vegetables is scanty, its effect on synthesis of the vitamin is known. According to Mengel (1979), glucuronic acid derived from glucose is a precursor of ascorbic acid.

Application of nitrogen, however, favours synthesis of amino acids more than glucose. It is, therefore, possible that application of N may reduce leaf ascorbic acid in vegetables. One of the earliest reports on reduction in leaf ascorbic acid of vegetables due to applied nitrogen is by Werner (1957). Nilsson (1979), however, reported non significant ascorbic acid changes in cabbage fertilized with 200 kg N ha^{-1} It is unknown whether this rate was sufficient to evoke a detectable response. The level of ascorbic acid in cowpea leaf is reported to be 310 mg (Oomen and Grubben, 1977),1706 mg (Maeda and Salunkhe, 1981), and 410 mg (Imungi and Potter,1983)

2.2.6. Minerals:

The levels of minerals in leafy vegetables may be regarded as a quality component (Mengel, 1979). Appendix 1 gives the recommended dietary allowances for selected minerals in human beings. Minerals whose levels have been reported to be high in cowpea leaves include potassium, calcium, magnesium, iron and manganese (Imungi and Potter, 1983). The level of minerals in leaf vegetables are, however, dynamic and change with fertilizer application (Mengel, 1979).

Potassium in man, is the principal intra-cellular cation that regulates osmotic pressure and pH equlibria, and a co-factor for some enzymes (Potter, 1986). Absorption of potassium by plants is luxurious (Mengel, 1979), but increases significantly with applied nitrogen (Werner, 1957). According to Werner (1957) and Hartman et al.(1986), leaf potassium levels increase significantly with nitrate nitrogen fertilization, whereas a suppression was observed from plants supplied with ammonium nitrogen.

Calcium is required in humans for bone and teeth formation, clotting of blood, enzyme functions and cell membrane integrity (Potter, 1986). The absorption and accumulation of calcium in vegetable leaves is influenced by plant species (Foy,1974), nitrate-ammonium nitrogen application (Claasen and Wilcox, 1974; Hartman et al.1986), and calcium : magnesium ratio in the soil (Lazaroff and Pitman, 1966).

Iron is a component of human blood haemoglobin that transports oxygen, and myoglobin which stores oxygen (Potter, 1986). Absorption of iron by plants is by active transport (Barber, 1984) and is influenced by levels of manganese, copper, and calcium in soil solutions (Shim and Vose, 1965). Iron deficiency in man leads to anaemia, a condition of low blood haemoglobin (Fairbanks, 1978).

There is no information available, on the effect of nitrogen application on the level of this nutrient in cowpea leaves.

Magnesium is required for muscle contraction in man,and acts as a co-factor of enzymes in Kreb's cycle (Aikawa, 1978). Manganese is important in bone structure and the functioning of nervous system enzymes. Information on the effect of nitrogen on the levels of magnesium and manganese in plants is scanty, but it is possible that application of nitrate-nitrogen may alter the levels of these nutrients in cowpea leaves.

2.3 The effect of storage temperature on water and ascorbic acid losses:

In the tropics vegetables are extremely perishable soon after harvest. This is due to high temperatures, fungal attack, and unsuitable methods of storage (FAO, 1969). The sale of leafy vegetables such as kale, spinach, lettuce and cowpeas in Kenya, is worsened by the fast onset of wilting, chlorosis and fungal attack. The looser forms of leafy vegetables become unsaleable after they lose 3-7% of their moisture (Robinson et al.,1975). Without improved storage methods, this loss can be attained within a few hours in the tropics, due to the high temperatures.Factors that increase water loss from leafy vegetables include high surface area to volume ratio,low leaf wax,and mechanical damage to tissues (Wills et al.1982). Burton (1982) suggested that wilting of leafy vegetables can be minimised through increasing storage humidity, reducing air movement, packaging or decreasing integument permeability to water loss. Loss of ascorbic acid in stored vegetables can be taken as a measure of the rate of deterioration loss in their general nutritional quality. Vitamin C in stored vegetables exist as ascorbic acid or dehydroascorbic acid both being anti scorbutic (Burton, 1982), or as oxidized forms such as gluconic and oxalic acid that have no Vitamin A activity (Ajayi et al., 1980).

In stored vegetables, ascorbic acid changes erratically. Mottram (1963) proposed that wilting, mechanical injury or exposure of cut vegetable surfaces to air lowers ascorbic acid significantly. Olliver (1967) observed that looser forms of green vegetables such as spinach lose ascorbic acid rapidly compared to tightly packed ones. These reports are in agreement with the findings of Nilson (1979) in which vitamin C of cabbage heads stored for 69 days remained unchanged. Ottoson (1979) reported that parsley and broccoli lost an average of 5-60 % of their vitamin C when stored for 0°C and 10°C, respectively.

2.4 Nitrate accumulation in vegetables:

The earliest and perhaps most authentic report of nitrate accumulation in plants was by Berthelot (1884) and has been the topic of numerous investigators since that time. Certain vegetables, because of a very efficient uptake system, inefficient reductive system,or unfavourable combination of both, tend to accumulate more nitrates than others (Maynard et al., 1976). Table 1 shows leaf, petiole and stem nitrate contents of some vegetables whose nitrate accumulation has been studied. Cabbage, lettuce and spinach top the list of such vegetables. Recent studies by Chweya (1984) and Abukutsa (1987) on kale and Kanampiu (1987) on collards indicate that the species accumulate nitrates. Although high soil nitrate levels are the principal causes of nitrate accumulation in vegetables, other environmental factors may contribute to this phenomemon. These factors include reduced light intensity or short photoperiods (Schuphan, et al., 1967; Cantliffe,1972), water stress and high relative humidity (Maynard and Barker, 1979), high soil temperatures (Cantliffe, 1972, Maynard et al.1976);and low carbon dioxide concentration (Maynard et al. 1976).

Accumulation of nitrates is not uniform within plant parts. It has for instance, been established that leaf petioles accumulate higher levels of nitrates than the blades (Pimpini et al.1970; Maynard et al.1976; Maynard and Barke

TABLE 1: Nitrate Nitrogen Concentration in fresh vegetables (Maynard, et al. , 1976)

Plant Part	Vegetable	Richardson (1907)	Wilson (1949) [a]	Jackson et al (1967)	Lee (1972)	Maynard and Baker (1972)
		ppm NO_3 -N, Fresh weight				
Leaves	Cabbage	46	43 - 276	72	207	165
	Lettuce	378	92	153	63	170
	Spinach	434	69 - 541 [b]	121	468	524
Petiole	Celery	340	743	641	226	535
	Rhubarb	-	230 - 1045	-	-	91
Stem	Asparagus	-	12	-	-	25

a= NO_3 - N concentration of expressed sap

b= Field sample

1979; Chweya, 1984; Abukutsa, 1987; and Kanampiu, 1987). There is variation in NO^{3-} accumulation even in cultivars of one species as is reported for smooth and savoyed spinach cultivars (Maynard and Barker, 1974). There is little information available on nitrate accumulation in tropical leafy vegetables (including cowpea), when supplied with high rates of nitrogen. This is perhaps due to the large number of tropical vegetable species whose research has not been undertaken .

2.5 Hazards of nitrates and nitrites to human health:

Most horticulturists use large quantities of N-fertilizers to promote fast vegetative growth of leafy vegetables. Studies on some vegetables indicate that they can accumulate nitrates when fertilized with high rates of nitrate-nitrogen (Maynardet al.,1976). The intake of vegetables that have accumulated nitrates may be harmful to humans, especially when there is gastro-intestinal disturbance (Mengel,1979). Toxicity to human is due to nitrites that arises from nitrate microbial reduction within the gastro- intestinal tract. This reduction is more likely in infants than adults, due to low acidity in their digestive tract, that allows the survival of coliforms and clostridial bacteria (Maynard et al.,1976).

Nitrites oxidizes ferrous iron in haemoglobin to the ferric form (methemoglobin) which cannot transport oxygen (Maynard et al.1976). Toxicity in man due to nitrite is shown by cyanosis, a bluish-purple colour of the skin and lips. This occurs when 15% haemoglobin is oxidized. Oxidation of 70% methemoglobin or more in human blood is fatal (Lee, 1970). The fatal doses for adults are reported as 15-70 mg of nitrate, or 20 mg of nitrite kg^{-1} of body weight (Burden, 1961; Lee, 1970). Nitrites and nitrosamines are linked to various types of cancer (Magee and Barnes 1956; Sen et al.1972; Tannaebaum et al.1978 and Cuzick and Babiker,1989).

CHAPTER THREE

3. MATERIALS AND METHODS

Two experiments were carried out between October and December 1988, and repeated between January and May 1989. The 1st and 2nd round of experiments are referred to as Season I and II, respectively.

3.1 Experimental site

The experiments were conducted at the Field Station, Faculty of Agriculture, Kabete Campus, University of Nairobi. The site stands at an altitude of 1940 metres above sea level, at latitude 1° 15' South and longitude 36° 44' East. It is located in a region that receive bimodal rainfall averaging 1000 mm annually. Long rains last over March to May, whereas short rains occur between October and December. Mean monthly maximum and minimum temperatures are 23 and 12°C' respectively. Table 2 shows some weather conditions at the site during the period of study.

The soil is a Nitosol according to FAO/UNESCO (1974) soil classification. It is derived from trachytic lava. The unit has deep and well drained soil profiles with thick acid topsoils that are resistant to erosion (Siderius, 1976). Soil samples were randomly taken within the experimental plots, at 0-15 and 15-30 cm

TABLE 2: Weather data at Kabete Field Station during the experimentation

		Rainfall (mm)	Average Relative Humidity (%)	Solar Radiation MJM^{-2}	Temperature (^{o}C) Max.	Min	Windrun (km/day)
			SEASON I				
1988	October	16.7	65	16.25	24.5	12.8	97.5
	November	105.3	71	15.07	22.1	13.5	112.2
	December	139.1	72	15.27	22.0	13.0	134.0
			SEASON II				
1989	January	134.6	67	16.57	23.3	13.0	75.1
	February	45.1	57	18.92	23.9	12.3	110.3
	March	93.1	61	18.87	24.9	13.7	87.9
	April	210.5	78	13.10	22.0	13.8	72.0
	May	497	74	14.38	22.0	13.6	25.9

soil samples were randomly taken within the experimental sites at 0-15 and 15-30cm depths. The samples were air-dried, ground and sieved for analyses of soil pH, organic carbon and total nitrogen. Results of chemical analyses of the soil from the two depths were averaged, because disc ploughing mixes the two during land preparation. Results of these analyses are shown in Table 3.

3.2 Seeds for planting

A dual purpose Vita-3 and the determinate K-80 varieties were used in this study. Seeds of Vita-3 were obtained from the Department of Crop Science, University of Nairobi whereas those of K-80 variety were obtained from the Dryland Research Station, Katumani, Machakos, Kenya. Both varieties give glossy and less hairy leaves.

3.3 Field experiments

3.3.1 Treatments and design:

Each of the two varieties was laid in a separate complete randomized block design experiment in which four nitrogen treatment (0, 60, 80 and 100 kg N ha^{-1}) were applied in three replicates. Only three replicates were used because the soil appeared uniformly fertile. Each treatment plot measured 3x6 metres. Three guard rows were maintained around each experimental plot.

