THE EFFECT OF NITROGEN RATES ON THE GROWTH, LEAF YIELD AND NUTRITIVE QUALITY OF THE BLACK NIGHTSHADE (*Solanum nigrum* L.)

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DEDICATION

To my Parents, Dickson

and Janet Murage.

DECLARATION

I, Ephraim Njagi Murage, declare that this thesis is my own work and has not been presented for a degree in any other University.

Ephraim Njagi Murage — 4/4/90 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, 1988 and March, 1989 at the Field station of the Faculty of Agriculture, Kabete Campus, University of Nairobi, to study the effect of nitrogen (N) rates on growth, leaf yield and nutritive quality of black nightshade (*Solanum nigrum* L.). Four N rates (O, 5, 10 and 15 g per plant) in the form of Calcium Ammonium Nitrate (26%N) were used. The plant growth was assessed by the determination of plant height number of leaves and branches per plant, while fresh weight of edible portions in tons per hectare was used to assess the plant yield. The plant height, number of leaves and branches per plant increased significantly ($P = 0.05$) with increasing rates of N fertilizer. However, plants top-dressed with N higher than 5g per plant did not show significant increase in growth. Nitrogen rates had significant effect on fresh weight with plants that were top-dressed with 5g of N per plant giving the highest yield of 44.5 tons per hectare.

The leaves were analysed for proximate chemical composition, ß-carotene, ascorbic acid and six minerals including calcium and iron. ß-carotene, total ash, crude fat, crude protein, potassium, calcium and magnesium levels increased with increasing rates of N application.

However the levels of ascorbic acid and crude fibre were significantly decreased by higher N rates. Sodium, iron and zinc contents in leaves tended not to be influenced by N-rates.

The evaluation of anti-nutrient factors in leaves involved the determination of nitrate-Nitrogen (NO_3-N), oxalates and total phenolics. Nitrate-nitrogen in the leaves increased significantly with increasing rates of N application while the oxalate content in leaves tended to decrease with increasing N rates. Nitrogen rates had no significant effect on the total phenolic content in the leaves.

INTRODUCTION

A number of wild plant species are used as source of leafy vegetables or pot-herbs by a large number of people of Kenya. It is not uncommon to find some of these plants being grown in home gardens. However, little attention has been given to these plants by agriculturists (Chweya, 1985), mainly due to the fact that most of them grow wild in forests, in waste and arable lands (Michieka, 1987; Mtotomwema, 1987). As a result, most emphasis has been laid on the production and consumption of exotic vegetables which are unfamiliar to majority of the rural people in Kenya.

In recent years, however, attempts have been made to identify some of the wild plant species which could be cultivated for vegetables. This has been prompted by the current realization of the possible role these wild leafy vegetables are likely to assume in providing nutrients such as vitamins and minerals in the predominantly vegetarian diets of especially the rural Kenyans (Gomez, 1981; Mtotomwema, 1987).

One such plant species is the black night shade (*Solanum nigrum*). The black night shade is a plant with wide ecological adaptability and grows all over the world, but mostly in the tropics between 0 - 2000 m above

sea level (Holm et al., 1977). It is a high moisture requiring plant and, therefore, thrives well in areas of high rainfall of about 1500 mm annually. The optimum temperatures for growth is between 20 - 30°C, whereas the soils should be very fertile and particularly high in organic matter, nitrogen and phosphorus (Epenhuijsen, 1974; Holm et al., 1977).

In Kenya, the black night shade occurs in many parts of the country and it is, therefore, known by different local names (Kokwaro, 1976). It is known as *mnavu, rinagu, managu, osuga* and *ndulu* by the Swahili, Gusii, Kikuyu, Luo and Kamba, respectively.

The black nightshade has high levels of ß-carotene (Provitamin A) and Vitamin C. It also has some proteins and minerals like calcium, potassium and iron (Imbamba, 1973; Chweya, 1985). Therefore, the vegetable can be used as a dietary supplement to the cereal-based diets of the rural Kenyans (Oke, 1973; Gomez, 1981).

