THE EFFECT OF NITROGEN SOURCES AND STORAGE ON NITRATE AND THIOCYANATE CONTENT OF KALE (*Brassica oleracea* var. *acephala* D.C.) LEAVES

BY

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D E D I C A T I O N

To my parents

Rose and Enos

DECLARATION

I, Mary Oyiela Abukutsa, declare that this is my own work and has not been presented for a degree in any other University.

Abukutsa.
Mary Oyiela Abukutsa

17/8/87
Date

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

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ABSTRACT

Two experiments were conducted between August 1986 and March 1987 at the Faculty of Agriculture Field Station, Kabete Campus, University of Nairobi to study the effects of nitrogen sources and leaf storage on the accumulation of nitrate-nitrogen (NO_3-N) and thiocyanate ion (SCN^-) content in leaves of "Thousand-headed" kale (Brassica oleracea var. acephala D.C.). Two nitrogen sources (Calcium ammonium nitrate CAN and sulphate of ammonia-SA) at the rate of 20 g N/plant and three storage conditions (shelf storage, storage with petioles immersed in water and refrigeration) were used. Leaves were analysed for NO_3-N and SCN^- before storing and after two, four and six days of storage.

Leaves from plants top-dressed with CAN accumulated significantly more NO_3-N than those top-dressed with SA in the second experiment. The effect of storage conditions on NO_3-N concentration was not significant. However, the leaves stored with petioles immersed in water had the lowest NO_3-N concentration. Nitrate nitrogen concentration in leaves decreased significantly with increasing storage. Interaction between nitrogen sources and storage

durations for NO_3-N concentration was significant. There was a general decrease in NO_3-N concentration with storage time but the decrease was more in leaves from plants top-dressed with CAN than those from plants top-dressed with SA. The petioles had significantly higher NO_3-N concentration than the laminae.

Leaves from plants top-dressed with SA yielded significantly higher SCN^- than those from plants top-dressed with CAN. Leaves stored on the shelf yielded the lowest SCN^- while those stored in the refrigerator yielded the highest. Thiocyanate ions in stored leaves decreased with increasing storage time with highest decrease being in leaves stored on the shelf and lowest in those stored in the refrigerator. Unstored leaves yielded significantly higher SCN^- than stored leaves. There was a significant interaction between storage conditions and durations for SCN^- content in leaves. Interaction between nitrogen sources, storage conditions and durations for SCN^- yield from leaves was significant. Top-dressing with CAN and storing the leaves on shelf for six days tremendously decreased SCN^- content.

1. INTRODUCTION

Besides cabbage (Brassica oleracea L. var. capitata) and spinach (Spinacia oleracea L.), kale (Brassica oleracea var. acephala D.C.) is at the moment the most important leafy vegetable in Kenya. Kale is an erect branched herb which does not form a head like cabbage. Enormous quantities of kales are grown by small holders both for human consumption and as livestock feed.

Kales, commonly known as "Sukuma wiki" meaning "that which helps one to carry on the whole week", is a popular vegetable whose popularity as a main vegetable diet has been attributed to its agronomic characteristics and its palatability. The vegetable is produced in diverse agro-ecological zones of Kenya in such areas as Kiambu, Nyeri, Kisii, Embu, Meru, Kakamega and South Nyanza districts and this makes the vegetable to be readily available in most markets in Kenya. The average yield of kale leaves is about 16 tonnes per hectare (Anon, 1984). Besides its growth versatility, the edible material is apparently more acceptable and palatable to a wide cross-section of consumers as compared to other vegetables.

Currently the consumption of "Sukuma wiki" by lower and middle income groups constitutes a significant portion of household diet both in rural and urban areas. Most of the institutions like schools, colleges and hospitals use it as the main relish.

Most consumers in the urban areas buy the leaves in bulk and store them on shelves or in refrigerators in their kitchens. In some cases leaves may turn yellow within a few days and may also become dehydrated. The consumers with refrigerators may extend the storage life of leaves. In urban markets, it is not uncommon to find leaves with petioles immersed in water to prevent them from dehydration. These methods of post-harvest handling of the leaves may affect their nutritive value and enhance the accumulation of some toxic substances like nitrates and nitrites (Maynard et al, 1976). The accumulation of nitrates and nitrites, and the presence of glucosinolates in plants may also be enhanced by a number of other factors. These factors include genetic, environmental (such as light and temperature) and nutrient supply (Maynard et al., 1976).

Objectives of the study.

1. To elucidate the possible effect of nitrogen sources on the accumulation of nitrate-nitrogen and thiocyanate ion yield from stored kale leaves.

2. To investigate the possible effect of different storage conditions (shelf storage, storage with petioles of leaves immersed in water and in the refrigerator) and period on the concentration of nitrate-nitrogen and thiocyanate ion content in stored leaves.

2. LITERATURE REVIEW

2.1 Nutritive value of kales.

The leaves of kale rank high among greens in their nutritive value (Yants, 1980). They contain a wide range of mineral elements and supply a large proportion of vitamins in our diet (Ang and Livingston, 1974; Salunkhe *et al.*, 1974). Of the seven *Brassica* species studied by Yants (1980), kale had the highest vitamin C content (134 mg per 100 g fresh weight).

The significance of kales as source of protein and energy is generally small as compared with that of cereals and products of animal origin (Ryall and Werner, 1972). Table 1 shows the importance of kale as a source of various nutrients. The table compares the food value of kales and other cruciferae vegetables.

Despite their high nutritive value, the leaves have been reported to accumulate nitrates (Maynard and Barker, 1979) and to contain glucosinolates which may be toxic (Johnston and Jones, 1966). Important factors affecting quality in fresh vegetables between harvest and use on the table include among others,

Table1: Food composition in terms of the retail weight (as purchased weight) (Chatfield, 1959).

Commodity description	Calories/ 100 gms	Protein (%)	Fat (%)	Calcium (mg)	Iron (mg)	Thiamine (mg)	Riboflavin (mg)
Broccoli	26	2.5	0.2	75	0.8	0.06	0.12
Brussel sprouts	36	3.6	0.4	26	1.0	0.06	0.12
Cabbage	17	1.1	0.1	35	0.3	0.04	0.03
Chinese cabbage	11	1.1	0.1	79	1.6	0.05	0.02
Cauliflower	13	1.3	0.1	12	0.6	0.06	0.05
Kale	27	2.5	0.4	145	1.4	0.07	0.16
Kohlrabi	15	1.1	0.1	24	0.3	0.03	0.03

the length of storage and temperature and humidity during storage (Robert et al., 1974).

2.2 Nitrogen and plant nutrition.

Nitrogen is central to the growth and development of all crop plants (Huffaker and Rains, 1978). Nitrogen nutrition is important because nitrogen is an essential nutrient for proper plant growth (Beevers, 1976; Mengel and Kirkby, 1979). It enhances vegetative growth (Barker and Maynard, 1972; Knight and Mitchell, 1983), proper root development and is utilised in the synthesis of protein and nucleic acids (Mengel and Kirkby, 1979).

Most plants prefer taking up nitrogen in the nitrate form even if it is applied in a different form (Maynard and Barker, 1979). The absorption of ammonium ions by plant root is rapid and its rate is markedly influenced by environmental factors such as temperature, aeration and water (Reisenauer, 1978). Nitrate-nitrogen (NO_3-N) nutrition produces better growth than ammonium form (Barker and Maynard, 1972). This is because ammonium ions suppress the absorption of metallic cations whereas the nitrate ions enhance the absorption and accumulation of most metallic ions

(Barker and Maynard, 1972). Fertilizers applied in ammonium form have to be taken up in nitrate form i.e. after nitrification. This process depends on soil factors such as pH, aeration, soil micro-organisms (Mengel and Kirkby, 1979) and temperature (Gardner and Pew, 1979). Nitrogen in plants is translocated through the xylem in form of nitrate or amino acid depending on the sources and root metabolism (Mengel and Kirkby, 1979).

Ammonium ions in the root arising either from direct absorption or from reduction of nitrate are converted by reductive amination of α-ketoglutamate to glutamate and by enzymatic synthesis to glutamine and asparagine (Reisenauer, 1978; Mengel and Kirkby, 1979). These amino acids are rapidly transported to the tops, where they are utilised in protein synthesis (Reisenauer, 1978).

After the nitrates have been taken up by plants, most of them are assimilated and metabolised to other nitrogen compounds like amino acids, proteins (Maynard et al, 1976) and amides (Beevers, 1976).