Nutrient/Reaction	Season I	Sea
pH Water	6.14 (0.26)	5.9
Total Nitrogen (ug/g soil)	4124 (105)	307
Available NH_4^+ (ug/g soil)	531.10 (394)	436
Available NO_3^- (ug/g soil)	1587 (882)	179
Carbon %	3.99 (0.33)	2.8

( ) Standard deviation (N = 8)

$\frac{1}{4124}$ →

u $\frac{\cancel{10000}}{1}$

1000,000 ⇒ 100

$\frac{4124}{1000}$

The source of N was calcium ammonium nitrate (26% N), topdressed six weeks after sowing. Each treatment plot received a basal application of 117 Kg ha^{-1} triple super phosphosphate(46% P_2O_5) at sowing. Application of nitrogen was followed by one hour of irrigation, due to inadequacy of rainfall during the study period. Each irrigation continued until the soil was wetted to a depth of 15 cm.

3.3.2 Cultural Practices:

The soil was ploughed with a tractor and disc harrowed twice before sowing. Seeds were planted at an inter-row spacing of 60 cm and an intra-row spacing of 30 cm. Three seeds were sown in each hill and thinned to one plant two weeks after seedling emergence. Plots were kept weed free until the plants formed a weed growth suppressing canopy.

Pests recorded in the experiment plots were aphids (Aphis craccicora); foliage beetles (Ootheca mutabilis); cutworms; (Argotis segetum), moles and birds. The foliage pests were controlled by spraying with an organo phosphorus insecticide (Gusathion) whereas moles and birds were controlled by trapping and scaring, respectively. No diseases were observed during the experimentation.

3.3.3 Observations and data collection:

Treatment plots had five rows of twenty plants per row. Observations were taken from randomly selected plants in the second and fourth rows, to avoid the edge effect on plant growth. Ten plants were taken from the second rows which were cut at ground level and entirely defoliated at 56 days after planting (DAP) for the first leaf harvests. The roots of the same were carefully uprooted and washed in tapwater, after which their nodules were removed,counted and weighed while fresh.

The fourth row in all treatment plots were used for selection of ten plants which were tagged for two successive leaf harvests, at 56 and 77 days after planting. At 56 DAP these plants were defoliated such that only four leaves from the tip remained on the main and lateral branches of each plant. The same plants were similarly defoliated three weeks later (77 DAP). The weight of leaves from the two harvests were taken as total yield. After the last set of data was collected (77 DAP) the remaining plants were left to form flowers and seeds for later use.

3.4 <u>Storage experiments</u>:

Freshly harvested leaves were placed in labelled perforated polythene bags for storage at room and refrigeration temperatures.

3.4.1 Treatments and design:

Vita-3 cowpea Variety was used for storage experiments. Storage treatments consisted of room temperature ($21\pm7^{\circ}C$) and refrigeration temperature ($4\pm1^{\circ}C$). The experiments were laid in a complete randomised block design similar and based on the field treatments. Leaf samples from each field treatments were harvested in duplicates at 77 days after planting, and placed in labelled perforated polythene bags. The bags were then loosely arranged in three cartons for room temperature storage and the other three for refrigeration storage.

3.4.2 Observation and data collection:

Water loss was determined daily from fresh weight differences of samples at the two storage temperatures. Weighing was discontinued at the third and fourteenth day for room and refrigeration temperatures, respectively. Ascorbic acid determinations were done for samples kept under the two storage conditions at harvest, day 1 and 3 for room storage whereas for refrigeration, two more analyses were done at day 7 and 14.

3.5 Laboratory analyses:

Laboratory analyses were carried out on freshly harvested and dry ground leaf samples. Fresh leaf samples were taken from the various plots and analysed immediately, or stored in a refrigerator while awaiting analysis. Dry leaf samples were prepared from fresh leaf harvests that were oven dried at 70°C to constant weight and then ground using a laboratory hammer mill (Glen Creston 48 Grinder) so as to pass through a one millimetre sieve.

3.5.1 Determination of dry matter:

Dry matter was determined by the AOAC (1984) recommended method. Two grammes of fresh leaf were weighed in duplicate and placed in moisture-free dishes previously dried, cooled and tared. The samples were placed in ovens at 105°C and dried to constant weight. The weight differences were used in the calculation of dry matter.

3.5.2 Determinationof total ash:

Ash was determined by the AOAC (1984) recommended method. Two grams of leaf dry matter were placed in weighed porcelain ashing dishes that were moisture-free, through previous oven drying at 105°C. These dishes were held in a muffle furnace at 600 °C for at least 4 hours so as to burn off organic material. They were then cooled to room temperature in a dessicator and weighed. The weight of residues were converted to ash.

3.5.3 Determination of ether extract:

Ether extract was determined by the AOAC (1984) recommended method. Two grammes of leaf dry matter were weighed in clean soxhlet thimbles, and covered with absorbent cotton wool. The soxhlet thimbles with the samples were then extracted using di-ethyl ether for at least 12 hours. Each ether extract was transferred to 250 ml round-bottomed flasks previously weighed. The extracts were dried at 100° C, to evaporate all ether, cooled and weighed. The weight of the residue was calculated as a percent ether extract.

3.5.4 Determination of total protein:

Total protein was determined using the macro Kjeldahl method (AOAC, 1984). The percentage of total nitrogen was multiplied by the factor 6.25 to convert it to percentage total protein. One gramme of the dry sample was weighed in a 200 ml Kjeldah flasks, with a blank prepared without a leaf sample. Each flask carefully received 20 ml of concentrated sulphuric acid and a tablet of selenium catalyst, before placement on a Kjeldhal heating assembly. Heating was done gently until frothing subsided, then strongly with constant rotation of flasks until all organic material was digested. The flasks were left to cool at room temperature. Distilled water, zinc powder (anti-frothing agent) and concentrated sodium hydroxide were added before transfering to Kjeldhal distillation unit. The distillate was collected in a 50 ml flat bottomed flask containing 25 ml of 0.01 N HCl and three drops of methyl indicator. The quantity of nitrogen in the distillate was determined by titration with 0.01 N NaOH.

3.5.5 Determination of crude fibre:

Crude fibre in dried samples was determined by AOAC (1984) recommended method. Two grammes of dry sample were weighed and boiled alternatively in 1.25% NaOH and 1.25% H_2SO_4. After digestion the residue were transferred with glass wool to silica dishes for oven drying at 105°C to constant weights, cooled in a dessicator then weighed. The dish and residue were then placed in a muffle furnace and ignited at 600°C to constant weight. The difference in dish weight (after drying digested samples at 105°C to constant weight) and the weight of the same after ashing at 600°C was calculated as percent fibre of the sample.

3.5.6 Determination of vitamin C:

Vitamin C was determined as reduced ascorbic acid using a modified titrimetric method of Barakat et al. (1955). For both fresh and stored leaf samples 50 g of leaf were weighed, chopped and blended with 100 ml of 10% trichloro-acetic acid for at least 5 minutes. The slurry was filtered using Whatman No. 41 filter paper. Ten millimetres of the filtrate was taken in a 100 ml conical flask and mixed with 5 ml of 4% KI and 1 ml of starch indicator. This mixture was titrated with a standard solution of N-Bromosuccinimide to a faint violet/blue endpoint which persisted for at least 15 seconds. Calculation of reduced ascorbic acid was based on the following equation:

$$\text{Vitamin C} = V.C. \times \frac{176}{178} \text{ (mg)}$$

Where V = VolumeofN-Bromosucinimide solution (ml)

C = Concentration of N-Bromosucinimide solution (mg/ml). The results were expressed on dry matter basis.

3.5.7 Determination of beta carotene:

Beta-carotene was determined by the AOAC (1984) commended method. Two grams of fresh leaf werethoroughly chopped and ground in a mortar. Twenty millilitres of acetone were used to extract the beta carotene from the sample. The extraction was carried out until the acetone was colourless. The combined extracts were made to 100 ml with acetone. 25 ml of the extract was evaporated to near dryness in a rotary evaporator. A solution of 1 ml was made from the dried acetone extract using 40-60°C petroleum ether and eluted through a column chromatograph packed with silica gel. The column was eluted with 40-60°C petroleum ether. Beta carotene appeared as the frost yellow fraction which was collected in a 25 ml flask and made to volume using petroleum ether. Beta Carotene absorbance was read at 440 nm. The concentration of the carotenoid was calculated from a standard curve prepared from solutions of pure beta- carotene in petroleum spirit. Values were expressed as mg/100g on dry matter basis.

3.5.8 Determination of minerals:

Specific minerals were determined on an absorption spectrophotometer of Perkin Elmer Company, 1982 Model. One gramme of ground dried leaf sample was placed in a small beaker into which 10 ml of concentrated nitric acid was added and digested until production of NO_2 fumes ceased.

The sample was cooled and 2-4 ml of 70% $HClO_4$ added followed by evaporative heating. The sample was transferred to 50 ml flask and diluted to volume with distilled water. Determination of mineral concentrations were read using specified absorption wavelengths for each mineral.

3.5.9 Determination of nitrate nitrogen:

Nitrate-nitrogen was determined using a colorimetric method of Cataldo et al. (1975) with slight modification according to Chweya (1985). A sample of 0.1g was incubated in 10 ml of distilled water at 45°C for 1 hour, and filtered using Whatman No. 41 filter paper. To 0.2 ml of the filtrate in a test-tube was added 0.8 ml of 5% salicylic acid in concentrated sulphuric acid. This was mixed thoroughly and allowed to cool. To each test tube was added 19 ml of 2N sodium hydroxide, allowed to cool for 30 minutes and the absorbance read at 410 nm, against a sample blank. Nitrate nitrogen was determined using a standard curve prepared using potassium nitrate whose concentrations ranged from 12.5 to 150 milligram of nitrates per millilitre of the solution.

3.6 <u>Data analyses</u>:

Collected data were subjected to analysis of variance using methods described by Steel and Torrie (1981) and means separated using Duncan's multiple range test (Duncan, 1954).

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CHAPTER FOUR

4. RESULTS AND DISCUSSION.

4.1 Effect of nitrogen rates on fresh leaf yield

Nitrogen application had no significant effect on cowpea leaf yield. Table 4 gives leaf yield of cowpea harvsted at 56 DAP, and total yield for plants that were tagged and harvested twice at 56 and 77 DAP. An inconsistent change in leaf yield was observed as nitrogen rates were increased. There was a significant difference in leaf yield for single and total harvests. On average leaf yield of single harvests (56 DAP) were two-fifth that of total yield. Differences in varietal leaf yield within a growth season were not significant.

The findings of this study show that cowpea leaf yield is not influenced by nitrogen application. It appears that application of up to 100 Kg of N ha^{-1} had little influence on leaf yield, perhaps due to the large amount of nitrogen that rhizobia are capable of fixing. Quantities of symbiotically fixed nitrogen in cowpea are reported to range between 150 to 354 kg of N ha^{-1} (Agboola and Fayemi, 1972; Nutman, 1976; Eaglesham et al.1977). The highest average leaf yield corresponded to a potential production of 1.75 and 4.24 tons ha^{-1} for single and total harvests, respectively. A trend of depressed leaf yield appear associated with application of 100 kg of N ha^{-1}.