Available information indicates presence of some undesirable antinutrient factors such as phenolic compounds, nitrates and oxalates in the leaves. These tend to give the vegetable an astringent taste and may sometimes lead to toxicity problems(Buck et al., 1966; Marshall et al., 1967; Carlsson, 1983). The levels of

these constituents together with the levels of vitamins and minerals, however, depend on such factors as growing conditions, climate and plant's genotype.

There has been no agronomic studies on the black nightshade in Kenya and, therefore, no information is available on the effect of environmental conditions and cultural practices on growth, yield and levels of both nutrients and anti-nutrients. It was, therefore, the purpose of this study to investigate the effects of nitrogen application on:

1. the growth of *Solanum nigrum* L.

2. leaf yield and

3. nutritive quality of the leaves.

LITERATURE REVIEW

1.1 Botany

Solanum nigrum L. is a member of the family *Solanacea* (Kokwaro, 1976). There are pronounced variants of the species that constitute a taxonomic complex comprising of three groups namely: *Solanum nigrum* L., *S. pseudonigrum Mtotomwema Ined.* and S. eldorettii Mtotomwema ined. (Mtotomwema, 1987). While *S. nigrum* has blackish purple fruits, *S. pseudonigrum mtotomwema ined.* has orange fruits and S. eldorettii Mtotomwema ined.has greenish fruits when mature. The *S. eldorettii* Mtotomwema ined. is slightly hairly on the stems and leaves, otherwise the three groups are relatively similar in other botanical aspects (Mtotomwema, 1987).

Solanum nigrum is a low spreading to erect annual although sometimes perennial, and grows as a weed naturally (Holm *et al.*, 1977). The plant has a primary root system with a stem that can grow to a height of 90 cm branching profusely to give round, smooth or sparsely hairly branches. The leaves can be alternate, simple or abovate, and are entirely toothed or irregularily lobed. The flowers are small and hang in clusters on a stalk from the stem internodes and are white or yellowish in colour. The fruits are multiseeded berries, spherical in shape and

may turn black when ripe (Epenhuijsen, 1974; Holms et al., 1977; Michieka, 1987).

1.2 Nitrogen Nutrition and Plant Growth

Nitrogen (N) is an intergral component of many compounds essential for plant growth processes, including synthesis of proteins and chlorophyll (Black, 1968). Therefore, N promotes vegetative growth and imparts a deep green colour and succulence to vegetative parts, qualities which are desirable in leafy vegetables (Brady, 1984; Salisbury and Ross, 1984; Tisdale et al., 1985).

Plants get their N from that which is available in the soil or from inorganic fertilizers added to the soil (Black, 1968). Both nitrate and ammonium forms of N can be taken up and metabolised by plants, depending on the species and environmental factors such as soil temperatures, aeration, pH and N source (Russel,1973; Buckman and Brady, 1968; Mengel and Kirkby, 1979). However, it is the nitrate form that is usually taken up mainly by arable crops so that even when ammonium fertilizer is applied, soil microorganisms oxidise the ammonium to the nitrate form (Hewitt and Smith, 1975; Huffaker and Rains, 1978; Mengel and Kirkby, 1979; Tisdale et al., 1985).

After absorption of nitrate ions by plant roots, a portion is reduced to nitrite ion, some of which

accumulates in the roots. The rest is translocated to the leaves (Huffaker and Rains, 1978). A further portion of the nitrate accumulates in the leaf cells, while the rest is assimilated (Reisenauer, 1978) into various organic substances to give the amino acids , glutamine and asparagine (Tisdale et al., 1985). It is from these amino acids that nucleic acids and proteins are synthesised (Hewitt and Smith, 1975; Tisdale et al., 1985).

1.3 Effect of N on Growth Rate and Yields of Leafy Vegetables

Sorensen (1984) working with cabbage (Brassica oleracea var. capitata L.) found that N tended to significantly improve the growth and development of plants thus increasing the total yield. Nitrogen application increased the mean fresh weight and therefore, yields of kale plants (Chweya, 1984). Similar findings have also been reported by Maynard and Barker (1979). Vegetables are fast growing, succulent crops that respond quickly to nitrogen application. The N response is generally favourable and in many cases results in improved yields and quality of the commodity (Maynard and Barker, 1979).