2.3 Nitrate accumulation in plant leaves.

Nitrate accumulation in plants is a natural phenomenon resulting from uptake of the nitrate ions in excess of their reduction and subsequent assimilation (Maynard and Barker, 1979). The concentration of nitrate in tissues is always in a dynamic state since it represents the difference between rates of absorption and assimilation within the plant (Maynard et al., 1976). A factor may modify tissue nitrate by affecting any one or all of the processes of absorption, assimilation and translocation (Maynard et al. 1976). Accumulation of nitrate ions in the plant tissues is dependent on and related to environmental factors, nitrogen supplying power of the soil under which the plant is grown (Maynard et al., 1976) and the genetic make up of the plant (August, 1974). The environmental factors include among others light, temperature (Cantliffe, 1972a; 1972b), carbon dioxide and water relations (August, 1974; Maynard et al., 1976).

Cantliffe (1972a) working with spinach grown under different light intensities found that concentration of nitrates in the leaves was increased

by the reduction of light intensity. Light is presumed to modify tissue nitrate concentration primarily by regulating nitrate reductase activity (Maynard et al., 1976). Steingrover et al. (1986) and Gysi et al. (1985) found the same effect. Working with table beets (Beta vulgaris), Cantiliffe (1972a) observed total absence of nitrate accumulation in leaves and roots of table beets at any photoperiod in unfertilized soils. However, in nitrogen fertilized soils, he noted an accumulation of nitrates in both leaves and roots. He also reported that nitrates decreased as the photoperiod extended from eight to twenty hours.

Harvesting of the leaves of both Radish (Raphanus sativus) and spinanch either at different hours of the day or further into the light period, did not seem to affect the nitrate concentration in the leaves (Cantliffe, 1971a). According to Cantliffe (1971b), when nitrogen fertilizer was added to spinach, the nitrate concentration decreased with a subsequent increment in temperature from 25°C to 30°C. Partial depletion of carbon dioxide and drought elevated nitrate concentration in leaves (Maynard et al., 1976).

Maynard and Barker (1974) found out that great differences in nitrate accumulation and critical nitrate concentration occurred among cultivars of spinach. Smooth leafed cultivars were lower in nitrate concentration than heavily savoyed ones (Barker et al., 1974; Maynard and Barker, 1974). Collards accumulated less nitrates than "Thousand-headed" kale (Chweya, 1986).

High nitrate concentrations accumulate in plants only if the nitrate supply in the nutrient medium was high or if there is interference with the normal nitrate metabolism within the plants (Maynard et al., 1976). High application of nitrogen may lead to high tissue nitrate accumulation (Schuphan, 1971; August, 1974; Splittstoesser et al, 1974 and Chweya, 1986). Chweya (1986) working with Collards and "Thousand-headed" kale found that top-dressing with CAN significantly increased NO_3-N content in both laminae and petioles of the two varieties. The source and amount of nitrogen applied, time and method of application govern the effects of nitrogen fertilizers on nitrate accumulation in vegetables (Maynard et al, 1976). Nitrate accumulation

increases as the rate of nitrogen fertilization increases (Lee et al, 1971; Pimpini et al, 1973; Lorenz, 1978; Chweya, 1986). Nitrate containing fertilizers result in higher nitrate accumulation than ammoniacal sources (Pimpini et al, 1973; Lorenz, 1978; Gardner and Pew, 1979). Barker et al (1971) showed that urea, ammonium nitrate and potassium nitrate side-dressed to rapidly growing spinach increased the nitrate content in its leaves. However, urea gave the least increase while potassium nitrate gave the highest. When pre-plant and side-dress application of equivalent amounts of nitrogen are compared respectively to plant nitrate accumulation, results vary depending on the species and on the time interval between side-dressing and harvest (Maynard and Barker, 1979). Spinach was found to accumulate more nitrate from a pre-plant broadcast application than from side-dressing nine days before harvesting (Maynard and Barker, 1979). Side-dressing with nitrogen under irrigation, increased NO_3-N in kale leaves (Chweya, 1984).

Accumulation of NO_3-N in leaves increased with age in spinach plants which were side-dressed with

nitrogen fertilizer but decreased with unfertilized plants (Aworh et al., 1980). Nitrates tend not to be uniformly distributed throughout the plant but rather do accumulate in certain parts (Maynard et al., 1976). Lorenz and Weir (1974) working with table beets, found highest nitrate concentrations in petioles than in roots and leaf blades in that order. Chweya (1987) found that petioles of kale had high NO_3-N than laminae.

2.4 Nitrate reduction to nitrites.

Nitrite is the first stable intermediate product in the assimilation of nitrate by plants (Hewitt et al., 1967). In leaves, the nitrate is reduced to nitrites by nitrate reductase (NR) (Maynard et al., 1976). The NR solely governs the nitrite-nitrogen (NO_2-N) accumulation in leaves (Maynard et al., 1976). However, the amount of NR in the plant is affected by many factors such as temperature (Hall and Hicks, 1977), photoperiodism, light duration and intensity (Cantliffe, 1972a; 1972b; 1972c), genetic factors (Barker et al., 1974 and Maynard and Barker, 1974), water content (Maynard et al., 1976) and age of the plant (Aworh et al., 1980). Other factors which also affect NR

include storage period and microbial activities during storage (Hall and Hicks, 1977).

The NR activity is more developed in younger sugar beet leaves than older ones (Van Agmond, 1972). Hence the accumulation of nitrites is higher in younger leaves than older ones (Aworh et al., 1980). Nitrites may be formed from nitrates before ingestion by animals (Lee et al., 1971; Maynard et al., 1976). Reduction of nitrates to nitrites may occur if absorption in the upper intestines is delayed by gastro-intestinal disturbances (Maynard et al., 1976). Reduction of nitrates to nitrites is more likely to occur in infants than in adults because of low acidity in the gastro-intestinal tract of infants which allows nitrate reducing bacteria (Coliform and Clostridial bacteriae) to survive (Maynard et al., 1976).

2.5 Hazards of nitrates and nitrites.

Nitrates in high concentration may cause great loss through illness or death (Maynard et al., 1976). The fatal adult dosage in human beings is in the order of 15-70 mg of NO_3-N per kilogramme body weight (Lee, 1970). Nitrates become toxic when converted

to nitrites (Maynard et al., 1976).

When the nitrite ion is absorbed into the bloodstream the ferrous iron of haemoglobin may be oxidised to the ferric form to form methaemoglobin which cannot transport oxygen and this causes a disorder known as methaemoglobinemia (Lee et al., 1971; Schuphan, 1971; Maynard et al., 1976. Acute nitrite toxicity is shown by cyanosis, a bluish discolouration of the skin and lips. This occurs when about 15% of the haemoglobin has been oxidised to methaemoglobin (Lee, 1970).

The presence of nitrite and secondary amines may lead to the formation of nitrosamines which are carcinogenic and mutagenic (Heisler, et al., 1974); Maynard et al., 1976; Mengel and Kirkby, 1979). Fatal adult dosage in humans of nitrites is 20 mg NO_2-N per kilogramme of adult body weight (Lee, 1970).

2.6 Glucosinolates in plants.

Glucocinolates are a unique class of plant constituent chemicals which occur primarily in plants of the cruciferae family (Johnston and Jones, 1966; Van Etten and Wolff, 1973; Tookey

et al., 1980). The distribution of individual glucosinolates in cruciferae family varies considerably (Olsson and Jeppsson, 1984; Michajlovskij, 1986; Hill et al., 1987). The three major glucosinolates which have been identified in kale are sinigrin (2-propenyl-GS), Glucoiberin (3-methyl sulphinyl-propyl-GS) and Glucobrassin (3-indolyl methyl-GS) (Michajlovskij, 1986).

Glucosinolates occurring in kale and other related vegetables are responsible for their pungent and characteristic flavours (Tookey et al., 1980). The flavours are not caused by the glucosinolates as such but by products from their enzymatic hydrolysis (Tookey et al., 1980 ; Olsson and Jeppsson, 1984). Glucosinolates occur throughout the plant and are always accompanied by an enzyme myrosinase (thioglucosidase) which is capable of hydrolysing them (Tookey et al., 1980; Michajlovskij, 1986) to give glucose, acid sulphate ion and one or more of the aglucon products (thiocyanates, isothiocyanates, progoitrin and nitrile) (Tookey et al., 1980); Carlson et al., 1985). Appendix 4 shows major product classes from the enzymatic hydrolysis of glucosinolates.

Myrosinase is localised separately in special cells and gets into reaction only when the plant tissue has disintegrated by for example maceration (Michajlovskij, 1986).

The factors regulating the level of glucosinolates in plant tissues are not well known. Environmental factors may play a role in determining glucosinolate level in plant tissue (Johnston and Jones, 1966). These include factors such as soil type (Neil and Bible, 1972; Bible and Chong, 1975), sulphur fertilization (Josefsson, 1970) and temperature and rainfall (Bible and Chong, 1975). Other factors include spacing (Chweya, 1984) and plant genetic factors (Johnston and Jones, 1966; Josefsson and Appelqvist, 1968; Carlson et al., 1987). Neil and Bible (1972) and Bible and Chong (1975) working with Raphanus sativus, found that root tissue of plants grown on organic soils had a higher quantity of thiocyanate ions than those grown on loam soils. They also found that low sulphur fertilization reduced glucosinolate content of seeds of

Rape kale (Brassica napus). The synthesis of glucosinolates is also related to nitrogen nutrition since the synthetic pathway for glucosinolates starts with amino acids like glutmate, alanine and serine (Fowden, 1967).

