"THE EFFECT OF NITROGEN RATES AND SOURCES ON LEAF PRODUCTION, NITRATE ACCUMULATION AND THIOCYANATE CONTENT IN KALE AND COLLARD (Brassica oleracea var. acephala, D.C.) LEAVES."

BY

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D E D I C A T I O N

To my mother

Rugina Kanampiu
and kid brother
Patrick Kanampiu

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Fred K. Kanampiu Date 1/9/87

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 Field Station of Faculty of Agriculture, Kabete Campus, University of Nairobi, to study the effect of rates and sources of nitrogen (N) on leaf production, thiocyanate content and nitrate accumulation in leaves of 'Thousand-headed' kale and 'Georgia' collards (both *Brassica oleracea* var. *acephala* D.C.). Four N rates (0, 10, 20 and 40 grams (g) per plant) and two sources (calcium ammonium nitrate-CAN and sulphate of ammonia-SA) were used.

Leaf yield was assessed by both cumulative leaf weight per 10 m^2 and leaf number per plant. The leaf yield increased as rates of N increased. Plants top-dressed with higher rates of N than 10 g per plant did not show significant increase in leaf weight. Nitrogen sources had no significant effect on leaf yield. However, varietal effect on leaf production was significant. 'Georgia' collards gave significantly higher leaf yield than 'Thousand-headed' kale did. Interactions between N rates and varieties on leaf weight and N sources and varieties on leaf number were significant during the second experiment. Interaction between N rates, sources and varieties on leaf number was also significant

during the second experiment.

Nitrate-nitrogen (NO_3-N) in the leaves increased with increasing rates of N fertilizer application. Leaves from plants top-dressed with CAN accumulated more NO_3-N than those top-dressed with SA. Leaves of 'Thousand-headed' kale accumulated significantly higher NO_3-N than those of 'Georgia' collards. Interaction between rates and sources of N on NO_3-N accumulation in leaves was significant during the first harvest of second experiment. The results also show that there was a positive correlation between NO_3-N in the soil and NO_3-N in leaves at harvest time and that petioles accumulated more NO_3-N than laminae.

Nitrogen rates had no significant effect on thiocyanate ion (SCN^-) content in leaves. However, the effect of N sources on SCN^- content was significant. Leaves from plants top-dressed with SA yielded significantly higher SCN^- than those top-dressed with CAN. Leaves of 'Thousand-headed' kale yielded significantly higher SCN^- than did those of 'Georgia' collards. Interaction between rates and sources of N on SCN^- content in leaves was significant. Interaction between N sources and varieties on SCN^- content in leaves was significant during second harvests on both experiments.

CHAPTER 1

INTRODUCTION

Kale (Brassica oleracea var. acephala D.C.) is a member of the Cruciferae family and is, most likely, native to eastern Mediterranean countries (Nieuwhof, 1969). Many types of kale are known, but the chief characteristic is that plants do not form heads or produce edible flowers. Some types are grown as ornamentals. The plants have glaucous green or blue-green foliage and open type of growth. Varieties vary in size and height, as well as in form and colour of their leaves (Nieuwhof, 1969; Ware and McCollum, 1980).

Kales differ from collards (also B. oleracea var. acephala D.C.) in that varieties of collards are less variable; plants are intermediate in size between the dwarf and tall kales; usually have a thick, stiff main stem which is terminated by a loose head of cabbage-like leaves; and growth is more upright in habit than is that of the dwarfer varieties in kale (Purseglove, 1968; Hawthorn and Pollard, 1954).

Kales and collards have a wide ecological adaptability (Anon., 1976). Kales were first introduced in Kenya as a fodder crop by the Europeans when they settled in the Kenya Highlands. The kales remained unknown as a vegetable for quite sometime. However, they have become increasingly important as a vegetable in most parts of Kenya.

Kales and collards are popularly referred to in Kenya as 'sukuma wiki' a Swahili phrase, which literally means 'a week pusher', which further means that kale and collard vegetables are eaten every day of the week. Kale and collard vegetables are important leafy vegetables that have high demand in the country. They have become staple vegetables amongst all categories of income groups in Kenya. Leaves are eaten as a green vegetable in making stews of varied kinds. The stews are used with other foods, especially grain products which are most popular amongst the low and middle class income groups in many parts of the country.

The vegetables are common in the backyard gardens of most homes in both urban and rural areas. Farmers living around urban areas grow them mainly for commercial purposes. Their supply to the urban markets is throughout the year. Many people have got

used to kales and collards and this trend is increasing as consumers' taste is changing in favour of these vegetables.

Kales and collards can be grown in a wide range of climatic conditions provided water is available. In Kenya, they are mainly grown in Kiambu, Kisii, Nyeri, Embu, Meru, South Nyanza, Nakuru, Narok and Bungoma districts (Anon., 1984).

Kales and collards have been reported to accumulate nitrates (Maynard et al., 1976; Chweya, 1986) and to contain glucosinolates (Michajlovskij, 1986; Gramberg et al., 1986; Chweya, 1987) in leaves. The accumulation of nitrates in plants is enhanced by a number of factors. These include, among others, vegetable varieties, sources and rates of nitrogen application and environmental factors such as light, temperature and rainfall. The use of nitrogen fertilizers has been on the increase, especially by vegetable growers, to obtain high yields on less acreage. Excessive quantities of nitrate-nitrogen in soil can lead to accumulation of nitrates by vegetable crops (Maynard et al., 1976; Gardner and Pew, 1979; Maynard and Barker, 1979).

The form of nitrogen supplied to plants has a

great influence on the absorption of nutrients. Ammonium ions are reported to suppress cation and enhance anion absorption (Barker and Maynard, 1972). Nitrate form gives a better vegetative growth and yield in leafy vegetables than ammonium form (Barker and Maynard, 1972).

Barker et al. (1974) working with eighteen spinach (Spinacia oleracea) cultivars found considerable differences in their ability to accumulate nitrates in leaves. Smooth-leafed cultivars of spinach are lower in nitrate concentration than heavily savoyed cultivars (Barker et al., 1974; Maynard and Barker, 1974).

Nitrates are converted to nitrites which, when consumed, may be toxic or carcinogenic to both humans and animals through the production of nitrosamines (Lee et al., 1971; Maynard et al., 1976; Wogan and Marletta, 1985). After absorption into the bloodstream, nitrite cause oxidation of active ferrous form to the ferric state found in haemoglobin which impairs oxygen carrying capacity of blood leading to methaemoglobinemia (Schuphan, 1971; Lee, 1970), a dangerous disorder which is particularly fatal to infants.

Glucosinolates occur in some cultivated plant species and are responsible for pungent flavours of cabbage (Brassica oleracea var. capitata L.) and related vegetables (Tookey et al., 1980; Wogan and Marletta, 1985). When the plant cells containing the glucosinolates are broken, say by marceration, the enzyme myrosinase hydrolyses the glucosinolates to yield bitter tasting substances and various derivatives including thiocyanate ions known to be goitrogenic (Appendix 5). Goitre in human beings has been attributed to the consumption of large amounts of cabbage and kales (Tookey et al., Johnston and Jones, 1966).

There are a number of factors that may affect the leaf yield and glucosinolates in the kale and collard leaves. These include environmental, genetic and cultural factors. Among these factors are vegetable varieties and sources and rates of nitrogen. Little work has been done on the influence of varieties, sources and rates of nitrogen on the leaf yield and levels of nitrates and thiocyanate in kale and collard leaves under Kenya conditions. It was therefore, the purpose of this study:

1. to investigate the effect of nitrogen rates on leaf yield, nitrate accumulation and thiocyanate

ion content in leaves of 'Thousand-headed' kale and 'Georgia' collard (both Brassica oleracea var. acephala D.C.) under Kabete conditions.

2. to investigate the effect of nitrogen sources on leaf yield, nitrate accumulation and thiocyanate ion content in leaves of 'Thousand-headed' kale and 'Georgia' collard.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrogen nutrition in plants.

Nitrogen is the key nutrient which promotes vegetative growth of a plant (Huffaker and Rains, 1978). Both nitrate and ammonium forms of nitrogen can be taken up and metabolised by plants (Huffaker and Rains, 1978; Mengel and Kirkby, 1979; Hewitt and Smith, 1975). However, nitrate form is often a preferential source for crop growth depending on species and other environmental factors (Mengel and Kirkby, 1979; Buckman and Brady, 1968; Hewitt and Smith, 1975). Ammonium ion absorption is best when its concentration is high (Beevers, 1976) and at neutral soil reaction (Mengel and Kirkby, 1979).

The form in which nitrogen is translocated depends on the source, uptake and metabolism (Reisenauer, 1978; Mengel and Kirkby, 1979). According to Reisenauer (1978), nearly all the ammonium-nitrogen is assimilated in root tissue and translocated as amino acids. Nitrate-nitrogen is translocated in nitrate ion form (Reisenauer, 1978;

Mengel and Kirkby, 1979).

Within plants, before metabolism, nitrate is reduced to nitrite then to amide (Sims et al., 1968). This is a key process in the nitrogen metabolism in plants (Hewitt and Smith, 1975). It is only at amide stage that branching of the metabolic network occurs, leading to formation of amino acids from which proteins and nucleic acids are synthesized (Sims et al., 1968; Tisdale and Nelson, 1971; Mengel and Kirkby, 1979).

2.2 Effect of Nitrogen on Yield of Kales and Collards.

2.2.1 Nitrogen rates.

Chweya (1984) working with 'Thousand-headed' kale found that nitrogen tended to increase the mean fresh weight, number of auxillary branches and leaves and, therefore, total yield per plant. Splittstoesser and van Mark (1974) working with several vegetables, including collards, found that the total protein and fresh weight of leaves increased with increasing rates of nitrogen. Barker et al. (1974) working with three spinach cultivars, found that leaf yield increased with increasing rates of nitrogen. These

authors further found that smooth-leafed cultivars yielded higher than savoyed or semi-savoyed cultivars and smooth-leafed cultivars responded better to nitrogen fertilization than savoyed cultivars.

Nitrogen induces leaf production and expansion in plants (Hewitt and Smith, 1975). Leaf size is primarily determined by cell expansion which is also influenced by nutrient supply amongst other factors (Cutter, 1971). Cultural practices that increase yield do so by wholly or mainly by influencing leaf growth (Watson, 1956). According to Watson (1956), nitrogen increases leaf area throughout the leaf growth period.

According to Hewitt and Smith (1975), cauliflowers (Brassica oleracea var. botrytis L.) chloroplast production is reduced when nitrogen is deficient. Chloroplasts are essential cell components and their reduction lowers the photosynthetic ability of the leaf (Cutter, 1971). In rape (Brassica napus L.), premature cell vacuolation and early differentiation or senescence occur if nitrogen is deficient (Hewitt and Smith, 1975).

2.2.2 Nitrogen sources.

Nitrate form of nitrogen is more easily leached in soil than ammonium form but is more readily available to the roots than the ammonium (Buckman and Brady, 1968). A combination of ammonium-nitrate forms, has been reported to increase dry weight in spinach (Cantliffe, 1972a; Aworh et al., 1980). Pew et al. (1979) found that nitrogen source in cabbage production did not affect the number of heads harvested.

When the amount of nitrate alone is increased, it enhances yield in lettuce (Knight and Mitchell, 1983). These authors further found that a combination of ammonium-nitrate forms gave higher yield than nitrate alone in nutrient solution containining same amoungts of nitrogen. Calcium ammonium-nitrate contains both the ammonium and nitrate forms of nitrogen whereas sulphate of ammonia contains the ammonium form only. It has also been reported (Jacob and van Uxekull, 1963) that using calcium ammonium-nitrate as the source of nitrogen, ensures a quicker response through nitrate

whilst ammonium prolongs its availability in soil.

2.3 Nitrate Accumulation in Plant Leaves and its Effect on Human Health.

Nitrite is the first stable intermediate in the assimilation of nitrate by plants (Hewitt et al., 1967; Maynard et al., 1976). Nitrites rarely accumulate in tissue of plants under normal conditions (Hewitt et al., 1967). Unlike nitrites, nitrates accumulation is frequent especially under conditions of molybdenum deficiency (Hewitt et al., 1967; Mengel and Kirkby, 1979). A few plant species, especially in Cruciferae family, have the ability to accumulate nitrates in the shoots (Salisbury and Ross, 1978; Maynard and Barker, 1979; Mengel and Kirkby, 1979). These authors attribute this condition to quicker rate of nitrate translocation from root to the shoot than assimilation in shoot and thus giving very little time for nitrates to be reduced and converted into amino acids.

2.3.1 Factors affecting Nitrate Accumulation in Plant Leaves.

Accumulation of nitrate in plants is dependent on the environmental conditions under which the plant

is grown (Cantliffe, 1972a, 1972b; Maynard et al., 1976). Plant part and genetic make up also have got a role to play on nitrate accumulation (Maynard et al., 1976; Barker et al., 1974; Maynard and Barker, 1974).

Nitrate supplying ability of the soil enhances nitrate accumulation in plants (Barker and Maynard, 1972; Asif and Greig, 1972; Maynard et al., 1976; Wolff and Wasserman, 1972). Light (Cantliffe, 1972a, 1972b, 1972c), water reactions and carbon dioxide concentration (Maynard et al., 1976) affect nitrate accumulation in plants. Wolff and Wasserman (1972) working with several vegetables, including kales and collards, found that nitrate accumulation was high under nitrogen rich soils and under reduced light and water. Cantliffe (1972b) working with spinach, showed that nitrate accumulation decreased as light intensity increased.