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Table 4: Cowpea fresh leaf yield tonnes/ha)

	SEASON I				SEASON II			
Variety	VITA-3		K-80		VITA-3		K-80	
Growth stage:	56 DAP	TOTAL	56 DAP	TOTAL	56 DAP	TOTAL	56 DAP	TOTAL
N-RATES Kg N/ha								
0	1.06	4.04	1.88	3.51	1.45	3.13	1.61	3.17
60	1.70	4.75	1.78	4.07	1.51	3.04	1.41	2.86
80	1.60	3.86	1.90	4.31	1.39	3.76	2.09	3.27
100	1.02	4.33	1.44	3.57	1.56	3.48	1.58	2.96
Mean	1.34	4.24	1.75	3.86	3.86	1.48	3.35	3.06

4 .2 The effect of nitrogen rates on leaf dry matter .

There was no significant effect of nitrogen application on leaf dry matter. The leaf dry matter during the experimentation is given in Table 5. Although the level of leaf dry matter was not significantly altered by application of nitrogen, it was, however, slightly higher in leaves harvested at 56 DAP than those at 77 DAP. Leaf dry matter contents for plants grown in season I were not significantly different from those of season II, although in the first they were lower. Varietal leaf dry matter differences were not significant.

Nitrogen application of upto 100 Kg ha^{-1} did not significantly alter leaf dry matter in cowpea. Similar findings have been reported by Chui (1988) for leaf dry matter of beans that were supplied with upto 130 Kg N ha^{-1}. It appears that although nitrogen contributes to synthesis of dry matter components applied nitrogen does not benefit cowpea. The findings of this study, however, are in sharp contrast to those of Haque et al. (1980), who found significant dry matter changes in whole cowpea seedlings that received 60 and 90kg N ha^{-1}. A higher N- rate was used in this study but cowpea leaf dry matter did not change significantly. The lack of response in cowpea with appllication of upto 100 kg N ha^{-1} imply that dry matter changes in leaves may be different from that of the whole plant.

Table 5 : Effect of nitrogen rates on (mean [a]) leaf dry matter

	SEASON I				SEASON II			
Variety	VITA - 3		K - 80		VITA-3		K-80	
Growth stage:	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Nitrogen (Kg N/ha)								
0	11.5	10.2	12.8	11.7	14.0	11.9	13.2	11.5
60	13.1	10.2	10.1	9.9	13.3	11.2	13.6	9.7
80	11.2	10.6	12.4	11.5	13.5	12.0	13.5	12.6
100	11.7	10.3	13.1	12.2	13.5	11.8	12.6	12.0
Mean [b]	11.9(1.3)	10.3(0.5)	12.1 (1.8)	10.3 (0.7)	13.6 (0.8)	11.7(1.3)	13.2(0.9)	11.4 (2.3)

b = mean N = 12

( ) standard deviation
DAP Days after planting

a = Mean leaf dry matter (g/100g fresh edible portions)

The difference in leaf dry matter of cowpea plants at 56 and 77 DAP may be attributed to the physiological maturity of leaves when harvested. Leaf harvests at 56 DAP were more mature and fully developed. Leaf harvests at 77 DAP, however,were young regrowths and relatively immature. The average leaf dry matter in cowpea plants was slightly lower in season I than in season II. This may be attributed to climatic variations during the two seasons. Differences in temperatures and rainfall in the two seasons may have caused the slight variations in leaf dry matter. The average monthly maximum temperatures for season I and II were 24.3 and 23.5°C; while average rainfall for the same was 87 and 196 mm, respectively.

The nutritional significance of dry matter in cowpea leaf vegetable, relates to provision of nutrients such as protein, lipids, organic acids and minerals. It also supplies cell wall material such as cellulose and lignin that contributes to dietary fibre (roughage). Although it may not be possible to know which of the dry matter component was high in cowpea leaf, harvests at 56 DAP had higher dry matter values than at 77 DAP. This may perhaps be due to the relatively more mature leaves that were harvested at 56 DAP relative to those at 77 DAP.

4.3 The effect of nitrogen on nodulation.

Application of nitrogen significantly affected nodule weight. The effect was significant in season I but a similar non-significant trend was noted in season II. The heaviest nodules were borne on plants that did not receive nitrogen. Table 6 shows that although root nodules from plants that received 60, 80 and 100 kg N ha^{-1} were not statisticaly different from one another, they were signicantly lower than those from controls without N. The number of root nodules per plant, however, were not significantly different at all nitrogen rates. The effect of applied N on cowpea root nodules is illustrated in Figure 1. Nitrogen application to cowpea plants decreased root nodule weight. Similar reports have been made for cowpea and other legumes that received nitrogen (Dart and Mercer, 1964; Ham et al. 1967; Haque et al. 1980; and Miller et al. 1982). According to Miller et al. (1982), nitrogen application to cowpea plants inhibits root nodule growth. Cowpeas,however, rely considerably on nitrogen fixation by root nodules and N application, therefore, may have adverse effects on the supply of the nutrient.

The reduction in root nodule weights due to applied N has important implications in cereal-cowpea intercropping. When cowpea and the cereal seeds are sown on the same hill

TABLE 6 : Effect of Nitrogen rates on mean[S] fresh nodule weight and number per

	SEASON I				SEASON II	
	VITA-3		K-80		vita-3	
Nitrogen (Kg/ha)	Weight	Numbers	Weight	Numbers	Weight	Numbers
0	145[a]	13	201[a]	26	157	5
60	59[b]	7	51[b]	21	75	6
80	48[b]	9	62[b]	22	51	6
100	66[b]	12	43[b]	19	74	6
Mean[t] + S.0	87(49)	10(5)	89(79)	22(6)	89(67)	6 (1)

S= Mean fresh nodule weight and numbers (N=3)

t= Mean fresh nodule weight and numbers (N=12)

( ) Standard deviation

Means followed by the same letter down the column are not signficiantly differe
using Duncan's Multiple Range.

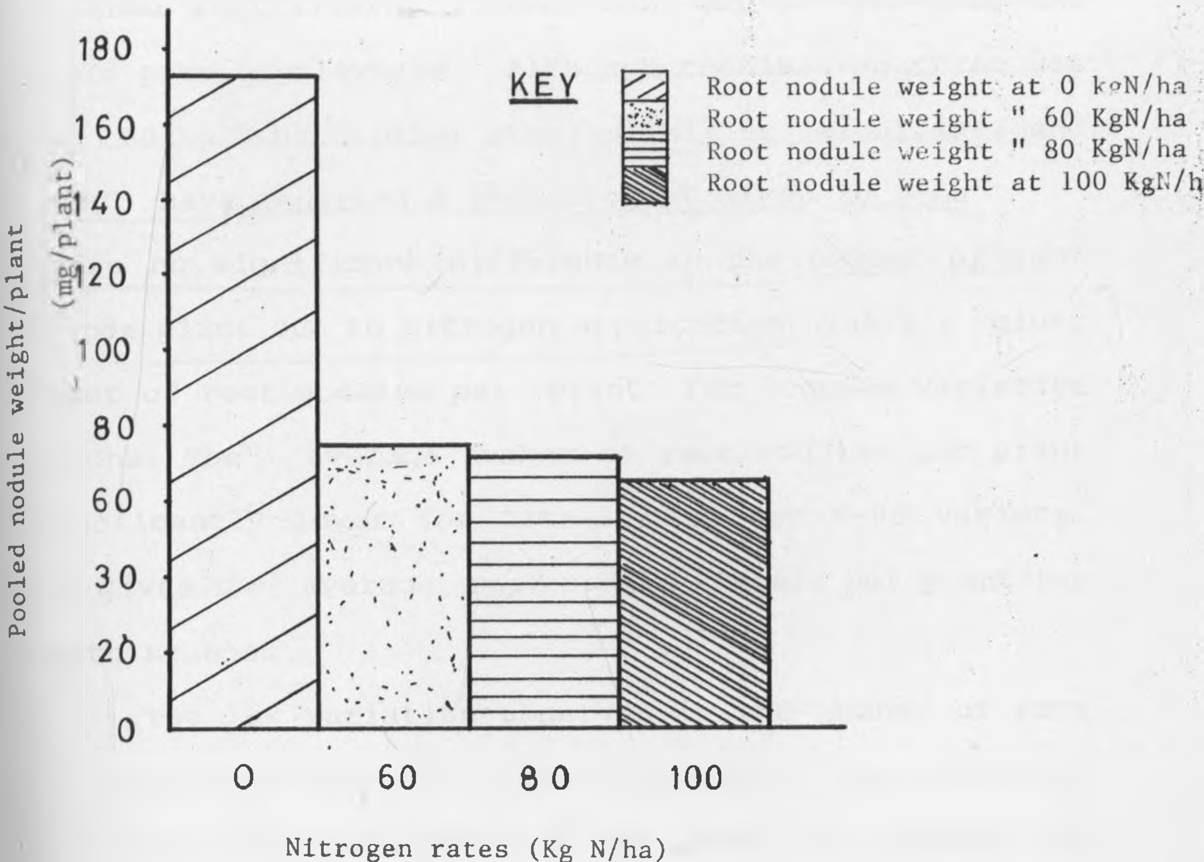

Fig. 1. Effect of nitrogen on fresh Cowpea nodule weight

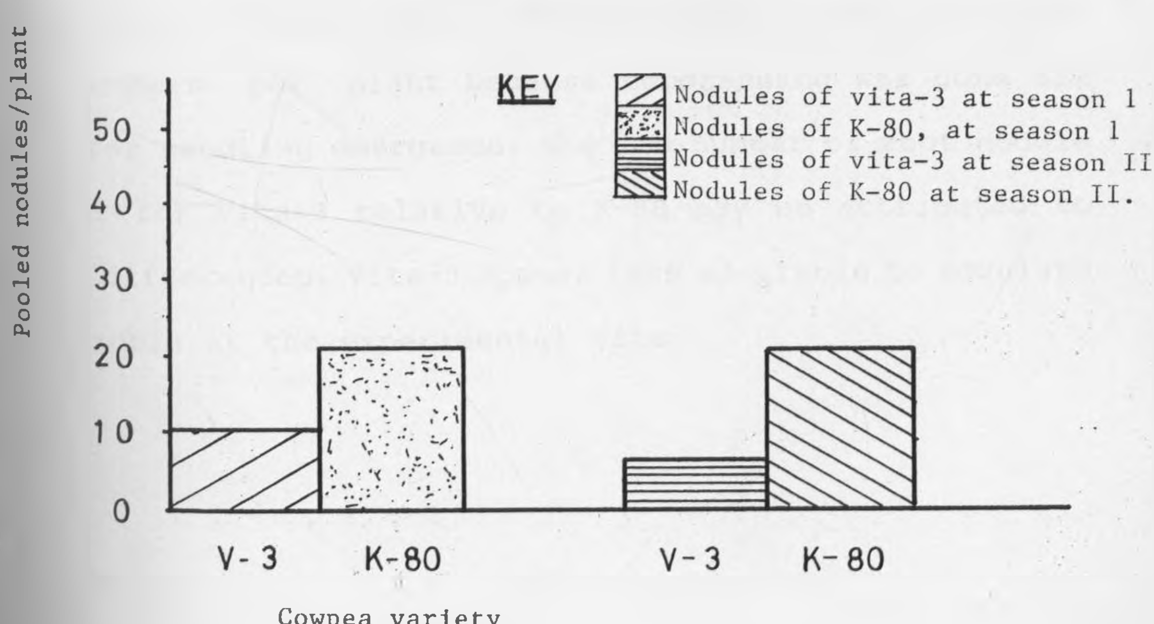

Fig. 2. Effect of nitrogen on fresh nodule numbers

with N-based fertilizers, nodulation may be affected and leaf yield possibly lowered. Although nodule reduction was noted at 60 Kg N ha^{-1} other studies (Miller et al.1982 and Chui, 1985) have reported a reduction at 11-50 kg N ha^{-1} There were no significant difference in the number of root nodules per plant due to nitrogen application. Table 6 gives the number of root nodules per plant for cowpea varieties and seasons. The average number of root nodules per plant was significantly lower for Vita-3 than for K-80 variety. Figure 2 gives the average root nodule numbers per plant for the growth seasons.