Root thiocyanate ion (SCN^-) content of Raphanus sativus was found to increase under cooler condition (Bible and Chong, 1975). They also found a positive correlation between SCN^- and rainfall in plants grown on loam soil, but a negative correlation with mean daily air temperature in plants grown on organic soils. It has been found also that close spacing tends to increase SCN^- content in kale leaves (Chweya, 1984).

It has been observed that SCN^- content in kale leaves is highest during the period of most active growth of the plant (Johnston and Jones, 1966). Thiocyanate production in kale leaves decreases with increasing age of the plant (Johnston and Jones, 1966) and decreases down the plant (Chweya, 1985). In leaves of the same age the lamina shows

consistently higher levels than petiole (Johnston and Jones, 1966; Paxman and Hill, 1974a).

Paxman and Hill (1974a) found that rape kale contained less than half the amounts of SCN^- found in 'Thousand-headed' kale and marrow stem. Bible *et al.* (1980) reported that early maturing cultivars of cabbage yielded less SCN^- than late maturing cultivars. Gramberg *et al.* (1986) found the total glucosinolate content of cooked Brussels sprouts to be significantly higher than that of cooked cauliflower. Working with six varieties of kale, Johnston and Jones (1966) found significant inter-varietal differences in SCN^- yield from leaves.

2.7 Hazards of thiocyanate ions.

Paxman and Hill (1974b) reported that the SCN^- content in kale was responsible largely if not wholly for goitrogenicity that was observed in rats. Thiocyanate ion lowers the iodine content of the thyroid or inhibits the organic binding of iodine (Neil and Bible, 1972). It also causes thyroid enlargement (Van Etten *et al.*, 1980), and

may lower fertility in livestock (Johnston and Jones, 1966). The development of goitre is dependent on dietary balance between iodine and thiocyanate. Its development is found by the determination of urinary iodine to SCN^- ratio, and goitre appears when the ratio reaches a critical threshold of about three (Michajlovskij, 1986).

2.8 Storage and nitrate and nitrite contents.

Nitrate accumulated in plant tissues may be reduced to nitrites during storage (Heisler et al., 1974). The reduction during storage is as a result of bacterial action or plant NR activity (Luhrs, 1973; Heisler et al., 1974). Heisler et al., (1974) reported that when spinach is stored at varying temperatures for different periods it produces different concentration of nitrites as shown in Table 2. Ten to fifteen percent of nitrate may be converted into nitrite after spinach has been held at room temperature for at least four days (Heinze, 1974). At 2°C the conversion is minimal (Lee et al., 1971, Heinze, 1974). The amount of nitrite accumulated in storage is not always related to the initial nitrate content in the leaf

Table 2: The effect of storage temperature and perio
the concentration of nitrites in spinach (H
et al., 1974).

Storage temperature	Period	Nitrite conc (mg/kg fres
Room temperture	3 days	140
5°C	29 days	10
25°C	2 days	10
Refrigerator	14 days	300

(Aworh et al., 1980). The mature leaves accumulated more nitrite during storage than immature leaves (Aworh et al., 1980).

According to Aworh et al. (1978), at 0°C, spinach can be stored as long as 40 days without showing significant changes in nitrogen components, but at 20°C substantial loss of NO_3-N and accumulation of NO_2-N can occur in spinach leaves within seven days. Aworh et al. (1980) concluded that the most effective means of minimising nitrite accumulation in spinach is to keep the temperature as close to 0°C as possible. Phillips (1968) had also observed some marked changes of NO_3-N and NO_2-N contents in spinach stored at room temperature compared to changes in samples stored under refrigeration. Hicks et al. (1975) noticed to increase in NO_2-N in carrot juice when bacterial growth was prevented. Nitrite-nitrogen is not detectable in freshly harvested vegetable leaves (Minotti, 1978).

2.9 Storage and thiocyanate ion content

Work done by Chong and Berard (1983) showed that there was a general moderate increase in SCN^-

between day 5 and 40 in cabbage heads stored at 1 ± 1°C.

Otherwise there is very little literature on the effect of storage on the content of SCN^- in Brassica vegetables.

3. MATERIALS AND METHODS

3.1 Experimental Site.

'Thousand-headed' kale was planted twice between August 1986 and March 1987. The two experiments were conducted on the experimental plots at the Field Station, University of Nairobi, Kabete Campus.

The area lies on the altitude of 1940 m above sea level and is within latitude 1° 15' S and longitude 36° 44' E. The average annual rainfall for Kabete is 1000 mm. There are two main rainy seasons, the long rains occuring during the months of March, April and May and the short rains in October, November and December. The experiments did not coincide with the rains therefore supplemental irrigation was necessary.

The mean monthly maximum temperature is 23°C and the minimum is 12°C. The details of the temperature, rainfall, relative humidity and cloudness during the experimental period is presented in Appendix 1.

3.2. Soil Characteristics

The site is under nitosol unit according to FAO/UNESCO (1974) classification. These soils are referred to as Kikuyu friable clay derived from tertiary trachytic lava which is very resistant to soil erosion. They are extremely deep and well drained. They are dark brownish in colour with a thick acid top-soil (Siderius, 1976).

Three random soil samples were collected from the entire experimental site at depths of 0-15 cm and 15-30 cm one day before the seedlings were transplanted. The soil samples were dried and analysed for pH, cation exchange capacity (CEC), organic carbon, available phosphorus and total nitrogen.

The pH, CEC, organic carbon, available phosphorus and total nitrogen were analysed according to the methods used in the Department of Soil Science, University of Nairobi (Ahn, 1973; 1975). Water content was determined by putting known weights of wet soil samples in the oven at 105°C for 48 hours, after which the dry weights were determined. The results of soil analyses are shown in Appendix 2.

3.3. Planting Materials

The seeds of kale variety "Thousand-headed" were obtained from East Africa Seed Company.

3.4. Treatments and Experimental Design

3.4.1 Field Treatments and Design

The field treatments consisted of two nitrogen sources (Calcium Ammonium Nitrate - CAN and Sulphate of Ammonia - SA). The amount applied was an equivalent of 1,111 kg N per hectare i.e. 20 g N per plant. This was done in a split application whereby one half of the fertilizer was applied three weeks after transplanting while the other half was applied three weeks later.

The experiments were set up in a completely randomised block design with three blocks each of size (12 x 2) m^2 with two plots of size (6 x 2) m^2 for the nitrogen sources.

3.4.2 Laboratory treatments and design

The laboratory treatments included three storage conditions and four storage durations. The storage conditions were shelf storage, storage of leaves with petioles immersed in water and refrigerator

at 6 ± 2 °C.

The leaves in each storage conditions were analysed immediately and after two, four and six days of storage.

Nitrogen sources were the main treatments, storage conditions and durations constituted the sub-treatments and sub-sub treatments respectively. The overall experimental design was a split - split plot design.

3.5. Cultural Practices

3.5.1. Nursery

A seedbed of one metre wide and four metres long was prepared to a fine tilth. Seeds were drilled in rows spaced 10 cm apart. The drilling was done after triple superphosphate (P_2O_5 = 46%) was applied and thoroughly mixed with the soil at the rate of 1 kg/4m^2. The drilled seeds were covered with a thin layer of soil and the seed bed mulched using dry grass. The seeds germinated after five to six days at which time the mulch was removed. The seed-bed was kept weedfree till the seedlings reached the transplanting stage. Watering was done every

morning. The frequency of watering was gradually reduced two weeks before transplanting, where watering was done every other day and once every two days one week prior to transplanting. Healthy seedlings were transplanted four weeks after germination.

3.5.2 Transplanting

The experimental plots were tractor ploughed and disc harrowed twice. Transplanting was done four days after the second harrowing. Triple Superphosphate (P_2O_5 - 46%) at the rate equivalent to 1,111 kg per hectare i.e. 20 g P_2O_5 per plant was applied. Furadan at the rate of 111 kg/ha 2 (g/plant) was also applied to control insect pests.

3.5.3 Agronomic practices

The plots were kept weedfree throughout the experimental period. This was done by hand using hoes and pangas.

Cutworms, aphids and moles which are the major kale pests at the University of Nairobi, Field Station were adequately controlled using Furadan, ripcord and trapping, respectively.

Ripcord was sprayed once every two weeks at a rate of 50 mls in 20 litres water to 1,000 m^2 area (0.5 litres per hectare). There was no disease incidence during experimental period. Supplemental irrigation by overhead sprinkler was done when needed to avoid water stress on the plants.