Cantliffe (1972c) reported that little nitrate-nitrogen accumulated in leaves and roots of beet (Beta vulgaris) at photoperiods of eight, twelve, sixteen or twenty hours when no nitrogen was added to the soil. He further found that even when nitrogen was applied, less nitrate-nitrogen accumulated as the photoperiod was extended from

eight to twenty hours. Therefore, nitrate concentration in shoots of plants is lower in the afternoon than in the morning and on sunny days than on cloudy days (Maynard et al., 1976; Minotti and Stankey, 1973). This shows that harvesting schedules could influence the nitrate concentration in kale and collard leaves.

The relative degree of absorption, translocation and assimilation of nitrate is also affected by temperature (Cantliffe, 1972a; Maynard et al., 1976). According to Cantliffe (1972a), the nitrate concentration in spinach leaves decreased with a subsequent increment in temperature, from 25°C to 30°C when the plants had been supplied with ammonium-nitrate fertilizer.

The amount and source of nitrogen may affect nitrate accumulation in vegetables (Maynard et al., 1976). Nitrate accumulation in leaves increases with increasing rates of nitrogen (Schuphan, 1971; August, 1974; Splittstoesser and van Mark, 1974; Maynard et al., 1976; Pimpini et al., 1973; Wehrmann and Hahndel, 1984). Heinze (1974), working with spinach, and Chweya (1986), working with kale and

collards, had similar findings. In the soil, organic nitrogen is converted to ammonium through mineralisation, whereas ammonium nitrogen is oxidised to nitrate through nitrification process (Russell, 1973). Therefore, within a certain period, as much nitrate may be accumulated from organic or ammoniacal fertilizers if sufficient time and favourable conditions for mineralisation to occur are availed (Barker, 1975).

Nitrate fertilizers result in higher nitrate accumulation than ammoniacal fertilizers (Pimpini et al., 1973; Lorenz, 1978; Gardner and Pew, 1979). Pimpini et al. (1973) found that, increasing quantities of fertilizers such as ammonium-nitrate, ammonium sulphate, calcium nitrate, urea or cyanamide caused an increase in total nitrogen and nitrate content in spinach, red beet, cabbage and cauliflowers. However, much higher nitrate accumulations in the plants were experienced when ammonium-nitrate, calcium nitrate or urea was applied than when ammonium sulphate or cyanamide was. This shows therefore, that the accumulation of nitrate in the plant can be influenced to a great extent by the source of nitrogen applied.

In spinach, petioles accumulate more nitrate than the laminae (Maynard et al., 1976). Chweya (1986) working with 'Thousand-headed' kale and collards had similar observations. This implies that, partial or complete removal of petioles prior to processing or preparation may reduce nitrate intake from vegetable leaves by humans (Maynard et al., 1976).

Nitrate accumulation differs among plant species and among cultivars within a species. Barker et al. (1974) and Maynard and Barker (1974) working with spinach found that, savoyed-leaf cultivars consistently had higher nitrate concentration than smooth-leafed cultivars. Fast growth rate, high yield, large protein content and high nitrate reductase activity are factors common to the smooth-leafed cultivars and these qualities may be the cause to their low nitrate content (Barker et al., 1974; Maynard and Barker, 1974).

Leaves of kales rank high among other vegetables within genus Brassica in their nutritive value (See Table 1). However, despite their nutritive value, the leaves have been reported to accumulate nitrates (Maynard et al., 1976; Chweya, 1986). Chweya (1986) found

Table1: Food composition in terms of the retail weight (as purchased per 100 grams fresh weight) (Chatfield, 1959).

Commodity description	Calories/ 100 gms	Protein (%)	Fat (%)	Calcium (mg)	Iron (mg)	Thiamine (mg)	Riboflavin (mg)	Ascobic (mg)	Vitamin A (i.u.)	Niacin (mg)
Broccoli	26	2.5	0.2	75	0.8	0.06	0.12	68	2030	0.6
Brussel sprouts	36	3.6	0.4	26	1.0	0.06	0.12	71	300	0.5
Cabbage	17	1.1	0.1	35	0.3	0.04	0.03	35	70	0.2
Chinese cabbage	11	1.1	0.1	79	1.6	0.05	0.02	20	2770	0.3
Cauliflower	13	1.3	0.1	12	0.6	0.06	0.05	37	50	0.3
Kale	27	2.5	0.4	145	1.4	0.07	0.16	76	4780	1.0
Kohlrabi	15	1.1	0.1	24	0.3	0.03	0.03	32	-	0.1

that 'Thousand-headed' kale accumulated more nitrates than collards.

2.3.2 Hazards of Nitrate to Human Health.

Nitrates are toxic to humans and animals like ruminants that eat vegetation which is high in nitrate (Salisbury and Ross, 1978). Nitrites formed from the reduction of nitrates pose health hazards (Maynard et al., 1976). In humans with healthy alimentary canals, nitrates are absorbed rapidly without reduction to nitrites (Maynard et al., 1976). Gastrointestinal disturbances may delay absorption of nitrates and hence increase the chances for them being reduced to nitrites (Maynard et al., 1976). After absorption into the bloodstream, nitrites cause oxidation of Iron II to Iron III found in haemoglobin hence imparing oxygen carrying capacity of the blood (Maynard et al., 1976; Maynard and Barker, 1974).

Burden (1961) and Lee (1970) stated that, in human, nitrates and nitrites toxicity appears to be in the order of 15 to 70 milligrams nitrate-nitrogen and 20 milligrams nitrite-nitrogen per kilogram

body weight, respectively. The presence of nitrites and secondary amines may lead to the formation of nitrosamines which can be carcinogenic compounds (Maynard et al., 1976; Mengel, 1979).

2.4 Thiocyanates

Glucosinolates occur in all Brassica vegetables and are responsible for their pungent or characteristic flavour (van Etten and Wolff, 1973; Tookey et al., 1980; Johnston and Jones, 1966). Within genus Brassica, the distribution of individual glucosinolates vary considerably (Olsson and Jeppson, 1984; Michajlovskij, 1986). Sinigrin (2-propenyl-GS), glucoiberin (3-methyl-sulphinyl-propyl-GS) and glucobrassicin (3-indolymethyl-GS) are the three major glucosinolates identified in kales (Michajlovski, 1986). Upon hydrolysis by the enzyme myrosinase (thioglucosidase), glucosinolates yield glucose, acid sulphate ion and one or more aglucon products namely iso-thiocyanate, nitriles and thiocyanates (Tookey et al., 1980; Carlson et al., 1985). These enzymatic products are the ones responsible for the pungent taste and contribute to the characteristic flavour of Cruciferae vegetables (Michajlovskij, 1986).

2.4.1 Factors Affecting Thiocyanate Content in Plants.

Neil and Bible (1972) noted that radish (Raphanus sativus L.) plants grown in organic soil yielded a significantly higher quantity of thiocyanate ion content than those grown on loam soil. Organic soils have good aeration and higher levels of sulphur than loam soils. Good aeration favours increased ions uptake from soil solution and this in turn may influence the level of glucosinolates in shoots (Neil and Bible, 1972).

The synthesis of glucosinolates is related to nitrogen nutrition since their synthetic pathway starts with amino acids such as glutamate, alanine or serine (Fowden, 1967; Mengel and Kirkby, 1979). Low sulphur fertilization reduces the glucosinolates content in rapeseed (Neil and Bible, 1972). Freeman and Mossadeghi (1972) reported a positive correlation between the amount of glucosinolates extracted from radish root tissue and sulphur nutrition. Sulphur is a major component of glucosinolates (Mengel and Kirkby, 1979; Whistler and Daniel, 1985). Hence, sulphur nutrition may have a considerable effect on the level of thiocyanate ion content in plants of Cruciferae

family.

Bible and Chong (1975), working with radishes found that thiocyanate ion content in roots increased under cooler conditions. They further found a positive correlation between thiocyanate ion content and rainfall for plants grown on loam soil, but a negative correlation with mean daily air-temperature for plants grown on organic soils. Chweya (1984) working with 'Thousand-headed' kale found that close spacing tended to increase thiocyanate content in leaves. This implies that temperature, rainfall and cultural practices could influence thiocyanate ion content in plants.

Thiocyanate ion content in leaves of kale is highest during the most active growth period of the plant (Johnston and Jones, 1966). Thiocyanate ion content in leaves of kale was observed to decrease with increasing age of plants (Johnston and Jones, 1966) and to decrease down the plant (Chweya, 1985). Johnston and Jones (1966) further observed in leaves of the same age that the laminae had consistently higher thiocyanate ion content than the petioles.

There is a genetic effect on thiocyanate ion

content in plants (Johnston and Jones, 1966; Michajlovskij, 1986; Paxman and Hill, 1974a; Carlson et al., 1987a, 1987b). Johnston and Jones (1966) working with six varieties of kale showed that important inter-varietal differences occur in their thiocyanate ion contents. Paxman and Hill (1974a) found that rape kale yielded less than half the amounts of thiocyanate ions found in 'Thousand-headed' and 'marrow-stem' kales. Gramberg et al (1986) found that glucosinolate contents of cooked Brussels sprouts (Brassica oleracea var. gemmifera L.) to be significantly higher than in cooked cauliflower.

There is very little work done on the effect of rates and sources of nitrogen on glucosinolates or thiocyanate ion contents in leaves of kale and collard vegetables.

2.4.2 Hazards of Thiocyanate ions to Human Health.

Paxman and Hill (1974b) reported that thiocyanate ion content in kale was responsible largely, if not wholly, for goitrogenicity that was observed in rats. Feeding kale to livestock can lead to reduced fertility and induce goitrogenic effects (Johnston

and Jones, 1966). In foods and feeds, isothiocyanates are decomposed to thiocyanates which cause thyroid enlargement (Tookey et al., 1980). Goitre in human beings has been attributed to the consumption of large amounts of cabbage and kale (Tookey et al., 1980). The development of goitre depends on dietary balance between iodine and thiocyanate which is found by determining urinary iodine to thiocyanate ions ratio. Goitre appears when the ratio reaches a critical threshold of about three (Michajlovskij, 1986). However, Wogan and Marletta (1985) feel that the role of this anti-thyroid substances in the etiology of human endemic goitre is apparently minimal.

CHAPTER 3

MATERIALS AND METHODS

Two experiments were carried between August 1986 and March 1987. First experiment was carried between August and November 1986, and the second experiment between December 1986 and March 1987.

3.1 Experimental Site.

The site selected for this study was at the Field Station of the Faculty of Agriculture, Kabete Campus, University of Nairobi. The Field Station is at an altitude of 1940 metres above sea level and lies at latitude 1^{o} 15' S and longitude 36^{o} 44' E with an average rainfall of about 1000 mm per year which is bimodal with peaks in April and November. The mean monthly maximum and minimum temperatures are $23^{o}C$ and $12^{o}C$, respectively. Appendix 1 shows the weather data during the experimental period.

3.1.1 Soil Characteristics

The site is under Nitosol unit according to FAO/UNESCO classification (FAO/UNESCO, 1974). These soils are referred to as Kikuyu friable clay derived

from tertiary trachytic lava 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 samples of soil were taken from the experimental site at two depths, that is, 0-15 centimetres (cm) and 15-30 cm from the surface of the soil a day before transplanting. The soil samples were then air dried and passed through a two millimetre sieve. The fraction which passed through the sieve was used to analyse for total nitrogen, cation exchange capacity (CEC), organic carbon, available phosphorus and pH water.

Organic carbon, CEC, available phosphorus and total nitrogen were determined using analytical methods used in the department of Soil Science, University of Nairobi (Ahn, 1973; 1975). For pH determination, one part of soil to two and half parts of water (w/v) were shaken vigorously for 30 minutes and pH measured using glass-electrode pH meter. The results of soil analyses performed prior to transplanting are presented in Appendix 2.

3.2 Planting Material

The seeds of 'Thousand-headed' kale and 'Georgia' collards were obtained from East African Seed Company. Seedlings were raised in a nursery using recommendations by the Ministry of Agriculture (Anon, 1981).

3.3 Treatments and Experimental Design

The treatments included four rates of nitrogen (0, 10, 20 and 40 grams (g) per plant), two nitrogen (N) sources (Calcium ammonium nitrate - CAN and sulphate of ammonia-SA) and two varieties of Brassica oleracea var. acephala D.C. ('Thousand-headed' kale and 'Georgia' collards). The treatments were arranged in a split-plot design with three replicates. The N rates were the main plots. The N sources and varieties were combined factorially to give four treatments which formed the sub-plots. The main plots and sub-plots were of size 13x5 metres (M) and 6x2 M, respectively. Split application of nitrogen was done, with half of the N applied three weeks after transplanting and the other half three weeks later, that is, six weeks after transplanting.

3.4 Cultural Practices.

3.4.1 Nursery

Fine tilth seedbeds of 1m wide and 4m long were prepared for each variety. One kilogram of tripple superphosphate (46 per cent P_2O_5), was uniformly applied on each seedbed and lightly mixed with soil using a rake. Seeds were sown in furrows, 10 cm apart and about 2.5 cm deep, and thinly covered with top soil. The seedbeds were then mulched using dry grass and then watered using cans every morning. Seedlings emerged after 5-6 days and the mulch was removed at the seventh day. The seedbeds were kept weedfree. Two weeks before transplanting, watering frequency was reduced to harden the seedlings. The seedlings were transplanted four weeks after emergence.