The low variation observed in the number of root nodules per plant for all nitrogen treaments, may mean that nodule initiation occured prior to the period of topdressing. According to Pawar and Ghulghule (1980), cowpea root nodule initiation starts seven days after seedling emergency. In the current study, N-application affected nodule sizes more than their numbers per plant because topdressing was done six weeks after seedling emergence. The low number of root nodule per plant for Vita-3 relative to K-80 may be attributed to varietal differences. Vita-3 appear less adaptable to nodulate with rhizobia at the experimental site.

Although one of the parents used to develop Vita-3 may have been of Kenya origin, the potential of the hybrid to freely nodulate with indigenous rhizobia as shown in this study seem to have declined. During the study, root nodules per plant for K-80 were more than twice those of Vita-3. The cause for the above variation is unknown but varietal preference in rhizobial nodulation may be a possible factor.

The effect of applied nitrogen on the absolute size of root nodules of cowpea plants is shown on Plates 1,2 and 3. One variety was used for this illustration, but the same effect was observed in the other. Nodules from plants grown in plots that had high N were tiny and in most cases dead. Such nodules sloughed off easily from the main and lateral roots. Controls however, had large nodules that were firmly attached to roots. Sections of root nodules from control plants revealed that they were alive and probably able to fix N. Plates 1, 2 and 3 show the relative sizes of the root nodules as nitrogen was varied. Nodule sizes tended to decrease with increased rates of nitrogen.

Plate 1: <u>The effect of application of 0 Kg of N per ha^{-1} on root nodule size and number</u>.

This Plate shows large nodules visible on both taproots and lateral roots of K-80 cowpea plants. Similar observations were noted on Vita-3 plants.

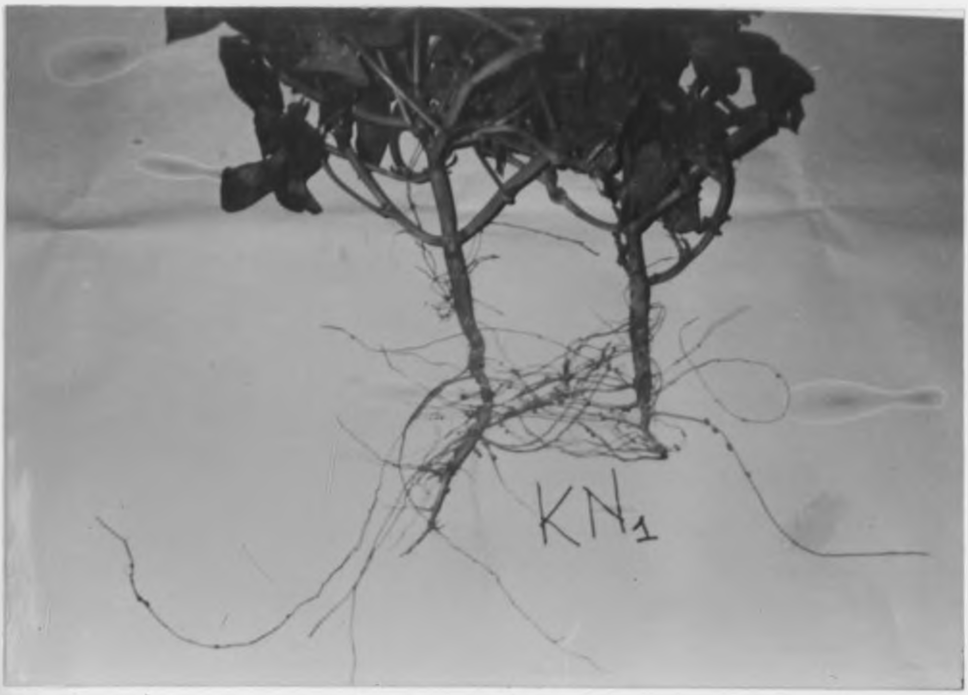

Plate 2: <u>The effect of application of 60 Kg of nitrogen per ha^{-1} on root nodule size and number</u>.

This plate shows fewer and smaller nodules on both taproots and laterals of cowpeas that received 60 Kg of N ha^{-1}

Plate 3: <u>The effect of application of 80 and 100 Kg N ha^{-1} on root nodule size and number</u>.

This plate shows that nodule size and number decreased even further at higher N-rates.

.4. The effect of nitrogen rates leaf nutritive quality.

.4.1 Total protein:

The effect of nitrogen application on leaf protein as significant only for K-80 cowpea variety, in season I at 7 DAP. Generally, there was an increase in leaf protein t higher rates of nitrogen. The highest average leaf protein as 29.9%. The protein of cowpea leaves at 77 DAP tended to e much higher than that at 56 DAP. There was little ifference in leaf protein of the two varieties. Table 7 ives the values of leaf protein from cowpea plants.

The significant increase in cowpea leaf protein due o applied nitrogen indicates that the crop synthesized more protein at higher N-rates. A similar trend in cowpea leaf protein has been reported from greenhouse experiments, in which application of 75 and 100 parts per million (ppm) of nitrogen, gave 12.8 and 20.46% leaf protein, respectively (Sasseville and Mills, 1979). Imungi and Potter (1983) reported a higher cowpea leaf protein (an average of 35%) by application of 536 ppm of nitrogen nutrient solution. Recent studies using N- rates of upto 40 kg N ha^{-1} for cowpea, gave non significant changes in leaf protein (Chui, 1985). It, therefore, appears that more leaf protein synthesis occur only at higher rates of applied nitrogen.

TABLE 7: Effect of Nitrogen rates on leaf protein (Mean^{S} total protein in grammes per 100 grammes of dry Matter

NITROGEN (Kg N/Ha)	SEASON I				SEASON II			
	VITA-3		K-80		VITA-3		K-80	
	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
0	18.69	27.2	19.4	24.2^{c}	21.8	28.4	20.61	28.5
60	19.91	27.3	18.86	25.3^{bc}	21.51	28.4	19.99	27.40
80	20.72	27.3	20.05	27.20^{ab}	20.20	29.30	20.61	28.0
100	20.38	27.6	21.59	28.60^{a}	21.53	28.50	22.61	29.90
Mean^{T}	19.92	27.3	19.98	26.45	20.99	28.65	21.46	20.22
	(1.6)	(0.9)	(2.3)	(1.9)	(1.4)	(0.7)	(1.7)	(1.54)

S = Mean for sample size of three (N=3)
T = Mean for sample size of twelve (N=12)
( ) = Standard deviation.

Means followed by same letter are not significantly different at 5% probability level using Duncan's New Multiple test.

The difference in leaf protein content of cowpea plants that received varied N-treatments is illustrated in Figure 3. Leaves that were harvested at 56 DAP were more mature than regrowths which were picked at 77 DAP. Younger leaves tend to have more protein than mature ones. Leaf harvests at 56 DAP were more mature than those re-harvested 3 weeks later (77DAP). This may be the cause of the higher leaf protein recorded for harvests at 56 relative to 77 DAP. According to Leopold and Kriedman (1985), young leaves are net importers of assimilates, whereas older ones export or retranslocate their assimilates. It is probable that leaf harvests of cowpea plants at 56 DAP had already started retranslocating their assimilates, hence their lower protein contents. Leaf harvests from the same plants at 77 DAP, were less mature, and this may have been the cause of their higher protein contents.

There appears to be slight increases in cowpea leaf protein due to applied N. The increase however, is too marginal to be worth of fertilizing.

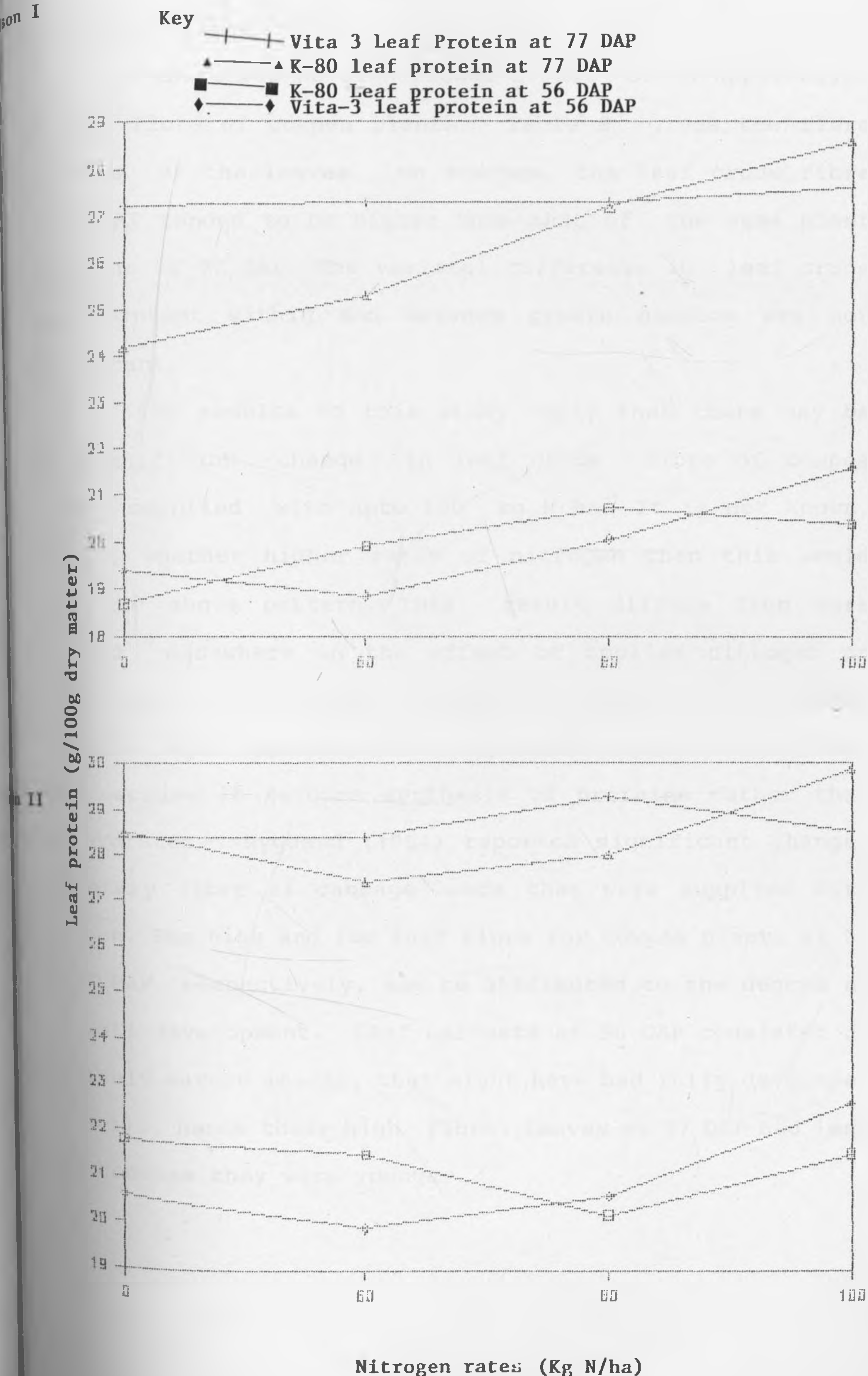

Fig 3 Effect of nitrogen rates on Cowpea leaf protein

4.4.2 Crude fibre

There was no significant effect of N-application on leaf fibre of cowpea plants. Table 8 gives the fibre contents of the leaves. On average, the leaf crude fibre at 56 DAP tended to be higher than that of the same plant regrowths at 77 DAP. The varietal difference in leaf crude fibre content within and between growth seasons was not significant.

