INFLUENCE OF NITROGEN FERTILIZATION AND PLANT AGE ON YIELD, QUALITY AND STORABILITY OF KALE AND COLLARD (*Brassica oleracea* var *acephala* D.C) LEAVES

BY

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DEDICATION

To my parents Grace and Sanya

DECLARATION

I, Salim Sanya Inzaule, declare that this is my own work and has not been presented for a degree in any other University.

Salim Sanya Inzaule — Date: 5/6/91

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

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ABSTRACT

Two experiments were conducted between June 1988 and July 1989 at the Faculty of Agriculture Field Station, Kabete Campus, University of Nairobi, to study the influence of nitrogen (N) application rates (0, 5, 10 and 15 gN per plant) on yield quality and storage ability of kale and collard (Brassica oleracea var. acephala D.C.) leaves. Calcium ammonium nitrate (CAN) was used as the N source. Leaves were harvested at intervals of 10 days for a period of upto 140 days after transplanting. Leaf yield at each harvest was determined and leaf samples stored on the shelf for 3 days and in a refrigetor for 21 days.

Leaves were analysed for crude protein, crude fibre, crude lipid, total ash, calcium, ascorbic acid and beta-carotene, after every other harvest. During storage, ascorbic acid retention was determined initially and at intervals of 2 days of storage for shelf and 7, 14, 21 days of storage for refrigeration.

Increasing N application significantly increased leaf yield. Applying more than 10g of N per plant, however, did not significantly increase yields. Leaf yield actually decreased. The results showed that leaf yield increased with plant age upto 60 days after transplanting, then remained almost constant upto 120 days after transplanting beyond which there was a drop of about 65%. per consecutive harvest.

Application of N significantly increased crude protein, crude lipid, total ash and Beta-carotene but decreased ascorbic acid and crude fibre. Calcium remained constant. Crude protein, ascorbic acid and Beta-carotene significantly decreased whereas crude fibre tended to increase with plant age. Crude lipid tended to increase with plant age upto 60 days after transplanting then significantly decreased for the rest of the harvest period. Total ash and calcium did not change significantly with plant age.

Ascorbic acid in stored leaves significantly decreased with increasing storage time. Highest decrease of about 72% being in leaves stored on the shelf and lowest of about 25% in the leaves stored under refrigeration were observed.

CHAPTER ONE

INTRODUCTION

Kale and collard (*Brassica oleracea* var. *acephala* D.C) both belong to the family Cruciferae and originated from Eastern Mediterranean countries (Nieuwhof, 1969). Besides spinach, kale and collard are the most important leafy vegetables in Kenya. The difference between kale and collard plants is that kale is erect and branched, and grows upto a height of 1m or over, while collard is an unbranched plant which looks like cabbage but does not form a head. In Kenya, the two crops are grown at altitudes of 700 m above sea level and above.

Kale and collard were first introduced in Kenya as fodder crops by the European settlers, in the Kenyan highlands. They remained unknown for sometime, but since three decades ago, they have become increasingly important both as food and fodder crops in many parts of the country.

Although kale and collard are mainly grown in Kenya in homegardens, a few farmers, especially near Nairobi, grow them for commercial purposes. These farmers supply the Nairobi market with fresh leaves of these vegetables. In the other parts of the

country, the vegetables are rarely grown as pure stands. Instead they are grown in mixtures with other local vegetables like Gynandropsis gynandra and Solanum nigrum. Kale and collard require very little technical know-how to grow them. They are therefore grown by many people even in towns in the backyards.

Leaves of kale and collard are eaten as green vegetables in stews especially with "ugali". In this form, the vegetables constitute part of a common dish for Kenyans of all income categories. This is because kale and collard leaves are among the cheapest vegetables available in the Kenyan markets as is evident from its local Swahili nickname "Sukuma Wiki" which means 'that which pushes the week'. They are common vegetables in most average households, especially at the middle of the month when there is little money available. However, even at other times, at least one or two meals eaten each day in most households include kale and/or collard leaves. In some parts of the country, however, especially within the nomadic tribes where eating of green vegetables is not common, kale and collard leaves are still unknown.

Little work appears to have been done on the cultural requirements for the two crops especially the nitrogen requirements and the effect of nitrogen application on the quality of the leaves. Growers therefore, apply fertilizer at rates that are not standardised. Also most growers, especially those who grow the vegetables for commercial purposes have no knowledge of how long they can continue harvesting leaves from the plants before economic yields start to decrease.

It is a common practice in urban areas that kale and collard leaves are harvested and taken for sale in open air markets where due to the high ambient temperatures, the leaves deteriorate quickly losing their sale value. Sometimes the consumers also buy excess of the leaves and either store them on shelves or in refrigerators. Although it is known that kale and collard leaves will stay at ambient temperatures for 2 - 3 days before they turn yellow (Aworh, et. al. 1978), it is not certain how long the leaves will keep in a domestic refrigerator.

The objectives of this study were therefore:

(1) To determine the effect of nitrogen rates and the plant age on yields of kale and collard.

(2) To determine the effect of nitrogen rates and plant age on the chemical composition of kale and collard.

(3) To determine the effect of nitrogen rates on the storage stability of leaves of kale and collard.

CHAPTER TWO

LITERARURE REVIEW

2.1 Kale and Collard

Kales and collards are representatives of Brassica oleracea, which closely resemble wild cabbage. They can be cultivated practically everywhere in the world (Nieuwhof, 1969). Collards are used as green vegetables in parts of Southern United States and curly kales in some German speaking countries (Nieuwhof, 1969).

Kale and collard leaves are rich in vitamin C, provitamin A, calcium, crude fibre, and protein (Anon. 1966; Neiuwhof, 1969; Crokett, 1972; Anon. 1976; Chweya, 1982; 1984; Tindall, 1983; Salunkhe, 1984; Anon, 1984).

2.2 History

Kales originated in the Mediterranean region from where they spread into Europe and parts of Asia (Nieuwhof, 1969). Collards are thought to have originated from deep South of the Mediterranean region because of their popularity there (Crockett, 1972).

Kales were introduced in Kenya by white settlers in the highlands as fodder crops (Ball, 1936) in some grassland and dairying areas. The main varieties introduced were 'Marrow-stem' and 'Thousand-headed' kales. Collard was introduced later as a vegetable. Kale and collard are normally grown from seeds but Gallop (1943) indicated that kale off-shoots and cuttings can also be used.

2.3 Botany

Kale and collard belong to the Cruciferae family genus Brassica, species oleracea, sub-species acephala (Nieuwhof, 1969; Salunkhe, 1973; Tindall, 1983; Crokett, 1972; Purseglove, 1974). The acephala group has many "sub-varieties" according to Nieuwhof (1969) as follows:

(a) Sub. var. medullosa Thell. Marrow stem kale;

(b) Sub. var. millecapitata (lev.) Thell. Thousand-headed kale;

(c) Sub. var. plana pterem. Smooth-leaved kale;

(d) Sub. var. palmifolia D.C. Tree kale;

(e) Sub. var. laciniata L. Curly kale.

Collard is a form of kale resembling non-heading cabbage (Nieuwhof, 1969; Tindall, 1983; Salunkhe, 1984). The alternative name, "tree cabbage" is quite descriptive, because when collards are young, they indeed look like cabbages, each collard plant having a rosette of handsome blue-green leaves but even as the plant matures, it does not form a head of tightly compacted leaves like cabbages do. Instead, its stem elongates in a tree-like fashion reaching 0.6 - 1.3 m in height (Crokett, 1972). The edible portion is a cluster of numerous, tender leaves at the top of the stalk (Salunkhe, 1984). 'Thousand-headed' kale has thick, smooth leaved and more branched plants and may attain a height of 2 m (Chweya, 1982). Kales do not form heads like cabbage or produce edible flowers like cauliflower and brocolli. Being variously curled and of beautiful colours, some kales are grown as ornamentals (Salunkhe, 1984).

Inflorescence of kale is the same as that of cabbage and needs a period of cool weather to initiate production of the flowering parts (Hawthorn and Pollard, 1954). Hawkins (1944) reported that at a high altitudes of 1670 m and above such as Molo in Kenya kales can flower. Earlier on, Hill (1942) did not consider that kales could flower in East Africa.

2.4 Production of Kale and Collard

Kale and collard are reliable croppers, which frequently do well on medium heavy fertile soils with a good moisture supply, but can also be grown on light soils (Nieuwhof, 1969; Salunkhe, 1983). Kale is more salt tolerant than cabbage and can be grown at pH of 7.5 - 8.0 (Nieuwhof, 1969; Crokett, 1972).

In Kenya *Brassica spp*, which include kale and collard, are planted on well drained soils with good crumb structure (Anon. 1976). In Southern United States Splittstoesser (1984) recommended fertile soils which are not prone to waterlogging.

Even though they are cool temperate crops, kale and collard have a wide ecological adaptability. They are the hardest of the cole crops and mature plants of hardy varieties can withstand temperatures of -10°C to 25°C (Nieuwhof, 1969). This is why the crops can even be grown in Southern Greenland. These crops require reliable water supply (Anon. 1976; Biggs, 1977).

In Kenya, kale and collard are grown at elevations of over 700 m above sea-level with rainfall of over 890 mm per annum (Anon. 1976, 1981).

2.5 Nutritive Value of Kale and Collard Leaves

Leaves of kale and collard rank high among greens in nutritive value (Anon. 1966; Yants, 1980; Chweya, 1982) (Table 1). They are rich in the minerals calcium and iron and vitamins especially vitamin C, thiamine and provitamin A (Nieuwhof, 1969; Crockett, 1972; Salunkhe, 1973, 1984; Chweya, 1982; Tindall, 1983). Of the seven Brassica species studied by Yants (1980), kale leaves were found to have the highest average vitamin C content (134 mg per 100g fresh weight). Crockett (1972) reported that collard leaves contain more vitamin A and C than cabbage leaves do. This was attributed to the fact that leaves of collards are fully exposed to the sun, and thus they become deep green in colour. Mengel and Feigenbaun (1979) reported that high vitamin C contents are found in Cruciferea family crops.

Brassicaceous vegetables which include kale and collard are also rich sources of proteins (Salunkhe et al. 1973). Nieuwhof (1969) reported that the protein contents of Brussels sprouts, kale, collard and young cabbage are high while the amount in sprouting broccoli is only appreciable and other cole crops contain much less. The protein levels for

Table 1: Composition of brassicaceous vegetables

Food	Water %	Energy Cal.	Protein %	Fat %	Carbohydrates %	Ca (mg)	P (mg)	Fe (mg)	Na (mg)	K P	Vit A IU	Thiamine (mg)	Vit C (mg)
Cabbage	92.4	24	1.3	0.2	5.4	49	29	0.4	20	233	130	0.05	47
Brocolli	89.1	32	3.6	0.3	5.9	103	78	1.1	15	382	2500	0.05	113
Brussel Sprout	85.2	45	4.9	0.4	8.3	36	80	1.5	14	390	550	0.1	102
Cauliflower	91.0	27	2.7	0.2	5.2	25	56	1.1	13	295	60	0.4	78
Kale	82.7	53	6.0	0.8	9.0	249	93	2.7	75	258	10000	0.16	186
Watercress	93.3	19	2.2	0.3	3.0	151	54	1.7	52	282	4900	0.08	79
Mustard	89.5	31	3.0	0.5	5.6	183	50	3.0	32	377	7000	0.11	97
Turnip green	90.3	28	3.0	0.3	5.0	246	58	1.8	40	250	7000	0.21	137
Chinese cabbage	95.0	14	1.2	0.1	3.0	43	40	0.6	23	253	150	0.05	25
Rutabaga	87.0	46	1.1	0.1	11.0	66	39	0.4	5	239	580	0.07	43
Collard	85.3	45	4.8	0.8	7.5	250	82	1.5	40	450	9300	0.16	152
Kolhrabi	90.3	29	2.0	0.1	6.6	41	51	0.5	8	372	20	0.06	66

Main Sources: Agricultural Handbook No. 8 USDA 1963 Washington, D.C., The Heinz Handbook of Nutrition (1959) McGra Hill Book Company New York; Nutrition Composition of Fresh California - Grown Vegetables (1962 California Agricultural Experiment Station Bulletin No. 788, Davis California cited from the Evaluati of Nutrition Value and Quality in Fresh Brassicaecous Vegetables, After Harvest, During Preparation, a Subsequent to Storage. D.K. Salunkhe and Kirti Salunkhe Symposium on Vegetable Storage (1973) pp. 143 178.

white cabbage and curly kale vary from 0.8 to 2.3 and from 1.9 to 8.8 per cent, respectively (Nieuwhof, 1969).

Very little work has been reported on the contents of crude fibre, total ash, and crude fat, in kale and collard leaves.

2.6 Nitrogen and Plant Growth

Huffaker and Rains (1978) reported that nitrogen is the central element to growth and development of all plant parts. Plants deprived of nitrogen show decrease in cell division and expansion, prolonged dormancy and therefore, delayed swelling of buds (Hewitt, et. al. 1975). Nitrogen enhances vegetative growth (Baker and Maynard, 1972; Knight and Mitchell, 1983) and is used in the synthesis of proteins (Mengel and Kirkby, 1979).

Nitrogen is absorbed by plants as nitrate or ammonium ions (Tisdale et. al. 1985), although most plants prefer taking nitrogen in nitrate form even when supplied in a different form (Maynard and Barker, 1979). It was reported by Hagema (1977) that nitrate is the predominant form of soil nitrogen

available to plants because soil organisms rapidly convert ammoniacal forms of nitrogen to the nitrate form.

Leaf size is determined by cell expansion which is influenced by nutrient supply amongst other factors (Mithorpe and Moorby, 1974; Cutter, 1971).

2.7 Nitrogen and Yield of Leafy Vegetables

Nitrogen increases yield of many leaf vegetables. Peck (1981) working with cabbage found that those plants which were grown with nitrogen fertilizer gave higher yields than those planted without. Chweya (1984) working with kale reported that nitrogen tended to increase mean fresh weight and number of axillary branches, and leaves, therefore yield per plant. Tisdale et al. (1985) reported that plants deficient in nitrogen became stunted and yellow in appearance. This is because nitrogen is involved in synthesis of chloroplasts. Allen (1972) reported that application of nitrogen in rapeseed plants led to more vigorous growth and development as was reflected by increase in stem length, number of flowering branches, total plant weight, leaf area index and hence high yield. This also agrees with the report by Eppendorfer (1978a)

that nitrogen application increased the yields of dry matter, especially in potatoes and kales. Peck (1981) working with cabbage also found an increase in yield due to application of nitrogen.

Heavy application of nitrogen fertilizer may not increase yields, but may check growth of the plant (Borna, 1971). Fertilizer applied in split doses may give higher yields than single application at the time of planting (Sinnandurai, 1973). In Kenya, split application in two equal portions of calcium ammonium nitrate are recommended for Brassicas (Anon., 1981). Borrel and Tendeschi (1972), working with cauliflower, found that two split applications of nitrogen gave higher yields than a single application of an equal amount.