3.4.2 Plots Preparation and Transplanting.

The trial field was ploughed and disc harrowed twice using a tractor before plots were marked out. A day before transplanting the field was watered. During transplanting, tripple superphosphate (46 per cent P_2O_5) at the rate of 20 g and 2 g of furadan per planting hole were applied

and mixed with soil. These rates are recommended by the Ministry of Agriculture (Anon, 1981). Seedlings of uniform size were then transplanted at various treatment plots. The spacing used was 60 x 30 cm giving a plant population of 55, 555 per hectare. The plots were kept weedfree throughout the experimental period by using 'pangas' and 'jembes' for weeding.

3.4.3 Pests and Diseases.

Aphids, cutworm and moles are the major pests at the Field Station. Aphids and cutworms were controlled using furadan and ripcord. Ripcord was sprayed every 2 weeks at a rate of 50 millilitres in 20 litres water to spray 1000 m^2 (0.5 litres per hect are). Moles were controlled by trapping. There were no disease incidences during the experimental period.

3.4.4 Irrigation.

Supplemental irrigation water was applied to avoid water stress in plants. This was done by using overhead sprinklers.

3.5 Observations during the Experiment

3.5.1 Soil Sampling

Each plot was sampled at two depths, that is 0-15 cm and 15-30 cm from the surface of the soil profile for the determination of available nitrogen. Sampling was done every time when there was leaves sampling. To minimise biochemical reactions by microbes and evaporation of water from the soil samples during transportation to the laboratory, the soil samples were put in polythene bags and placed in cool-box. The samples were then analysed for available nitrogen and water content determined. Available nitrogen was analysed using analytical methods used in the Soil Science department, University of Nairobi and values expressed as μg NO_3-N per g dry soil. Water content was determined by gravimetric method, that is, weights of oven dried ($105^{o}C$) samples subtracted from their respective wet weights over oven dry weights. This was necessary in order to calculate NO_3-N concentration on dry soil basis for the purpose of standardization.

3.5.2 Leaf Sampling

Ten plants per sub-plot were randomly selected and tagged. The tagged plants were used to

determine the number of leaves per plant at every harvesting time which was done six, eight and ten weeks transplanting. All the leaves were counted except the top four. To determine the yield (fresh weight) per unit area, plant leaves from an area of 5.76 m^2 per sub-plot were harvested (except the top four hence making all the plants uniform) and weighed. After the determination of the number of leaves and fresh weights in the first and second harvesting, ten leaves per sub-plot were picked at random, put in polythene bags and taken to the laboratory for dry weight, nitrate-nitrogen and thiocyanate content determinations. Sampling was always done between 8 and 9 a.m. After leaf harvesting and yield determination, all the guard rows were stripped leaving the top four leaves so that all the plants were uniform for the next harvesting.

3.6 Laboratory Analysis

3.6.1 Dry Matter Determination.

Petioles and laminae were separated using scapel knife. Known weights of petioles and laminae were put in paper bags and dried in the oven at 70°C for 96 hours after which the samples

were weighed. The dried samples were ground using a grinding machine (Glen Creston Stanmore England, DFH 48 - Type) and passed through 1 millimetre sieve. Ground samples were stored in plastic bottles, closed tightly and preserved for nitrate-nitrogen analysis.

3.6.2 Nitrate-nitrogen Determination

Nitrate-nitrogen (NO_3-N) in petioles and laminae was determined using the colorimetric method described by Caltado et al. (1975) with a slight modification according to Chweya (1985); that is, after incubation instead of centrifuging, the mixture (distilled water and sample) was filtered using Whatman's filter paper number 41. The filtrate was used for NO_3-N determination with values expressed as per cent (%) NO_3-N. Nitrate-N accumulation in leaves was estimated by averaging amounts from petioles and laminae because the weight ratio of petioles to laminae was about one to one (Chweya, 1985).

3.6.3 Thiocyanate ion content Determination.

Thiocyanate ion (SCN^-) content in petioles and laminae was determined using the colorimetric

method described by Bible et al., (1980) with slight modification according to Chweya (1985); that is, one part sample to five parts distilled water (w/v) was used for laminae and one part sample two parts distilled water (w/v) for petioles instead of one part sample to one part distilled water (w/v). Values were expressed as micrograms Potassium thiocyanate per gram dry weight (µg KSCN/g dry wt – ppm). Thiocyanate ion content in leaves was estimated by averaging amounts from petioles and laminae because the weight ratio of petioles to laminae was about one to one (Chweya, 1985).

3.7 Data Analysis.

Data obtained were subjected to analysis of variance (ANOVA) using methods illustrated in Steel and Torrie (1981). Separation of means was done using Duncan's multiple range test at 5% probability level ($P \leq 0.05$) as illustrated by the same authors.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effects of Rates and Sources of Nitrogen, and Varieties on Leaf Yield.

Effect of N rates on cummulative leaf weight per 10 m^2 and leaf number per plant was significant ($P \leq 0.05$). Table 2 shows that both weight and number of leaves increased with increasing rates of N. Plants that were not top-dressed with N had significantly lower leaf weight and number than those top-dressed. However, plants top-dressed with higher rates of N than 10 g per plant did not show significant increase in leaf weight. Plants top-dressed with 40 g N per plant gave significantly higher number of leaves than those top-dressed with 10 g N per plant but not with those top-dressed with 20 g N per plant.

The findings that the two yield parameters were increased with increasing N rates could be due to the fact that N induces leaf production and increases leaf area surfaces throughout growth. Leaf size is primarily determined by cell expansion which is also influenced by N nutrient supply amongst other factors

Table 2: Effect of nitrogen rates on cumulative leaf weight and number of *Brassica oleracea* var. *acephala* D.C.

Nitrogen rates (gN per plant)	Total leaf weight ($kg/10m^2$)		Total leaf number per plant	
	First experiment	Second experiment	First experiment	Second experiment
0	37.15^{a}	34.38^{a}	30^{a}	27^{a}
10	78.44^{b}	69.61^{b}	32^{b}	29^{ab}
20	83.25^{b}	77.81^{b}	33^{bc}	30^{bc}
40	77.28^{b}	79.81^{b}	34^{c}	32^{c}

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

(Hewitt and Smith, 1975; Watson, 1956). The present results of yield increase with increasing N rates is similar to early findings of Chweya (1984) and Splittstoesser and van Mark (1974). Chweya (1984) found that N tended to increase the mean fresh weight and number of leaves and, therefore, total yield per plant of kale. Splittstoesser and van Mark (1974) who had worked with lettuce (Lactuca sativa L.), cabbage, collards and mustard (Brassica juncea L.) also found that leaf fresh weight increased with increasing rates of N.

The effect of N sources on cumulative leaf weight per 10 m^2 and leaf number per plant was only significant ($P \leq 0.05$) for leaf number during second experiment (See Table 3). Though nitrate form is more readily available to the roots than ammonium form (Buckman and Brady, 1968) in the soil, ammonium nitrogen is oxidised to nitrate through nitrification process with course of time (Russel, 1973). May be due to longer availability of ammonium-nitrogen in soil, the resulting leaf yield does not differ from that of plants top-dressed

Table 3: Effect of nitrogen sources on cumulative leaf weight and number of Brassica oleracea var acephala D.C.

	Total leaf weight ($kg/10m^2$)		Total leaf number per plant	
Nitrogen Sources	First experiment	Second experiment	First experiment	Second experiment
CAN	69.90	60.93	32	28[a]
SA	68.50	63.89	32	30[b]

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

with nitrate-nitrogen. Therefore, this may explain why N sources tended to have no significant effect on the two yield parameters. Except for the findings of Gardner and Pew (1979) on lettuce, very little work has been done on the effect of N Sources on 'Thousand'headed' kale and 'Georgia' collards. However, the two workers found that ammonium sulphate, ammonium nitrate, calcium nitrate or urea had no effect on the number of heads harvested in lettuce.

Results in Table 4 show the effect of varieties on cumulative leaf weight per 10 m^2 and leaf number per plant. In both experiments, the two yield parameters were significantly higher ($P \leq 0.05$) for 'Georgia' collards than 'Thousand headed' kale. 'Georgia' collards are faster growing and greener than 'Thousand-headed' kale (Anon, 1975) hence they could be photosynthetically more efficient in CO_2 fixation than 'Thousand'headed' kale. This would result in more photosynthates which are utilised in nitrate reductase synthesis and hence more NO_3 assimilation. This may result in more growth hormones synthesis which increase cell division and hence more photosynthates sinks which may

Table 4: Effect of varieties on cummulative leaf weight and number of *Brassica oleracea* var. *acephala* D.C.

Varieties	Total leaf weight ($kg/10m^2$)		Total leaf number per plant	
	First experiment	Second experiment	First experiment	Second experiment
'Thousand-headed' kale	64.18[a]	60.27[a]	27[a]	23[a]
'Georgia' collards	73.87[b]	70.55[b]	37[b]	35[b]

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

lead to high leaf yields. This could be one of the reasons why 'Georgia' collards yielded more than 'Thousand-headed' kale within the ten weeks period (after transplanting) of the experiement. Varietal differences in leaf yield were also observed by Chahira (1982) and Barker et al. (1974). Chahira (1982) found that collards gave higher leaf weight and number than 'Thousand-headed' and 'Marrow-stem' kales. Barker et al. (1974) working with spinach found that smooth-leafed varieties yielded higher than sayoyed or semi-savoyed varieties. These authors further found that smooth-leafed varieties responded better to N fertilization than savoyed varieties.

Interactions between rates and sources of N on both yielde parameters and between N rates and varieties for cumulative leaf number per plant were not significant. Interaction between N sources and varieties on cumulative leaf weight per 10 m^2 was also not significant. Interactions between N rates and varieties on cumulative leaf weight pr 10 m^2, and between N sources and varieties on cumulative leaf number per plant were significant only during the second experiment. Figure 1 shows that leaf

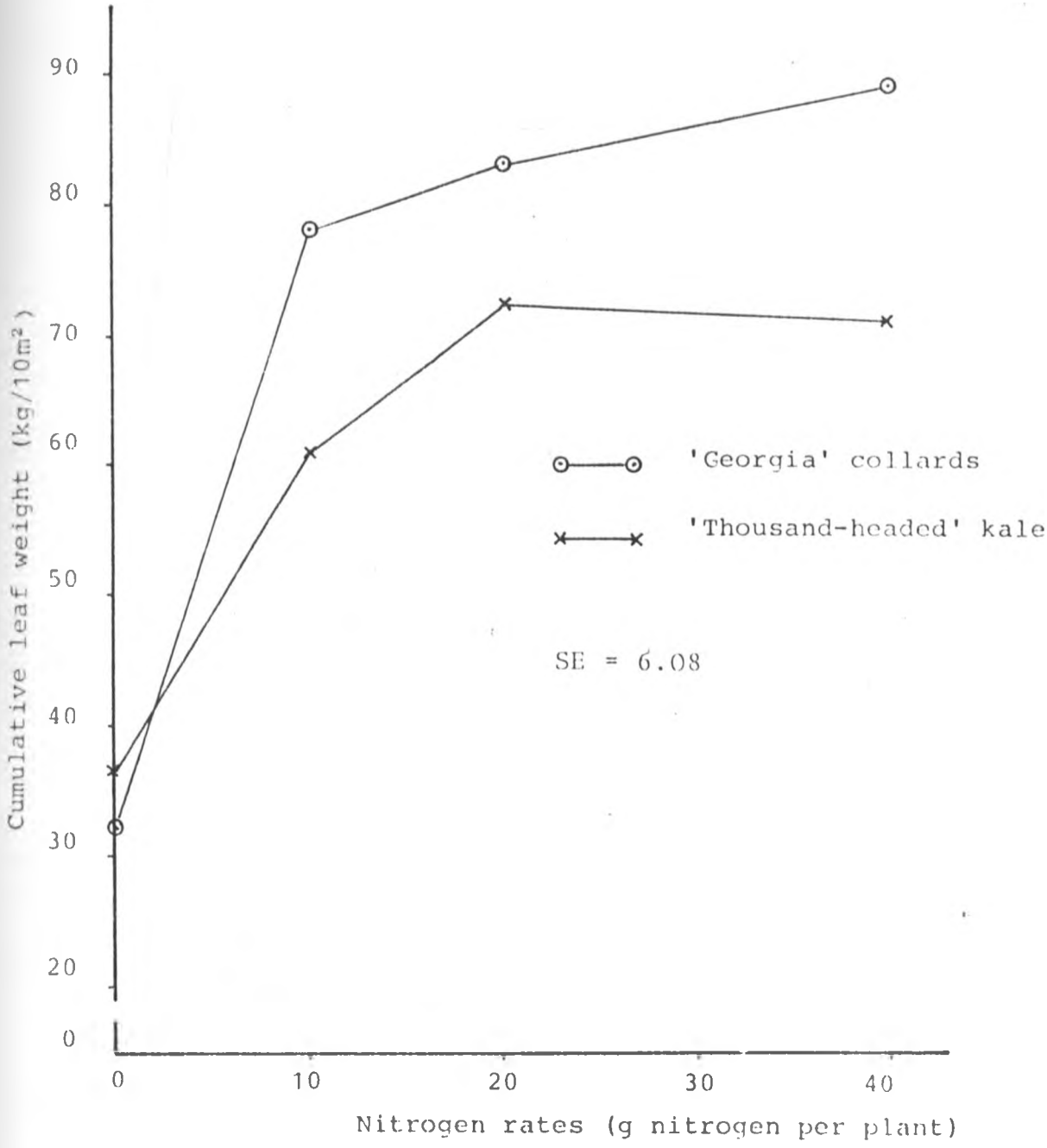


Figure 1: Interaction between nitrogen rates and varieties on cumulative leaf weight (kg/10m²) of Brassica oleracea var acephala D.C. - Second experiment

weight for both varieties increased with increasing N rates. Increase in leaf weight with increasing N rates appeared to be higher for 'Georgia' collards than for 'Thousand-headed' kale. 'Georgia' collards are faster growers than 'Thousand-headed' kale (Anon., 1975) and that can be the reason why they gave more yields than 'Thousand-headed' kale as N rates increased within the ten weeks period (after transplanting) of the experiment. Figure 2 shows that 'Georgia' collards responded more in cumulative leaf number per plant when top-dressed with SA than when top-dressed with CAN whereas 'Thousand-headed' kale did not respond more when top-dressed with SA than when top-dressed with CAN. Interaction between N rates, sources and varieties were only significant on cumulative leaf number per plant in the second experiment (See Table 5). As N rates increased, there was a tremendous increase in cumulative leaf number per plant for 'Georgia' collards top-dressed with CAN. Perhaps this was so because effects of N rates and variety also increased the cumulative leaf number per plant.