The results of this study imply that there may be no significant change in leaf crude fibre of cowpea plants supplied with upto 100 kg N ha^{-1} It is not known, however, whether higher rates of nitrogen than this would alter the above pattern. This result differs from work reported elsewhere on the effect of applied nitrogen on crude fibre of some leafy vegetables. According to Mengel (1979) nitrogen application to vegetables leads to less leaf fibre because it favours synthesis of proteins rather than carbohydrates. Nygaard (1984) reported significant changes in dietary fibre of cabbage heads that were supplied with nitrogen. The high and low leaf fibre for cowpea plants at 56 and 77 DAP, respectively, may be attributed to the degree of cell wall development. Leaf harvests at 56 DAP consisted of relatively mature leaves, that might have had fully developed cell walls, hence their high fibre. Leaves at 77 DAP had less fibre because they were younger.

Table 8: Cowpea leaf Ether extract in g/100 grammes of dry matter

	SEASON I				SEASON II			
	VITA -3		K -80		VITA-3		K-80	
Growth stage	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Nitrogen (Kg N/ha)								
0	16.1	15.6	16.4	13.5	16.5	14.6	15.6	13.5
60	13.9	14.4	12.5	13.7	15.9	15.3	14.4	13.7
80	15.3	14.6	15.2	14.9	15.5	14.4	14.3	14.9
100	14.2	14.8	15.0	14.1	16.5	15.2	14.8	14.1
Mean (N=12)	16.2	16.8	14.8	14.0	16.1	14.9	14.8	14.0
	(1.06)	(1.3)	(2.0)	(0.9)	(1.02)	(0.8)	(1.3)	(0.9)

a= Means of three samples

( ) = standard deviation

4.4.3. Ether extract

There was a significant effect of nitrogen rates on ether extracts. Table 9 gives the levels of ether extract of leaf harvests. Highest values of leaf ether extract were recorded from plants that received 100 kg N ha^{-1}. A similar pattern was observed in other experimental treatments. Ether extract consists of a large number of organic compounds, some of whose levels may have increased due to application of nitrogen. Mengel (1979) for instance suggested that the levels of chlorophyll and carotenes increase in leafy vegetables due to application of nitrogen. It may be possible that the increase in ether extracts due to applied nitrogen was due to increase in the synthesis of such compounds.

Young leaves contain more protoplasmic components compared to mature ones (Leopold and Kriedmann 1985). Most of the ether extract components are protoplasmic in nature. Since leaves at 56 DAP were more mature than those at 77 DAP, the former may, therefore, have contained less protoplasmic but more cell wall components. This may be the reason for their lower ether extracts relative to those of leaves harvested at 77 DAP. On the other hand, it would be argued that partial defoliation at 56 DAP left fewer leaves per cowpea plant, that were turned into sinks of photosynthates which may have led to higher ether extract values at 77 DAP.

Table 9: Effect of nitrogen rates on ether extracts of cowpea leaves (Mean[p] Ether Extract in g/100 g dry matter)

	Season I				Season II			
Variety:	VITA-3		K-80		VITA-3		K-80	
Growth stage:	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Nitrogen (kg N/Ha)								
0	2.5	4.2^{b}	2.2^{b}	4.0^{c}	2.9	6.0^{a}	2.7	4.7^{b}
60	2.5	4.9^{ab}	3.0^{b}	6.0^{a}	2.8	4.4^{b}	2.5	5.0^{b}
80	2.8	4.6^{b}	2.9^{b}	4.8^{b}	3.1	6.6^{a}	2.4	5.5^{ab}
100	3.0	5.7^{a}	3.2^{a}	6.4^{a}	3.0	6.7^{a}	2.8	6.0^{a}
Mean[q]	2.7 (0.26)	4.85 (0.70)	2.83 (0.40)	5.73 (0.70)	2.95 (0.30)	5.95 (1.16)	2.62 (0.25)	5.30 (0.70)

p= Mean for three samples (N =3)

q= Mean for twelve sample N(=12)

() Standard deviation

Means followed by the same letter down the column are not significantly different using Duncan's New Multiple Range Test

Irrespective of the specific components that led to significant increases in leaf ether extracts of cowpea plants due to applied N, the increase per se has an important nutritional importance, because most of the components in ether extract are nutrients in human diet. It is probable therefore, that defoliation in cowpea leafy vegetable, improves the nutrient quality of subsequent regrowths.

Neither varieties nor seasons had any significant differences in cowpea leaf ether extracts. The optimal rate of N for cowpea leaf ether extracts in season I and II were 60 and 80 kg ha^{-1} respectively.

4.4.4 Total ash

Application of nitrogen significantly increased the leaf total ash contents of cowpea plants as shown in Table 10. Total ash tended to increase with higher rates of nitrogen. The leaves of plants at 56 DAP had lower leaf ash than of the same at 77 DAP. During both seasons significance in leaf ash content was detected only at 77 DAP.

Nitrogen application to developing cowpea plants increased total ash contents of the leaves. According to Robb and Pierpoint (1983), young leaves are net importers of mineral ions whereas mature ones retranslocate most of their cations. Total ash is predominantly made of mineral ions.

Table 10: Effect of nitrogen rates on total ash of cowpea leaves (g/100 g dry matter).

	Season I				Season II			
Variety:	VITA-3		K-80		VITA-3		K-80	
Growth stage:	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Nitrogen rate (kg N/Ha)								
0	7.30	11.40	6.0	6.30^{c}	8.70	11.30^{b}	10.4	12.70^{c}
60	7.40	10.60	7.60	11.40^{a}	10.01	13.72^{b}	9.04	17.10^{bc}
80	7.60	11.20	8.0	10.80^{a}	9.65	22.40^{a}	10.26	21.10^{ab}
100	9.00	18.8	8.20	8.90^{b}	9.40	16.90^{a}	10.46	21.70^{a}
Mean (N=12)	7.8 (0.79)	11.24 (1.75)	7.45 (1.0)	9.35 (2.4)	9.25 (0.95)	16.07 (4.9)	10.04 (1.13)	18.08 (4.47)

( )= Standard Deviation.

Means followed by the same letter down the column are not significantly different at 5% probability level using Duncan's Multiple Range Test.

Leaves from plants at 77 DAP had higher ash contents perhaps due to the accumulation of mineral ions in the few leaves that remained after the first defoliation. These leaves were probably net importers of mineral cations. It is possible that the CAN fertilizer use may have been accompanied by passive uptake of mineral cations. Such cations possibly accumulated in leaves of cowpea plants, resulting in higher leaf ash contents at 77 DAP growth stage.

The level of leaf ash contents in plants at 56 DAP were consistently lower than of the same at 77 DAP. Leaves harvested at 77 DAP had significantly higher levels of ash as N-rates were increased, probably due to accumulation of cations in fewer leaf sinks that remained after the first defoliation.

Leaves that were harvested at 56 DAP had lesser total ash contents perhaps due to retranslocation of their cations to meristematic tissues. Figure 4 shows the level of total ash in the leaves from the two growing seasons and the two growth stages. It can be seen that application of nitrogen had more influence on leaf ash at 77 than at 56 DAP. Varieties, however, did not exhibit much difference in their leaf ash at any one harvest. Increases in leaf ash

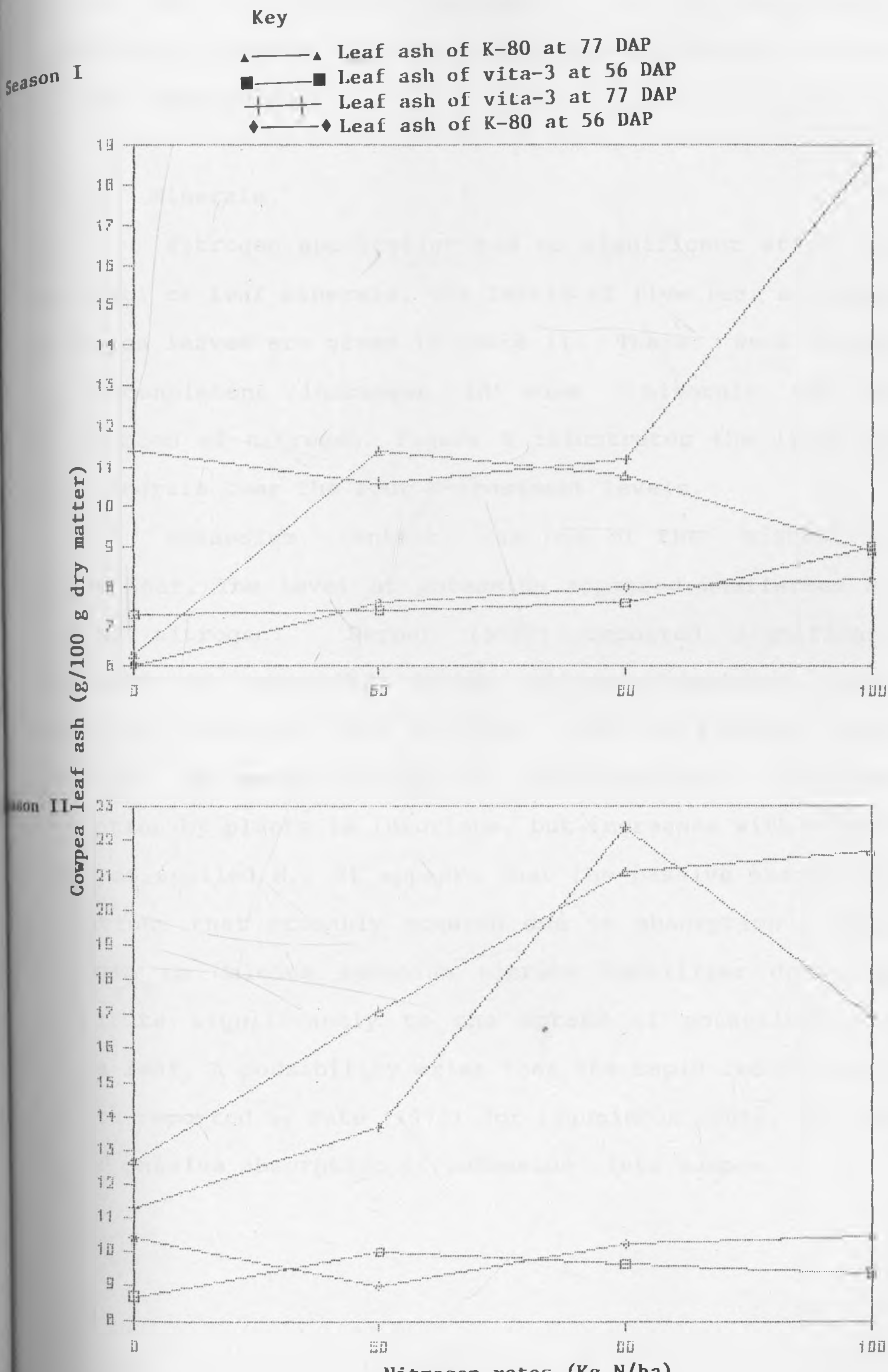

Fig 4: Effects of nitrogen rates on cowpea leaf ash

ontent due to applied nitrogen is of nutritional ignificance because it implies increase in minerals content f such vegetables.