2.8 Effect of Continuous Harvesting on Yield of Leafy Vegetable Crops

One way of harvesting kale is by regularly picking leaves from the plants (Thompson and Kelly, 1957). The leaves are normally picked before they become tough (Ware and McCollum, 1975). This type of harvesting is common for kale grown in homegardens (Chweya, 1982). This implies that plants are being regularly defoliated. There is hardly any literature

on the effect of defoliation on total yield of kale plants. However, reduction in the leaf area of the plants may lead to adverse effects on their growth.

Leaf picking reduces the photosynthetic area, effective plant population pressure and competition at a given plant density. Evans (1975) stated that provided partial defoliation of a plant leaves sufficient leaf area for full light interception, there would be no reduction in total yield. This was attributed to mobilization of reserves or increased photosynthetic rate on the remaining leaves which compensates for the removed leaves. According to the same author, regular removal of lower leaves weakens roots in plants. This would imply that frequent defoliation would shorten the life span of such plants. Chweya (1982) reported that defoliation of kale did not significantly affect various quality aspects of the leaves whether kale plants were widely or closely spaced. The same author further found that wide spacing, however, and high nitrogen application significantly increased dietary fiber in both intact and defoliated plants; most probably due to increased growth in the plants.

From the above discussion, it can be concluded that defoliation would be expected to have an adverse effect on the growth and yield of kale and collard plants. However, since the removed leaves are part of the final yield, defoliation may not significantly reduce the total leaf yield of kale and collard plants. Defoliation may actually reduce yield losses which could be due to senescent leaves.

2.9 Effect of Age of Plants on Leaf Yield and Composition

There is very little information on the relationship between total yield and plant age in leafy vegetables like kale and collard. Itulya (1985) worked with kale and found that overall, leaf yield remained stable upto 90 days after transplanting after which there was a drop of 47%. This might have been due to the fact that as the plants grew, their ability to put on more leaves decreased (Salisbury and Ross, 1986)

The vitamin content at harvest can vary with maturity of the crop as noted with ascorbic acid and Beta-carotene (Brechti *et al.* 1976). Beta-carotene changes with maturation of tomatoes (Watada *et al.*, 1976). In *Gynandropsis ovandra* the highest levels of vitamin C were obtained 50 days after planting, after

which the vitamin contents declined steadily throughout the remaining period of growth (Abe et al., 1977). In cabbage, the dry matter content increased with time up to a maximum then it fell (Nilson, 1981). The cease in growth was accompanied by a fall in the dry matter content. Work done by Vereeke et al. (1971) revealed that total nitrogen content decreased with the age of the leaves.

Nieuwhof (1969) working with cole crops reported that vitamin C content per unit fresh-weight of cabbage decreased with plant age and growth and in some cases there was a negative correlation between vitamin contents and the size of the head. The same author also reported that increasing nitrogen content of the soil caused an increase in the nitrogen contents in the plants, but often this effect was slightly small, and variation brought about by season and habitat were much more important than increasing nitrogen. The author further reported that with advancing age of the plant, the protein content in cole crops generally fell off. Barta (1975) reported that levels of fatty acids and protein decreased as plants aged.

Carbohydrates accumulate in leaves of crops during growth. Thus young plants are lower in carbohydrates than older plants. The contents of carbohydrates in plants therefore depend very much on plant age (Mengel, 1979). Chweya (1982) who worked with kale found that there was a tendency for total plant nitrogen to decline with harvesting time. Decline in total nitrogen is due to dilution of accumulated nitrogen in the plant because of increased growth (Barker, et al. 1971). Calcium remains constant throughout the harvesting period (Chweya, 1982).

2.10 Effects of Nitrogen on the Nutritive Value

Literature on the effects of fertilization upon the nutrient contents of leafy crops is voluminous and generally contradictory, probably because of variations in soil chemistry, water availability, and other environmental factors. Work has been done by Barker (1975); and Chweya (1984) on the effect of fertilization on nutritive value of kale leaves. Peron et al. (1984) working on lettuce found that when ammoniacal nitrogen was used instead of nitrate nitrogen, there was increased ascorbic acid content. This vitamin content increased also when the ratio of k:mg went up to six (Peron et al. 1984). This is

contrary to the findings by Mengel (1979) that high nitrogen contents tend to decrease vitamin C. The same author reasons out that it is because of competition of photosynthates between carbohydrates and amino acids metabolism that also affect vitamin C. When nitrogen supply is high, more photosynthates are used for synthesis of amino acids and less photosynthates for synthesis of hexoses, disaccharides and polysaccharides. This is also supported by Lewandowska and Skapski (1977) who found that while nitrogen fertilizer increased yields and carotene contents of kale plants, the content of dry matter, ascorbic acid and total sugars were reduced. However, some work done by Aberg and Ekdahl (1948) was contradictory. They found less ascorbic acid in kale with suboptimal nitrogen application than in those with adequate application. Also Burrell et al. (1940) working with cabbage, and Sheets et al. (1954) working with collards observed that ascorbic acid contents increased with N-fertilization.

Nitrogen is an exogenous factor which influences protein/carbohydrate ratio in the leaves. Suboptimal nitrogen supplies result in high contents of carbohydrates due to lack of nitrogen. This restricts protein synthesis so that more of photosynthates are available for carbohydrates

(Mengel, 1979). Increasing rates of nitrogen increase protein contents (Eppendorfer, 1984b Breteler, 1982). Nitrogen fertilization also has influence on quantity and quality of protein. The quantity of protein increases with increasing levels of nitrogen but the quality decreases especially above levels of about 62.4 kgN per hectare (Fritz et al. 1971a). Quality of protein is also influenced by locality and variety of the crop (Fritz 1971b). Blamey (1973) who worked with sunflower and Chweya (1984) who worked with kale, found that protein content in kale leaves increases with increasing rate of nitrogenous fertilizer application. The rate of protein synthesis depends on nitrogen supply (Mengel, 1979). Abundant nitrogen supply and light intensity favour formation of chloroplasts. As these organelles contain much protein and carotene, they raise the protein content as reported by Mengel (1979). Increase in percentage protein contents of kale with increased N-fertilization has been reported by Harmmerton (1967) who worked with kale and Oloya (1976) and Del Valle (1971) who worked with collard.

Although there are no reports on the influence of nitrogen on carotene levels in Brassicas, reports from other crops exist. Working with carrots Nowakuwski (1971) reported that increasing nitrogen

levels caused increase of carotene in the roots of carrots. Similar results were also reported by Venter (1979) and Vereeke et al. (1979) who both worked with carrots.

Fritz et al. (1971a) found that the increase in carotene with amount of nitrogen fertilizer applied to spinach crop, even after optimum yields were decreasing, was due to nutrient imbalance. This could also be the case with kales.

Leaf lipids are closely related to the formation of chloroplasts, due to the fact that chloroplasts are rich in membranes (Thylakoids), and hence nitrogen application promoted the synthesis of leaf lipids (Barta, 1975).

In general, it is evident that nitrogen application not only increases yields but also has marked effects on nutrient contents like lipids, ascorbic acid, protein, etc. (Salunkhe, 1973). However, this was contradicted by Fritz et al. (1971b) who reported that accumulation of vitamins depends more on genetic and ecological factors than on nitrogen fertilization.

2.11 Storage of Kale and Collard Leaves

During storage of freshly harvested vegetables, the living plant material is expected to alter (Norman, 1973). After harvest the vegetables are still physiologically active and enzymatic respiratory processes may bring about the profound changes. Changes in the composition of raw vegetables are accompanied by decrease in their nutritive value. The changes occur after harvest, during transportation, holding and subsequent storage. Norman (1973) reported that vitamin A was sensitive to acid, air, light and heat, while vitamin C was sensitive to alkalinity, air, heat and light. Once the vegetables have been cooled, the maintenance of the correct temperatures is probably the most important factor in reducing deterioration during cold storage and distribution (Dennis, 1984). Many crops should be stored at temperatures approaching 0°C, because produce rapidly deteriorates at high temperatures. The deterioration includes loss of nutritive value.

Ottosson (1979) reports that the vitamin C contents of Brussel sprouts and cauliflower rapidly decrease when stored at 20°C. The same author further reports that brocolli and lettuce stored at

2°C lost 50% of ascorbic acid and dehydroascorbic acid within seven days. Ottosson (1979) also found that ascorbic acid in brocolli was relatively stable at 0°C but showed a marked decline at 10°C and 20°C. The author also reports similar results for parsley and kale. Burton (1982) reported that loss of ascorbic acid from leafy vegetables and similar commodities is variable. Olliver (1967) had observed that the ascorbic acid contents of the looser forms of green vegetables, such as spinach, decreased rapidly after harvest, but that a head of cabbage with tightly packed leaves could be stored for several days without significant losses in the vitamin. Persson (1979) confirmed high retention of ascorbic acid in cauliflower at 0°C and rapid loss at 20°C, whereas for cabbage and rutabaga, virtually no change in ascorbic acid contents occured at 0°C or 20°C over four weeks of storage. Berg et al. (1973) reported that storage of cabbage at a relative humidity near saturation (98 - 100%) reduced decay, moisture loss and colour loss. As a result cabbage remained firm and crisp, and retained the green colour longer during storage. Salunkhe et al. (1973) found that if cabbage was stored for along time it accumulated high concentrations of sulphur compounds but ascorbic acid and other water soluble vitamins were reduced.

Factors like relative humidity, temperature, exposure to air and physical condition of leafy vegetables are important for preservation of ascorbic acid. Zepplin and Elvenjem (1944) working with brocolli found that the ascorbic acid in brocolli was destroyed rapidly at room temperature, but was significantly retained under refrigeration. Ezell and Wilcox (1959) reported that wilting of cabbage, cauliflower, kale, collard and turnip greens appreciably reduced the ascorbic acid contents. Temperature and relative humidity have been reported to be the primary factors in preserving carotene in kales, collards and turnip greens (Ezell and Wilcox, 1959). The authors further reported that when kale, collard, turnip greens, spinach, rape, cabbage, and snap beans were subjected to a slow moderate and rapid wilting at 0, 10, 21.1 or 23.0°C, the vegetables that lost moisture faster and therefore wilted more rapidly tended to be affected more by humidity and to lose vitamin C more rapidly than those resistant to wilting. Prepackaging of leafy vegetables in plastic films effectively reduced loss of moisture and preserved a fresh and crisp appearance (Salunkhe, 1984). The same author however, reported that low temperatures were found to preserve better the beta-carotene levels in leafy greens.

Norman (1973) lists the factors which cause food deterioration to include (a) growth of microorganisms (bacteria, yeasts and molds) (b) activities of natural food enzymes (c) insects, parasites and rodents, (d) temperature (e) moisture and dryness (f) air especially oxygen (7) light and (h) time. Working with sweet corn, the author found that in just 24 hours at room temperature, 26% of the total sugars were lost with a comparable loss of sweetness in the corn. Peas and lima beans can lose over 50% of their sugar in just one day at room temperature. Losses are, however, slower under refrigeration but there is still great change in vegetable sweetness and freshness within 2 or 3 days (Norman, 1973). Burton (1982) recommended that leafy materials, because of liability to water loss, must be stored at 100 per cent relative humidity which unfortunately is ideal for microbial growth. The author further states that the temperature for storage must be lowered sufficiently to reduce the risk microbial damage to a minimum. A temperature of 0°C is desirable as found by Burton (1982).

In general, the lower the temperature of storage, the less the abnormalities developed, and therefore the less loss of nutritive value and quality of vegetables. The extent of deterioration

depends on the type of vegetable, its anatomical and morphological structure, rate of respiration, substrates, such as sugar and organic acids and external conditions (Salunkhe, 1973).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

Two experiments were conducted on experimental plots at the Field Station of the University of Nairobi, Kabete campus, between July 1988 and July, 1989.

Kabete Campus lies within latitude 1° 15' and longitude 36° 44'E at an altitude of 1940m above sea level. The area has an average rainfall of about 1000 mm per year which is bimodal, with the long rains coming in March, April and May and the short rains coming in October, November and December.

The mean monthly maximum temperature is 23°C, while the mean monthly minimum is 12°C. The details of temperature and rainfall during the experiment are presented in appendix I.

3.1.1 Soil Characteristics

The site is under nitosol unit according to classification (FAO/UNESCO, 1974). The soils are friable brown of the Kikuyu friable clay derived from tertiary trachytic lava. They are deep and well drained (Siderius, 1976).

Three soil samples were taken at random at a depth of 0 - 15 cm and 15 - 30 cm one day before the seedlings were transplanted. The samples were dried and analysed for pH, organic carbon, total nitrogen, calcium and available phosphorus - by the methods of Ahn (1973, 1975). These results are shown in appendix II.

3.2 Planting Materials

Seeds of "Thousand headed" kale and "Georgia" collard were obtained from the East Africa Seed Company Ltd., Kijabe street, Nairobi.

3.3 Experimental treatments and design

3.3.1 Field treatments and design

The field treatments consisted of four levels of nitrogen (0, 5, 10, and 15g per plant) as calcium ammonium nitrate (26% N) and two varieties.

Each experiment was set up in a split plot design with three blocks. The varieties formed the main plots each of 10 m x 4.8 m, while the N rates formed the subplots each measuring 4.8m x 2.5m.

3.3.2 Nitrogen application

Nitrogen fertilizer was applied in split application whereby half the amount was applied three weeks after transplanting, and the other half three weeks later as recommended by the Ministry of Agriculture (Annon. 1981). The application was carried out at 5cm from the stem to avoid scotching of the plants.

3.4 Cultural Practices

3.4.1 Nursery

A raised seedbed of 8m length and 1m width was prepared to fine tilth, then divided into two. Triple superphosphate (46% P_2O_5) was mixed with the soil at the rate of $1kg/m^2$. Drills were made at 20cm apart. The seeds were sown thinly and covered lightly with soil then mulched with dry grass mulch. The mulch was removed after eight days when the seeds had emerged. The seedbed was kept weed free by manual weeding.

Two weeks after sowing, seedlings were thinned out to 2 - 3 cm apart in order to produce sturdy seedlings. Watering was done every morning but the frequency was reduced two weeks before transplanting, such that during the last week before transplanting, watering was done after every other day in order to harden the seedlings (Anon. 1981). The day before transplanting watering was done very early in the morning in the nursery so that seedlings could retain some soil at the root zone when being transplanted. Healthy seedlings of uniform size were transplanted four weeks after sowing.

3.4.2 Transplanting

The experimental plots were ploughed by a tractor then disc harrowed. The blocks were marked out, then triple superphosphate and Furadan 5% applied at the rate of 20 g, and 2 g to each planting hole, respectively. Furadan was for controlling cutworms and other insects. The seedlings were planted at a spacing of 60 cm between and 30 cm within rows.