4.2 Effects of Rates and Sources of Nitrogen, Varieties on Nitrate Accumulation in Leaves.

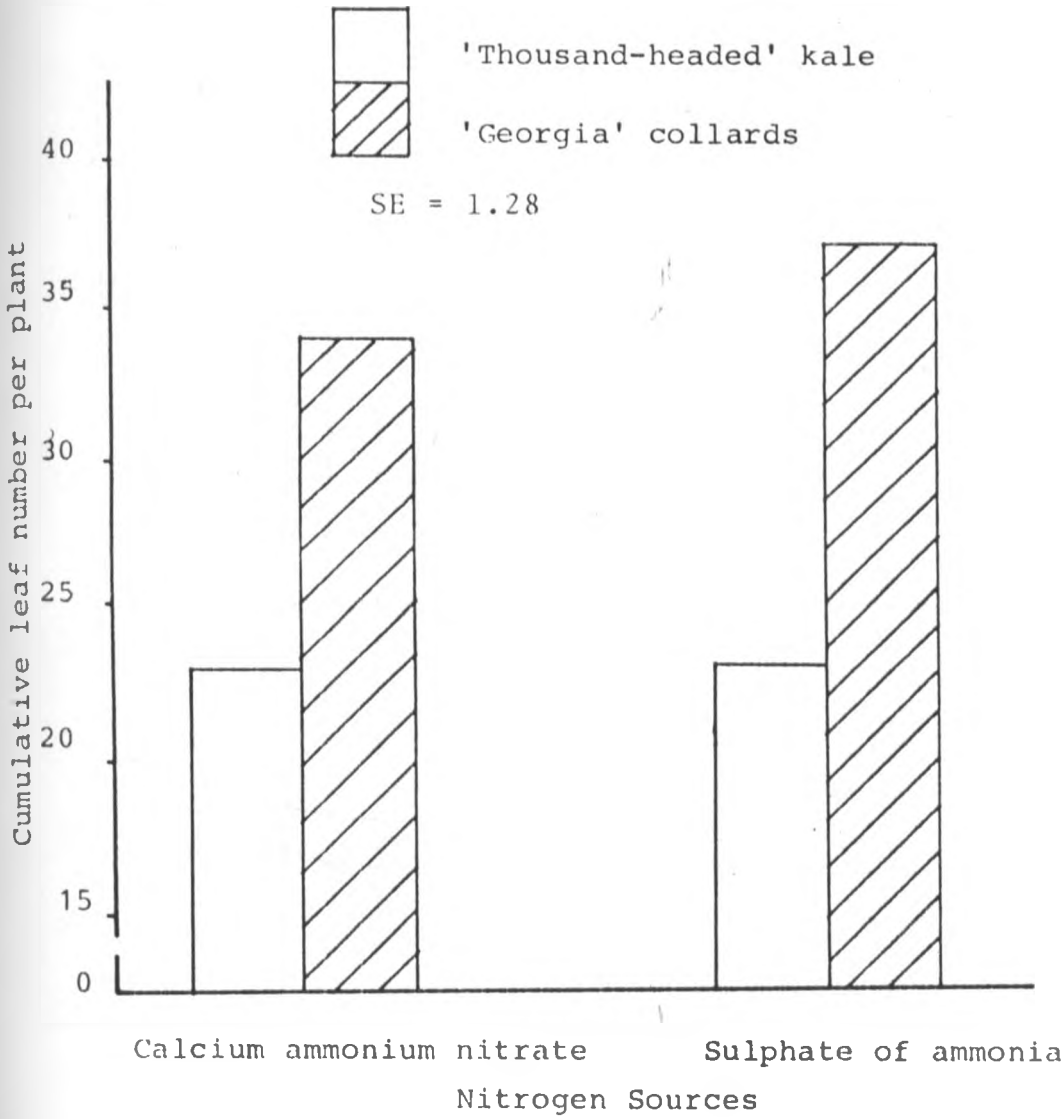


Figure 2: Interaction between nitrogen sources and varieties on cumulative leaf number per plant of Brassica oleracea var acephala D.C. - Second experiment

Table 5: Effect of nitrogen rates, sources and varieties on cumulative leaf number per plant of Brassica oleracea var. acephala D.C. - Second experiment.

Nitrogen Sources / Varieties	Total leaf number per plant — Nitrogen rates (g nitrogen per plant)								Varieties means
	0		10		20		40		
	CAN	SA	CAN	SA	CAN	SA	CAN	SA	
'Thousand-headed' kale	21	21	24	23	23	23	25	26	23
'Georgia' collards	30	35	34	34	33	40	38	40	35
Nitrogen sources means	25	28	29	33	28	31	31	33	
Nitrogen rates means	27		29		30		32		

S.E. = 1.38

Effect of N rates on NO_3-N accumulation in leaves, petioles and laminae was significant ($P \leq 0.05$). Table 6 shows that NO_3-N accumulation in leaves, petioles and laminae increased with increasing rates of N. In both experiments, leaves from plants that were not top-dressed with N had significantly lower NO_3-N accumulation than those top-dressed. The differences in NO_3-N accumulation between leaves from plants top-dressed with 10 g N and those top-dressed with 40 g N per plant were significant. Whereas in the first experiment top-dressing with more than 20 g N per plant had no significant effect on NO_3-N accumulation in leaves, it had significant effect in the second experiment. In the second experiment, differences between all N rates on NO_3-N accumulation in leaves were significantly different from each other.

The accumulation of nitrate in plant tissues is always in a dynamic state since it represents the difference between rates of absorption and rates of assimilation within the plant. At high rates of N may be more nitrates are taken up by plants than can be assimilated and hence resulting in NO_3-N accumulation

Table 6: Effect of nitrogen rates on nitrate-nitrogen accumulation in leaves of *Brassica oleracea* var. *acephala* D.C.

	% Nitrate-nitrogen											
	First experiment						Second Experiment					
	First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)			First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)		
Nitrogen rates (gN per plant)	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
0	0.69^{a}	0.91^{a}	0.37^{a}	0.47^{a}	0.62^{a}	0.32^{a}	0.70^{a}	1.01^{a}	0.40^{a}	0.57^{a}	0.96^{a}	0.18^{a}
10	2.14^{b}	3.33^{b}	0.94^{a}	2.15^{b}	3.59^{b}	0.75^{b}	1.66^{b}	2.78^{b}	0.54^{ab}	1.37^{b}	2.40^{b}	0.34^{a}
20	2.70^{bc}	4.26^{b}	1.09^{b}	2.61^{c}	4.15^{b}	1.06^{c}	2.10^{c}	3.51^{c}	0.70^{b}	1.83^{c}	3.09^{c}	0.56^{ab}
40	2.86^{c}	3.95^{b}	1.77^{b}	3.04^{c}	4.70^{b}	1.21^{c}	2.71^{d}	4.31^{d}	1.11^{c}	2.47^{d}	4.14^{d}	0.80^{b}

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

as the rates of N increases. Probably at high N rates the NO_3 level in leaves was very high and may be not all could be reduced by nitrate reductase. The increase of NO_3-N accumulation in 'Thousand-headed' kale and 'Georgia' collard leaves with respect to rates of N increase is a case very common with various vegetables (Pimpinini et al., 1973; Heinze, 1974; Schuphan, 1971; Splittstoesser and van Mark, 1974; Aworh et al., 1980; Chweya, 1986). Pimpini et al. (1973) found that increasing rates of N caused an increase in NO_3-N accumulation in spinach, redbeet, cabbage and cauliflower. Chweya (1986) working with 'Thousand-headed' kale and collards, and Splittstoesser and van Mark (1974) working with several vegetables including collards found that increasing rates of N increased NO_3-N accumulation.

As shown in Table 6, NO_3-N accumulation in petioles and laminae as N rates increased had a similar pattern as in the leaves. Petioles had higher NO_3-N accumulation than laminae and leaves, however, laminae had lower NO_3-N accumulation than leaves. Perhaps there is less nitrate assimilation in petioles than in laminae and this, therefore, could account for the higher NO_3-N accumulation in petioles

than in laminae. The present results of petioles having higher NO_3-N accumulation than laminae is supported by early findings of Chweya (1987) and Maynard et al. (1976). Chweya (1987) working with 'Thousand-headed' kale and collards found that petioles accumulated more NO_3-N than laminae.

Nitrate in high concentrations may cause a great economic loss through illness or death of human and animals. Nitrate toxicity is relatively low and varies widely. The fatal adult dose in humans is in the order of 15 to 70 milligrams of NO_3-N per kilogram of body weight (Lee, 1970). However, an adult of about 70 kilograms has to consume about 0.5 - 2.7 kilograms of leaves at ago from plants top-dressed with about 10 g N per plant to ingest the fatal dose. Since an adult rarely consumes this amount of leaves at ago, ingestion of the toxic dosage is unlikely.

Effect of N sources on NO_3-N accumulation in leaves, petioles and laminae was only significant ($P \leq 0.05$) during the first harvest of second experiment, and for laminae during the first harvest of first experiment. However, it was observed that in both experiments the leaves from plants top-dressed with CAN tended to have consistently higher NO_3-N accumulation than those top-dressed with SA (See Table 7).

Table 7: Effect of nitrogen sources on nitrate-nitrogen accumulation in leaves of Brassica oleracea var. acephala D.C.

	% Nitrate-nitrogen											
	First Experiment						Second Experiment					
	First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)			First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)		
Nitrogen sources	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
C A N	2.26	3.27	1.23[a]	2.17	3.43	0.93	2.16[a]	3.45[a]	0.87[a]	1.63	2.78	0.47
S A	1.93	2.96	0.86[b]	1.92	3.10	0.74	1.43[b]	2.35[b]	0.51[b]	1.49	2.51	0.48

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

The observation may be explained by the fact that when the two nitrogen sources are supplied to plants, the nitrate form is readily available whereas ammonium form is oxidised to nitrate through nitrification process within a certain period (Russel, 1973). However, both nitrate and ammonium ions are absorbed by plants. Nitrate nitrogen is absorbed and translocated as nitrate ions whereas for ammonium nitrogen, the ions are assimilated in the root tissue and translocated as amino acids (Reinsenaur, 1978; Salisbury and Ross,1978). This shows therefore that nitrate accumulation in leaves of 'Thousand-headed' kale and 'Georgia' collard can be influenced to a great extent by the sources of N applied.

The difference in NO_3-N accumulation in leaves with respect to N sources (that is, nitrate and ammoniacal) is a case very common with various vegetables (Pimpini et al., 1973, Gardner and Pew, 1979; Lorenz, 1978). Pimpini et al. (1973) found that ammonium nitrate caused a much higher nitrate accumulation than ammonium sulphate in spinach, redbeet, cabbage and cauliflower. Gardner and Pew (1979) found that NO_3-N accumulation in midribs of wrapper leaves of lettuce was higher for plants fertilized with calcium nitrate than those fertilized

with ammonium sulphate.

Petioles and laminae from leaves of plants top-dressed with CAN had higher NO_3-N accumulation than those from plants top-dressed with SA (See Table 7).

Table 8 shows that NO_3-N accumulation in leaves petioles and laminae of 'Thousand-headed' kale was higher than those of 'Georgia' collards. Except during the second harvest of second experiment, leaves and petioles of 'Thousand-headed' kale had significantly ($P \leq 0.05$) higher NO_3-N accumulation than those of 'Georgia' collards. Effect of variety on NO_3-N accumulation in laminae appeared not to be significant. 'Georgia' collards are more improved than 'Thousand-headed' kale in terms of selection towards fast growth, early maturity and high yield (Anon, 1975). Due to fast growth, 'Georgia' collards could be having a higher nitrate reductase activity (which reduces nitrates) than 'Thousand-headed' kale, which may lead to their better nitrate assimilation than 'Thousand-headed' kale.

From these findings, 'Georgia' collards seems to be a better variety in terms of nitrate assimilation than 'Thousand-headed' kale because

Table 8: Effect of varieties on nitrate-nitrogen accumulation in leaves of Brassica oleracea var. acephala D.C.