.4.5 Minerals

Nitrogen application had no significant effect on he level of leaf minerals. The levels of five leaf minerals n cowpea leaves are given in Table 11. There were slight ut inconsistent increases in some minerals due to pplication of nitrogen. Figure 5 illustrates the level of eaf minerals over the four N-treatment levels.

Potassium content was one of the highest in owpea leaf. The level of potassium appeared unaffected by pplied nitrogen. Werner (1957) reported significant ncreases in potassium cation of non-leguminous leafy egetables supplied with nitrogen. Similar findings were eported by Mengel (1979) who indicated that potassium bsorption by plants is luxurious, but increases with higher ates of applied N. It appears that the passive absorption f cations that probably occured due to absorption of NO_3^- itrogen in Calcium Ammonium nitrate fertilizer does not ontribute significantly to the uptake of potassium into owpea leaf. A possibility exist that the rapid reduction of itrate reported by Pate (1973) for leguminous roots, did not avour passive absorption of potassium into cowpea.

Table 11: The effect of nitrogen rates on mineral contents of cowpea.

(Mean [a] levels of minerals in mg per 100 g Dry matter)

	Season I										Season II									
VARIETY:	VITA-3					K-80					VITA-3					K-80				
N-RATES (kg/Ha)	K^{+}	Ca^{++}	Mg^{++}	Fe^{++}	Mn^{++}	K^{+}	Ca^{++}	Mg^{++}	Fe^{++}	Mn^{++}	K^{++}	Ca^{++}	Mg^{++}	Fe^{++}	Mn^{++}	K^{+}	Ca^{++}	Mg^{++}	Fe^{++}	Mn^{++}
0	3017	2030	370	177	57	3417	2437	410	107	43	3110	3550	460	280	93	3426	3504	410	230	100
60	3500	2397	410	123	53	3283	2287	427	103	37	3017	3587	543	620	153	3450	3580	450	266	113
80	3500	2420	420	120	67	3371	2650	457	90	47	3170	3553	477	293	110	3445	3560	470	268	115
100	3453	2593	487	137	60	3467	2613	433	100	50	2927	3553	483	330	120	3490	3565	475	252	126
Mean	3362	2360	422	139	56	3371	2509	432	100	44	3055	3561	491	381	119	3453	3552	451	254	113
	(334)	(361)	(75)	(40)	(13)	(280)	(317)	(26)	(25)	(8)	(227)	(138)	(83)	(191)	(41)	(240)	(170)	(72)	(100)	(50)

a =Mean of three samples

() Standard deviation

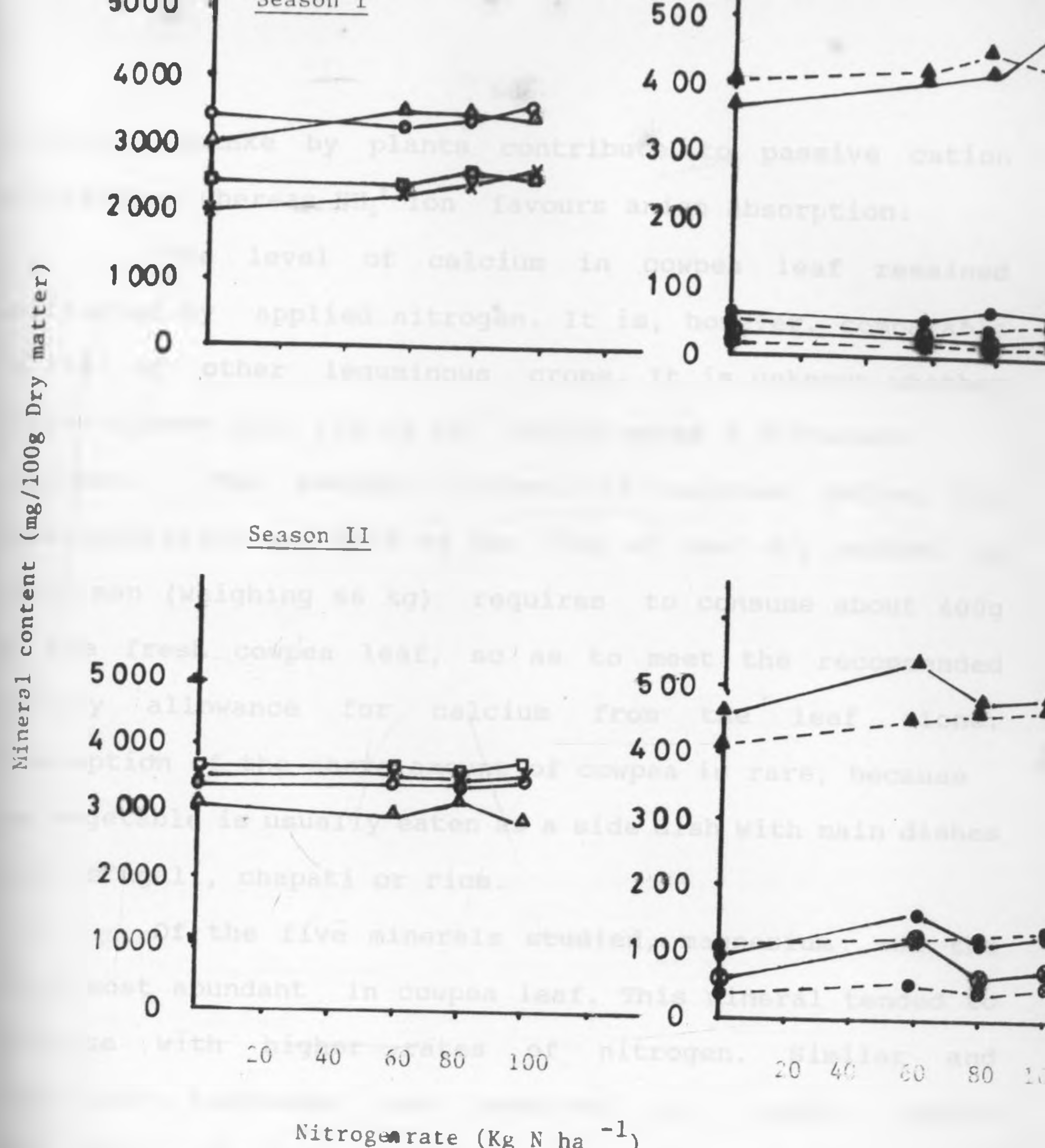

Fig.5. Effect of nitrogen on some mineral contents of Cowpea

K^{+} of vita-3

Ca^{++} of vita-3

K^{+} of K-80

Ca^{++} of K-80

Mg^{++} of vita-3

Fe^{++} of K-80

Mg^{++} of K-80

Fe^{++} of vita-3

Mn^{++} of vita-3

Mn^{++} of K - 80

Nitrate uptake by plants contribute to passive cation absorption whereas NH_4^+ ion favours anion absorption.

The level of calcium in cowpea leaf remained unaffected by applied nitrogen. It is, however, comparable to that of other leguminous crops. It is unknown whether levels higher than 100 kg ha^{-1} would evoke a different response. The average content of calcium during the experimentation was 2995 mg per 100g of leaf dry matter. An adult man (weighing 66 kg) requires to consume about 400g of the fresh cowpea leaf, so as to meet the recommended dietary allowance for calcium from the leaf alone. Consumption of the above amount of cowpea is rare, because the vegetable is usually eaten as a side dish with main dishes such as ugali, chapati or rice.

Of the five minerals studied, magnesium was the third most abundant in cowpea leaf. This mineral tended to increase with higher rates of nitrogen. Similar and significant increases are reported for tomato leaves that received higher rates of nitrogen (Hartman et al.,1986). According to Tisdale and Nelson (1956), nitrogen and magnesium form the chlorophyll molecule of green plants. Absorbed N probably contributed to more synthesis of chlorophyll molecules but there is no evidence to confirm that it significantly increased leaf Mg^{2+} contents.

The average content of magnesium in cowpea leaf was 449 mg per 100g of dry matter. To meet the recommended dietary allowance for calcium from cowpeas alone, an adult man would need to consume 1022 g of fresh leaves per day. This is impractical, but the deficit may be met by other food sources.

The levels of iron and manganese in cowpea leaf were low compared to the other three elements. These two nutrients exhibited inconsistent changes with applied nitrogen. Their absorption is probably not associated with uptake of nitrate nitrogen.

4.4.6 Ascorbic acid.

The effect of nitrogen application on cowpea leaf ascorbic acid was not significant (Table 12). Leaf ascorbic acid, however, tended to be lowerat 56 DAP than at 77 DAP. application to vegetables promotes protein, at the expense of ascorbic acid synthesis. In the current study, however, there were non-significant changes in leaf ascorbic acid

The findings of this study are of contrast to those reported on the effect of N-application on ascorbic acid synthesis. According to Mengel (1970), nitrogen with applied nitrogen. Slightly higher ascorbic acid values were observed at 77 DAP. This may be due to accumulation of the synthesized vitamin in the few leaves left after the first defoliation.

Table 12: Effect on nitrogen rates on ascorbic acid and B-Carotene in cowpea leaves

Mean P Ascorbic acid and Beta-Carotene in milligrammes per 100 grammes dry matter)

	Mean^{p} Ascorbic acid and B-Carotene.							
	Season I				Season II			
Nutrient:	Ascorbic Acid		B-Carotene		Ascorbic Acid		B-Carotene	
Growth stage	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Nitrogen (kg N/Ha)								
0	323	357	26	56	400	486	36	48
60	300	412	30	52	413	521	26	57
80	439	421	24	52	401	453	39	44
100	457	403	29	67	398	402	25	59
Mean^{q}	380 (123)	398 (45)	27 (7)	57 (17)	400 (90)	465 (100)	31 (17)	52 (18)

p = means of three samples
q = means of twelve samples
() = standard deviation
DAP = Davs after planting

Ascorbic acid is synthesised from glucoronic acid that is derived from glucose. The reduction in ascorbic acid due to applied N in vegetables is attributed to more protein synthesis at the expense of glucose. Since application of upto 100 kg N ha^{-1} to cowpeas had no significant change on the level of the vitamin, two suppositions may be made from the study. The first one is that the rates used were probably low to ellicit a significant response. Alternatively the crop under study may be self sustained with fixed N to the extent that an external source does not alter its metabolism.