3.4.3 Field practices

The plots were kept weed-free by manual weeding. Insect pests such as cutworm (*Agrotis spp*), were controlled by using furadan 5% and cabbage sawfly (*Athalia spp*) and aphids (*Brevicoryne brassicae*) were controlled by using Ripcord 0.25%. Ripcord was applied at the rate of 50 ml in 20 L water to spray $1000m^2$ (i.e. 0.5 litre per hectare). Moles were controlled by trapping. Some of the serious weeds recorded were such as blackjack (*Bidens pilosa*), fathen (*Chemopodium album* L) and nutgrass (*Cyperus rotundus*).

Irrigation was carried out to avoid water stress in plants whenever there was drought. In the second experiment the seedlings were transplanted when it was dry and the irrigation system broke down. In later stages, rot disease caused by Xanthomonas campestris was spotted in kale plants. This was confirmed by the pathology department of the University of Nairobi. The disease is either seedborn or soilborn and so it was difficult to control. Collards were not affected by the disease.

3.5 Harvesting of the Leaves

Harvesting started 40 days after transplanting and continued for 140 days at intervals of 10 days. Each time all the plants from a centrally placed area of $1m^2$ from each subplot were harvested leaving only the top four leaves. This implied that only two fully expanded leaves and the bud were left on the plant. Guard rows were also harvested in the same manner to make the field uniform. Harvesting was done early in the morning between 8.00 and 9.30 a.m. Leaves from each subplot were harvested separately and weighed. The sum of the weights was used to calculate the yield per plot and hence per unit area.

3.5.1 Sampling of the leaves for laboratory analyses

After weighing, the leaves from each subplot were mixed thoroughly then sampled for laboratory analyses and storage. The samples were put in polythene bags to minimise water loss.

3.5.2 Storage study

Leaf samples weighing 500 g from each subplot were taken, placed in polythene bags and stored on a shelf at ambient temperature of about 20°C ± 2 and in a refrigerator at temperature of 6 ± 2°C. The polythene bags were perforated to allow air circulation. For shelf storage, leaves were analysed for vitamin C every day and every seven days for the leaves stored in the refrigerator until the leaves turned yellow.

3.6 Proximate Analyses

The leaves were separated into petioles and laminae. The laminae were put in paper bags, weighed and then dried in an oven at temperatures of 80°C for 96 hours. The dried samples were weighed again to determine dry matter, then ground using laboratory

Hammer 8 Labmill (Miller Christy 8000 RPM) with a sieve of 2mm diameter. The ground samples were kept in tightly closed bottles and saved for analyses.

3.6.1 Determination of crude protein

Hundred grams of the grounded sample were placed in each of three Kjeldahl flasks. A fourth flask was a blank. Half (1/2) selenium tablet was added into each flask, then 10ml of concentrated sulphuric added to each flask and the flasks placed in a digestion rack. Heat was applied until the frothing subsided and the solution became clear. The sample was cooled and diluted using distilled water. The sample was then transferred into a distilling flask and 4 - 5 drops of phenolpthalein indicator were added. The distilling flask was connected to a Hoskin's apparatus which was steam generator. Twenty-five mls of boric acid were added into 150ml Erlenmeyer flask and set under a condenser with the tip beneath the surface of the solution. Steam was left to pass through the flask carrying the sample then carefully 50% NaOH was added until the solution turned pink. Distillation was continued until two thirds (2/3) of flask was filled. The ammonia collected was titrated with standardized HCl (0.01N). The blank value was subtracted from the sample value to get the true amount of boric acid neutralised by ammonia.

3.6.2 Determination of crude fibre

Two grams of grounded sample were put in a beaker and 25ml of 2.05N sulphuric acid added and the volume increased to 200ml with boiling water. The sample was boiled for 30 minutes being stirred occasionally. The sample was filtered using filter sticks packed with glass wool then washed with distilled water three times and filtered with a vacuum. The residue was boiled with 25ml of 1.78N sodium hydroxide after increasing the volume to 200ml with boiling water. Boiling was continued for thirty minutes. The boiled sample and base solution were filtered as above and the residue and glass wool were transferred using distilled water into silica dishes, then the residue was washed with 15ml of 95% ethyl alcohol. The residue was dried in an oven (105°C) for two hours then cooled in a desiccator. The dish and residue were ignited in a muffle furnace (600°C) for 2 hours. The part burned off was crude fibre. The remaining ash was cooled in a desiccator and weighed. The difference between these weights was the weight of the crude fibre.

3.6.3 Determination of crude lipid (Ether extract)

Two grams of grounded sample were placed in a clean Soxhlet thimbles and the thimbles covered with absorbent cotton wool. The thimble and contents were placed in Soxhlet extractor and a collection flask was inserted. Di-ethyl ether was added into the extractor high enough to syphon out until the flask was half-full (75ml). Then the sample and di-ethyl were placed on a condenser, and water was turned on to cool the condenser. The extraction process was left to continue for 12 hours before evaporating ether in the flask. The content was dried in dry flask in 105°C oven for 30 minutes, then cooled to room temperature and weighed.

3.6.4 Determination of total ash

Two grams of grounded sample were placed into dishes and the dishes and the sample placed into a muffle furnace and the temperature raised to 600°C for 24 hours. The furnace was cooled to 100°C, the dishes with samples placed in a desiccator to cool to room temperature and then weighed as quickly as possible to prevent moisture absorption. The weight of the ash was calculated as percent of air-dried sample.

3.6.5 Determination of calcium

Calcium was determined by a wet digestion method. One gram of the total ash sample from (3.6.4) above was placed into a small beaker. Ten mls of concentrated nitric acid was added and allowed to stand overnight. The mixture was then heated on a hot plate until the production of red NO_2 fumes ceased. The beaker was cooled and a small amount (2 - 4ml) of 70% perchloric acid was added and heated again and allowed to evaporate to a small volume. The sample was transferred to a 50ml flask and was diluted to volume with distilled water. To overcome interferences, 1% (W/V) lanthanum was added. The sample was placed on a perkin Elmer atomic absorption spectrophotometer and compared to a standard solution which was made up of calcium carbonate (250mg/L), 25ml of deionized water and 5ml of hydrochloric acid. Calcium was expressed on dry weight basis in percentage.

3.7 Determination of ascorbic acid

Determination of reduced ascorbic acid was carried out by using a modified method of Barakat *et al.* (1955). Thirty grams of chopped leaves were blended with 60 ml of 10% trichloroacetic acid, then filtered through Whatman No. 41 filter paper. Ten ml

of the filtrate were pipetted into a 150 ml conical flask and 5 ml of potassium iodide added then titrated with a standard solution of N-bromosuccinimide using starch as an indicator. Results were expressed in mg/100g of dry matter.

3.8 Determination of beta-carotene

Two grams of fresh material were chopped finely and placed in a mortar with about 10ml of acetone. The material was ground and transferred into a 100 volumetric flask. The residue was extracted again with 10ml of acetone and the extract was transferred to the volumetric flask. The extraction was continued till the residue no longer gave colour to the acetone. The combined extract was made up to 100ml. Twenty-five ml of the extract was evaporated to dryness on a rotary vacuum evaporator. The residue was dissolved in 1ml of petroleum ether and the solution introduced onto a chromatography column prepared from a mixture of 20ml of benzene, 7ml of ethanol, 100ml of petroleum ether, silica gel and sodium sulphate. Beta-carotene went through the column as a yellow pigment very quickly. The beta-carotene was made to the volume in the 25ml volumetric flask with petroleum ether and its

absorbance at 440nm read. The concentration of beta-carotene in the solution was read from the standard curve and calculated per 100 grams of dry material.

3.9 Data analysis

Data was subjected to analysis of variance (ANOVA) using the method of Gomez et al. (1984) and Steel and Torrie (1981). Mean separation was by Duncan multiple range test at probability level $P \leq 0.05$ (Gomez et al. 1984).

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Nitrogen, Variety and Plant Age on Leaf Yield

4.1.1 Effect of nitrogen

Fig. 1 shows the cumulative leaf yield during both experiments. In both experiments, as nitrogen rate increased the yield increased reaching maximum at the rate of 10g N per plant then started to decrease. Yields in the first experiment were higher than in the second experiment. There was no significant difference between the leaf yield from plants top dressed with 0g, 5g and 15g per plant. Top-dressing with 10g N per plant gave the highest cumulative leaf yield.

4.1.2 Effect of varieties

The cumulative leaf yields differed significantly between the two varieties as shown in Fig. 1. Collard outyielded kale during the two experiments by about 20%.

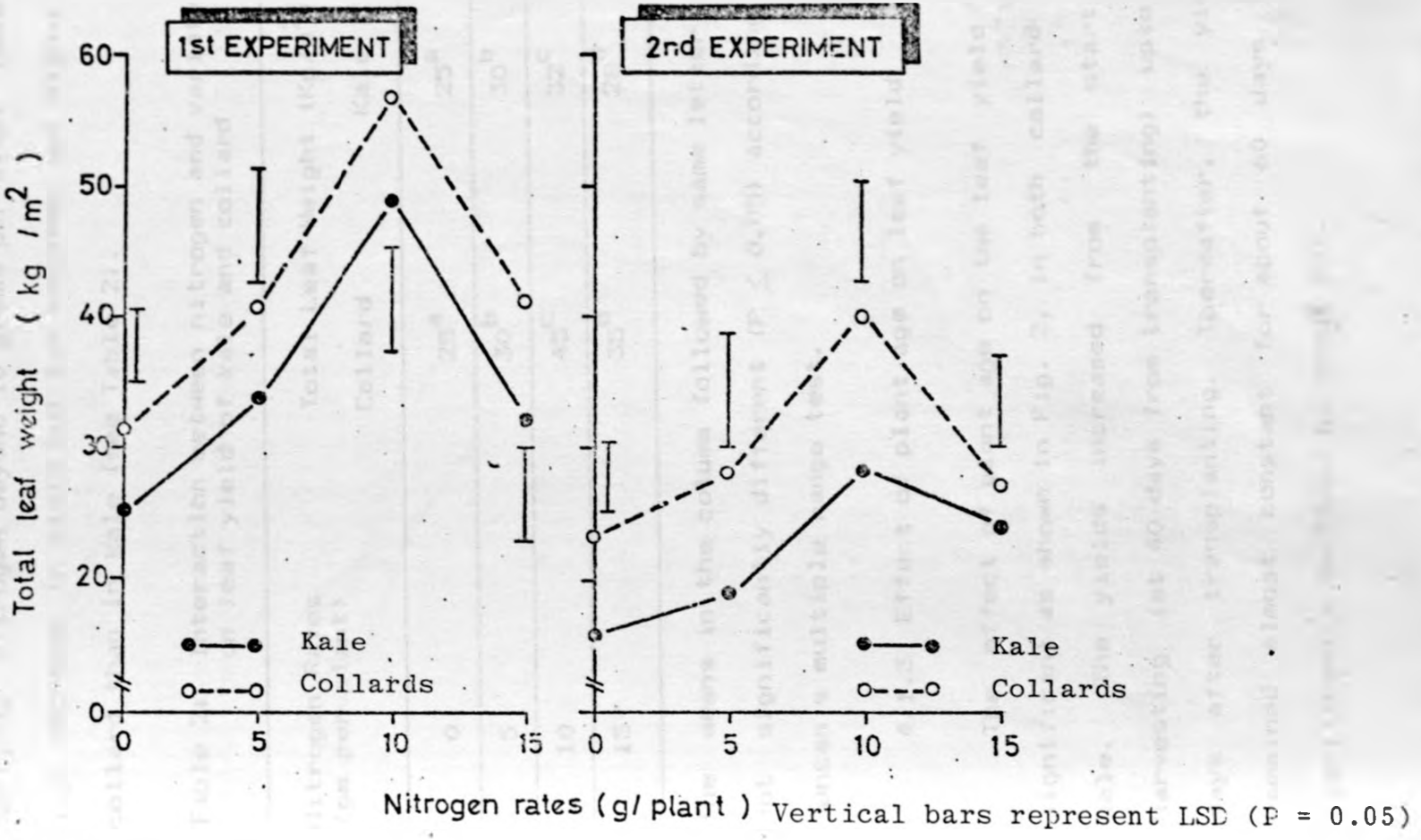


Fig.1. : Effects of nitrogen on total leaf yield of collards and kale

The interaction of nitrogen and variety on leaf yield was significant during the second experiment. Applying nitrogen beyond 10 grams per plant gave a high decrease in yield but the response was higher in collard than in kale (see Table 2).

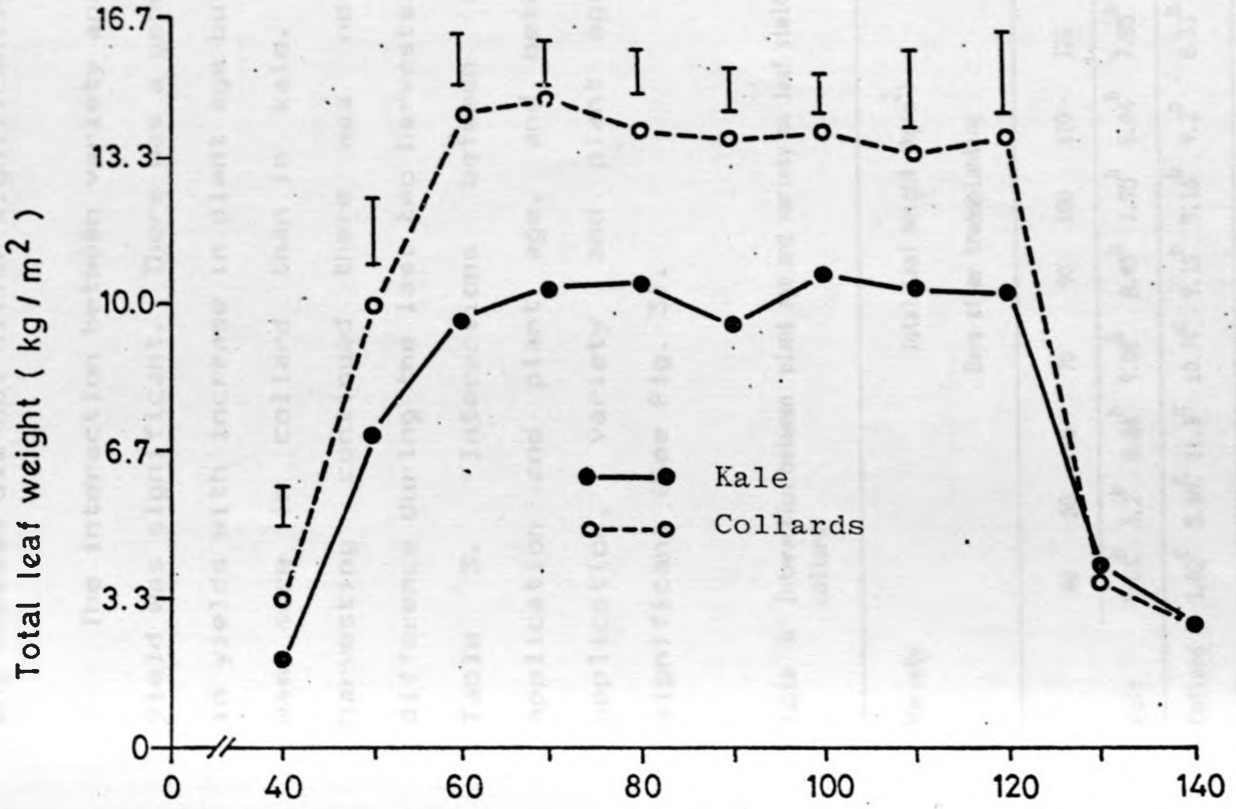
Table 2: Interaction between nitrogen and variety on leaf yield of kale and collard

Nitrogen Rates (gm per plant)	Total Leaf Weight (Kg/m^2)	
	Collard	Kale
0	25^a	25^a
5	30^b	30^b
10	45^c	32^c
15	35^d	28^d

The means in the column followed by same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

4.1.3 Effect of plant age on leaf yield

The effect of plant age on the leaf yield was significant as shown in Fig. 2, in both collard and kale. The yields increased from the start of harvesting (at 40 days from transplanting) upto 60 days after transplanting. Thereafter, the yields remained almost constant for about 60 days then significantly declined by about 65%.