Varieties	% Nitrate-nitrogen											
	First Experiment						Second Experiment					
	First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)			First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)		
	leaf	Petiole	Lamina	leaf	Petiole	lamina	leaf	Petiole	lamina	leaf	Petiole	lamina
'Thousand-headed' kale	2.50[a]	3.70[a]	1.24[a]	2.24[a]	3.68[a]	0.81	1.94[a]	3.20[a]	0.68	1.54	2.67	0.40
'Georgia' collards	1.69[b]	2.52[b]	0.85[b]	1.86[b]	2.85[b]	0.86	1.64[b]	2.59[b]	0.69	1.59	2.63	0.54

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

'Georgia' collards accumulate less nitrate ions than 'Thousand-headed' kale. Results from this study are in line with the findings of Chweya (1986) that leaves of 'Thousand-headed' kale had higher NO_3-N accumulation than those of collards. Maynard and Barker (1974) and Barker et al. (1974) also observed that savoyed-leaf cultivars of spinach consistently had higher nitrate accumulation than smooth-leafed cultivars.

Interaction between N rates and sources on NO_3-N accumulation in leaves was not significant except during the first harvest of second experiment only. Therefore, interaction between N rates sources for NO_3-N accumulation appears not to be significant. As N rate increased, plants top-dressed with CAN responded more to NO_3-N accumulation in leaves than those top-dressed with SA (See Figure 3). May be this was so because rates and sources of N had an effect on NO_3-N accumulation in leaves. Interactions between N rates and varieties, N sources and varieties, and between N rates, sources and varieties on NO_3-N accumulation in leaves were not significant.

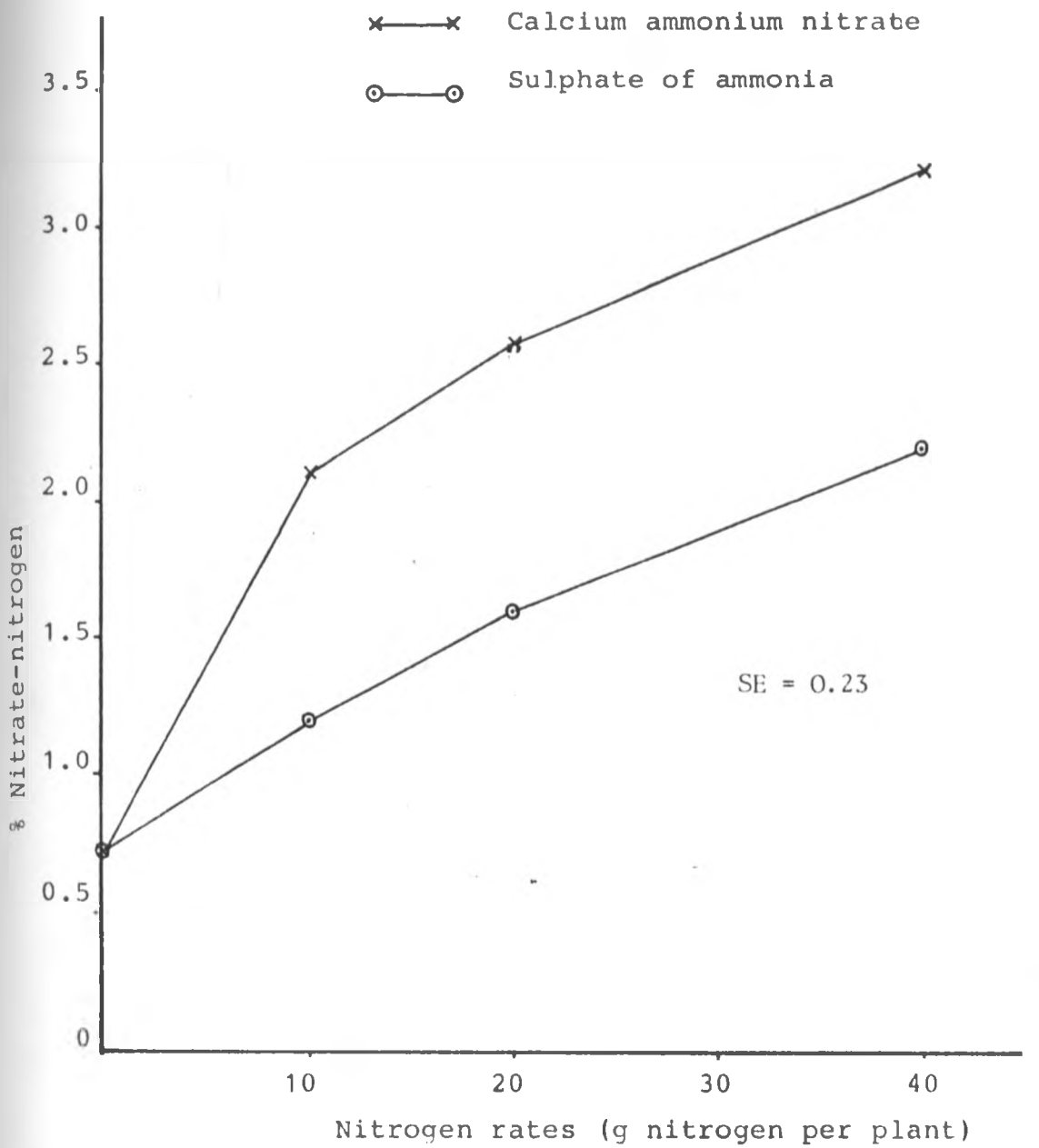


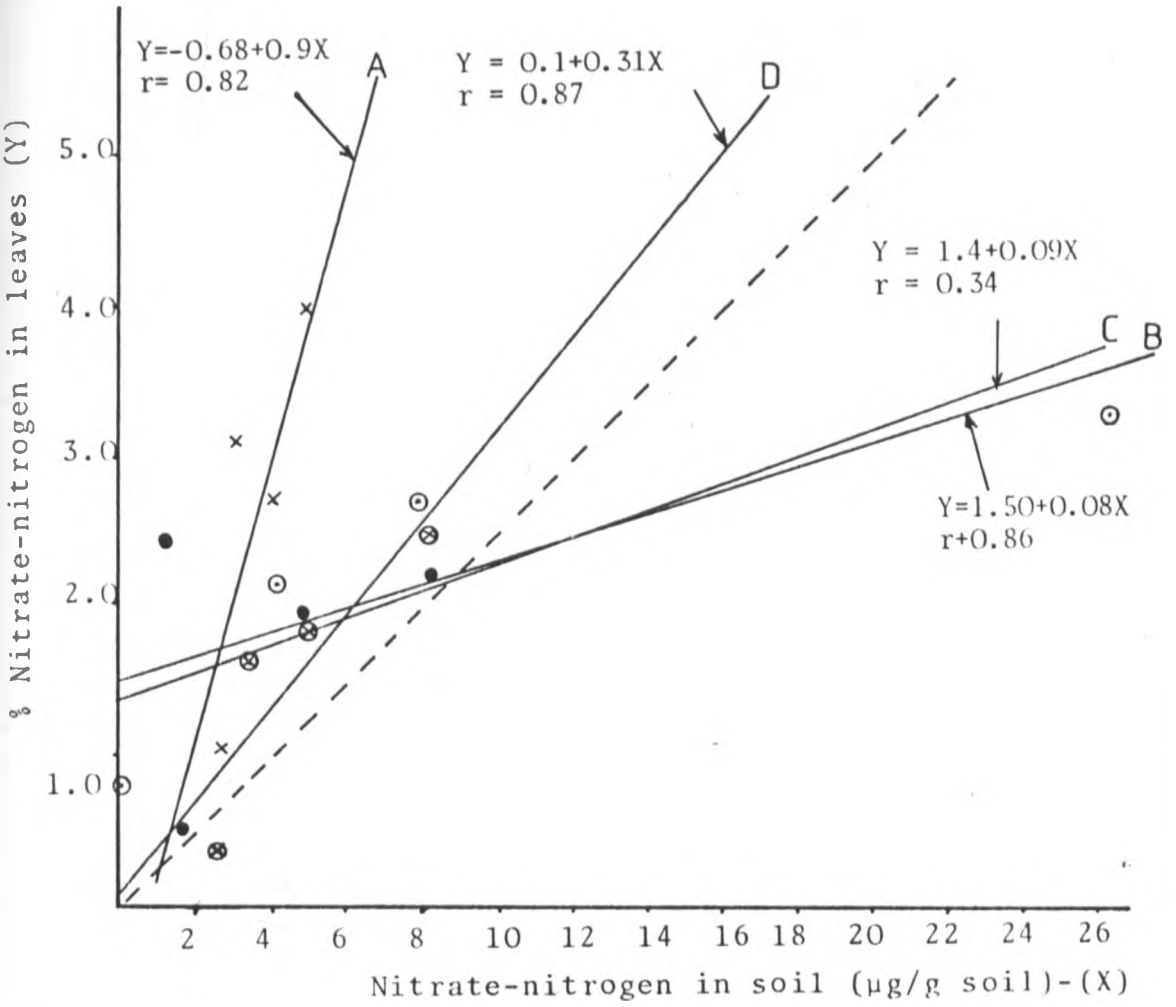
Figure 3: Interaction between rates and sources of nitrogen on nitrate - nitrogen accumulation in leaves of Brassica oleracea var acephala D.C. - first harvest of second experiment

4.2.1 Nitrate-nitrogen Accumulation in Leaves as Related to Nitrate-nitrogen Levels in Soil.

There was a positive correlation of NO_3-N levels in soil and NO_3-N accumulation in leaves of 'Thousand-headed' kale and 'Georgia'collard at harvest time (See Figure 4). The strongly positive correlation coefficients (r) were in the range of 0.76 - 0.96 whereas the weakly positive correlation coefficients were in the range of 0.06 - 0.39. Appendix 4 shows the correlation coefficients (r) and NO_3-N accumulation in leaves of 'Thousand-headed' kale and 'Georgia' collard as related to NO_3-N levels in soil. No NO_3-N was traced in the soil during the second hervest of second experiment. The positive correlation between NO_3-N levels in soil and NO_3-N accumulation in leaves, agreed with the above observation that leaves from plants top-dressed with CAN (which contains nitrate-nitrogen) accumulated increasing NO_3-N as N rates increased (See Figure 3).

4.3 Effect of Rates and Sources of Nitrogen, and Varieties on Thiocyanate ion content in Leaves.

Nitrogen rates had no significant effect on



r = Correlation coefficient

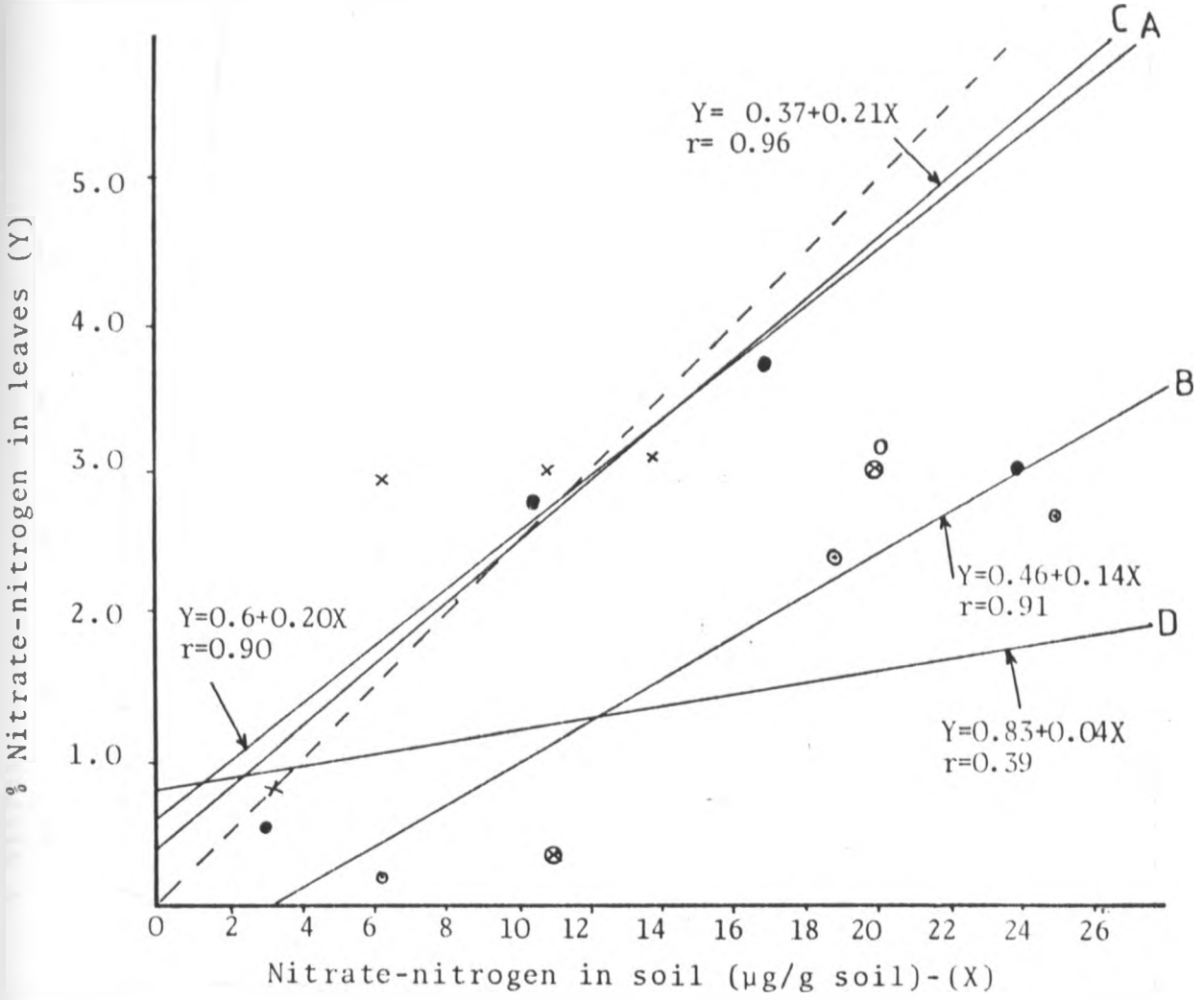
x A = 'Thousand-headed' kale top-dressed with CAN

o B = 'Thousand-headed' kale top-dressed with SA

● C = 'Georgia' collard top-dressed with CAN

⊗ D = 'Georgia' collard top-dressed with SA

Fig 4a: Correlation between nitrate-nitrogen in soil and nitrate-nitrogen in leaves of Brassica oleracea var acephala D.C. - First harvest of first experiment.