3.6 Observations During the Experiment

3.6.1 Sampling of the leaves

Harvesting was done six and eight weeks after transplanting. Each time, all the plants were harvested from each plot covering an area of 5.76 m^2. Guard rows were not harvested. Harvesting was done by removing all fully expanded leaves except the top four. Later the guard rows were also stripped except the top four leaves hence making all the plants uniform. Harvesting was done between 8.00 and 9.00 a.m. Leaves from each plot were harvested separately and thoroughly mixed before being taken to the laboratory for further treatments.

The harvested leaves were put in labelled polythene bags to minimise water loss from the transpiring leaves. These were transported to the laboratory.

The leaves from the same plots were completely mixed. Leaf samples were randomly picked for SCN^- and dry matter determination. Samples used for dry matter determination were also used for NO_3-N and NO_2-N determinations. The remaining leaves were divided into three equal portions which were stored in three different conditions. Ten leaves were randomly picked from each condition after two, four and six days of storage and analyses continued as for the unstored leaf samples.

3.7 Laboratory Analyses

3.7.1 Dry matter determination

The sampled leaves were separated into lamina and petiole. Known weights of fresh petiole and lamina were dried in an oven at 70°C for 96 hours to determine the dry weights. The dried samples were ground using a Glen Creston 48 Grinder to pass through a 1 mm sieve. The ground samples were kept in tightly closed plastic bottles for NO_3-N analyses.

3.7.2. Nitrate - nitrogen determination.

Nitrate - nitrogen in petioles, laminae and water in which petioles were immersed was determined using Cataldo *et al.* (1975) method with slight modifications for petioles and laminae according to Chweya (1985). Instead of centrifuging the incubated samples, they were filtered through Whatman paper No. 41 and the filtrate used for the analysis and values expressed as NO_3-N%. The NO_3-N concentration in the leaf was estimated by averaging values obtained for petiole and lamina. Chweya (1985) had found that the proportion of petiole to lamina was about one to one (1:1). The water in which the petioles of leaves were immersed in was also analysed for NO_3-N.

3.7.3 Nitrite-nitrogen determination.

Nitrite-nitrogen determination was done for the leaves stored on the shelf and with petioles immersed in water. This was done using modified Griess Ilosvay method (Bremmer, 1965) with slight modification. For extraction, 100 mg of ground sample were suspended in 100 ml of deionized water and incubated at 45 °C for one hour. The mixture was filtered through Whatman paper No. 41 and the filtrate used for the analysis with values expressed in NO_2-N %. Since both nitrate and nitrite are very soluble in water it was thought that nitrite can also be extracted using water just as the nitrates.

3.7.4 Thiocyanate ion determination.

Thiocyanate ions in petioles and laminae were determined using Bible et al. (1980) method but with some slight modification according to Chweya (1985). One part sample to five parts distilled water (w/v) was used for laminae and one part sample to two parts distilled water (w/v) for petioles instead of one part sample to one part distilled water (w/v) and values expressed in µg KSCN per g d.w. (ppm). The SCN^- content in leaf was estimated by

averaging values obtained for petiole and lamina. This was done because the proportion of petiole to lamina is about one to one (Chweya, 1985).

3.8 Data Analysis

Data obtained were subjected to analysis of variance (ANOVA) using methods of Little and Hills (1978). Mean separation was done using Duncan's New Multiple Range Test at probability level of $P \leq 0.05$ (Steel and Torrie, 1981).

4. RESULTS AND DISCUSSION

4.1 The Effects of Nitrogen Sources, Storage Conditions and Durations on Nitrate-nitrogen Accumulation.

4.1.1 Nitrogen sources.

The effect of nitrogen sources on NO_3-N accumulation in leaves, petioles and laminae was significant in the second experiment. Results in Table 3 show that leaves, petioles and laminae from plants top-dressed with CAN accumulated significantly more NO_3-N than those top-dressed with SA. This finding that leaves from plants top-dressed with CAN accumulated more NO_3-N than those from plants top-dressed with SA could be explained by the fact that, nitrates absorbed by the roots are translocated in nitrate form whereas ammonium form is assimilated in the root tissue and translocated as amino acids (Reisenauer, 1978). This indicates therefore that nitrogen in nitrate form is not assimilated quickly in the plant tissues as compared to ammonium form, The nitrate form requires more time to be assimilated in the plant and this

Table 3: The effect of nitrogen sources on the concentration of nitrates in Kale leaves.

	(NO_3 - N %)											
	First experiment						Second experiment					
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)		
Nitrogen Source	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
C A N	2.62	4.17	1.07	2.41	3.77	1.05	2.6[a]	4.10[a]	1.07[a]	2.6[a]	4.10[a]	1.0[a]
S A	2.58	4.14	1.02	2.39	3.71	0.99	2.0[b]	3.20[b]	0.73[b]	2.0[b]	3.23[b]	0.7[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.

therefore necessitates its accumulation in leaves. Another possible explanation is the fact that most plants prefer taking up nitrogen in the nitrate form (Maynard and Barker, 1979). Plants top-dressed with CAN take up the nitrates rapidly hence resulting in their accumulation in the leaves.

The high NO_3-N accumulation in leaves with respect to nitrogen sources is a case very common with various vegetables (Gardner and Pew, 1979; Barker and Maynard, 1972). These results are comparable to the report of Gardner and Pew (1979) who found that NO_3-N accumulation in wrapper leaves of head lettuce was highest for plants fertilized with calcium nitrate and lowest for plants top-dressed with ammonium sulphate. Barker and Maynard (1972) working with cucumber (Cucumis sativus L.) and pea (Pisum sativum) also found that plants top-dressed with ammoniacal fertilizer accumulated significantly less NO_3-N than those top-dressed with nitrate fertilizer.

The results show that petioles had significantly higher NO_3-N concentration than the laminae (Table 3 - 5). This is probably due to the

Table 4: The effect of storage conditions on nitrate concentration in Kale leaves.

	(NO_3 - N %)											
	First experiment						Second experiment					
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)		
Storage Conditions	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
Shelf Storage	2.76	4.46[a]	1.05	2.45	3.75	1.02	2.33	3.80[a]	0.83	2.30	3.76[a]	0.79
Storage of leaves with Petioles immersed in water	2.22	3.45[b]	0.99	2.31	3.62	1.00	2.07	3.23[b]	0.88	2.06	2.86[b]	0.82
Refrige-rator	2.83	4.55[a]	1.10	2.46	3.86	1.05	2.45	3.97[a]	0.99	2.45	3.94[a]	0.91

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

fact that NO_3-N assimilation could be occurring in the laminae faster than petioles. The lamina is much more greener than petiole meaning that it has more chlorophyll which traps light for photosynthesis. Photosynthesis supplies carbohydrates that are oxidised and provide energy for NR production (Salisbury and Ross, 1978), hence used in nitrate assimilation. The finding that petioles had higher NO_3-N concentration than the laminae is in line with the report of Chweya (1987) that petioles of kale accumulated more nitrates than laminae. If petioles are removed from kale leaves intended for use by human beings a substantial amount of NO_3-N could be done away with. Toxic dosage is at 15 - 70 mg NO_3-N/kg body weight (Lee, 1970) which means a 70 kg adult will require to consume 0.6 to 2.7 kg of fresh leaves from plants top-dressed with 20 g N per plant to get the toxic dosage. However there is no available data to show per capita consumption in Kenya.

4.1.2 Storage conditions

Table 4 shows the results of the effect of storage conditions on NO_3-N concentration in leaves,

Petioles and laminae. The effect was not significant ($P \leq 0.05$) for the leaves and laminae but was significant for petioles. However, NO_3-N concentration tended to be lower in leaves, petioles and laminae stored with petioles immersed in water than in those leaves stored either on the shelf or in the refrigerator.

Leaves stored with petioles immersed in water had the lowest NO_3-N concentration presumably due to nitrate leaching into the water. This was partly confirmed by analysing NO_3-N in water in which the petioles had been immersed for six days. The analysis showed traces of NO_3-N in the water. The possible explanation for low NO_3-N in leaves could be due to the fact that NO_3-N could have been assimilated into other nitrogenous compounds. The leaves were observed to be turgid and therefore, it is possible that metabolic processes were taking place in the leaves and hence the assimilation of nitrates into other nitrogenous compounds (Hewitt and Smith, 1975; Salisbury and Ross, 1978; Faragher *et al.*, 1983). Leaves stored on shelf were wilted and had become dehydrated after about two days of storage. This may have affected the metabolic processes in leaves and hence little assimilation of nitrates

into other nitrogenous compounds. The assimilation of NO_3-N involved enzymes like NR and nitrite reductase which do not function actively under conditions of water stress in the tissues (Hewitt and Smith, 1975; Maynard et al., 1976). The higher NO_3-N content in refrigerated leaves than in leaves on shelf could be explained by the fact that, in the refrigerator metabolic processes which are catalysed by enzymes are slowed down because of the low temperatures which inactivate the enzymes (Mazur and Harrow, 1971; Burzo, 1981).