Vertical bars represent LSD (P = 0.05

Yields of collard were significantly higher than those of kale by about 20%, from the time at which harvesting started upto 120 days after transplanting. Thereafter the mean yields of the two varieties at any harvest did not differ significantly.

The interaction between variety and plant age on yield was significant. There was a general increase in yields with increase in plant age but the increase was more in collard than in kale. However, as harvesting continued there was no significant difference during the last two harvests, as shown in Table 3. Interactions between nitrogen and application and plant age, and between nitrogen application, variety and plant age were not significant (See Fig. 3).

Table 3: Interaction between plant age and variety on leaf yield of kale and collard

Variety	Total Leaf Weight (kg/m^2)									
	Days after transplanting									
	40	50	60	70	90	100	110	120	130	140
Kale	2.03^b	3.2^b	8.84^b	9.08^b	8.45^b	7.95^b	8.04^b	7.85^b	6.95^b	4.3^b
Collard	5.02^c	5.68^c	11.3^c	10.74^c	9.15^b	9.16^b	9.2^b	8.33^b	7.85^b	5.2^b

Means in the column followed by same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

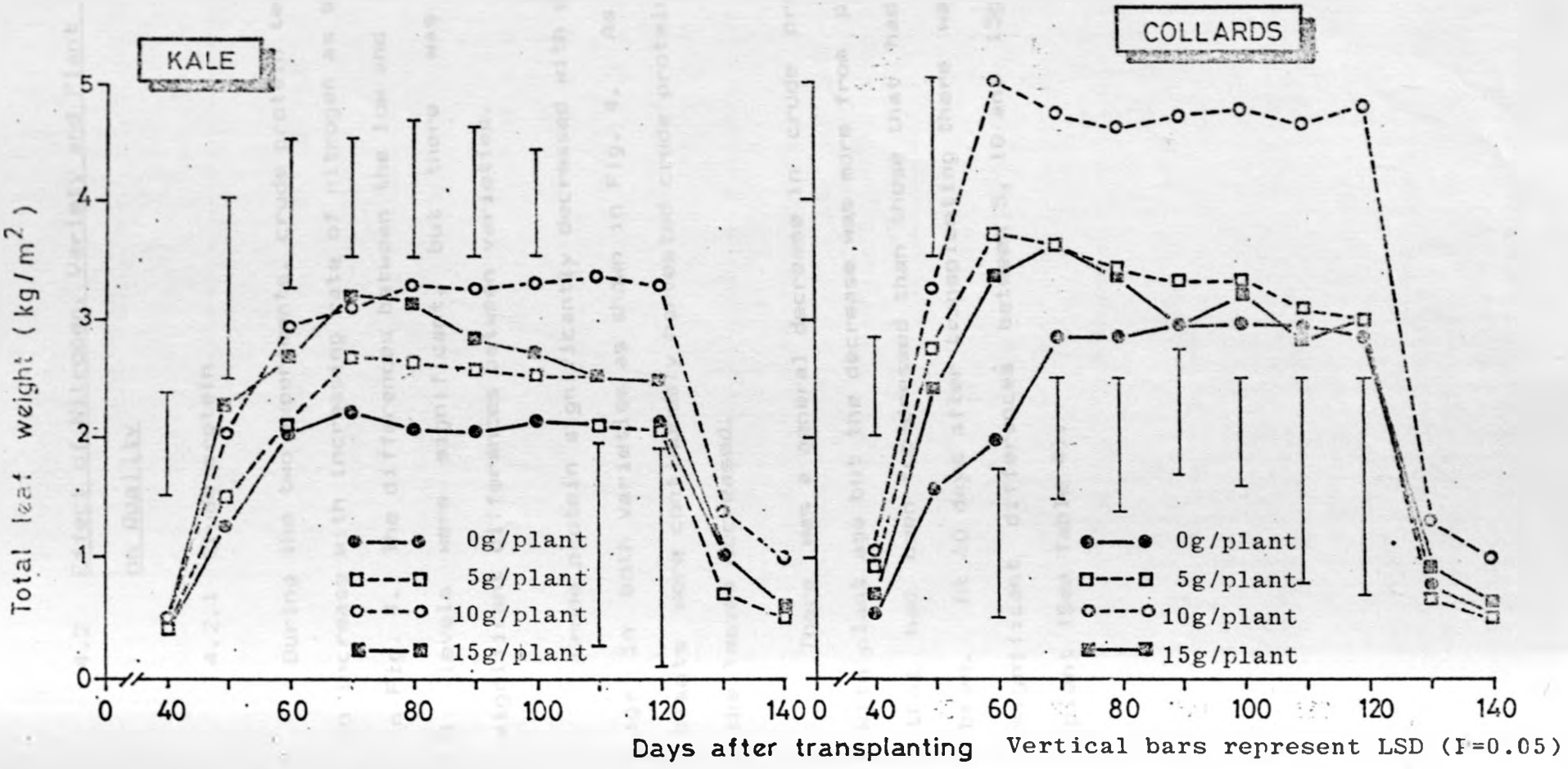


Fig. 3: Effect of Nitrogen rates and plant age on the leaf yields of kale and collards - . Second Experiment.

4.2 Effect of Nitrogen, Variety and Plant Age on Quality

4.2.1 Crude protein

During the two experiments, crude protein tended to increase with increasing rate of nitrogen as shown in Fig. 4. The differences between the low and high N levels were significant, but there was no significant differences between varieties.

Crude protein significantly decreased with plant age in both varieties as shown in Fig. 4. As the plants were continuously harvested crude protein in the leaves decreased.

There was a general decrease in crude protein with plant age but the decrease was more from plants that had been top-dressed than those that had not been. At 60 days after transplanting there was no significant differences between 5, 10 and 15g per plant (See Table 4).

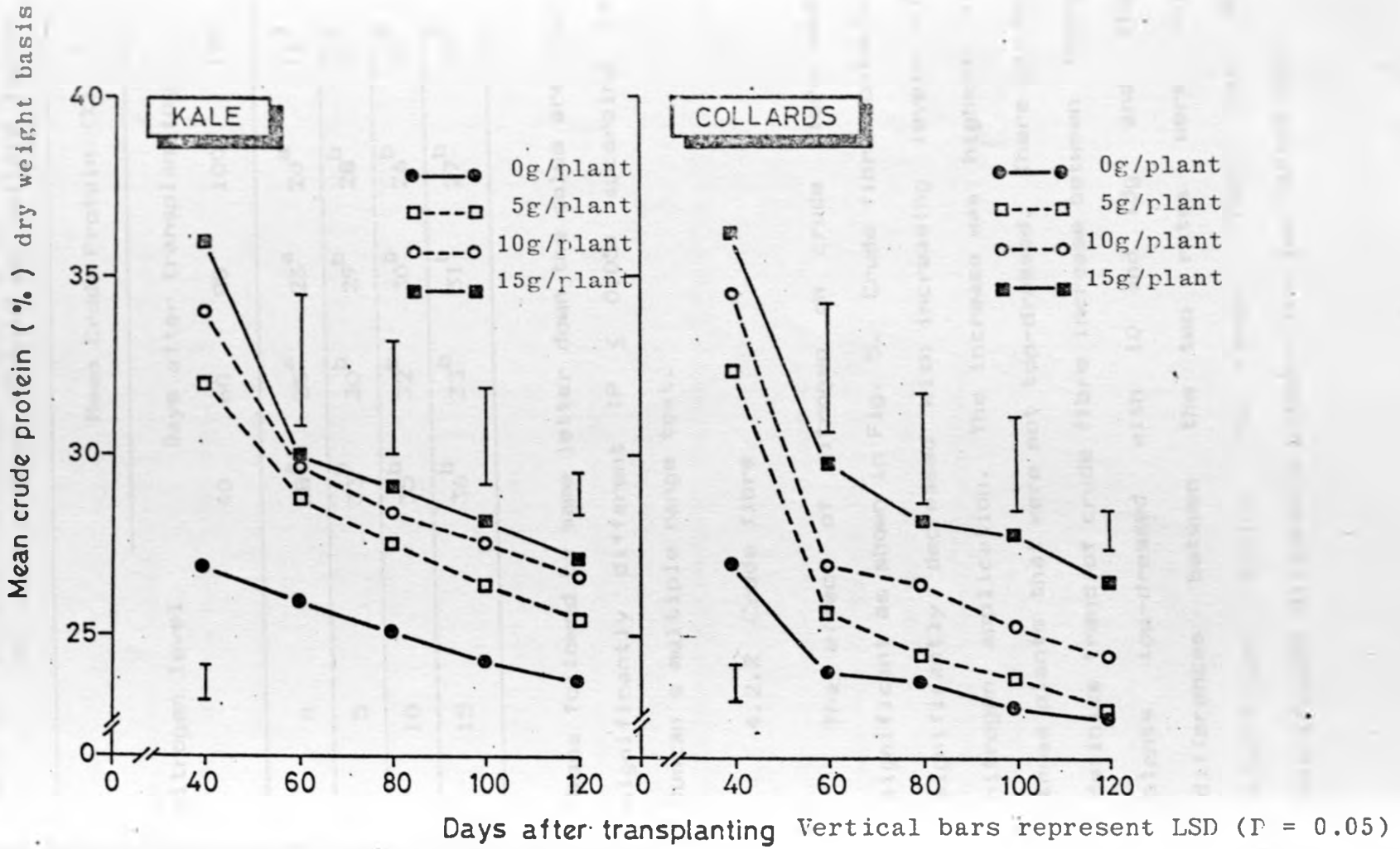


Fig. 4: Effect of Nitrogen rates and period of plant age on crude protein in kale and collard leaves - First Experiment

Table 4: Interaction between nitrogen and plant age on crude protein of kale and collard leaves

Nitrogen level	Mean Crude Protein (%)				
	Days after transplanting				
	40	60	80	100	120
0	28[a]	25[a]	23[a]	20[a]	16[a]
5	33[b]	30[b]	29[b]	28[b]	20[b]
10	35[b]	32[b]	30[b]	26[b]	21[b]
15	36[b]	33[b]	31[b]	27[b]	21[b]

Means followed by same letter down the colum are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

4.2.2 Crude fibre

The effect of nitrogen on crude fibre was significant as shown in Fig. 5. Crude fibre contents significantly decreased with increasing levels of nitrogen application. The increase was highest in those plants that were not top-dressed. There was no definite trend of crude fibre increase between those plants top-dressed with 10 and 15g and the differences between the two rates were not significant during the study. There was no significant difference between the two varieties.

The proportion of crude fibre in the leaves increased with the age of the plants for the period of 80 days of harvest as shown in Fig. 5. At 100 days after transplanting the proportion of crude fibre was almost constant till the end of the experimentation.

The interactions between nitrogen and plant age, nitrogen and variety and nitrogen, variety and plant age were not significant.

4.2.3 Crude lipids

The effects of nitrogen on crude lipids were significant as shown in Fig. 6, but there was no significant difference between the two varieties. Crude lipid significantly increased with increasing level of nitrogen application. The increase was highest in those plants that were top-dressed with 15g N. The rate of increase of cruid lipid with increasing rate of nitrogen was lowest between 5 and 10g per plant.