r =Correlation coefficient

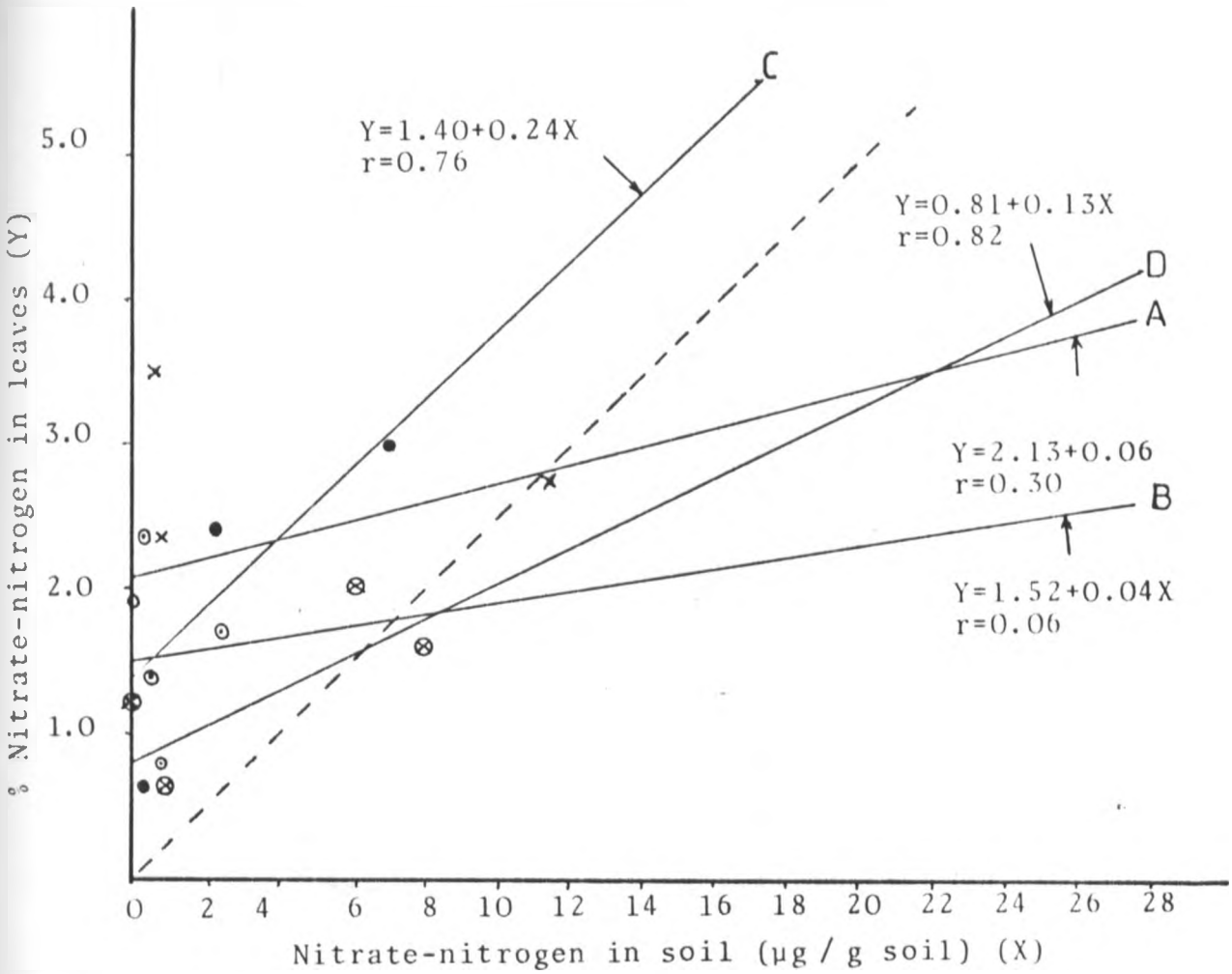
× A ='Thousand-headed' kale top-dressed with CAN

○ B ='Thousand-headed' kale top-dressed with SA

● C ='Georgia' collard top-dressed with CAN

⊗ D ='Georgia' collard top-dressed with SA

Fig. 4b: Correlation between nitrate-nitrogen in soil and nitrate-nitrogen in leaves of Brassica oleracea var acephala D.C. (Second harvest of first experiment).



r = Correlation coefficient

× A = 'Thousand-headed' kale top dressed with CAN

○ B = 'Thousand-headed' kale top dressed with SA

● C = 'Georgia' collard top-dressed with CAN

⊗ D = 'Georgia' collard top-dressed with SA

Figure 4c: Correlation between nitrate-nitrogen in soil and nitrate-nitrogen in leaves of Brassica oleracea var acephala D.C. (First harvest of second experiment).

SCN^- content in leaves. However, the effect of N sources on SCN^- content in leaves was significant ($P \leq 0.05$). Table 9 shows that leaves, petioles and laminae from plants top-dressed with SA (which contains 21% sulphur) had significantly higher SCN^- content than those top-dressed with CAN. This indicates that sulphur has a big role to play in SCN^- content in plant tissues. Sulphur is a major component of glucosinolates (Mengel and Kirkby, 1979; Carlson et al., 1985; Josefsson, 1970). This explains why leaves from plants top-dressed with SA had higher SCN^- content than those from plants top-dressed with CAN. The present results with leaves from plants top-dressed with sulphur containing fertilizer (SA) yielding more SCN^- than those from plants top-dressed with CAN, is supported by Josefsson (1970) who found that rapeseed plants supplied with sulphur containing fertilizer synthesized more glucosinolates than those not supplied with sulphur.

Except during first harvest of second experiment, petioles yielded more SCN^- than leaves and laminae, and the laminae yielded less SCN^- than leaves (See Table 9). These results were contrary

Table 9: Effect of nitrogen sources on thiocyanate ion content in leaves of *Brassica oleracea* var. *acephala* D.C.

	KSCN ppm											
	First Experiment						Second Experiment					
	First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)			First harvest (six weeks after transplanting)			Second harvest (eight weeks after tansplanting)		
Nitrogen sources	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
C A N	3087[a]	4566[a]	1608[a]	2973[a]	4359[a]	1586[a]	350[a]	337[a]	362[a]	292[a]	306[a]	277[a]
S A	5289[b]	7272[b]	3305[b]	6401[b]	9336[b]	3470[b]	710[b]	710[b]	710[b]	669[b]	678[b]	659[b]

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

to the findings of Johnston and Jones (1966) who working with kales found that laminae yielded higher SCN^- than petioles. In the first harvest of second experiment, laminae had higher SCN^- than petioles, this agreed with findings of Johnston and Jones (1966). Sulphur, which is a major component of glucosinolates, is metabolised in the leaves, entirely in the chloroplasts (Salisbury and Ross, 1978). Since laminae has more chloroplasts than petioles, more glucosinolates (precursors of SCN^-) are expected to be synthesized in laminae than petioles.

Table 10 shows the effect of varieties on SCN^- content in leaves, petioles and laminae. Leaves, petioles and laminae of 'Thousand-headed' kale yielded significantly ($P \leq 0.05$) higher SCN^- than those of 'Georgia' collards. Leaves of 'Georgia' collard may have been physiologically more mature than those of 'Thousand-headed' kale because the former had started bolting six weeks after transplanting whereas the latter had not started even by the end of the experiment. Another reason could be that young leaves are photosynthetically more efficient than old ones. This could provide more glucose which could be used in glucosinolates synthesis

Table 10: Effect of varieties on thiocynate ion content in leaves of *Brassica oleracea* var. *acephala* D.C.

	KSCN ppm											
	First Experiment						Second Experiment					
	First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)			First harvest (six weeks after transplanting)			Second harvest (eight weeks after transplanting)		
Varieties	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina	Leaf	Petiole	Lamina
'Thousand-headed' kale	4747[a]	6264[a]	3230[a]	6229[a]	9310[a]	3140[a]	677[a]	632[a]	722[a]	554[a]	559[a]	549[a]
'Georgia' collards	3629[b]	5774[b]	1683[b]	3145[b]	4385[b]	1912[b]	350[b]	347[b]	350[b]	406[b]	424[b]	387[b]

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

in young leaves than in old leaves. Late maturing cabbage cultivars yielded more SCN^- than early maturing ones (Bible et al., 1980). 'Georgia' Collard being a faster maturing variety than 'Thousand-headed' kale appears to be a good variety for consumption as it yields less SCN^-. Differences in SCN^- content in leaves with respect to variety is common with various vegetables (Johnston and Jones, 1966; Paxman and Hill, 1974a; Gramberg et al., 1986; Carlson et al., 1987a, 1987b). Johnston and Jones (1966) who worked with six varieties of kale, showed important inter-varietal differences in their SCN^- content. Paxman and Hill (1974a), working with rapeseed, 'Thousand-headed' and 'Marrow-stem' kales, found that rapeseed contained less than half the amount of thiocyanates found in 'Thou-' sand-headed' and 'Marrow-stem' kales.

Figure 5 shows the interactions between rates and sources of N on SCN^- content in leaves which was significant. SCN^- content in leaves of of plants top-dressed with SA increased with increasing rates of N (hence sulphur increased) whereas it decreased for those from plants top-dressed with CAN. Results of this study are supported by Josefsson (1970)

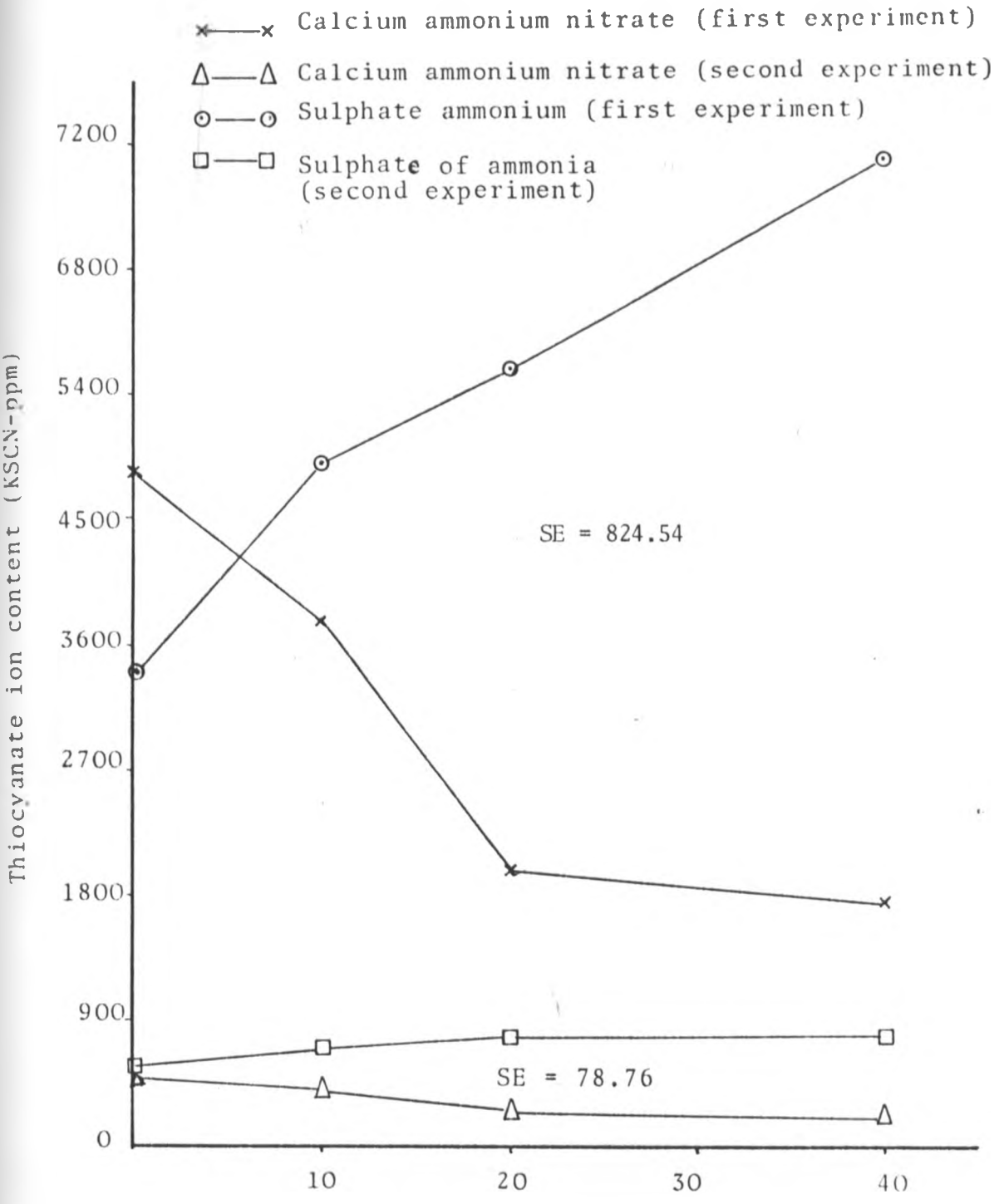


Fig. 5a: Interaction between rates and sources of nitrogen for thiocyanate ion content in leaves of Brassica oleracea var. acephala D.C. - First harvests of first and second experiments (six weeks after transplanting).