Barker et al. (1974) and Peck (1974) reported increased yields in spinach (Spinacia oleracea L.) and cabbage with increased N application. Sorensen (1984),

while working with cabbage reported that the supply of N had significant effect on the growth and development of cabbage heads. The yields increased from 13.2 to 70.8 tonnes/ha due to application of 600 kg N/ha. However, the dry matter content of the heads decreased significantly from 11.8 to 10.2% with increasing application of N from 0 to 600 kg N/ha.

Deficiency of N in the soil results in reduced photosynthetic ability of the plant due to reduced production of chloroplasts. This leads to early flowering, cell vacuolation, early differention and/or senescence (Bunt, 1973; Hewitt and Smith, 1975).

1.4 Effect of N on Plant Quality

Freshness is an important quality aspect of leafy vegetables. Freshness is attributed to the water status of the plant (Mengel, 1979) or the turgidity of the plant tissues. The turgor of plant cells is mainly a question of osmotic potential in the cells which depends on the concentration of osmotically active solutes in the cell vacuoles (Mengel, 1979). The osmotically active solutes may be organic like sugars and amino acids, or inorganic like the nitrates. Increased N application will raise the level of organic solutes and also provide nitrates for improved osmotic potential (Wenzel and Michael, 1966).

Therefore, increased N application to vegetables improves the water status of the plant leading to an increase in fresh weight and better vegetable quality (Peck, 1974; Sorensen, 1984; Mengel, 1979).

1.4.1 Chemical Composition

The protein:carbohydrate ratio in leaves is influenced by N availability to vegetable plants (Mengel, 1979). Limited N supply results in higher levels of carbohydrates, and restricted protein synthesis as more photosynthates are used for carbohydrate synthesis (Hehl and Mengel, 1972; Mengel, 1979). Hence a plant deficient in N will have higher contents of sucrose, starch and fructosans than crude protein (Blanny and Chapman, 1974; Mengel, 1979). Similar findings were reported for spinach and cauliflower by Fritz and Habben (1973) and Pimpini et al. (1973) respectively. However, as the amount of reducing sugars, total N and crude protein get enhanced by N fertilization; the crude fibre content, which includes cellulose, hemicellulose, lignin and pentosans decrease (Mengel, 1979).

Nitrogen supply and high light intensity enhance formation of chloroplasts, which contain thylakoid membranes that synthesise fatty acids (Mengel, 1979). Therefore, an increase in N supply to vegetable plants may raise the level of fatty acids (Tisdale et al., 1985).

1.4.2 Vitamin A

Tisdale et al. (1985) reported that N promotes vegetative growth and imparts a deep green colour to the vegetative parts. The deep green colour is mainly due to chlorophyll pigment which is synthesised in the chloroplasts. The chloroplasts are also rich in proteins and contain ß-carotene which is a pro-vitamin A (Black, 1968; Mengel, 1979). Nitrogen supply to plants raises the amount of chloroplasts hence, increases the content of Vitamin A (Mengel, 1979). Work done by Habben (1973) on carrots (Daucus carota L.), showed that nitrogen fertilization increased the level of vitamin A. Similar findings were reported by Fritz and Habben (1973) while working with spinach.

1.4.3 Vitamin C

Vitamin C is found in plants as either reduced ascorbic acid or the oxidized form dehydroascorbic acid. Its deficiency in human nutrition results in scurvy (Birch and Parkers, 1974). The content of vitamin C in plants is influenced mainly by the light intensity and N level (Mengel, 1979). Vitamin C synthesis is associated with carbohydrate metabolism since its primary precursor is

glucose which is activated by uridine triphosphate or adenine triphosphate, then oxidized to glucoronic acid, the direct precursor of vitamin C (Mengel, 1979). Thus light intensity which is directly involved in the photosynthetic ATP and glucose synthesis will determine the level of vitamin C in plants (Salisbury and Ross, 1984; Mengel, 1979).