The average ascorbic acid was 411 mg 100 g^{-1} of dry matter. This correspond to 49 mg ascorbic acid per 100 g of raw edible portions. According to Imungi and Potter (1983), cooked cowpea leaf retain an average of 13.2% of their total ascorbic acid . Assuming the same losses, cowpea leaf would supply 6.5 mg ascorbic acid for every 100 g of cooked edible portion consumed. From appendix 1 on Recommended Dietary Allowances (Anon 1980), a daily consumption of 538 and 923 g of cooked cowpea leaves would be needed for infants and adults respectively to meet the daily nutritional requirements of ascorbic acid. Although this consumption may not be possible, the potential contribution of cowpea leaf to the daily requirement of the vitamin in man cannot be totally ignored.

4.4.7. Beta carotene.

The effect of nitrogen application on beta carotene in cowpea leaf was not significant (Table 12). There were,however, higher levels of the carotenoid in leaves harvested at 77 DAP relative to those at 56 DAP. This differ from work reported by Fritz and Habben (1971) and Mengel (1979) that nitrogen application to vegetables increases the level of carotenoids. Synthesis of carotenoids requires two molecules of Acetyl-co-Enzyme A (Salisbury and Ross 1979). There is no nitrogen in the molecular structure of carotenoids. Since nitrogen application, however, promotes synthesis of more chlorophyll, photosynthesis may also have increased. This then made carbohydrates more available. Catabolism of carbohydrates generate Acetyl-Co-Enzyme A, a precursor of beta carotene. The lack of change statistically in beta carotene during this study implies that the crop may not have benefited from applied nitrogen with respect to this constituent. Higher levels of beta carotene observed at 77 relative to 56 DAP, suggest that the pro-vitamin A increased as the leaf matured. A similar observation is reported for leafy vegetables that were harvested at increasingly mature growth stages (Abe and Imbamba,1970). The increases may have been due to acummulation of synthesised carotenoids as plant leaves matured.

.8. Nitrate Accumulation.

Nitrogen application had no effect on nitrate mulation in leaves of cowpea plants. Table 13 shows the els of leaf nitrates during the study. NO_3^- nitrogen was her in leaves at 56 DAP relative to those at 77 DAP. The rage NO_{3}. nitrogen range in cowpea leaf was 23- 86 parts million (ppm). This shows that cowpea leaf does not mulate NO_3^- nitrogen when supplied with upto 100kg N ha^{-1}. ever, at 100 kg N the level of leaf nitrate nitrogen was sistently higher than for lower rates.

The level of the anion decreased substantially when ves were analysed at 77 DAP, perhaps due to leaching of the on, or, reduction and incorporation of absorbed nitrate tissue proteins. The findings of this study therefore, that cowpea is not a high accumulator ofNO_3^- nitrogen.

. The effect of room and refrigeration storage on water and ascorbic acid losses..

.1 Water loss.

There was a significant water loss at both room and igeration storage temperatures. The effect of storing a leaves at either room temperature or in a refri-

Table 13: Effect of nitrogen rates on nitrate accumulation in cowpea leaves

(NO_3^--Nitrogen in mg per 100 g Dry matter).

	Season I				Season II			
Variety:	VITA-3		K-80		VITA-3		K-80	
Growth stage:	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
N-Rate (kg/Ha)								
0	61	15	59	49	43	39	46	38
60	68	18	73	49	60	38	56	38
80	71	22	74	50	52	49	63	50
100	72	23	83	53	66	49	70	62
Mean (N=12)	68	19	72	50	54	44	59	47
	(11)	(9)	(13)	(15)	12	11	15	14

( ) = Standard Deviation

on water loss is shown in Table 14. Figure 6 the rate of water under the two storage ures. The average water loss was 17.3 and 8.11 for refrigeration temperatures, respectively. It is Figure 6) that water loss was more rapid in leaves t room than at refrigeration temperature. Leaves temperature storage turned chlorotic by the third eas those refrigerated remained green upto fourteen torage.

The results of this study show that cowpea leaves er leafy vegetables lose water and quality rapidly torage. Robinson et al. (1983) reported that 3-7 % oss in leafy vegetables makes them unsaleable. , a common problem in vegetables stored at room ure may be attributed to chlorophyll breakdown. The is thought to be hastened by production of gas.

e persistency of the green colour for cowpea leaves geration temperature may be due to reduced rates of cal processes, especially those that are catabolic. uction, therefore, either lowers the rate of yll breakdown, or decreases ethylene production. refrigeration is an expensive storage method, it is e-less quite appropriate in prolonging the freshness leaf vegetable.

& water loss and mean Ascorbic acid content/mg/100g)

	SEASON I				SEASON II			
	Room Storage		Refrigeration		Room Storage		Refrigeration	
	% wt loss	vit. C changes	% wt loss	Vit. C changes	% wt loss	Vit.C changes	% wt loss	Vit C chamges
Storage period								
Day 0	0((0)^{b}	398(45)^{a}	0(0)^{C}	398(45)	0(0)^{b}	465(100)^{a}	0(0)^{c}	465(100)
Day 1	4.8(1.6)^{b}	329(42)^{a}	1.6(.7)^{bc}	------	6.2(1.2)^{b}	387(80)^{a}	1.9(1.5)^{c}	------
Day 2	22.5(3.5)^{a}	-------	5.1(1.1)^{bc}	------	18.5 (4)^{a}	-------	3.4 (1)^{bc}	------
Day 3	35(5)^{a}	169(24)^{b}	9.8(1)^{ab}	378(43)	27(5)^{b}	220(49)^{b}	6.7(.8)^{abc}	341(109)
Day 7	------	-------	15.5(4.8)^{a}	372(41)	-----	-------	10.2(3.5)^{ab}	327(97)
Day 14	----	-------	16.4(3.5)^{a}	368(40)	------	-------	12.5(2.4)^{a}	323(96)

( ) refers to standard deviation

Means followed by the same letter down the column are not significant different from each other at 5% probability level using Duncan's Multiple Range Test.

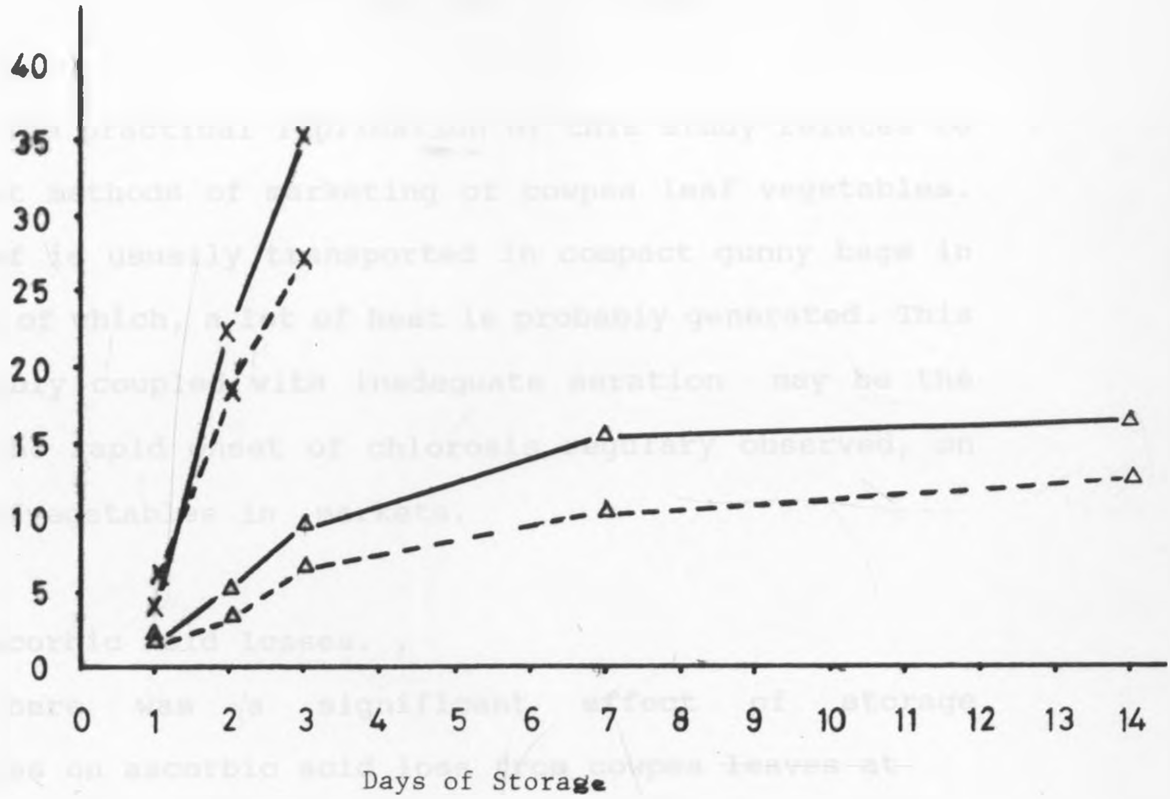

Fig. 6. Effect of room and refrigeration storage on water loss

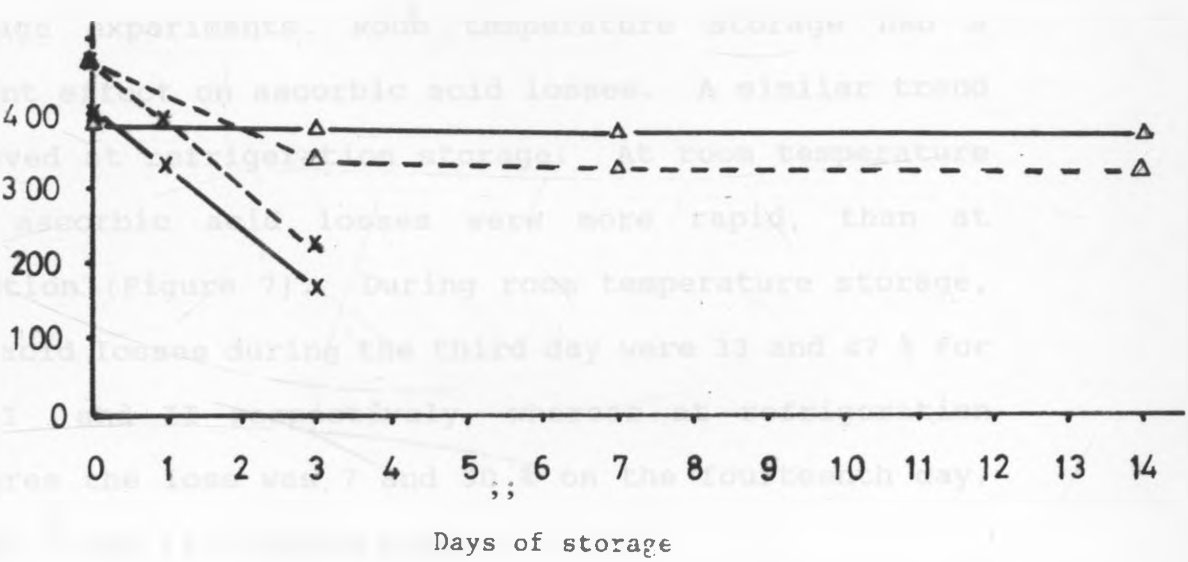

Fig. 7. Effect of room and refrigeration storage on vitamin C losses

X——X Water loss at room temperature.