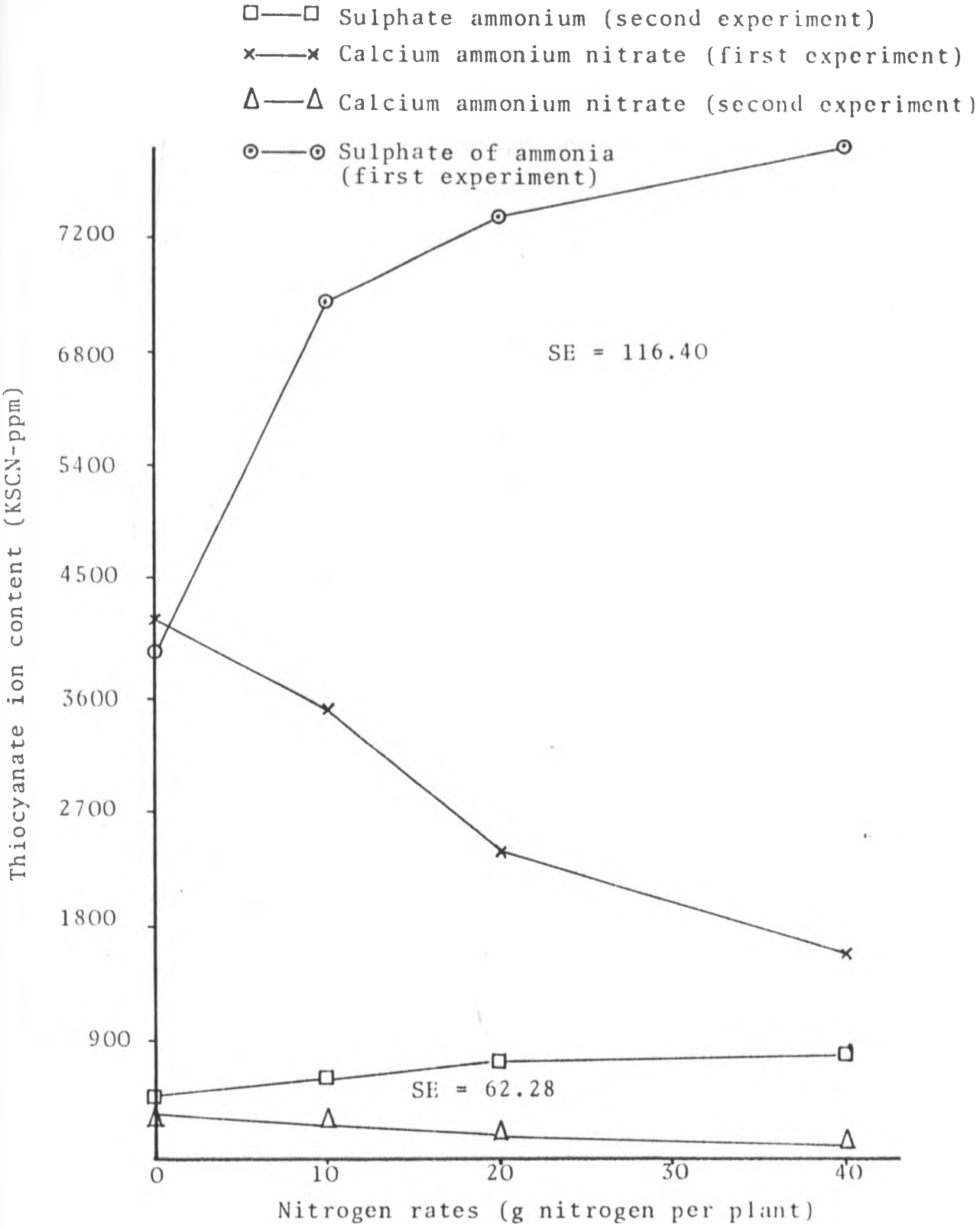


Fig. 5b: Interaction between rates and sources of nitrogen for thiocyanate ion content in leaves of Brassica oleracea var. acephala D.C. - Second harvests of first and second experiments (eight weeks after transplanting).

who found a decrease in glucosinolate when ammonium-nitrate nitrogen was increased and an increase when sulphur nutrition was increased in rapeseed. Interaction between N sources and varieties on SCN^- content in leaves was significant only during the second harvests of both experiments. Figure 6 shows that the difference between 'Thousand-headed' kale and 'Georgia' collard in SCN^- content in leaves was higher when they were top-dressed with SA than when top-dressed with CAN. This happened probably because SA increases SCN^- content in leaves whereas CAN decreases it in both varieties. The pattern shown in Figure 6 during second harvest of first experiment was similar to that of second harvest of second experiment. Interactions between N rates and varieties, and between N rates, sources and varieties on SCN^- content in leaves were not significant.

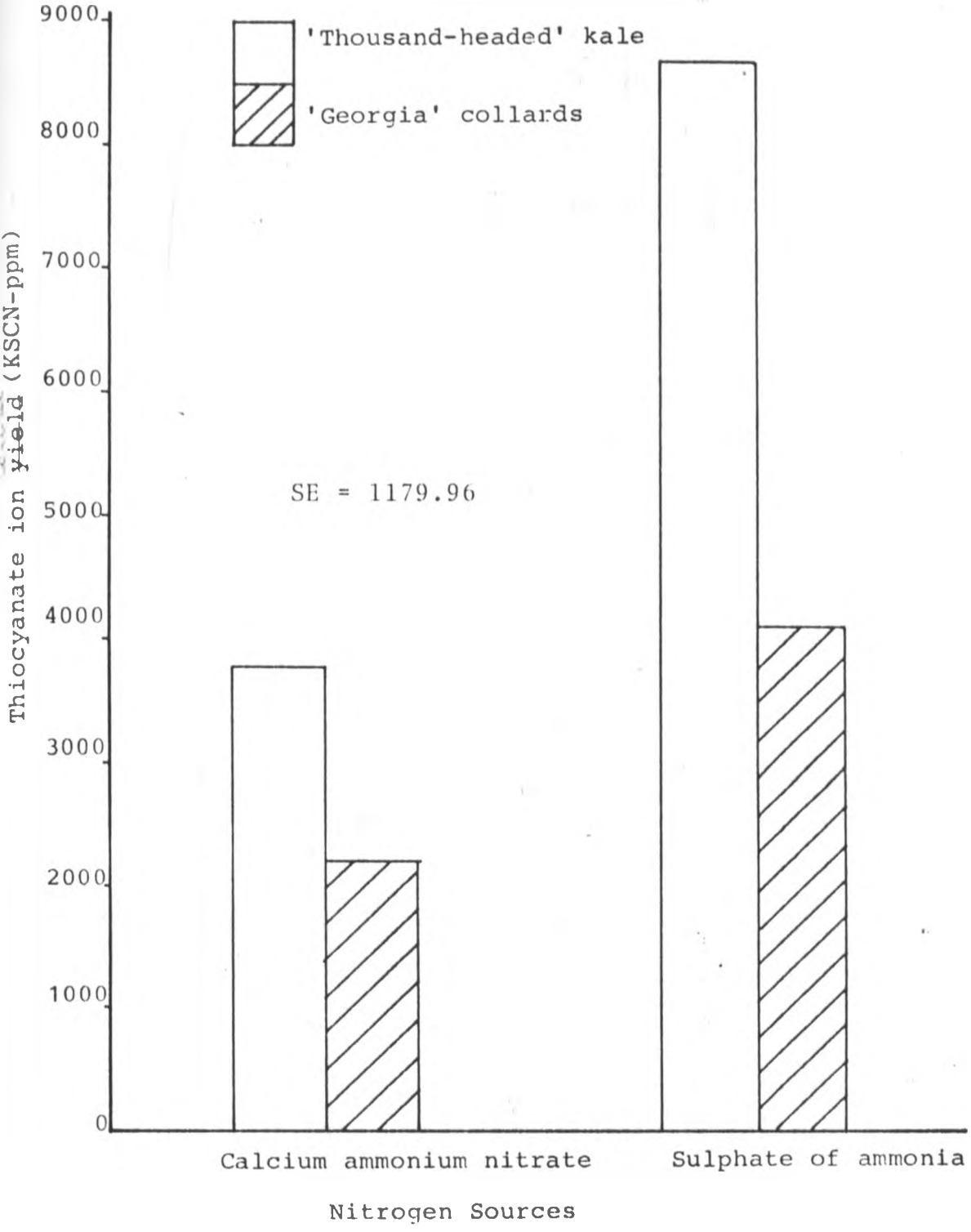


Figure 6: Interaction between nitrogen sources and varieties on thiocyanate ion content in leaves of Brassica oleracea var acephala D.C. - second harvest of first experiment

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH WORK.

5.1 Conclusions

From the results of these experiments and the ensuing discussion, the following conclusions can be made.

Increasing rates of nitrogen, increased cumulative leaf weight per 10 m^2 and leaf number per plant. However, plants top-dressed with higher rates of nitrogen than 10 g per plant did not show significant increase in leaf weight. Whereas plant top-dressed with 40 g nitrogen per plant yielded significantly more leaves than those top-dressed with 10 g , they did not yield significantly more than those top-dressed with 20 g. I would recommend, Kenyan markets where 'Thousand-headed' kale and 'Georgia' collard are valued or sold in terms of both weight and number, it would not be economical to farmers from areas with soils and climate similar to Kabete to apply nitrogen rates more than 10 g per plant. Nitrogen sources seemed to have no significant

effect on cumulative leaf weight and number. However, 'Georgia' collard gave significantly higher cumulative leaf weight and number than 'Thousand-headed' kale. Therefore, it appears that 'Georgia' collard could be a better variety in terms of leaf yield than 'Thousand-headed' kale which (collard) I would recommend farmers to grow.

Whereas cumulative leaf weight from plants top-dressed with increasing nitrogen rates from 10 to 40 g per plant did not increase significantly, NO_3-N accumulation in leaves tended to increase significantly. Therefore, top-dressing 'Thousand-headed' kale and 'Georgia' collard with low rates of nitrogen, in areas with similar soils and climate like Kabete, would give reasonable yield and at the same time help to reduce NO_3-N accumulation in Leaves and hence reduced NO_3-N accumulation in leaves and hence reduced NO_3-N intake. Nitrogen sources had an influence on NO_3-N accumulation. Leaves from plants top-dressed with CAN accumulated consistently more NO_3-N than those top-dressed with SA. Therefore, top-dressing kales and collards with CAN would help to reduce NO_3 accumulation in leaves. Leaves of 'Georgia' collard accumulated significantly lower NO_3-N than 'Thousand-headed' kale. Collard appear to be a better variety than kale in terms of NO_3

accumulation. Petioles accumulated more NO_3-N than laminae, hence removal of petioles from leaves prior to processing or preparation would reduce NO_3-N intake.

Nitrogen rates were found to have no significant effect on SCN^- content in leaves. However, effect of nitrogen sources was significant. Leaves from plants top-dressed with SA (which contains 21% sulphur) yielded significantly higher SCN^- than those top-dressed with CAN. Therefore, the use of nitrogen fertilizers without sulphur can be useful in reducing glucosinolates in Brassica vegetables and hence reduced SCN^- content. Significant inter-varietal differences in SCN^- content in leaves were found with 'Thousand-headed' kale yielding more than 'Georgia'. 'Georgia' collard appeared to be better than 'Thousand-headed' kale in that their leaves yielded less SCN^- than those of 'Thousand-headed' kale.

Therefore at low nitrogen rates, regardless of nitrogen sources, 'Georgia' collard gave reasonable cumulative leaf weight and number, and low NO_3-N accumulation and SCN^- yield from leaves. On the whole, 'Georgia' collard appears to be a better variety than 'Thousand-headed' kale for farmers to grow.

5.2 Suggestions for Further Research Work.

From this study, top-dressing Brassica oleracea var. acephala D.C. with more than 10 g nitrogen per plant did not give significant increase in cumulative leaf weight whereas NO_3-N accumulation increased significantly. Farmers in areas with similar soils and climate like Kabete need not apply nitrogen rates beyond 10 g per plant for it would not be economical. From this study, it was not clear how leaf yield and NO_3-N accumulation would change between nitrogen rates of 0 - 555 kilograms per hectare (0 - 10 g nitrogen per plant at the spacing of 60 x 30 cm). Therefore, more investigation on the effect of nitrogen rates between 0 - 10 g per plant on leaf yield and NO_3-N accumulation should be done.

Leaves from plants top-dressed with CAN accumulated more NO_3-N than from plants top-dressed with SA. Leaves from plants top-dressed with SA yielded more SCN^- than those top-dressed with CAN. Therefore, there is need to investigate on the effect of more nitrogen sources on NO_3-N accumulation and SCN^- yield from leaves. The nitrogen sources should include ammoniacal fertilizers without nitrate and sulphur.

'Georgia' collard gave more leaf yields, less NO_3-N accumulation and less SCN^- content in leaves than 'Thousand-headed' kale. Therefore, more varieties in the country like 'marrow-stem' and 'Dwarf Siberian' kales should be tested to see which variety accumulate less NO_3-N and yield less SCN^- and at the same time give good leaf yield.