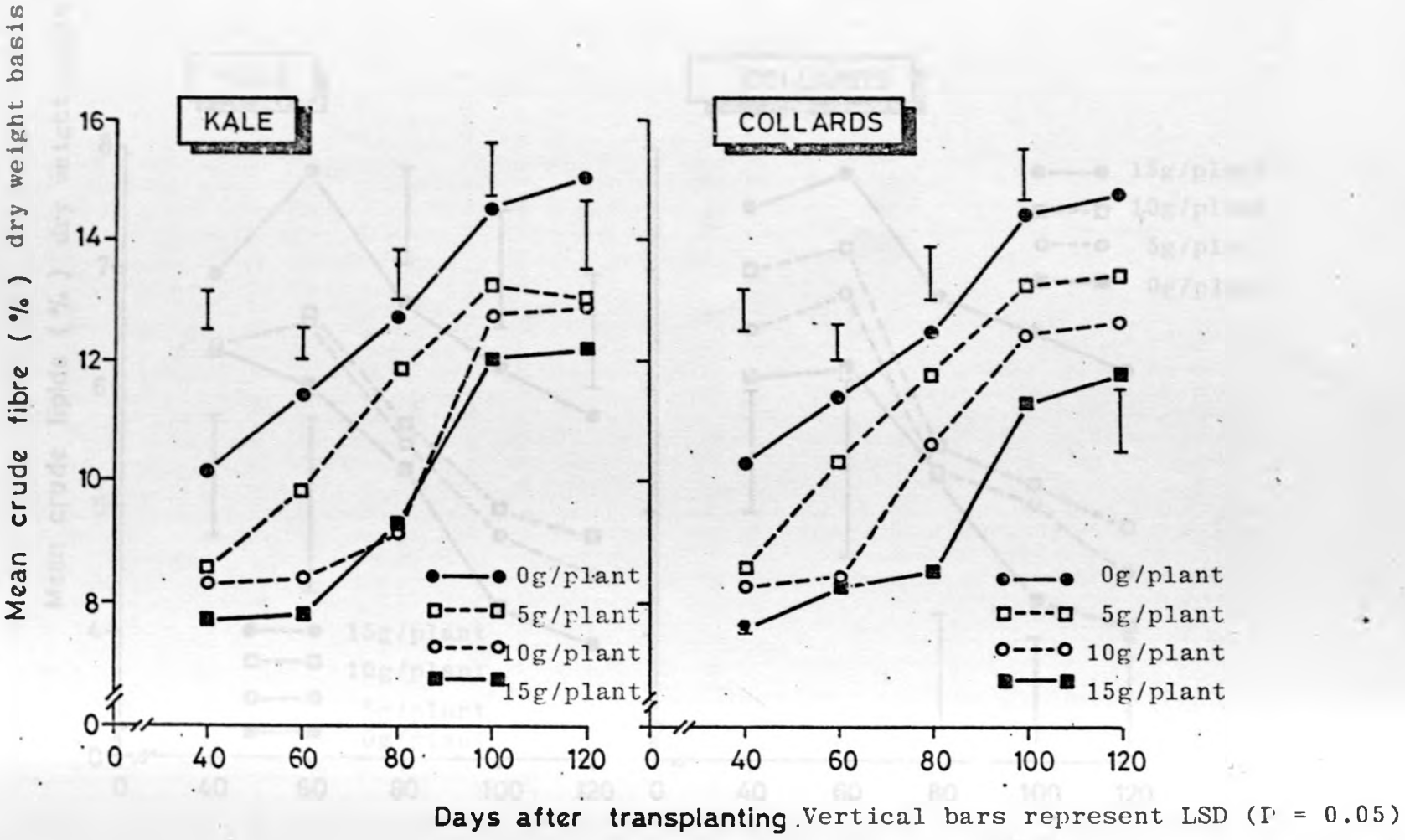
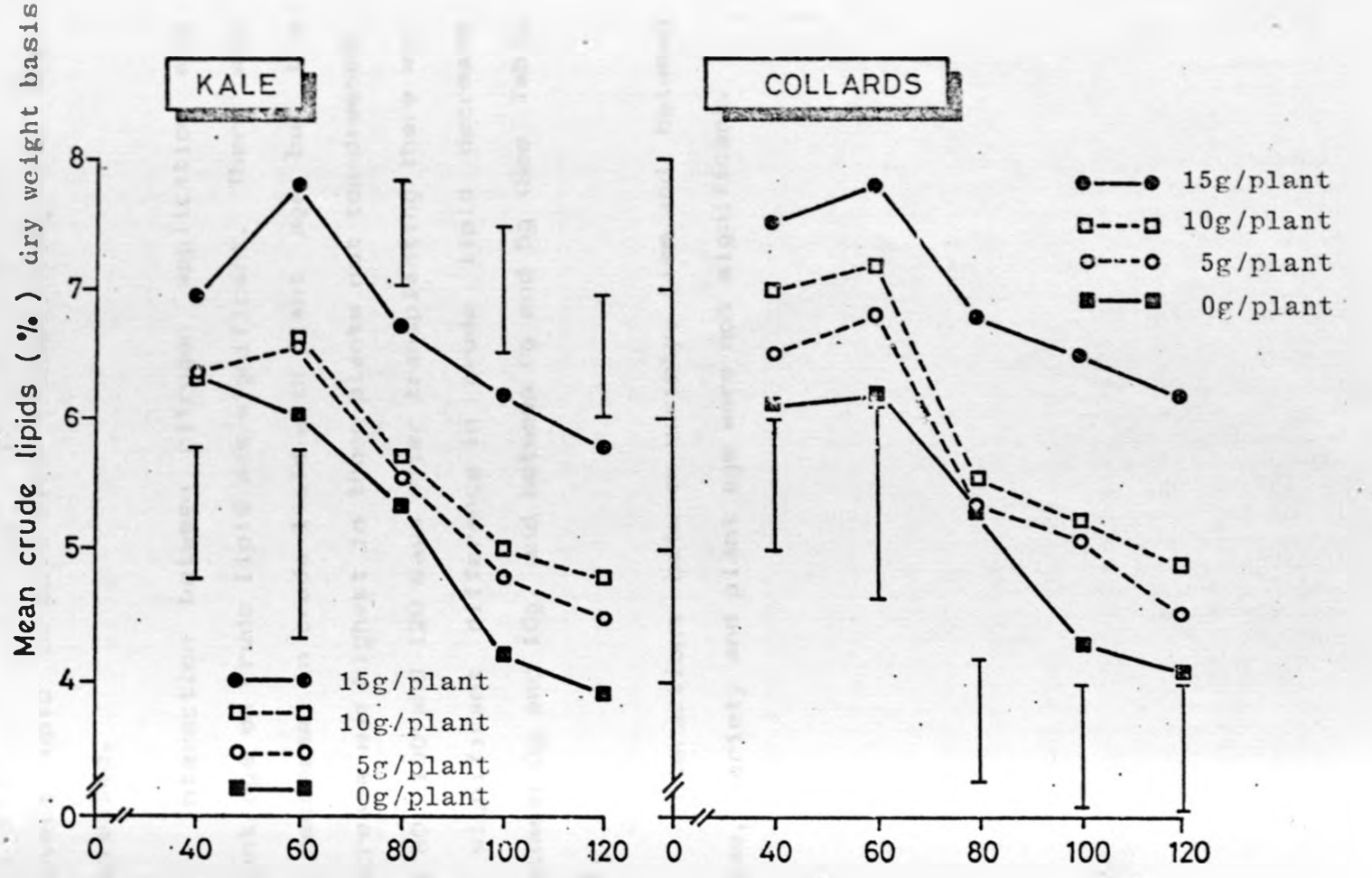


Fig. 5: Effect of Nitrogen rates and plate age on crude fibre in collards and kale leaves - Second Experiment



Vertical bars represent LSD (P = 0.05)

Lipids tended to increase with the period of harvest upto 60 days after transplanting and then decreased.

Interaction between nitrogen application and plant age on crude lipid was significant. There was a decrease in crude lipid with plant age but the decrease was highest in those plants not top-dressed. At 80, 100 and 120 days after transplanting there was a significant difference in crude lipid decrease between 0g and 10g, and between 0g and 5g (See Table 4).

Interactions between variety, time and between time, variety and plant age were not significant.

Table 5: Interaction between nitrogen rates and plant age on crude lipids of kale and collard leaves

Nitrogen Level	Mean Crude Lipid (%)				
	Days after transplanting				
	40	60	80	100	120
0	6.5[a]	5.5[a]	5.0[a]	4.0[a]	3.0[a]
5	6.5[a]	5.6[a]	5.4[b]	4.4[b]	3.8[b]
10	6.5[a]	5.4[a]	5.3[b]	4.5[b]	4.0[b]
15	7.0[b]	6.5[b]	5.7[c]	5.2[c]	5.0[c]

Means in the column followed by same letter(s) are not significant different ($P \leq 0.05$) according to Duncan's multiple range test.

4.2.4 Total ash

The effect of nitrogen on total ash was significant as shown in Table 6. Total ash significantly increased with increasing level of nitrogen. The difference between the two varieties was not significant.

The effect of age on total ash was not significant. Total ash remained constant with harvesting period.

4.2.5 Calcium

There was no significant effects of N levels, period of harvest and varieties on calcium content.

Table 6: Effects of nitrogen levels on total ash of kale and collard leaves

N-Levels	Total Ash
Kale	
0	13.65[a]
5	15.12[b]
10	16.13[c]
15	17.30[d]
Collard	
0	13.60[a]
5	15.11[b]
10	16.13[c]
15	17.09[d]

Means followed by the same letter down the column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

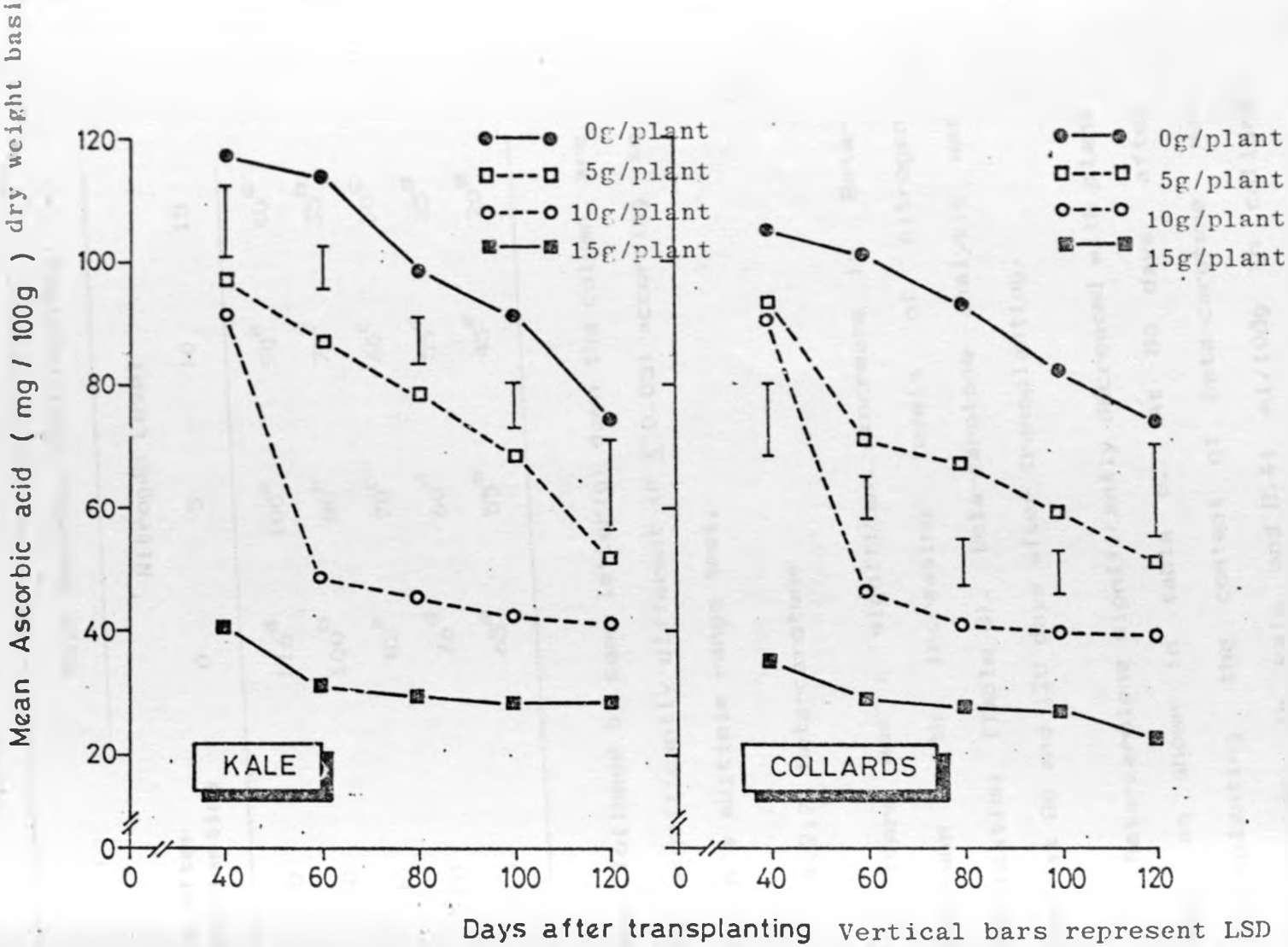
4.2.6 Ascorbic acid

The effect of nitrogen application in influencing ascorbic acid accumulation was significant in both varieties. Ascorbic acid significantly decreased with increasing levels of nitrogen (Fig. 7).There was no significant difference between the two varieties.

Ascorbic acid significantly decreased with the age of the plant as shown in Fig. 7.

Interaction between plant age and nitrogen application on ascrobic acid on the leaves was significant. Throughout the harvesting period all the N rates gave significantly different ascorbic acid values except at 40 and 120 days after transplanting. At 40 and 120 days after transplanting there was no significant differences in ascorbic acid content from plants top-dressed with nitrogen rates of 5 and 10g N. The decrease in ascorbic acid with plant age was highest in those plants top-dressed with 10g N per plant (See Table 7).

Interactions between variety and plant age, and between variety, nitrogen application and plant age were not significant.



Vertical bars represent LSD (P = 0.05)

Fig. 7:- Effect of Nitrogen and plant age on ascorbic acid in collards and kale leaves - Second Experiment

Table 7: Interactions between nitrogen rates and plant age on ascorbic acid of kale and collard leaves

	Mean Ascorbic Acid (mg/100g)			
	Nitrogen Level			
Days After Transplanting	0	5	10	15
40	116[a]	100[a]	98[a]	40[a]
60	100[b]	80[b]	75[b]	35[b]
80	82[c]	70[c]	60[c]	30[c]
100	76[d]	60[d]	55[d]	25[d]
120	65[e]	55[e]	45[e]	20[e]

Means followed by same letter(s) down the column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

4.2.7 Beta-carotene

There was a significant increase in Beta-carotene with increasing levels of nitrogen application (Table 8). Beta-carotene analysis was done at 80 and 120 days after transplanting.

Beta-carotene significantly decreased with plant age as shown in table 8. At 80 days after transplanting the content of Beta-carotene was 8.68ml/100g in kale and 8.41 ml/100g in collard leaves while at 120 days after transplanting the

Table 8: Effects of nitrogen levels on beta-carotene (mg/100g) in kale and collard leaves (Second experiment)

N-Levels	Beta-carotene (mg/100 DM)	
Kale	120 days	80 days
0	4.95[a]	6.27[a]
5	5.71[b]	7.23[b]
10	6.85[c]	8.68[c]
15	7.56[d]	9.58[d]
Collard		
0	4.85[a]	6.15[a]
5	5.53[b]	7.00[b]
10	6.64[c]	8.41[c]
15	7.17[d]	9.08[d]

Means followed by same letter down the column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

Beta-carotene content was 6.85 ml/100g in kale and 6.64 m/100g in collard leaves at Nitrogen level of 10g. The decrease on the average was 21%.

There was no significant difference between the two varieties. Interactions between variety and nitrogen and nitrogen and plant age and nitrogen, plant age and varieties were not significant.

4.3 The Effect of Storage Condition and Time on Ascorbi Acid

4.3.1 Storage condition

Effect of storage condition on ascorbic acid content in leaves was significant. Figure 8 shows that leaves stored on the shelf lost ascorbic acid at higher rate than those stored in a refrigerator. Thus those leaves stored in a refrigerator retained ascorbic acid for a longer period i.e. at two days of shelf storage and fourteen days of refrigerator storage the retention of ascorbic acid was almost the same.

4.3.2 Storage duration

Figure 9 shows the effect of storage and nitrogen application on ascorbic acid under refrigeration. Ascorbic acid significantly decreased with the period of storage in the refrigerator at the rate of 25%.