4.1.3 Storage durations

Effect of storage durations on NO_3-N concentration in leaves, petioles and laminae was significant ($P \leq 0.05$) in all cases except for leaves of first harvest from first experiment. Table 5 shows that NO_3-N concentration in unstored leaves was significantly higher than that in the stored ones. The table also shows that NO_3-N concentration decreased with increasing storage time. The decrease in NO_3-N concentration during storage could be explained by the fact that leaves were still active and so NO_3-N assimilation into other nitrogenous compounds may have been taking place. This conclusion

Table 5: The effect of storage durations on the concentration of nitrates in Kale leaves.

	(NO_3 - N %)											
	First experiment						Second experiment					
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)		
Storage Durations	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
Unstored Leaves	3.16	5.13[a]	1.21[a]	3.12[a]	5.01[a]	1.23[a]	2.85[a]	4.38[a]	1.31[a]	2.78[a]	4.33[a]	1.22[a]
Two days of Storage	2.76	4.45[b]	1.05[b]	2.52[b]	3.96[b]	1.08[b]	2.55[b]	3.95[ab]	1.08[b]	2.54[a]	4.03[a]	1.02[b]
Four days of Storage	2.39	3.77[c]	1.01[bc]	2.17[c]	3.33[c]	1.00[b]	2.21[c]	3.65[b]	0.72[c]	2.18[b]	3.57[b]	0.66[c]
Six days of Storage	2.10	3.27[c]	0.91[c]	1.81[d]	2.68[d]	0.78[c]	1.55[d]	2.67[c]	0.49[d]	1.58[c]	2.62[c]	0.47[d]

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

was also reached by Aworh et al. (1978) who found that there was a substantial decrease of NO_3-N in spinach leaves held for 3-7 days at 20 °C.

Nitrite-nitrogen determination in stored leaves was done in the second harvest of second experiment to indicate whether some of the NO_3-N was being converted to NO_2-N during storage. Samples from leaves stored on shelf and those stored with petioles immersed in water were used for the determination. It was observed that in the two storage conditions there were no traces of NO_2-N in unstored leaves. The determination also showed that NO_2-N concentration in the stored leaves were in traces. During nitrate assimilation, nitrite is a stable intermediate compound which is further reduced to ammonia (Salisbury and Ross, 1978). This probably explains why the traces of NO_2-N could not account for the reduction of NO_3-N though that possibility cannot be ruled out. These results are supported by the study of Lee et al. (1971) in which it was observed that there were traces of NO_2-N in table beets and spinach which had been stored for a period of 60 days at 20 °C. They however reported a decrease in NO_3-N concentration

with storage time and observed that the NO_2-N traced in the leaves could not account for the losses in NO_3-N.

Kale leaves stored on the shelf deteriorated rapily and were considered unfit for human consumption (yellow and wilted) after 2 - 3 days. This was in contrast with those stored with petioles immersed in water which took a longer period before they were considered unfit for human consumption i.e. yellow with off odours, after 4 - 5 days. The refrigerated leaves were still fresh by the time the experiment was terminated (6 days of storage). These observations of usability of stored leaves are in line with the findings of Aworh et al. (1978) who observed that spinach stored at 20 °C deteriorated more rapidly and was considered unmarketable after 2 days and unusable after 3 days than those held at 0° C which were still fresh by the 15th day of the experiment. From these observation the 2 and 4 days storage periods could be considered maximum for shelf storage and for leaves whose petioles were immersed in water respectively. Refrigerated leaves could be stored for a much longer period without going bad.

4.1.4 Interactions.

The interaction between nitrogen sources and storage durations for NO_3-N concentration was significant for the second experiment. There was a general decrease in NO_3-N concentration with storage durations but the decrease was more in the leaves from plants top-dressed with CAN than those plants top-dressed with SA (Fig. 1). The rapid decrease of NO_3-N in plants top-dressed with CAN could be due to the general phenomenon of enzyme induction. Synthesis of NR can be stimulated in whole or excised plant parts by various factors such as nitrate (Salisbury and Ross, 1978). There is a maximum amount of nitrate which must be present in the plant before NR synthesis is induced (Salisbury and Ross, 1978). It is possible that this critical level was more easily reached in plants that were top-dressed with CAN than those top-dressed with SA, because the former accumulated significantly higher NO_3-N than the latter. Hence more NR was synthesised and more nitrate reduction occurred. Interactions between nitrogen sources and storage conditions, between storage conditions and durations and between nitrogen sources, storage conditions and durations for NO_3-N concentration were not significant.

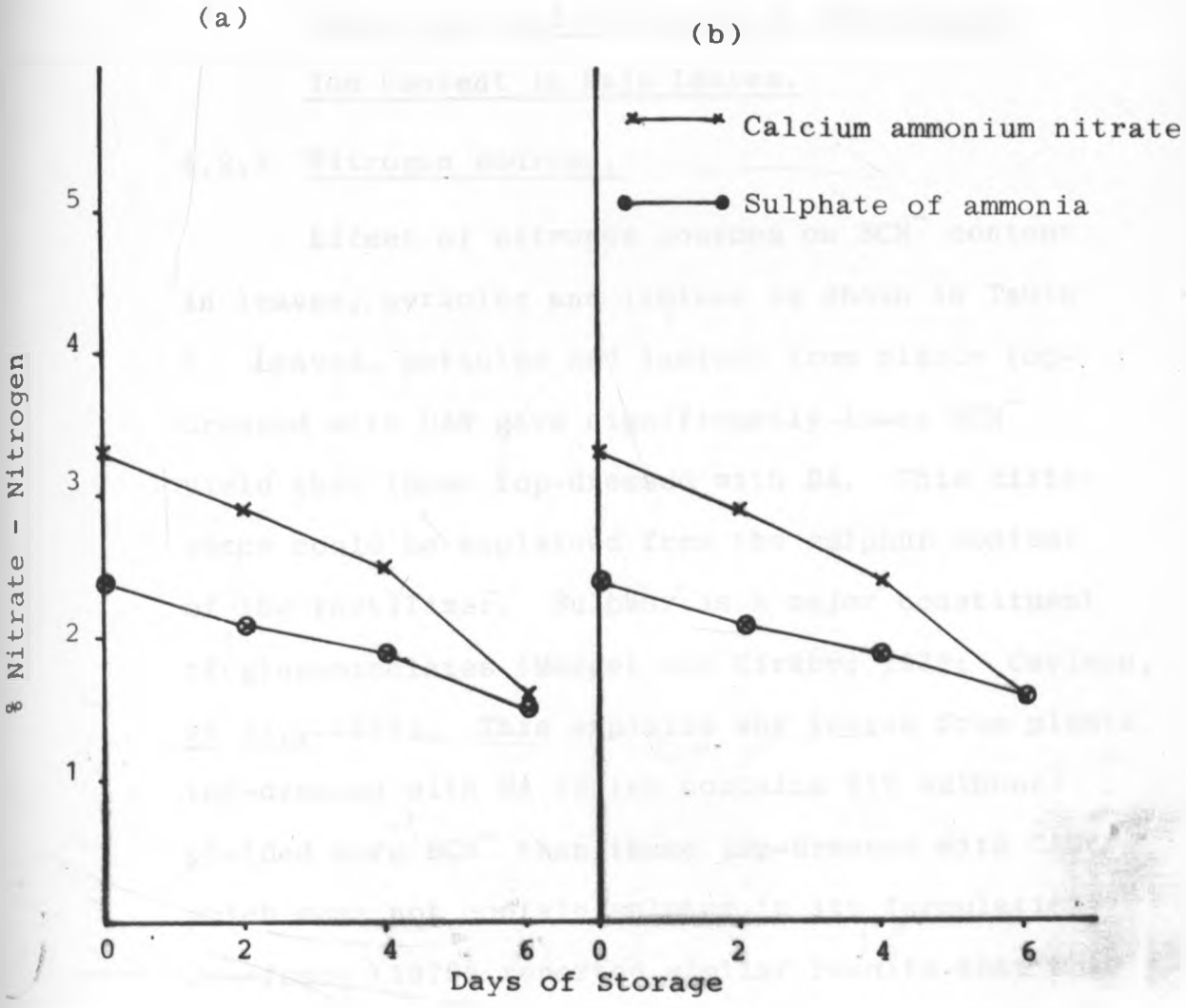

Figure 1: Interaction between nitrogen sources and storage durations on nitrate concentration in the leaves

(a) Second experiment, first harvest S.E. = 0.18

(b) Second experiment, second harvest S.E. = 0.12

4.2 The Effects of Nitrogen Sources, Storage Conditions and Durations on Thiocyanate Ion Content in Kale Leaves.

4.2.1 Nitrogen sources.

Effect of nitrogen sources on SCN^- content in leaves, petioles and laminae is shown in Table 6. Leaves, petioles and laminae from plants top-dressed with CAN gave significantly lower SCN^- yield than those top-dressed with SA. This difference could be explained from the sulphur content of the fertilizer. Sulphur is a major constituent of glucosinolates (Mengel and Kirkby, 1979; Carlson, et al., 1985). This explains why leaves from plants top-dressed with SA (which contains 21% sulphur) yielded more SCN^- than those top-dressed with CAN which does not contain sulphur in its formulation. Josefsson (1970) reported similar results that when a sulphur containing fertilizer was applied to rape seed, more glucosinolates were synthesised than when sulphur was missing.