'Thousand-headed' kale and 'Georgia' collard leaves are consumed by livestock when raw and by humans when cooked. It would be therefore interesting to investigate the effect of cooking on NO_3-N concentration in leaves.

However, to get conclusive results, the cases suggested should be carried out in various ecological zones of Kenya and over a number of seasons.

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

MONTH	Mean Radiation MJM^{-2}	Mean Sunshine hrs/day	Temperature ^{o}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
Sept.	20.02	5.7	23.2	11.1	17.2	4.3	4.2	63	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-Jan	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: Some soil chemical characteristics before planting.

Nutrient/ Soil reaction	First Experiment	Second Experiment
pH water	6.6. (0.26)	5.9 (0.21)
CEC (me/100g soil)	29.1 (3.54)	24.2 (2.52)
P (ug/100 soil)	27.60 (17.78)	7.78 (3.60)
Total N (ug/g soil)	3072 (218)	2921 (369)
% Carbon	2.64 (0.19)	1.78 (0.61)

( ) Standard deviations

Appendix 3.1: ANOVA for cumulative leaf weight per 10 m^2 and leaf number per plant

Source of variation	degrees of freedom	Mean sum of squares			
		A	B	C	D
Blocks	2	683.25*	123.75$^{n.s.}$	4.16$^{n.s.}$	3.8$^{n.s.}$
Rates	3	5,501.91**	5,344.3**	46.86**	63.03*
Mainplot error	6	68.68	156.6	1.39	8.18
Variety	1	1,125.20**	1,269.3**	1,333.50**	1,788.5**
Source	1	11.21$^{n.s.}$	110.9$^{n.s.}$	2.54$^{n.s.}$	38.5*
Variety X Source	1	268.86$^{n.s.}$	23.3$^{n.s.}$	11.04$^{n.s.}$	38.6*
Rates X Variety	3	7.61$^{n.s.}$	313.3*	2.07$^{n.s.}$	6.37$^{n.s.}$
Rates X Source	3	16.77$^{n.s.}$	86.8$^{n.s.}$	1.18$^{n.s.}$	6.8$^{n.s.}$
Rates X variety X Source	3	9.45$^{n.s.}$	110.4$^{n.s.}$	0.69$^{n.s.}$	70.03**
Subplot error	24	69.49	95.6	3.06	4.94
Total	47				

A - Firstexperiment (Leaf weight). B - Second experiment (Leaf weight).

C - First experiment (leaf number). D - Second experiment (Leaf number).

n.s. - not significant.

* - Significant at 5% probability level.

** - Significant at 1% probability level.

Appeindix 3.2: ANOVA for NO_3 accumulation (% NO_3-N) in leaves.

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	$0.47^{n.s.}$	$0.20^{n.s.}$	$0.19^{n.s.}$	$0.54^{n.s.}$
Rates	3	11.74^{**}	14.52^{**}	8.57^{**}	7.69^{**}
Mainplot error	6	0.32	0.63	0.11	0.18
Variety	1	7.98^{**}	1.74^{**}	1.07^{*}	$0.03^{n.s.}$
Source	1	$1.28^{n.s.}$	$0.78^{n.s.}$	6.39^{**}	$0.21^{n.s.}$
Variety X Source	1	$0.26^{n.s.}$	$0.005^{n.s.}$	$0.03^{n.s.}$	$0.006^{n.s.}$
Rates X Variety	3	$0.26^{n.s.}$	$0.14^{n.s.}$	$0.05^{n.s.}$	$0.02^{n.s.}$
Rates X Source	3	$0.06^{n.s.}$	$0.19^{n.s.}$	0.74^{*}	$0.18^{n.s.}$
Rates X Variety X Source	3	$0.68^{n.s.}$	$0.10^{n.s.}$	$0.01^{n.s.}$	$0.05^{n.s.}$
Subplot error	24	0.44	$0.20^{n.s.}$	0.17	0.33
Total	47				

A - First harvest of first experiment. B - Second harves of first experiment.

C - First harvest of second experiment. D - Second harvest of second experiment.

n.s. - not significant,

*- Significant at 5% probability level. ** - Significant at 1% probability level.

Appendix 3.3: ANOVA for NO_3 accumulation (% NO_3-N) in petioles.

Source of Variations	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	$3.43^{n.s.}$	$0.38^{n.s.}$	$0.26^{n.s.}$	3.10^{*}
Rates	3	27.69^{**}	39.78^{**}	23.83^{**}	21.79^{**}
Mainplot error	6	2.19	1.83	0.33	0.37
Variety	1	16.77^{**}	8.30^{**}	4.49^{**}	$0.02^{n.s.}$
Source	1	$1.16^{n.s.}$	$1.36^{n.s.}$	14.40^{**}	$0.88^{n.s.}$
Variety X Source	1	$0.13^{n.s.}$	$0.21^{n.s.}$	$0.47^{n.s.}$	$0.05^{n.s.}$
Rates X Variety	3	$1.05^{n.s.}$	$0.59^{n.s.}$	$0.19^{n.s.}$	$0.20^{n.s.}$
Rates X Source	3	$0.38^{n.s.}$	$0.58^{n.s.}$	2.68^{**}	$0.93^{n.s.}$
Rates X Variety X Source	3	$1.76^{n.s.}$	$0.65^{n.s.}$	$0.03^{n.s.}$	$0.15^{n.s.}$
Subplot error	24	1.04	0.55	0.31	0.71
Total	47				

A - First harvest of first experiment. B - Second harvest of first experiment.

C - First harvest of second experiment. D - Second harvest of second experiment.

n.s. - not significant.

* - Significant at 5% probability level.

** - Signficant at 1% probability level.

Appendix 3.4: ANOVA for NO_3 accumulation (% NO_3-N) in laminae.

Source of variation	degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	$0.36^{n.s.}$	$0.06^{n.s.}$	0.39^{*}	$0.13^{n.s.}$
Rates	3	3.93^{*}	1.87^{**}	1.15^{**}	0.86^{*}
Mainplot error	6	0.61	0.09	0.05	0.16
Variety	1	1.82^{*}	$0.02^{n.s.}$	$0.003^{n.s.}$	$0.22^{n.s.}$
Source	1	1.64^{*}	$0.47^{n.s.}$	1.57^{*}	$0.0002^{n.s.}$
Variety X Source	1	$0.56^{n.s.}$	$0.08^{n.s.}$	$0.19^{n.s.}$	$0.12^{n.s.}$
Rates X Variety	3	$0.56^{n.s.}$	$0.01^{n.s.}$	$0.10^{n.s.}$	$0.07^{n.s.}$
Rates X Source	3	$0.41^{n.s.}$	$0.02^{n.s.}$	$0.10^{n.s.}$	$0.04^{n.s.}$
Rates X Variety X Source	3	$0.25^{n.s.}$	$0.08^{n.s.}$	$0.07^{n.s.}$	$0.01^{n.s.}$
Subplot error	24	0.34	0.13	0.21	0.35
Total	47				

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

Appendix 3.5: ANOVA for SCN^- content (ppm) in leaves.

Source of variation	Degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	16,328,071.1*	21,358,845.8*	264,829.8**	41.028.6$^{n.s.}$
Rates	3	972.948.8$^{n.s.}$	2,400,853.0$^{n.s.}$	4,979.7$^{n.s.}$	1,711.6$^{n.s.}$
Mainplot error	6	1,791,417.3	2,425,395.5	17.400.7	13,810.3
Variety	1	15.016.981.4*	114,077,166.8**	1,288,057.7**	263,292.2**
Source	1	58,185,648**	139,291,788.1**	1,086,309.2**	1,517,629.7**
Variety X Source	1	373,121.3$^{n.s.}$	26,892,107.9*	29,750.5$^{n.s.}$	99,099.2**
Rates X Variety	3	287,746.8$^{n.s.}$	619,606.5$^{n.s.}$	2,112.4$^{n.s.}$	8,255.1$^{n.s.}$
Rates X Source	3	26.252,612.3**	22,313,851.2**	280.954.5**	224,289.2**
Rates X Variety X Source	3	48,276.$^{n.s.}$	4,457,970.4$^{n.s.}$	12,506.4$^{n.s.}$	18,956.5$^{n.s.}$
Subplot error	24	2,122,322.63	4,176,918.6	19,009.4	10,911.6
Total	47				

A - First harvest of first experiment. B - Second harvest of first experiment.

C - First harvest of second experiment. D - Second harvest of second experiment.

n.s. - not significant.

* - Significant at 5% probability level. ** - Significant at 1% probability level.

Appendix 3.6: ANOVA for SCN^- content(ppm) in petioles.

Source of variation	Degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	24,085,803.15$^{n.s.}$	44,080,410.6*	73,387.8$^{n.s.}$	20,290.8$^{n.s.}$
Rates	3	922,283.27$^{n.s.}$	7,835.810.5$^{n.s.}$	8,956.7$^{n.s.}$	87,120.8$^{n.s.}$
Mainplot error	6	4,793,724	7,605,522	23,273.1	34,862.6
Variety	1	5,701,476.2$^{n.s.}$	290,969,008.3**	964,750.5*	318,699.95**
Source	1	87,888,175.2**	297,256,302.1**	773,430.2**	1,296,261.3**
Variety X Souce	1	5,348,677.5$^{n.s.}$	93,660,468.8*	31,672.7$^{n.s.}$	105,468.8$^{n.s.}$
Rates X Variety	3	737,320.9$^{n.s.}$	2,538,053.3$^{n.s.}$	6,537.3$^{n.s.}$	28,617.1$^{n.s.}$
Rates X Source	3	42,235,697.8**	44,471,579.2*	195,921.7**	328,448.5**
Rates X Variety X Source	3	940,142.2$^{n.s.}$	13,978,900.3$^{n.s.}$	29,846.6$^{n.s.}$	13,992.7$^{n.s.}$
Subplot error	24	2,820,312.7	12,610,171.1	15,143.1	25,865.7
Total	47				

A - First harvest of first experiment. B - Second harvest of first experiment.

C - First harvest of second experiment. D - Second harvest of second experiment.

n.s. - not significant.

* - Significant at 5% probability level. ** - Significant at 1% probability level.

Appendix 3.7: ANOVA for SCN^- content (ppm) in laminae.

Source of variation	Degrees of freedom	Mean Sum of Squares			
		A	B	C	D
Blocks	2	13,082,287.2**	10,850,804.8**	597,816.1**	68,965.8**
Rates	3	2,269,548.7n.s.	303,341.4n.s.	8,058.9n.s.	71,641.1**
Mainplot error	6	847,507.5	590.061.2	16,402.3	5,679.2
Variety	1	28,751,004.2**	18,092,124.2**	1,659,864.1**	312,987**
Source	1	34,589,958.5**	39,859,897.5**	1,451,856.3**	1,756,440.1**
Variety X Source	1	12,491,941.1n.s.	482,202.6n.s.	27,744.1n.s	92,576.3*
Rates X Variety	3	1,354,952.2n.s.	574,907.5n.s.	10,656.7n.s	5,373.8n.s.
Rates X Source	3	14,321,307.1*	9,477,635.3**	407,620.7**	141,293.0**
Rates X Variety X Source	3	700,210.4n.s.	629,355.8n.s.	8,853.0n.s.	30.935.3n.s.
Subplot error	24	3,204,449.8	1,645,922.9	43,866.2	17,461.3
Total	47				

A - First harvest of first experiment. B - Second harvest of first experiment.

C - First harvest of second experiment. D - Second harvest of second experiment.

n.s. - not significant

* - Significant at 5% probability level. ** - Significant at 1% probability level.

Appendix 4: Correlation Coefficients (r) and NO_3-N accumulation in leaves of Brassica oleracea var. acephala D.C. as related to NO_3-N levels in Soil (a) First harvest of first experiment

'Thousand-headed' Kale				'Georgia' Collard			
C A N		S A		C A N		S A	
NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf
2.6	1.05	0	0.81	1.2	2.44	2.5	0.56
3.1	3.15	4.3	2.16	1.7	0.52	3.4	1.66
4.1	2.77	8.0	2.77	4.8	1.96	5.1	1.86
5.1	3.99	26.4	3.33	8.3	2.18	8.2	2.50
r = 0.82		r = 0.86		r = 0.34		r = 0.87	

(b) Second harvest of first experiment

'Thousand-headed' Kale				'Georgia' Collard			
C A N		S A		C A N		S A	
NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf
3.3	0.81	6.2	0.21	3.0	0.54	11.2	0.33
6.3	2.47	19.1	2.40	5.3	1.94	19.8	3.07
11.8	2.98	20.3	3.21	10.7	2.75	34.2	2.00
13.7	3.11	25.9	2.69	17.1	3.78	34.8	1.77
r = 0.90		r = 0.91		r = 0.96		r = 0.39	

(c) First harvest of second experiment

' Thousand-headed' Kale				'Georgia' Collard			
CAN		SA		CAN		SA	
NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf	NO_3-N in Soil (µg/g soil)	% NO_3-N in leaf
0	0.76	0.34	2.36	0	1.9	0	1.02
0.78	3.47	0.50	1.37	0.60	0.64	0.8	0.59
0.86	2.34	0.85	0.83	2.34	2.43	6.20	2.02
11.48	2.73	2.50	1.69	7.0	2.99	7.9	1.56
r = 0.30		r = 0.06		r = 0.76		r = 0.82	

Appendix 5 : Major product classes from the enzymatic hydrolysis of glucosinolates (Carlson et al., 1987).