X- - -X Vitamin C loss at room temperature.

△——△ Water loss at refrigeration temperature

△- - -△ Vitamin C loss at refrigeration temperature.

The practical implication of this study relates to current methods of marketing of cowpea leaf vegetables. pea leaf is usually transported in compact gunny bags in course of which, a lot of heat is probably generated. This t probably coupled with inadequate aeration may be the se of the rapid onset of chlorosis regulary observed, on pea leafvegetables in markets.

.2. Ascorbic acid losses.

There was a significant effect of storage peratures on ascorbic acid loss from cowpea leaves at n room and refrigeration storage temperatures. Table 14 es the level of ascorbic acid loss from cowpea leaf during storage experiments. Room temperature storage had a nificant effect on ascorbic acid losses. A similar trend observed at refrigeration storage. At room temperature rage, ascorbic acid losses were more rapid, than at rigeration (Figure 7). During room temperature storage, orbic acid losses during the third day were 33 and 47 % for son I and II respectively, whereas at refrigeration peratures the loss was 7 and 30 % on the fourteenth day, season I and II, respectively.

The findings of this study indicate that cowpea leaf ; a short shelf-life under room temperature storage, ·ing which ascorbic acid undergoes significant losses. se findings are in agreement with observations made by iver (1967), that looser forms of green vegetables lose orbic acid rapidly during storage. During the experi- ntation, higher ascorbic acid losses were associated with h water losses. Although the effect of water loss on corbic acid in vegetables is unknown, it is possible that lting exposed leaf tissues to air, which then increased idation of the vitamin.

There was no significant change in ascorbic acid of pea leaf at refrigeration storage. This perhaps, was due a reduction in bio-chemical processes in leaf tissues at e low temperatures. Stability of ascorbic acid at figeration storage has been reported for other leaf getables (Nilson,1979;and Otosson,1979).

CHAPTER FIVE

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

.1 Conclusions.

The effect of applied nitrogen on leaf dry matter nd yield was not significant. Little variation was observed mong N treatments . It may be concluded that the supply of itrogen through rhizobium fixation in Kabete is sufficient or cowpeas to grow without additional nitrogen. Most leafy egetables are known to increase yield with applied nitrogen. he findings of this study, however, show that cowpeas may ot benefit from additional nitrogen if leaf yield is the nly objective for fertilizing.

Nodulation was significantly influenced by itrogen application. All rates of applied nitrogen reduced odulation in comparison with controls. Unless the soil is eficient in suitable rhizobia it appears uneconomical to opdress cowpeas with nitrogen. If,however, high quality leaf egetable is the main aim of producing a cowpea crop, it may e necessary to suppliment the fixed nitrogen with an external ource . Application of nitrogen influenced some nutritive omponents of cowpea leaf vegetables. Leaf protein, ether xtract and total ash contents increased signicantly with igher rates of nitrogen whereas, crude fibre ascorbic acid nd beta carotene ined almost unaltered. Minerals did not alter ificantly with applied nitrogen. It can be concluded that a few leaf nutrients are altered by nitrogen applicatio

There was no evidence from this study that cowpea be among the nitrate accumulators if fertilized with N. is possible that cowpeas have high nitrate- reductase ivities that enable them to have negligible leaf NO_3^- rogen accumulation.

Storage experiments showed that significant water occured at both room and refrigeration storage. The ter method of storage, however, had lesser water loss from vegetable. The water loss in cowpea leaves may be due the thin blade and the looseness of the leaf,that promoted movement of water from tissues to the surrounding air. loss in weight at refrigeration storage was rather sual,but may be due to evaporation caused air movement in coldroom. Ascorbic acid decreased significantly at room perature but remained relatively constant at refrigeration rage. It is possible that the vitamin in cowpea sold by vegetable vendors in open air markets get quite low due hih prevailing temperatures.

Suggestions for further research

This study was conducted under soils rich in organic ter and probably indigenous rhizobia. It would be of erest to establish how the crop grown would respond at ilar N in marginal rainfall areas where cowpea is ularly grown.

Although the effect of nitrogen alone has been sidered in this study, it is known that other elements are uired for normal growth of all crops. It may therefore be thwhile to study the effect of a combination of N-P-K tilisers on growth ,yield and nutritive quality of cowpea ves. The dual purpose cultivars used in these experiments e a lesser leaf yield compared to those exclusively for f. The latter produce leaves for a relatively longer period ause they are indeterminate. A study on the effect of N lication on such varieties is likely to give usefull dings as to whether the crop really need any supplimentary rces of the nutrient.

Nodulation as reported in this study was only fined to indigenous soil rhizobia. The presence of suitable ains of indigenous rhizobia to nodulate with the many tivars of cowpea currently developed through breeding can ,however be gauranteed. Studies therefore, need to be ertaken on peat based inoculants

r cowpea so that inoculation programmes can be effecte l
ere necessary.

The findings on nitrate accumulation in this stud, quire further research especially with leafy cowpea ltivars whose response to nitrogen in form of vegetative owth is high. Higher levels of nitrogen would probably be ed than those for this study. Since organic matter also lease substantial quantities of nitrates during trification, it is justifiable to include manure as one of e treatments when determining nitrate accumulation in wpea plants.

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APPENDIX 1: RECOMMENDED DIETARY ALLOWANCES FOR SELECTED NUTRIENTS (Anno. 1980)

Category	Age (Years)	Weight (Kg)	Protein (g)	Vitamin A[a] (μ R.E)	Vitamin C (mg)	Calcium (mg)	Magnesium (mg)	Iron (mg)	Potessium (mg)	Manganese (mg)
Infants	.0-0.5	6	kg x 2.2	420	35	360	50	10	350-925	.5-1.0
	.5-1.0	9	kg x 2.0	400	35	540	70	15	425-1275	.5-.7
Children	1-3	13	23	400	45	800	150	15	550-1650	.7-1.0
	4-6	20	30	500	45	800	200	10	775-2325	1.0-1.5
	7-10	26	34	700	45	800	250	10	1000-3000	1.5-2.0
Males	15-18	66	56	1000	60	1200	350	18	1525-4575	2.0-3.0
	19-22	70	56	1000	60	800	350	10	1875-5625	2.5-5
	23-50	70	56	1000	60	800	350	10	1875-5685	2.5-5
	51+	70	56	1000	60	800	350	10	1875-5685	2.5-5
Females	11-14	45	46	800	50	1200	300	18	1525-4575	2.0-3.0
	15-18	55	46	800	60	1200	300	18	1525-4575	2.5-5.0
	19-22	55	44	800	60	800	300	18	1875-5625	2.5-5.0
	51+	55	44	800	60	800	300	10	1875-5525	2.5-5.0

[a] Retinol equivalents: 1 retinol equivalent = μg retinol or 6 μg B-carotene

For

APPENDIX 2: ANOVA FOE EFFECT OF NITROGEN RATES ON FRESH ROOT NODULE WEIGHT AND NUMBERS:

Mean sum of squares

Source of variation	Degree of freedom	SEASON I				SEASON II			
		VITA-3		K-80		VITA-3		K-80	
		Weight	No.	Weight	No.	Weight	No.	Weight	No.
N-Rates	3	0.148*	2219.4 n.s	1.68*	3334 n.s	0.65 n.s	2567.5 n.s	0.142 n.s	89.6 n.s
Blocks	2	0.028	2868.1	.067	5188	0.42	11161	.104	254.3
Error	6	0.026	2242.1	.295	3509	0.35	899	.113	226.9

* Significant at 5% probability level

n.s. not significant.

APPENDIX 3: ANOVA FOR EFFECT OF NITROGEN RATES ON COWPEA LEAF PROTEIN

Mean sum of squares

SEASON I

		VITA-3		K-80		VITA-3
		56 DAP	77 DAP	56 DAP	77 DAP	56 DAP
Source	d.f					
N-rates	3	2.38 n.s	0.11 n.s	4.10 n.s	9.57	1.94 n.s
Blocks	2	1.61	0.27	0.26	0.33	1.56
Error	6	2.87	1.35	7.60	1.76	2.19

* = Significant at 5% probability level using Duncan's Multiple Range Test.

n.s = Not significant.

APPENDIX 4: AVONA FOR EFFECT OF NITROGEN RATES ON COWPEA LEAF ETHER EXTRACT:

Mean sum of squares

		SEASON I				SEASON II			
	VARIETY:	VITA-3		K-80		VITA-3		K-80	
	GROWTH STAGE:	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Source	Degree of freedom								
N-rates	3	0.12 n.s	1.26*	0.487**	1.480**	0.050 n.s	3.295*	0.13 n.s	1.03 n.
Blocks	2	0.083	0.238	0.010	0.195	0.095	0.60	0.111	0.29
Errors	6	0.037	0.201	0.041	0.075	0.087	0.61	0.035	0.29

n.s = Not significant

* = Significant at 5% probability level using Duncan's Multiple Range Test.

** = Significant at 1% Probability level using Duncan's Multiple Range Test.

APPENDIX 5: ANOVA FOR EFFECT OF NITROGEN RATES ON LEAF ASH CONTENT.

(Mean sum of squares)

		SEASON I				SEASON II			
VARIETY:		VITA-3		K-80		VITA-3		K-80	
GROWTH STAGE:		56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP	56 DAP	77 DAP
Source	df								
N-rates	3	3.63 n.s	0.06 n.s	4.80 n.s	16.80**	1.04 n.s	69.53*	1.37 n.s	61.00**
Blocks	2	1.86	3.60	0.70	4.70	1.84	2.11	0.29	5.90
Errors	6	1.51	2.12	2.60	0.48	0.54	9.04	1.55	4.21

** Significant at 1% probability level using Duncan's Multiple Range Test.

* Significant at 5% probability level using Duncan's Multiple Range Test.

n.s not significant.

APPENDIX 6: ANALYSIS OF VARIANCE FOR DURATION OF ROOM STORAGE ON WEIGHT LOSS IN COWPEA LEAVES.

Source of	df	Mean sum of squares	
		Season I	Season II
Days of storage	2	2610.468 **	1452.370 **
Blocks	2	6.886 n.s	4.456 n.s
Error	4	7.146	1.854

** Significant at 1% probability level using Duncans' Multiple range test.
* " 5% " " " " " " "

n.s Not significant.

APPENDIX 7: ANALYSIS OF VARIANCE FOR EFFECT OF DURATION OF REFRIGERATION STORAGE ON WEIGHT LOSS.

Source of variation	df	Mean sum of squares	
		Season I	Season II
Days	4	595.887**	291.950**
Blocks	2	.014n.s	.326n.s
Error	8	1.077	1.106

** Significant at 1% Probability.

* Signicant at 5% Probability

n.s Not Significant

APPENDIX 8: <u>ANALYSIS OF VARIANCE FOR EFFECT OF DURATION (DAYS) OF ROOM STORAGE ON ASCORBIC ACID LOSSES.</u>

Source of variation	df	Mean sum of squares	
		Season I	Season II
Days of storage	2	461327.694	160774.731
Blocks	2	244746.528	5249.817
Error	4	244946.909	5249.817

** Significant at 1% probability level.
 * Significant at 5% probability level.
n.s = not significant