In the first experiment, SCN^- content in petioles was significantly higher than that from laminae. This is contrary to the findings of Johnston and Jones (1966) who reported that laminae

Table 6: The effect of nitrogen sources on thiocyanate ion content in kale leaves.

	KSCN (ppm)											
	First experiment						Second experiment					
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)		
Nitrogen Sources	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
C A N	2139	3576	754[a]	3800[a]	6392	2048	447[a]	465[a]	490[a]	475[a]	455[a]	488[a]
S A	2253	3676	830[b]	5076[b]	7736	2414	771[b]	618[b]	868[b]	742[b]	617[b]	867[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.

yielded significantly higher SCN^- than petioles from kale leaves. However in the second experiment first harvest, there was no significant difference between SCN^- content in petioles and laminae. In the second harvest, laminae yielded higher SCN^- than petioles. This finding is simililar to that of Johnston and Jones (1966) and Chweya (1987) who working with kales found that SCN^- content in laminae was significantly higher than from petioles. Petioles could therefore be containing less SCN^- content than laminae.

4.2.2 Storage conditions

Effect of storage conditions on SCN^- content in leaves, petioles and laminae was significant. Table 7 shows that in the first experiemnt leaves stored on the shelf yielded a significantly lower SCN^- than either those stored with petioles immersed in water or those in the refrigerator. However, during the first harvest of the second experiment, the leaves stored on the shelf and those stored with petioles immersed in water yielded significantly lower SCN^- than those refrigerated. During the second harvest of the second experiment, leaves

Table 7: The effect of storage conditions on thiocyanate ion content in kale leaves.

	KSCN (ppm)											
	First experiment						Second experiment					
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)		
Storage conditions	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
Shelf storage	2031[a]	3453[a]	705[a]	4020[a]	6188[a]	1750[a]	532[a]	482[a]	582[a]	530[a]	480[a]	580[a]
Storage of leaves with petioles immersed in water	2351[b]	3663[ab]	764[b]	4580[b]	7062[b]	2098[b]	564[a]	552[a]	669[ab]	609[b]	490[a]	665[ab]
Refrigerator	2335[b]	3763[b]	907[c]	4712[b]	7943[c]	4602[c]	730[b]	674[b]	787[b]	687[c]	588[b]	786[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.

from all the storage conditions had significantly different SCN^- contents. Leaves stored on the shelf yielded the lowest SCN^- values while refrigerated leaves yielded the highest. The lowest SCN^- content in shelf stored leaves may be due to a thigh rate of metabolism accelerated by high temperatures. Carbohydrates were therefore depleted and hence glucosinolates, being sugar compounds, could have been utilised as substitutes (Langer and Greer, 1977). Storing leaves with their petioles immersed in water may allow the leaves to photosynthesise for sometime (Faragher et al., 1983). This then may delay the use of glucosinolates in metabolism as has been proposed for shelf stored leaves. For leaves stored in the refrigerator, there was low or hardly any biochemical processes taking place because of the inactivation of enzymes by low temperatures (Mazur and Harrow, 1971). Therefore there could have been very minimal depletion of glucosinolates than were in the leaves at harvesting.

4.2.3 Storage durations

Table 8 shows that SCN^- content in leaves, petioles and laminae decreased with increasing storage time. Unstored leaves, petioles and laminae yielded significantly higher SCN^- than stored ones. The decrease of SCN^- content with storage time could have been due to the effect of respiration (Henze and Hunter, 1981). Respiration is thought to utilise glucosinolates after depletion of carbohydrates hence the decrease. The observed decrease of SCN^- content in leaves with increasing storage time is contrary to observations of Chong and Berard (1983) who reported a general moderate increase of SCN^- content between day 5 and 40 from cabbage leaves stored at $1 \pm 1^{\circ}C$.

These results, indicate that there is an effect on SCN^- content when leaves are stored for different periods. Storage could help reduce the amount of glucosinolates in leaves but this reduction could only be valid if the leaves were still fit for human consumption. This is because it was observed that leaves stored on the shelf and those stored with petioles immersed in water passed the

Table 8: The effect of storage durations on thiocyanate ion content in

	KSCN (ppm)							
	First experiment						Seco	
	First harvest (6 weeks after transplanting)			Second harvest (8 weeks after transplanting)			First harvest (6 weeks afte transplantin	
Storage durations	Leaf	Petiole	Lami-na	Leaf	Petiole	Lami-na	Leaf	Petiole
Unstored leaves	2427	3917	936[a]	5462[a]	8091[a]	2832[a]	720[a]	649[a]
Two days of storage	2354	3826	883[b]	5177[b]	7653[b]	2612[a]	640[b]	602[a]
Four days of storage	2051	3460	760[c]	4411[c]	6826[c]	1938[b]	594[bc]	510[ab]
Six days of storage	1654	3302	589[d]	3547[d]	5688[d]	1542[c]	544[c]	516[b]

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

usable stage after 2 - 3 and 4 - 5 days, respectively. The leaves under the refrigerator were still fresh by the end of the experiment.

4.2.4 Interactions

There was a significant interaction, in the second harvest of the first experiment and the first harvest of the second experiment, between storage conditions and storage durations for SCN^- content in leaves. The SCN^- content decreased with storage time but the decrease was highest in the shelf stored leaves and lowest in the refrigerated ones (Fig. 2). This rapid decrease in shelf stored leaves could be associated with senescence of the leaves according to the report of Chong and Berard (1983) which stated that the consistently rapid decline in the glucosinolate seemed to be associated with increasing senescence as evidenced by the marked decrease in quality during the same period.

Interaction between nitrogen sources, storage conditions and durations for SCN^- content in leaves was significant during first harvest of second experiment. Table 9 shows the interaction

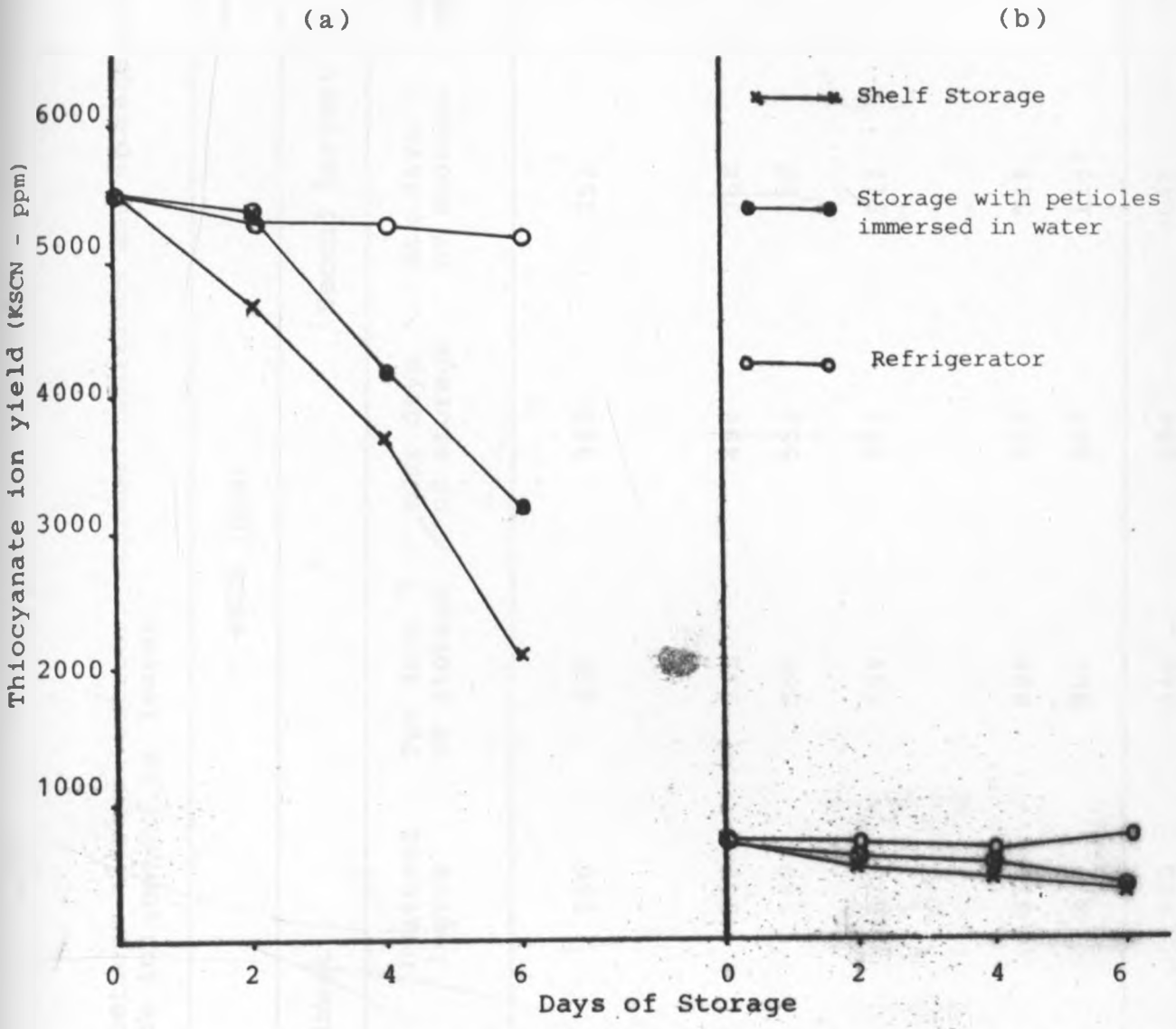

Figure 2: Interaction between storage conditions and durations on thiocyanate ion content in the leaves