Pflugar and Mengel (1972) reported that potassium supply to plants enhances synthesis of vitamin C. However generous applications of N fertilizer to vegetable plants decrease the content of vitamin C because there results an increase in the competition for photosynthates for amino acids and carbohydrate synthesis (Werner, 1957). This results in the decline of vitamin C synthesis as was observed in cabbage and spinach by Sorensen (1984) and Fritz and Habben (1973), respectiely. In kale (*Brassica oleracea var acephala* D.C.) vitamin C content decreased from 113 to 99 mg/100 g fresh matter by applying 0.5 and 2g N per plant respectively(Werner, 1957).

1.4.4 Minerals

Minerals like potassium, calcium, iron and magnesium are important quality components in leafy vegetables (Mengel, 1979). Leafy vegetables are rich sources of these minerals (Gomez, 1982). Iron, which is important in the structure and formation of red blood cells (Simon, 1966; Maynard et al., 1976) is found in high

amounts in Solanum nigrum (Gomez, 1982). Gomez (1982) estimated that leaves of this vegetable may provide about 12 mg of iron and about 290 mg of calcium per 100 g fresh weight the latter which is crucial for the formation of bones. However, the levels of minerals in plants depend on the minerals availability from the soil. Thus mineral accumulation can be enhanced by application of appropriate fertilizers (Mengel, 1979). Even though plants will tend to absorb various minerals present in the nutrient medium, there is some selectivity which varies with plant species (Hewitt and Smith, 1975; Mengel, 1979). Therefore, it is unlikely that N will have any effect on the uptake of various minerals.

1.5 Antinutrient Factors

1.5.1 Nitrates

Many plant species have a tendency to accumulate nitrate-N in their vegetative parts. This is so especially with the members of the Brassicaceae (Cruciferae) and Chenopodiceae families (Salisbury and Ross, 1978; Maynard and Barker, 1979; Mengel and Kirkby, 1979; Kunsch et al., 1983). According to Mengel and Kirkby (1979), accumulation of nitrates occurs if the rate of nitrate translocation from root to shoot is faster than the assimilation into the shoot. The genetical make up of a plant and the environmental factors surrounding the plant influence the

accumulation of nitrates in plants (Maynard et al., 1976; Maynard and Barker, 1979; Mengel, 1979; Carlson, 1983; Kunsch et al., 1983). Plants grown in soils rich in nitrate-N; coupled with conditions of water stress, limited concentration of carbon dioxide and high relative humidity; have a great tendency to accumulate nitrate in their vegetative parts (Maynard et al., 1976; Aworh et al., 1980).

Light intensity, quality and duration (photoperiodism) affect nitrate accumulation (Cantliffe, 1972 a,b,c). In spinach, increased light intensity tends to decrease nitrate accumulation (Cantliffe, 1972a). Minotti and Stankey (1973) and Cantliffe (1972b), while working with beet root plants (*Beta vulgaris L*) found that there was a diurnal variation in the accumulation of nitrates, such that the levels of nitrate accumulated decreased as the photoperiod was extended from 8 through 12, 16 to 20hrs.

Low temperatures affect soil nitrification, root growth, tissue permeability and active absorption of nitrates from the soil (Cantliffe, 1972c; Maynard et al., 1976). Cantliffe (1972c) working with spinach showed that the concentration of nitrate in the leaves increased with rise in temperatures from 5^{o} to $25^{o}C$, whether or not N fertilizer was applied.

The rate, source and time of application of N

fertilizer tends to influence the accumulation of nitrates in plants (Maynard and Barker, 1971; Pimpini et al.,1973; Maynard et al., 1976; Carlsson, 1983). The rate of accumulation in leaves is enhanced by increase in the rates of N fertilizer (Fritz and Habben, 1973; Kunsch et al., 1983; Peck et al., 1971). Chweya (1986) and Kanampiu (1987) while working with kale and collards respectively reported similar findings. When the interval between side dressing with N and harvest was short in spinach, nitrate accumulation was greatest due to potassium nitrate followed by ammonium nitrate and the least due to urea (Barker et al., 1971). Therefore, application of nitrate fertilizers enhance nitrate-N accumulation more than application of ammoniacal fertilizers (Pimpini et al., 1973; Kanampiu, 1987).

Plants of different species, varieties and age accumulate nitrate-nitrogen differently (Aworh et al., 1980; Barker et al., 1971). Cantliffe (1972a) observed differences in nitrate accumulation between savoy and smooth leafed spinach. Similar findings were reported by Kanampiu (1987) while working with kale and collards. The author further reported that nitrate accumulation varied with age and different parts of the plant leaves.