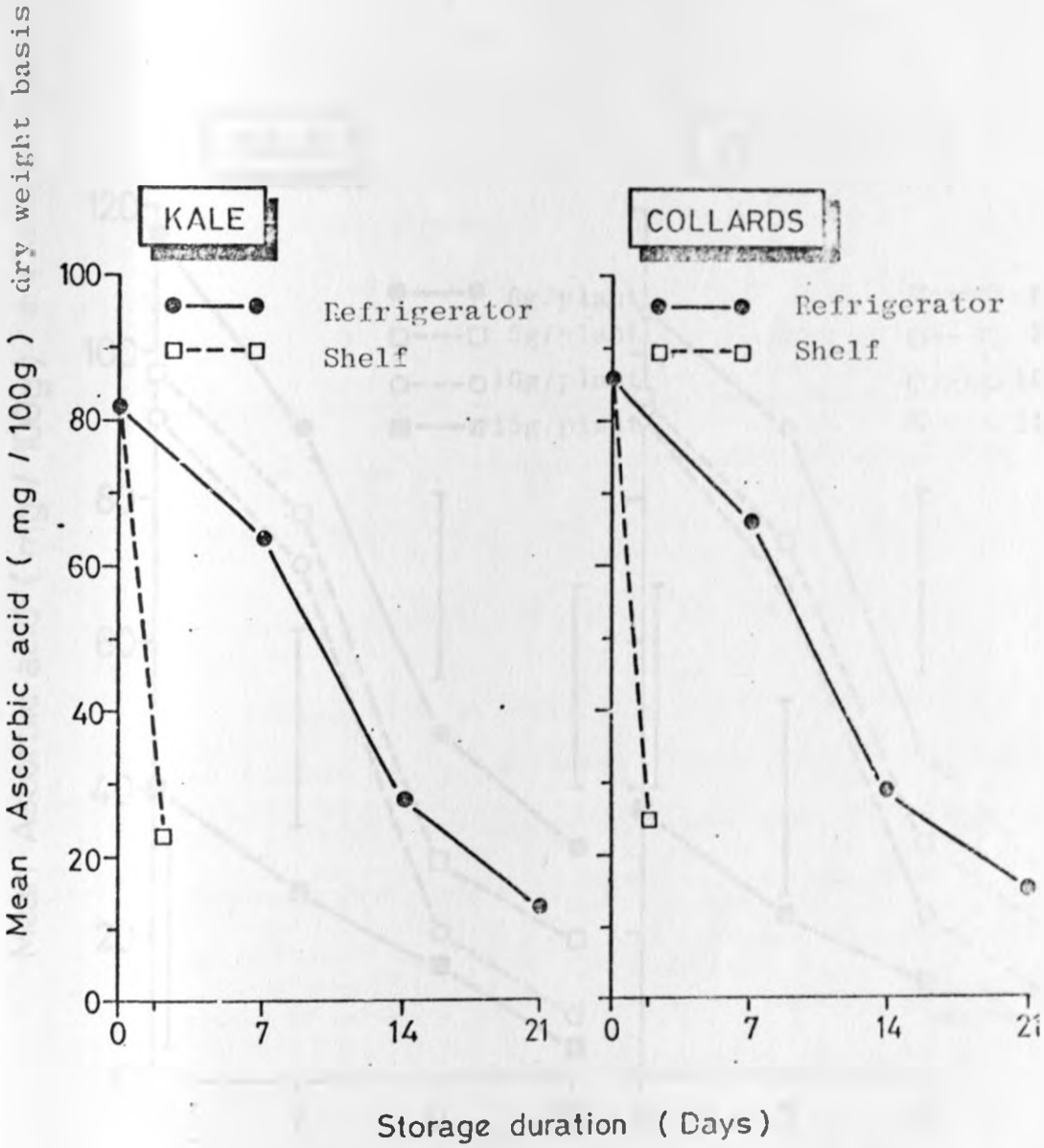


Fig. 8: Effect of storage time on ascorbic acid in the leaves of kale and collards - Second Experiment

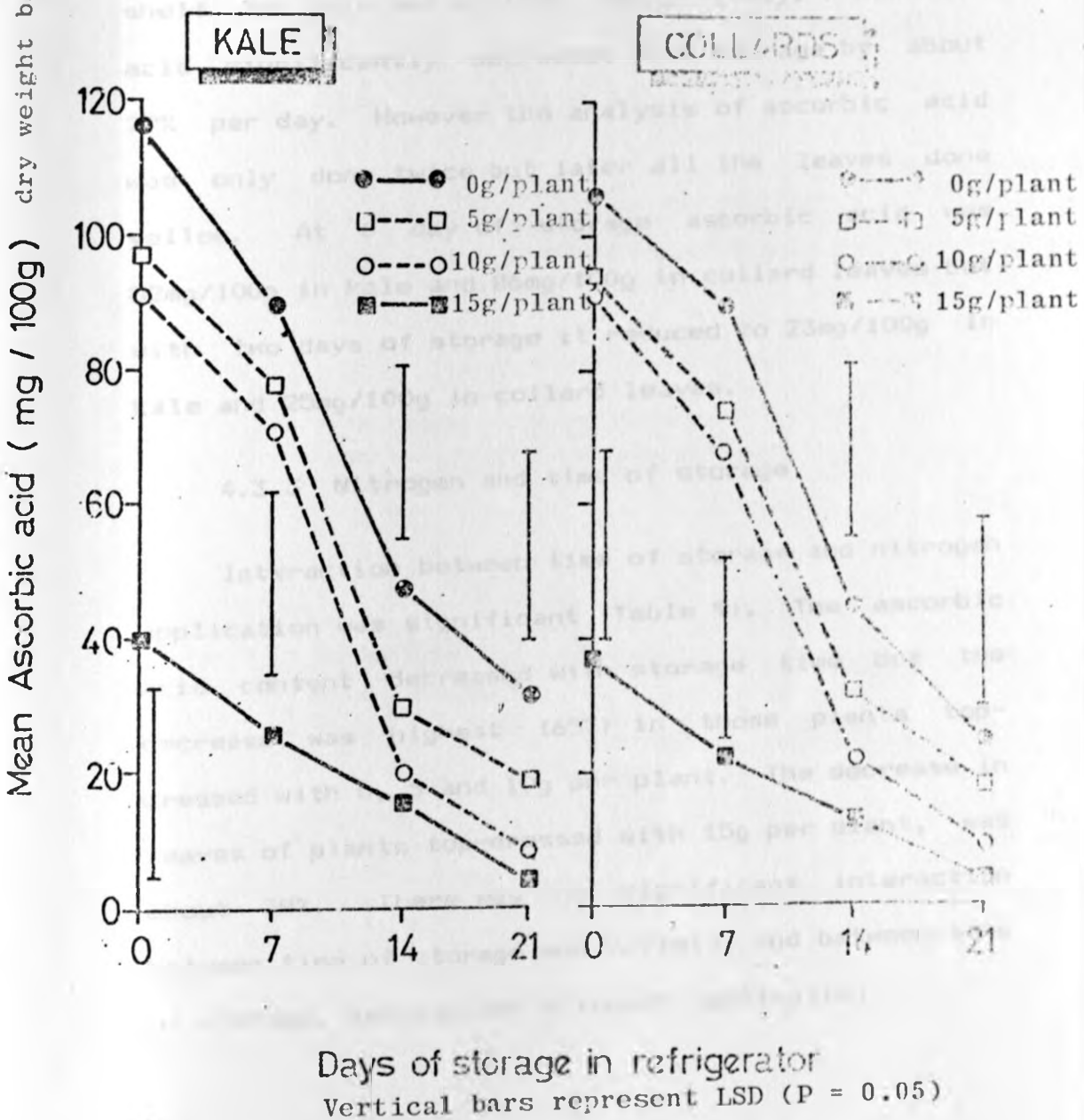


Fig. 9: Effect of Nitrogen and storage period in a refrigerator on ascorbic acid in kale and collard leaves - Second Experiment

Fig. 10 shows the effect of storage and nitrogen application on ascorbic acid in leaves stored on the shelf for kale and collard respectively. Ascorbic acid significantly decreased with storage by about 72% per day. However the analysis of ascorbic acid was only done twice but later all the leaves done yellow. At 0 day of storage ascorbic acid was 82mg/100g in kale and 86mg/100g in collard leaves but with two days of storage it reduced to 23mg/100g in kale and 25mg/100g in collard leaves.

4.3.3 Nitrogen and time of storage

Interaction between time of storage and nitrogen application was significant (Table 9). The ascorbic acid content decreased with storage time but the decrease was highest (65%) in those plants top-dressed with 0, 5 and 10g per plant. The decrease in leaves of plants top-dressed with 15g per plant, was about 38%. There was no significant interaction between time of storage and variety, and between time of storage, variety and nitrogen application.

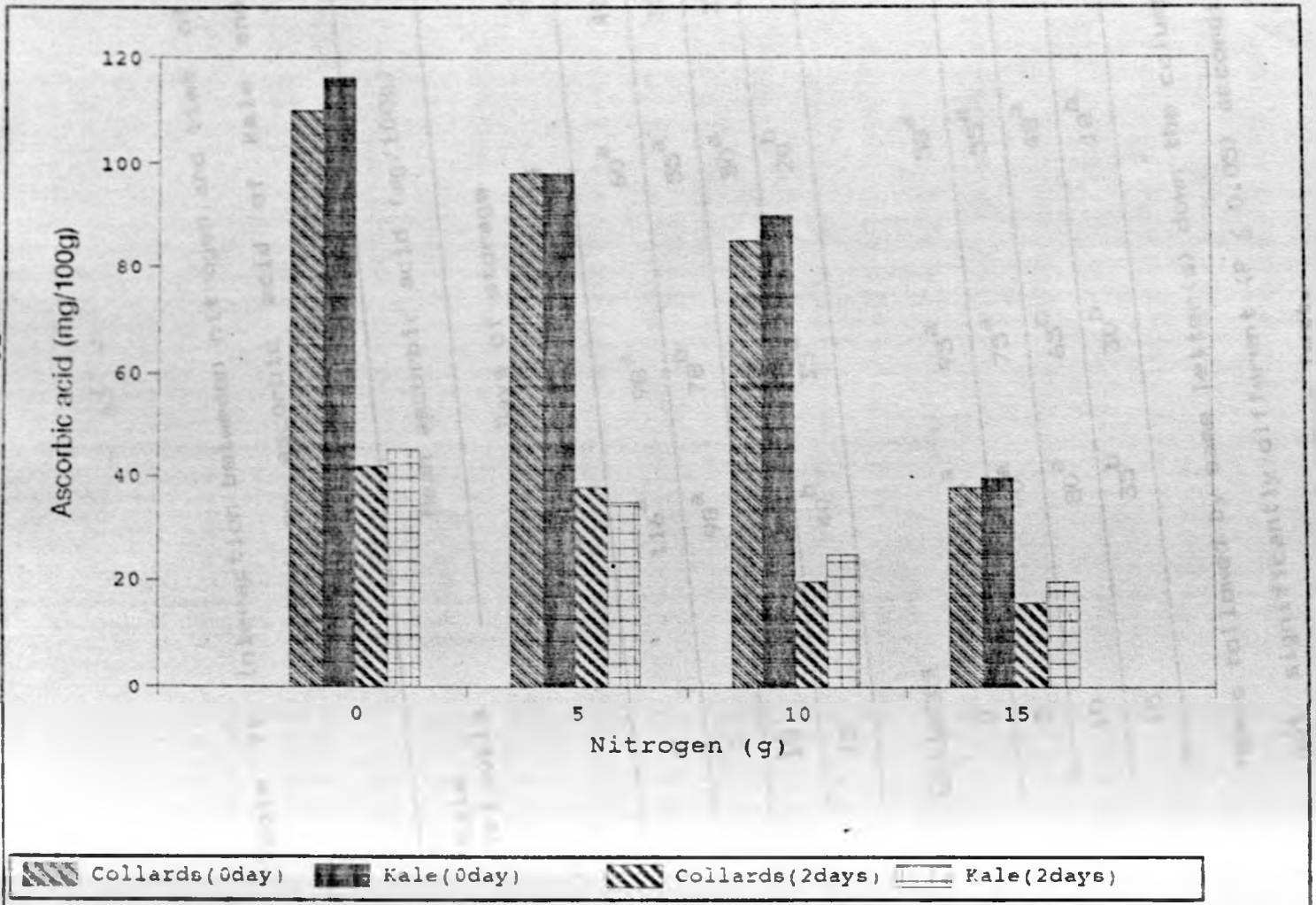


Fig 10: Effect of Nitrogen and Storage Period on Ascorbic Acid (Shelf Storage)

Table 9: Interaction betweeen nitrogen and time of storage on ascorbic acid of kale and collard leaves

Kale N-Levels	Mean ascorbic acid (mg/100g)			
	Days of storage			
	0	7	14	21
0	116^{a}	98^{a}	60^{a}	40^{a}
5	98^{a}	78^{b}	55^{a}	35^{a}
10	85^{a}	68^{c}	50^{a}	25^{a}
15	40^{b}	35^{d}	20^{b}	5^{b}
Collards				
0	110^{a}	95^{a}	58^{a}	40^{a}
5	90^{a}	75^{a}	55^{a}	35^{a}
10	80^{a}	65^{c}	48^{a}	22^{a}
15	35^{b}	30^{b}	18^{b}	4^{b}

Means followed by same letter(s) down the column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Nitrogen, Variety and Plant Age on Leaf Yield

5.1.1 Effect of nitrogen

From the results, it is evident that leaf yield increased with increase in nitrogen application. These results agree with the findings of Splittsoeseer, et. al. (1974) and Chweya (1984). Splittsoesser et. al. (1974) while working with kale among other crops had also found that increasing nitrogen rates increased the fresh weight of the leaves. Chweya (1984), who worked with kale found that nitrogen tended to increase fresh weight and number of leaves, therefore, yield. The increase in cumulative leaf yield with increasing nitrogen rates may be due to the fact that nitrogen induces leaf production and increases leaf cell expansion (Hewitt and Smith, 1975 and Huffaker and Rains, 1978). Yields of collards, a very close relative of kale, have been increased appreciably by application of nitrogen fertilizer (Del Valle, 1971; Oloya, 1976).

Nitrogen application increases yields because leaf size is determined by cell expansion which is influenced by nitrogen supply among other factors (Mithorpe and Moorby, 1974; Cutter, 1971). Allen (1972) reported that application of nitrogen in rapeseed plant led to more vigorous growth and development as was reflected by increase in stem length, number of flowering branches, total plant weight, leaf area index and hence high yield.

The lower yields at rates of nitrogen above 10g per plant was probably due to the fact that high levels of nitrogen checked the growth of the plants. This is because nitrogen is very soluble in water and not absorbed by the soil, thus it raises the osmotic pressure of the soil solution around the plants to a damaging level if used at too high levels (Russell, 1973). These results agree with the findings of Borna (1971), who, while working with lettuce and cabbage, found that heavy application of nitrogen fertilizer caused increase in yield but only upto certain maximum, further increase in fertilizer gave no further increase in yield, and even checked growth of the plant. High application of calcium ammonium nitrate decreased the yields because high levels of calcium ions created ionic imbalance in the soil rendering other ions like mg^{2+} to become unavailable.

Mengel and Kirkby (1979) reported that too much calcium ammonium nitrate applied in the soil changes the environment around the roots of a plant. Jacob and Van Uxekull (1963) stated that in many crops, yield increases with increasing amount of nitrogen applied upto a certain level. They further stated that higher increase of nitrogen above this level does not result in higher increase and very high levels may reduce yields. However, these authors further stated that the optimum amount varies with the plant species, soil nitrogen level, and the balance of nitrogen and other plant nutrients. According to these authors high levels of nitrogen above the optimum would not show any response in yield increase. From the findings of other workers cited above the levels of 15g N per plant used in this study were too high.