(a) First experiment second harvest S.E. = 170

(b) Second experiment first harvest S.E. = 40

Table 9: Interaction between nitrogen sources, storage conditions and s on thiocyanate ion content in leaves.

		KSCN (ppm)			
	Second experiment				Second ha
Source	Condition	Unstored leaves	Two days of storage	Four days of storage	Six da of sto
C A N	Shelf storage	560	439	383	252
	Storage with petioles immersed in water	560	533	498	368
	Refrigerator	560	569	553	450
S A	Shelf storage	879	669	561	511
	Storage with petioles immersed in water	879	826	701	519
	Refrigerator	879	803	867	115
Mean of durations		720	640	594	542

S.E. = 76

between nitrogen sources, storage conditions and durations during second harvest of second experiment. Top-dressing with CAN and storing leaves on the shelf for six days tremendously decreased SCN^- content in the leaves.

5. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH WORK.

5.1 Summary and Conclusions.

Leaves from plants that were top-dressed with CAN accumulated more NO_3-N than those from plants which were top-dressed with SA. This shows that the NO_3-N accumulated in kale leaves could be influenced by source of nitrogen used. A fertilizer which provides nitrogen to the plant in the nitrate form may increase the accumulation of NO_3-N in the leaves. Therefore, NO_3-N accumulation could be minimised by use of nitrogen sources which do not contain nitrate.

Leaves from plants that were top-dressed with SA yielded more SCN^- than those from plants top-dressed with CAN. This high SCN^- content in leaves with SA may be attributed to the fact that sulphur is a constituent of glucosinolates which upon hydrolysis yield SCN^-. Hence the use of nitrogen fertilizers without sulphur can be useful in reducing glucosinolates in *Brassica* vegetables.

Storage of leaves may reduce NO_3-N concentration in the leaves. This reduction may not necessarily be accompanied by NO_2-N accumulation. This is because in nitrate assimilation, nitrite is the first stable product which is further reduced to other nitrogenous compounds such as amides and amino acids. Leaves stored with petioles immersed in water reduced the NO_3-N concentration than those stored on the shelf though this was not significant. Even a period longer than six days, no reduction of NO_3-N during refrigeration occurred.

The shelf storage could reduce SCN^- content in leaves more than when petioles of leaves were immersed in water or refrigerated. However, leaves stored on shelf deteriorated rapidly and turned yellow after 2 - 3 days. Storage of leaves with petioles immersed in water could reduce the amount of both NO_3-N and SCN^- content in leaves and it can also make the leaves remain fit for use for a relatively longer period of time (4 - 5 days). The length of time that stored leaves may remain fresh could depend on the storage method and its environment. The leaves stored on shelf, with petioles

immersed in water and refrigerator remained fresh for 2 - 3, 4 - 5 and more than 6 days respectively.

The petioles had significantly higher NO_3-N concentration than the laminae. Complete or partial removal of petioles prior to preparation of kale leaves may lead to a reduction of NO_3-N concentration in the leaves.

5.2 Suggestions for Further Research Work.

From this study leaves from plants top-dressed with CAN accumulated more NO_3-N than those top-dressed with SA. However, leaves from plants top-dressed with SA yielded more SCN^- than those top-dressed with CAN. Therefore it would be interesting to find out how fertilizers without nitrate and sulphur would influence NO_3-N concentration and SCN^- content in leaves.

In most cases farmers do not use fertilizers to raise kale in their small farms. Natural fertility may suffice but this may also influence NO_3-N and SCN^- content in leaves, for instance, during the

upsurge of NO_3-N (birch effect) or deposition of sulphur from the atmosphere especially in industrialised areas. A study can be done to find out how natural fertility would influence NO_3-N and SCN^- in leaves.

Leaves stored with petioles immersed in water had the highest reduction in NO_3-N while those stored on the shelf had the highest reduction of SCN^- with storage time. However the usability period was 2 - 3 and 4 - 5 days respectively. Although the leaves in the refrigerator were still fresh by the end of the experiment, there was no reduction in NO_3-N and SCN^- with storage time. Shelf storing accompanied with sprinkling of water, which is another common practice in Kenyan markets should be investigated and compared with storage of leaves with petioles immersed in water to see if usability period could be extended and NO_3-N and SCN^-

content reduced. In the refrigerator where there was minimal reduction in NO_3-N and SCN^- the usability period seemed to be longer. A study should therefore be done to find the length of time the leaves can be stored in the refrigerator before they get spoiled, and to study how chopping leaves and freezing them would affect their composition compared to freshly analysed leaves.

Another very important factor which could be investigated is the effect of storage on the micro-nutrients of the leaves. Dennis (1979) reported that all stored vegetables are subject to the same type of deterioration, physical, physiological, biochemical and pathological. However, the relative importance of deterioration varies considerably between different vegetables. It may be possible to reduce NO_3-N and SCN^- yield by storing but at the same time reducing vitamins and micro-nutrients and hence

making the leaves to have a less nutritive value.

Kale leaves are normally consumed after cooking. It would be useful to investigate the effect of cooking leaves on the NO_3-N concentration and SCN^- content in leaves.

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Appendix 1: Mean monthly weather record - Field Station, Kabete, between July 1986-March 1987.

Month		Mean Radiation MJ M^{-2}	Mean Sunshine hrs/day	Temperature °C Max.	Min	Mean	Total Rainfall mm/month	Mean Evaporation mm/day	% RH Mean	Total wind Run km/day
1986	July	17.16	4.95	20.7	9.5	15.1	6.2	2.8	73	48.4
	August	16.18	7.3	22.7	9.4	16.1	2.3	3.8	65	82.6
	September	20.06	5.7	23.2	11.1	17.2	4.3	4.2	64	99.6
	October	21.99	7.7	25.1	13.1	19.1	40.4	5.1	64	132.8
	November	18.62	5.7	21.8	13.5	17.7	202.0	3.3	77	108.1
	December	23.63	7.5	22.8	12.5	17.7	91.5	4.3	70	108.7
1987	January	24.39	8.9	23.8	12.7	18.3	79.5	4.7	67	118.9
	February	25.72	9.2	25.4	12.5	19.0	95.5	5.3	60	128.7
	March	25.66	9.2	26.8	10.5	18.7	15.4	5.8	57	127.1

Appendix 2: Laboratory Soil Analysis data, prior to transplanting

Nutrient Soil reaction	Experiment 1	Experiment 2
PH Water	6.6 (0.26)	5.9 (0.21)
CEC (Me/100g Soil)	29.1 (3.54)	24.2 (2.54)
Available phosphorus (µg/100g soil)	27.60 (17.78)	7.78 (3.6)
Total Nitrogen (µg/g dry soil)	3072 (218)	2821 (369)
Carbon (%)	2.64 (0.19)	1.78 (0.61)

( ) Standard deviations

Appendix 3: Analyses of variance (ANOVA) tables.

Appendix 3.1: ANOVA for Nitrate-Nitrogen concentration in leaves. (NO_3- N%)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	$679.46^{n.s.}$	$0.84^{n.s.}$	$0.16^{n.s.}$	$0.10^{n.s.}$
Sources (S)	1	$698.13^{n.s.}$	$0.008^{n.s.}$	7.58^{**}	6.42^{**}
Mainplot error	2	748.68	0.81	0.057	0.0089
Storage Conditions (C)	2	$760.43^{n.s.}$	$0.16^{n.s.}$	$0.94^{n.s.}$	$0.90^{n.s.}$
SXD	2	$695.65^{n.s.}$	$0.007^{n.s.}$	$0.81^{n.s.}$	$0.57^{n.s.}$
Subplot error	8	725.79	0.22	0.30	0.26
Storage duration (D)	3	$735.02^{n.s.}$	5.57^{**}	5.76^{**}	4.91^{**}
SXD	3	$705.20^{n.s.}$	$0.20^{n.s.}$	0.52^{*}	0.70^{*}
CXD	6	$699.98^{n.s.}$	$0.048^{n.s.}$	$0.22^{n.s.}$	$0.22^{n.s.}$
SXCXD	6	$701.96^{n.s.}$	$0.067^{n.s.}$	$0.19^{n.s.}$	$0.15^{n.s.}$
Sub-subplot error	36	697.93	0.13	0.18	0.17
Total	71				

A - First experiment, First harvest.
B - First experiment, Second harvest.
C - Second experiment, First harvest.
D - Second experiment, Second harvest.
n.s. - not significant.
* - Significant at 5% probability level.
** - Significant at 1% probability level.

Appendix 3.2: ANOVA for Nitrate-Nitrogen concentration in petioles. (NO_3-N%)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks (B)	2	$0.83^{n.s.}$	$3.29^{n.s.}$	$0.48^{n.s.}$	$0.24^{n.s.}$
Sources (S)	1	$0.02^{n.s.}$	$0.06^{n.s.}$	14.13^{**}	12.75^{**}
Main plot error	2	2.93	3.57	0.12	0.049
Storage conditions (C)	2	8.96^{**}	$0.34^{n.s.}$	3.52^{*}	3.44^{*}
SXC	2	$1.09^{n.}$	$0.05^{n.s.}$	$2.48^{n.s.}$	$2.38^{n.s.}$
Subplot error	8	0.64	0.73	0.63	0.65
Storage durations (D)	3	11.77^{**}	17.62^{**}	9.65^{**}	10.06^{**}
SXD	3	$1.21^{n.s.}$	$0.46^{n.s.}$	1.93^{*}	1.66^{*}
CXD	6	$1.60^{n.s.}$	$0.08^{n.s.}$	$0.64^{n.s.}$	$0.42^{n.s.}$
SXCXD	6	$0.20^{n.s.}$	$0.17^{n.s.}$	$0.52^{n.s.}$	$0.49^{n.s.}$
Sub-subplot error	36	0.93	0.53	0.49	0.44
Total	71				

A - First experiment, First harvest. B - First experiment, Second harvest.

C - Second experiment, First harvest. D - Second experiment, Second harvest.

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

Appendix 3.3: ANOVA for Nitrate-Nitrogen Concentration in laminae. (NO_3-N%)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	$0.11^{n.s.}$	$0.05^{n.s.}$	$0.04^{n.s.}$	$0.04^{n.s.}$
Sources (S)	1	$0.04^{n.s.}$	$0.08^{n.s.}$	2.00^{*}	2.17^{*}
Mainplot error	2	0.01	0.21	0.07	0.08
Storage Conditions (C)	2	$0.7^{n.s.}$	$0.01^{n.s.}$	$0.15^{n.s.}$	$0.09^{n.s.}$
SXC	2	$0.03^{n.s.}$	$0.0009^{n.s.}$	$0.06^{n.s.}$	$0.05^{n.s.}$
Subplot error	8	0.05	0.03	0.09	0.06
Storage durations (D)	3	0.29^{*}	0.63^{**}	2.33^{**}	2.07^{**}
SXD	3	$0.01^{n.s.}$	$0.07^{n.s.}$	0.34^{*}	$0.21^{n.s.}$
CXD	6	$0.01^{n.s.}$	$0.02^{n.s.}$	$0.09^{n.s.}$	$0.07^{n.s.}$
SXCXD	6	$0.01^{n.s.}$	$0.03^{n.s.}$	$0.07^{n.s.}$	$0.07^{n.s.}$
Sub-Subplot error	36	0.05	0.03	0.09	0.07
Total	71				

A - First experiment, First harvest.
B - First experiment, Second harvest.
C - Second experiment, First harvest.
D - Second experiment, Second harvest.
n.s. - not significant.
* - Significant at 5% probability level.
** - Significant at 1% probability level.

Appendix 3.4: ANOVA for thiocyanate ion content in leaves (KSCN - ppm)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	2,071,856$^{n.s.}$	376,128$^{n.s.}$	64,837$^{n.s.}$	15,875^{n}
Sources (S)	1	517,504$^{n.s.}$	13,090,180*	1,551,146*	1,278,134**
Mainplot error	2	778,624	537,792	36,078	14,395
Storage Conditions (C)	2	575,216*	10,652,160**	238,412**	147,502**
SXC	2	3,456$^{n.s.}$	344,384$^{n.s.}$	44,616$^{n.s.}$	8,966^{n}
Subplot error	8	107,620	272,016	17,690.25	12,942.
Storage durations (D)	3	691,754.7$^{n.s.}$	13,253,160**	101,132**	167,206*
SXD	3	51,605.33$^{n.s.}$	24,277.34$^{n.s.}$	18,514$^{n.s.}$	6,148^{n}
CXD	6	151,125.3$^{n.s.}$	3,003,029**	63,701**	26,341.34
SXCXD	6	109,973.2$^{n.s.}$	254,549.3$^{n.s.}$	31,826.67*	6,721^{n}
Sub-subplot error	36	378,331.6	140,174.2	10,825.83	18,453.
Total	71				

A - First experiment, First harvest.
B - First experiment, Second harvest.
C - Second experiment, First harvest.
D - Second experiment, Second harvest.
n.s. - not significant.
* - Significant at 5% probability level.
** - *Significant at 1% probability level.*

Appendix 3.5: ANOVA for thiocyanate ion content in petiole (KSCN-ppm)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	8,901,984$^{n.s.}$	4,066,944$^{n.s.}$	10,373$^{n.s.}$	33,698$^{n.s.}$
Sources (S)	1	295,168$^{n.s.}$	32,510,210$^{n.s.}$	833,340*	431,212$^{n.s.}$
Mainplot error	2	4,171,744	3,387,136	20,911	60,535
Storage Conditions (C)	2	753,440*	18,478,590**	205,176*	71,552*
SXC	2	180,352$^{n.s.}$	787,584$^{n.s.}$	52,842$^{n.s.}$	12,207$^{n.s.}$
Subplot error	8	150,256	466,560	27,355	12,760.5
Storage durations (D)	3	1,339,371$^{n.s.}$	20,114,770**	77,204$^{n.s.}$	210,714.7**
SXD	3	89,237.34$^{n.s.}$	114,432$^{n.s.}$	92,273.34*	101,746.7*
CXD	6	114,485.3$^{n.s.}$	5,951,830**	101,122**	18,817.67$^{n.}$
SXCXD	6	49,770.67$^{n.s.}$	661,930.7$^{n.s.}$	45,988.67$^{n.s.}$	6,150.33$^{n.}$
Sub-Subplot error	36	2,075,531	304,256	30,349.39	30,013.89
Total	71				

A - First experiment, First harvest.
B - First experiment, Second harvest.
C - Second experiment, First harvest.
D - Second experiment, Second harvest.
n.s. - not significant.
* - Significant at 5% probability level.
** - Significant at 1% probability level.

Appendix 3.6: ANOVA for thiocyanate ion content in laminae (KSCN - ppm)

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	4,654$^{n.s.}$	7,335,984$^{n.s.}$	193,787$^{n.s.}$	39,810$^{n.s.}$
Sources	1	104,192**	2,407,169$^{n.s.}$	2,568,506*	2,689,266**
Mainplot error	2	800	1,361,808	98,917	18,654
Storage Conditions (C)	2	258,642**	7,509,696**	255,779*	286,695*
SXC	2	8,144$^{n.s.}$	70,752$^{n.s.}$	56,731$^{n.s.}$	43,985$^{n.s.}$
Subplot error	8	2,724.5	288,516	48,610.5	48,555
Storage durations (D)	3	425,802.7**	6,401,963**	142,727.3**	142,834.7*
SXD	3	3,289.33$^{n.s.}$	22,517.34$^{n.s.}$	36,503.33$^{n.s.}$	32,668$^{n.s.}$
CXD	6	63,980.67$^{n.s.}$	1,859,067**	40,394.33$^{n.s.}$	41,931.33$^{n.s}$
SXCXD	6	3,040.67$^{n.s.}$	107,733.3$^{n.s.}$	36,268.33$^{n.s.}$	34,385.33$^{n.s}$
Sub-subplot error	36	2,955	150,488.9	22,649.11	33,278.56
Total	71				

A - First experiment, First harvest.
B - First experiment, Second harvest.
C - Second experiment, First harvest.
D - Second experiment, Second harvest.
n.s. - not significant.
* - Significant at 5% probability level.
** - Significant at 1% probability level.

Appendix 4 : Major product classes from the enzymatic hydrolysis of glucosinolates (Carlson et al., 1987).