There is also possibility of plant/plant interactions. As plants grow at a fast rate because of nitrogen, they start competing for light and water and hence these two factors become limiting and, therefore, the plants may put on less leaves and hence lower yields (Salisbury and Ross, 1986). However, biomas and plant yield height was not measured during the study.

5.1.2 Effect of plant varieties

The cumulative leaf yields did not differ significantly between the two varieties, even though the total yield overall was significant. Overall collard outyielded kale by about 20%. This may be due to the fact that collard have more erect leaves with a darker green colour than kale. Thus the former carry out photosynthesis per plant at higher rates than the latter, therefore grow faster (Annon. 1975).

Difference in total yields between varieties may be attributed to varietal differences in their ability to use environmental resource. Differences in their ability to differentiate leaves at the stem apex could also contribute to yield differences. Leaf angle and size could also contribute to differences in yield (Allesi and Powell, 1975). Collard had more dense canopy cover and more erect leaves than kale. Light interception by less erect leaves was shown to be greater in beans by (Gardiner et. al., 1978). Contribution towards yield by dense canopy cover and leaf angle cannot be deduced from this study since no leaf angles, size and light interception were measured. Dense canopy cover gave a better ground cover which suppressed weeds and also reduced evaporation and soil temperature.

Chahira (1982) reported significant differences between kale varieties. He found that dwarf-Siberian kale was significantly more superior to 'Thousand headed' and 'Marrow-stem' kales in terms of yield. Kanampiu (1987) while working with collard and kale found that 'Georgia' collards yielded significantly more than 'Thousand heaed' kale.

5.1.3 Effect of plant age

The effect of plant age on the leaf yield was significant in both collard and kale leaves. The yields increased upto 60 days after transplanting then remained almost constant for about 60 days then significantly declined by about 65%.

The increase in yield with age of the plant may be due to the fact that as the plant grows, it puts on more vegetative materials before reproductive phase starts (Bleasdale, 1980). Overall, leaf yields remained stable till 120 days after transplanting beyond which time, there was a drop of 65%. These findings agree with those of Itulya (1985), who while working with kale, found that overall, leaf yield remained stable till 90 days after transplanting beyond which there was a drop of 47% for each subsequent harvest. The decrease in yield may be due to the fact that as the plant grows old it starts to senescence thus yields decrease (Sulisbury

and Ross, 1986; Goldsworthy et. al., 1984). The decrease in yield also can be attributed to the fact that as the picking of leaves, especially the lower leaves, progresses, the roots of the plant get weakened resulting in reduced growth, especially in plants with erect leaves (Evans, 1975). In this study leaves were continuously harvested, so a lot of nitrogen was removed from the plant which led to decrease in growth and hence low yields. This was in line with the findings of Hewitt and Smith (1975) who, while working with cauliflower (B. oleracea var botrytis L.) found that chloroplast production was reduced when nitrogen is deficient. Chloroplasts are essential cell components and their reduction lowers the photosynthetic ability of the leaf (Cutter, 1971). Hewitt and Smith (1975) also found that in rape (Brassica napus L.) premature cell vacuolation and early differentiation or senescence occur if nitrogen is deficient. As leaves are picked, there are less leaves left and hence photosynthesis ability is reduced. Thus there is less photosynthates which are translocated to roots, and hence less roots are formed to explore the soil volume for nutrients. The yields decrease after 60 days of harvesting perhaps because of lack of nitrogen which is required for vegetative growth (Tisdale et. al., 1985).

Yields of 'Georgia' collards were significantly higher than those of 'Thousand headed' kale by about 20% from the time at which harvesting started upto 120 days after transplanting. Thereafter the mean yields of the two varieties at any harvest did not differ significantly. This was due to the fact that 'Georgia' collards started bolting so that most of the photosynthates were channelled to formation of flowers rather than to formation of new leaves. 'Thousand headed' kale was attacked by rot disease caused by Xanthomonas compestris, hence reducing the yields.

5.1.4 Nitrogen and variety interaction

The interaction of nitrogen and variety on leaf yield was significant during the second experiment. The analysis of variance was calculated on total yield basis. Applying nitrogen beyond 10g per plant gave a high decrease in yield but the response was higher in collard than in kale. This is because collard seems to be more responsive to high nitrogen levels than kale. This could also be explained by their genetic make up differences. As explained earlier in this chapter, collard grow faster than kale so plant/plant competition is higher in collard than in kale.

5.1.5 Plant age and variety interaction

The interaction between variety and plant age on yield was significant. There was a general increase in yields with increase in plant age but the increase was more in collard than in kale. Collard grow faster than kale and hence the response was high (Anon. 1976). Collard have erect leaves which intercepts high amounts of sunlight so the rate of photosynthesis is high (Bleasadale, 1984).

5.2 The Effect of Nitrogen and Plant Age on the Nutritive Value

5.2.1 Crude protein

The effect of nitrogen application on leaf crude protein was however significant between those plants top-dressed and those that were not. Crude protein tended to increase with increasing rates of nitrogen. Nitrogen influences protein/carbohydrate ratio in the leaves (Mengel et. al., 1979). Suboptimal nitrogen supplies result in high contents in high contents of carbohydrates due to lack of nitrogen. This restricts protein synthesis so that more photosynthates are available for synthesis of carbohydrates. Eppendorfer (1978a) while working with kale and spinach and Breteler (1982) while working with maize reported that low nitrogen

restricts synthesis of protein. Work done by Blamey who worked with sunflower and Chweya (1984) who worked with kale found that protein levels increased with increasing rates of nitrogen application. The amount of protein synthesized seems to depend on nitrogen supply. Abundant nitrogen supply and light intensity favour the formation of chloroplasts (Barta, 1975). As these organelles contain much protein and carotene the protein content increases. The increase in protein contents in kale and collard leaves was also reported by Hammerton (1967) who worked with kale and De Valle (1971) and Oloya (1976) who both worked with collard.

Crude protein significantly decreased with plant age in both varieties. As the plants were harvested continuously, most of the nitrogen may have been removed from the plants. This may have affected the synthesis of proteins since nitrogen is an element required in the synthesis of proteins. Chweya (1982), while working with kale found that total nitrogen declined with harvesting time. The decline in proteins may also have been due to dilution of accumulated nitrogen in the plant because of increased growth (Barta, 1975). The decline in total nitrogen has also been reported by Barker et. al. (1971), and Lawrence et. al. (1982).

The interaction between nitrogen application and plant age on crude protein was significant. There was a general decrease in crude protein with plant age but the decrease was more from plants that had not been top-dressed than those that were top-dressed. The rapid decrease of crude protein in plants that were not top-dressed could be due to nitrogen supply. These plants lacked nitrogen and since nitrogen is involved in synthesis of crude protein, there was a high decrease. As explained earlier in this chapter nitrogen and light are the major factors required for protein synthesis (Barta, 1975). Thus the rapid decrease of crude protein in those plants that were not top-dressed was due to lack of nitrogen. Nitrogen is involved in synthesis of chloroplasts which are rich in proteins (Mengel, 1979). Interaction between nitrogen, variety and plant age on crude protein was not significant.

5.2.2 Crude fibre

The effect of nitrogen on crude fibre was significant. Crude fibre contents decreased with increasing levels of nitrogen application.

The decrease in crude fibre with increase in rate of nitrogen application may be attributed to the fact that nitrogen is an "exogenous" factor. Nitrogen is one of the exogenous factors which

influences carbohydrate ratio in the leaves (Mengel, 1979). Low levels of nitrogen result in high proportion of crude fibre (Salukhe, 1973). Further, lack of nitrogen restricts protein synthesis so that more photosynthates are available for proportion of carbohydrates synthesis (Mengel, 1979). Eppendorfer (1978a) who worked with spinach, kale, cauliflower and potatoes also reported that high levels of nitrogen decreases proportions of carbohydrates.

The proportion crude fibre in leaves significantly increased with the age of the plant. Crude fibre increases with age of the plant due to the fact that as the plant grows old, most of the carbohydrates are stored as crude fibre thus the plant puts on thick leaves (Norman et. al., 1985). As the plant grows old, it exhausts most of the nutrients especially nitrogen from the soil so that there is a decrease in photosynthesis. This leads to few roots being formed and hence the plant can not exploit the soil volume for water (Kramer, 1972). When plants lack water most of the cellwall thickens with cellulose to prevent water loss (Kramer, 1972). Thus the highest proportion of crude fibre is cellulose and hence percentage crude fibre in the leaves increases. The findings of crude fibre increasing with age agrees with the findings of

Mengel (1979) and Nieuwhof (1969) who reported that the content of crude fibre in the leaves depends very much on plant age.

5.2.3 Crude lipid

The effect of nitrogen on crude lipids were significant. Crude lipid significantly increased with increasing levels of nitrogen application. The method used for determination of lipids also includes chlorophyll in the results. Any factor contributing towards increase in chlorophyll and chloroplasts will show increase in crude lipids (Barta, 1975). Since nitrogen increase the rate of chloroplasts formation, it can be reasoned out that it also increases leaf lipids.

Lipid tended to increase with period of harvest upto 60 days after transplanting and then decreased. The increase was highest in those plants that were top-dressed. The initial increase may be due to the fact that since the plants were still young, there were still high levels of nitrogen so plants were forming leaves which had a lot of chloroplasts. This was also reported by Collins (1988) who workied with alfafa, red clover and birds foot. Sixty days after transplanting, lipids decreased after each consecutive harvest. The decrease could be due to the fact that since lipids are associated with

chloroplasts, as the plant becomes old, it starts to senescence and hence few chloroplasts are manufactured leading to decrease in lipids (Barta, 1975). Lipids decrease with age because of dilution of nitrogen in the plant and nitrogen is a major precussor of lipids in the leaves. This agrees with the findings of Mengel (1979).

There was a significant interaction, in both experiments, between nitrogen rates and plant age for crude lipid content in leaves. The crude lipid content decreased with plant age but the decrease was highest in those plants which were not top-dressed than those plants which were top-dressed. This rapid decrease in those plants which were not top-dressed was associated with depletion of nitrogen in these plants according to reports by Barta (1975) which stated that the consistently rapid decline in the crude lipid seem to be associated with rapid dilution of nitrogen in these plants. As explained earlier in this chapter lipids are associated with chloroplasts and chloroplasts are manufactured from nitrogen. Interactions between variety, time and between time, variety and plant age were not significant.

5.2.4 Total ash

The effect of nitrogen on total ash in collards and kale leaves was significant. Total ash significantly increased with increasing level of nitrogen. The increase may be attributed to increase in growth because of increase in nitrogen supply. This agrees with the finding of Mengel and Kikby (1979) who reported that with increasing level of nitrogen there is an increase in plant growth and hence higher mineral uptake. Nitrogen uptake also enhances the uptake of other nutrients. This conclusion was also reached by Lawrence et. al. (1982) who found that nitrogen fertilizer increased the percentage of phosphorus and potassium of the forage. Higher uptake of other minerals due to increased nitrogen was also reported by Walter (1973), who, while working with potatoes found that there was high uptake of potassium when nitrogen supply was high.

5.2.5 Calcium

There was no significant increase in calcium due to increase in N levels or plant age. Most soils contain enough calcium for crop growth if the crop is not affected by soil acidity (Walter, 1973). Since Brassicas falls in the catagory of crops which are not affected by soil acidity then it can be reasoned

out the kale and collard never responded to additional of calcium ions in the soil. Lawrence et. al. (1982) and Chweya (1982) reported similar results that calcium remained constant throughout the harvesting period.

5.2.6 Ascorbic acid

Ascorbic acid significantly decreased with increasing levels of nitrogen. The synthesis of vitamin C is associated with carbobydrate metabolism. The precursor is glucose which is activated by uridine trisphosphate (UTP) and then oxidized to the activated glucoronic acid the direct precursor of vitamin C. Thus all processes, which promote the synthesis of UPT or adenine trisphosphate (ATP) and glucose, favourably influence synthesis of vitamin C. Increasing nitrogen application decreases vitamin C. This is probably results from competion for photosynthates between glucose and amino acid metabolism (Peck, 1981). When nitrogen supply is high more photosynthates are used for the synthesis of amino acids and thus less photosynthates are available for synthesis of glucose (Mengel, 1979). Lewandowka and Skapski (1977) reported similar results that increasing nitrogen application in kale increased yields, but the content of ascorbic acid tended to decrease. However, these results are

contrary to those of Aberg and Edkahl (1948) who found less ascorbic acid in kales supplied with sub-optimal nitrogen application than those supplied with adequate nitrogen. This could have been so because the soils used were so low in nitrogen such adequate nitrogen supplied was not high to depress the synthesis of vitamin C.

Ascorbic acid varied with the age of the plant, such that as the plant aged ascorbic acid decreased. This could be due to the fact that as the plant aged, it exhausted most of the nutrients needed to synthesis vitamin C. This is supported by Brechti, et. at. (1976) who worked with tomatoes. Similar results were reported by George et. al. (1970) who worked with sorghum-sudangrass hybrid and found that plant ascorbic acid decreased over the 7-week period suggesting that the soil nutrient supply was being depleted. Abe et. al. (1977) who worked with Gynandropsis gynandra found that the highest levels of ascorbic acid were achieved after 50 days of harvest, then declined steadily throughout the remaining period.

There was a significant interaction, between nitrogen rates and plant age for ascorbic acid in the leaves. The ascorbic acid content decreased with the plant age but the decrease was highest in those

plants that were not top-dressed The rapid decrease in those plants not top-dressed could be associated with nutrient supply which was depleted as explained elsewhere in this chapter.

5.2.7 Beta carotene

There was a significant increase with increasing levels of nitrogen application. Beta-carotene analysis was done at 80 and 120 days after transplanting. There was, however, no significant difference between the two varieties. Beta-carotene is closely associated with chloroplasts so any factor which increases chloroplasts formation favours increase of Beta-carotene. Beta-carotene increases with level of nitrogen application since nitrogen encourages chloroplasts formation (Fritz et. al. (1971a); Mengel 1979). Fritz et. al. (1971a) who worked with spinach, Nowakowski (1971), Venter (1979) and Vereeke et. al. (1979) who all worked with carrots, reported that Beta-carotene increased with increasing rates of nitrogen fertilization.

Beta-carotene significantly decreased with plant age. As the leaves are harvested most of the nutrients were removed from the soil and this limited root growth (Nieuwhof, 1969). The results agree

with reports by Brechti et. al. (1976) who worked with tomatoes and found that Beta-carotene decreased with the age of the plant.

5.3 The Effect of Storage Condition and Time on Ascorbic Acid

5.3.1 Storage condition

The leaves stored on the shelf lost ascorbic acid at a high rate than those stored in a refrigerator.

Leaves stored on the shelf deteriorated rapidly and were considered unfit (yellow and wilted) for human consumption after 2-3 days. This was in contrast with those which were stored in a refrigerator, which turned yellow after 21 days. These observations are in line with the findings of Aworh et al. (1978) who observed that spinach leaves stored at 20°C deteriorated rapidly and were considered unmarketable after 2 days, while those stored at 0°C still looked fresh after 15 days of storage. Ezell and Wilcoz (1959) working with kale also reported that at the slow wilting rate of 0°C the loss of ascorbic acid was lower than at 21.1°C. All stored vegetables are subjected to the same type of deterioration physiologically and biochemically. However, the relative importance of deterioration

varies considerably between different vegetables and storage conditions (Dennis, 1979). In a refrigerator, microbial and enzyme activities are low compared to their activities at ambient conditions. This is supported by Mazur and Harrow (1971) who reported that for leaves stored in refrigerator, there was low biochemical processes taking place because of inactivation of enzymes by low tempratures.

Thus those leaves stored in refrigerator retained ascorbic acid for a longer period i.e. at two days of shelf storage and fourteeen days of refrigerator storage the retention of ascorbic acid was almost the same.

5.3.2 Storage duration

Ascorbic acid significantly decreased with period of storage in the refrigerator at the rate of 25%. This was because ascorbic acid is sensitive to alkalinity, air, heat and light (Norman, 1973). As the leaves are exposed to such conditions, ascorbic acid is lost (Norman, 1973). Changes in the composition of raw vegetables after harvest may decrease their nutritive values. These changes occur during transportation, holding and subsequent storage. The decrease in ascorbic acid with storage agrees with reports by Salunkhe (1973) who reported

that if cabbage is stored for a long time it could have a high concentration of sulphur compounds but less ascorbic acid and other water soluble vitamins. In a refrigerator, the microbial activities still take place but at lower rates. The decrease in ascorbic acid may also be due to respiration of plant cells which could be taking place thus breaking down the ascorbic acid (Henz and Hunter, 1981).

Leaves stored on the shelf were only analysed for two days before they turned yellow. Ascorbic acid significantly decreased with storage by about 72% per day for kale and collard respectively. The decrease may be because of microorganisms and respiration of the plant cells which are very high at shelf storage. The rapid decrease in shelf stored leaves could be associated with senescence of the leaves according to reports by Chong and Berard (1983). High temperature on the shelf could also have contributed to high decrease of ascorbic acid. Burton (1982) recommended that a temperature of 0°C is desirable for leafy vegetables, because of their liability to water loss. At 0 day of storage ascorbic acid was 82 mg/100g in kale and 86 mg/100g in collards but with two days of storage it reduced to 23 mg/100g in kale and 25 mg/100g in collards.

5.3.3 Nitrogen and time of storage

Interaction between time of storage and nitrogen application was significant for refrigeration storage. The ascorbic acid content decreased with storage time but the decrease was highest in those plants top-dressed with 0, 5 and 10 g per plant. The less decrease in those plants top-dressed with 15 gN per plant could be associated with high content of proteins in the leaves. One of the factors which lowers the rate of respiration is low starch, fructose or sugars (Salisbury and Ross, 1986). Respiration is one of the factors which cause ascorbic acid to decrease in stored leaves (Norman, 1973). Salisbury and Rose (1986) report that when a plant is starved of the respiration substrates like sugar, proteins may be oxidized. It is also possible that when proteins are involved in respiration, the H^+ ions are released which increase acidity and hence lowering the rate of enzyme activity. During the last days of storage there was no significant difference between the various rates. This could be due to lack of substrate in the leaves of plants top-dressed with 0, 5 and 10 gN per plant since most of it had been used. There was no significant interaction between time of storage and variety, and between time of storage, variety and nitrogen application.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1. Summary and Conclusions

Increasing rate of N increased cumulative leaf yield. Plants that were top-dressed with more than 10g N per plant significantly increased leaf weight. Thus it can be recommended that those farmers with soils similar to Kabete soils, should not apply more than 10g N per plant. There was also a significant difference between 'Georgia' collard and 'Thousand headed' kale in terms of yield. 'Georgia' collard yield was higher than that of 'Thousand headed'kale for the period of harvesting considered. Thus for the period considered (140 days after transplanting) it can be recommended that farmers could grow 'Georgia' collard.

Cumulative yields significantly increased upto 60 days after transplanting after which they remained almost constant upto 120 days after transplanting, beyond which, there was a drop of about 65%.

From the data, it can also be concluded that nitrogen application has a significant effect on the nutritive value of 'Georgia' collard and 'Thousand headed' kale. Crude protein, lipids, total ash, and Beta-carotene increased with increasing levels of nitrogen application.

Time of harvest had a significant effect on nutritive value. Crude protein significantly decreased with period of harvest. Crude fibre significantly increased with period of harvest, whereas lipids tended to increase upto 60 days after transplanting then started to decrease for the rest of the harvesting period. Total ash remained almost constant during the harvesting period. Ascorbic acid and Beta-carotene decreased throughout the harvesting period.

The effects of storage on ascorbic acid in the leaves was significant. Ascorbic acid decreased with storage period. However, leaves stored on the shelf deteriorated rapidly and turned yellow only after 2-3 days. Storage of leaves in refrigerator reduced loss of ascorbic acid and also allowed the leaves to remain fit for use for a relatively longer period than storage on shelf. In the refrigerator the loss of ascorbic acid was low but after one month all leaves had turned yellow.

6.2 Suggestions for Further Research Work

Based on this study, farmers should not apply more than 10g N per plant. Thus it can be of importance if research is carried out by applying between 5g and 10g N per plant to establish the best rate. The research should be carried out in various ecological zones in the country. Since fertilizers are becoming expensive, trials should be carried out using farmyard manure which is less expensive, where it is available.

Another important factor which could be investigated is whether it is possible to lengthen the period of harvesting by applying nitrogen fertilizer in three or more splits. In this study yields started decreasing after 140 days after harvesting.

During the experiment, storage was done but only ascorbic acid was analysed. It can be of importance to analyse changes of other nutrients such as total ash, beta-carotene and calcium which constitute the high nutritive value of kale and collard leaves.

Kale and collard leaves are normally consumed after cooking. It could be useful to investigate the effect of cooking on nutritive value of the leaves.

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Appendix 1: Mean monthly weather record - field station, Kabete between June, 1988 and July, 1989

Month	Mean radiation	Mean sunshine	Temperature (°C)			Total rainfall mm/month	%RH mean	Total wind run km/day
			Max.	Min.	Mean			
1988								
June	15.85	-	21.3	12.6	16.9	50.9	75.5	56.3
July	15.58	3.3	20.7	11.6	16.1	18.7	77	48.2
August	10.89	3.7	20.9	11.9	16.4	46.9	76	50.6
September	13.15	4.6	22.6	12.1	17.3	27.1	71	67.9
October	16.27	7.6	24.5	12.8	18.5	16.7	64.5	97.5
November	15.07	6.9	22.1	13.5	17.8	105.3	72.5	112.2
December	15.27	7.8	22.0	13.0	17.5	139.1	72	134.0
1989								
January	16.57	7.5	23.3	13.0	18.1	134.6	67.5	75.1
February	18.92	9.4	23.9	12.3	18.1	45.1	57	110.3
March	18.87	8.8	24.9	13.7	19.3	93.1	61	87.9
April	13.10	5.4	22.0	13.8	17.9	210.5	78	72.0
May	14.38	6.1	22.0	13.6	17.8	497.0	74.5	25.9
June	12.40	5.2	20.8	12.3	16.5	27.5	73	26.4
July	10.01	2.8	20.8	11.2	15.6	44.2	77	14.6

Appendix 2: Some soil chemical characteristics at the field station, University of Nairobi before planting

Nutrient/Soil reaction	First experiment	Second experiment
pH Water	6.13 (0.216)	6.6 (0.26)
Calcium m.e%	7.85 (0.96)	6.5 (1.0)
Carbon %	1.55 (0.072)	1.4 (0.18)
Phosphorus (ppm)	11 (1)	8 (1.2)
Total N (vg/g soil)	1950 (295.8)	2500 (215)

( ) Standard deviations

Appendix 3: Analyses of variance (ANOVA) tables

Appendix 3.1: ANOVA for yields (Kg/m^2) throughout the year

Source of variation	Degrees of freedom	Mean sum of squares	
		A	B
Blocks	2	14.157363^{ns}	5.41^{**}
Variety	1	130.8764^{**}	123.51^{**}
Main plot error	2	10.0062955	0.117
Rates	3	221.221181^{**}	80.33^{**}
Rates x Variety	3	1.42155^{ns}	7.23^{**}
Subplot error	12	2.5969	0.95
Time	10	145.58294^{**}	106.3^{**}
Time x Variety	10	4.515444^{**}	3.5^{**}
Rates x Time	30	3.89127^{ns}	2.3^{ns}
Time x Rates x Variety	30	1.01006^{ns}	1.2^{ns}
Sub-Subplot error	160	1.46817	1
Total			

A - First experiment (yields)

B - Second experiment (yields)

ns - Not significant

* - Significant at 5% probability level

** - Significant at 1% probability level

Appendix 3.2: ANOVA for crude protein and crude fibre in the leaves

Source of variation	Degree of freedom	Mean sum of squares			
		A	B	C	D
Blocks	2	2.8970^{ns}	5.0019^{ns}	2.35^{ns}	1.66^{ns}
Variety	1	0.524041^{ns}	0.9364^{ns}	1.06^{ns}	0.234^{ns}
Main Plot error	2	4.4451296	0.2903	0.231	0.024
Rates	3	182.8718**	52.7100**	108.67**	55.0**
Rates x Variety	3	1.2660^{ns}	0.0067^{ns}	0.137^{ns}	0.11^{ns}
Sub-plot error	12	7.6277	2.0457	2.36	0.72
Time	4	117.8608**	189.7291**	0.87^{ns}	91.5**
Time x Variety	4	2.6866^{ns}	6.8922^{ns}	0.824^{ns}	0.60^{ns}
Rates x Time	12	8.7315*	1.2308^{ns}	0.572^{ns}	1.1^{ns}
Time x Rates x Variety	12	2.7047^{ns}	0.3050^{ns}	0.645^{ns}	0.27^{ns}
Sub-Subplot error	64	4.6677	0.2631	1.00	0.62
Total	119				

A - First experiment (crude protein)

B - First experiment (crude fibre)

C - Second experiment (crude protein)

D - Second experiment (crude fibre)

ns - Not significant

* - Significant at 5% level

** - Significant at 1% level

Appendix 3.3: ANOVA for total ash and fat in the leaves

Source of variation	Degree of freedom	Mean sum of squares			
		A	B	C	D
Blocks	2	4.1971*	11.16*	2.13^{ns}	0.100^{ns}
Variety	1	0.9470^{ns}	0.236^{ns}	0.086^{ns}	0.146^{ns}
Main Plot error	2	0.1755	0.386	1.26	0.028
Rates	3	38.5696**	20.84**	68.9**	18.21**
Rates x Variety	3	0.8974^{ns}	0.463^{ns}	0.06^{ns}	0.129^{ns}
Sub-plot error	12	3.7119	2.36	0.1	0.253
Time	4	44.6070^{ns}	33.82**	3.5^{ns}	9.58**
Time x Variety	4	2.204775^{ns}	0.32^{ns}	0.13^{ns}	0.189^{ns}
Rates x Time	12	1.3540^{ns}	2.5**	0.4^{ns}	0.189^{ns}
Time x Rates x Variety	12	0.9381^{ns}	9.22^{ns}	0.14^{ns}	0.106^{ns}
Sub-Subplot error	64	0.7387	0.113	0.4	0.468
Total	119				

A - First experiment (total ash)

B - First experiment (fat)

C - Second experiment (total ash)

D - Second experiment (fat)

ns - Not significant

* - Significant at 5% level

** - Significant at 1% level

Appendix 3.4: ANOVA for ascorbic acid in the leaves

Source of variation	Degrees of freedom	Mean sum of squares	
		A	B
Blocks	2	80^{ns}	158*
Variety	1	72^{ns}	215^{ns}
Mainplot error	2	329	83.52
Rates	3	25283**	22250.73**
Rates x Variety	12	12^{ns}	14.78^{ns}
Subplot error	4	102	54.38
Time	4	7409**	3975.55**
Time x Variety	12	135^{ns}	30.73^{ns}
Rates x Time	12	407^{ns}	131.77**
Time x Rates x Variety	12	24^{ns}	35.21^{ns}
Sub-Subplot error	64	129	115.28
Total	119		

A - First experiment (ascorbic acid)

B - Second experiment (ascorbid acid)

^{ns} - Not significant

* - Significant at 5% probability level

** - Significant at 1% probability level

Appendix 3.5: ANOVA for calcium and beta-carotene in the leaves

Source of variation	Degrees of freedom	Mean sum of squares		
		A	B	C
Blocks	2	0.064^{ns}	3.7082^{ns}	0.0016^{ns}
Variety	1	0.0054^{ns}	0.04497^{ns}	0.0078^{ns}
Mainplot error	2	0.0013	0.1778	0.01782
Rates	3	0.016^{ns}	21.7397^{**}	0.0111^{ns}
Rates x Variety	1	0.02^{ns}	0.7451^{ns}	0.0198^{ns}
Subplot error	12	0.015	0.9285	0.0229
Time	1	0.0035^{ns}	45.7666^{**}	0.00630^{ns}
Time x Variety	1	0.0015^{ns}	2.3986^{ns}	0.0001^{ns}
Rates x Time	3	0.0043^{ns}	0.3281^{ns}	0.0091^{ns}
Time x Rates x Variety	3	0.0024^{ns}	0.21044^{ns}	0.01858^{ns}
Sub-Subplot error	16	0.17	0.8572	0.00814
Total	47			

A - First experiment (calcium)

B - Second experiment (beta-carotene)

C - Second experiment (calcium)

* - Significant at 5% probability level

** - Significant at 1% probability level

ns - Not significant

Appendix 3.6: ANOVA for ascorbic acid in the leaves during refrigerator storage

Source of variation	Degrees of freedom	Mean sum of squares
		A
Blocks	2	319.0^{ns}
Variety	1	74.00^{ns}
Mainplot error	2	35.00
Rates	3	98.00**
Rates x Variety	3	15.0^{ns}
Subplot error	12	40.00
Time	3	24554.0**
Time x Variety	3	16.0^{ns}
Rates x Time	9	944.8**
Time x Rates x Variety	9	11.7^{ns}
Sub-Subplot error	48	26.60
Total	95	

A - Second experiment 1st harvest

ns - Not significant

* - Significant at 5% probability level

** - Significant at 1% probability level

Appendix 3.7: ANOVA for ascorbic acid in the leaves during shelf storage

Source of Variation	Degrees of Freedom	Mean Sum of Squares
		A
Blocks	2	103.4^{ns}
Variety	1	81.12^{ns}
Mainplot error	2	38.4
Rates	3	52.91^{**}
Rates x Variety	3	56.2^{ns}
Subplot error	12	33.2
Time	1	43248^{**}
Time x Variety	1	15.9^{ns}
Rates x Time	3	1549.6^{**}
Time x Rates x Variety	3	7.7^{ns}
Sub-Subplot error	16	45.9
Total	47	

A - Second experiment (shelf storage ascorbic acid)

^{ns} - Not significant

* - Significant at 5% probability level

** - Significant at 1% probability level