Nitrates have toxic effects on humans and animals only when they are in their reduced nitrite form (Mengel,

1979; Maynard et al., 1976). Reduction is caused by the enzyme nitrate reductase. When nitrite ions are absorbed into the blood stream, they oxidise the ferrous ion of the haemoglobin to the ferric form, producing methaemoglobin and therefore lowering the oxygen-carrying capacity of the blood (Simon, 1966; Maynard and Barker, 1974; Maynard et al., 1976). This leads to a condition known as methaemoglobinemia (Canfliffe, 1972). In an acidic medium such as the one in the intestines, the nitric acid formed may be split into hydroxyl and nitrosyl ions the latter which reacts with secondary amines to form nitrosamines that are carcinogenic (Mengel, 1979).

Lee (1970) reported that nitrate toxicity is relatively low and with a fatal dose varying from 15 to 70 mg per kg body weight as for adult humans, while the lethal dose for nitrite is around 20 mg per kg body weight.

1.5.2 Oxalates

Oxalates occur naturally in many cultivated and wild leafy vegetables (Buck et al., 1966; Robinson, 1973; Carlsson, 1983). Oxalates are synthesised as by-products of photosynthesis, a process during which the leaf synthesises energy and the carbon-skeletons of the organic substances (Carlsson, 1983).

Amaranthus spp, Chenopodium album L, Solanum nigru Brassica oleracea, Spinacia oleracea and *Beta vulgaris* are among the leafy vegetables which have been reported to have high levels of oxalates (Buck *et al.*, 1966; Marshall *et al.*, 1967; Robinson, 1973; Carlsson, 1983). Buck *et al.* (1966) and Marshall *et al.* (1967) reported that *Amaranthus retroflexus* contains as high as 12.60% to 30.75% oxalates expressed as equivalent oxalic acid on dry weight basis.

Although little information is available on factors that enhance synthesis of high levels of oxalates in plants, Carlsson (1983) attributed it to genetic and environmental factors. Therefore, different plant species and varieties will have varying amounts of oxalates. The leaves tend to have higher levels of oxalates than stems due to the role of the leaf as a major photosynthetic organ (Carlsson, 1983; Robinson, 1973). Carlsson (1983) while working with *Amaranthus hypocondriacus, Brassica oleracea var acephala* D.C and *Chenopodium album* reported that an increase in N fertilizer tended to decrease the contents of oxalates.

Work done by Abd-El-Hadi *et al.* (1985) on spinach showed that an increase of N fertilizer rates from 0 to 150 mgN/kg soil increased the total oxalate content to 11%, especially when a nitrate fertilizer like Calcium Ammonium Nitrate was used. The worker further showed that the total oxalate contents decreased with maturity of

plants. Abd-El-Hadi et al., (1985) also found that low temperatures and short day lengths are favourable for enhanced oxalic acid production and accumulation of total oxalates. High oxalic acid contents in spinach were matched by accumulation of high levels of nitrate.

Osweiler et al. (1969), Hill and Rawate (1982) reported that, plants with high oxalic acid contents have also high levels of nitrates. Hence, these two compounds have been implicated in numerous cases of especially livestock poisoning (Simon, 1966). In pigs they tend to cause a lethal disease called perirenal edema, especially when the pigs ingest large amounts of plants like pig weed (*Amaranthus* spp), nightshade (*Solanum nigrum*) or the Buffalo burr (*Solanum rostratum*) (Buck et al., 1966; Marshall et al., 1967).

Excess amounts of oxalic acid in food interferes with calcium absorption in the gastrointestinal tract. This is because oxalic acid precipitates calcium ions to give an insoluble calcium oxalate (Carlsson, 1983). This results in calcium deficiency in the body even when the nutrient is present in the diet in sufficient amounts (Robinson, 1973; Carlsson, 1983). Work by Robinson (1973) showed that oxalates also cause gastrointestinal upsets, hematuria, non-coagulability of blood, and convulsions that can lead to death.

Analyses of several fractions of raw, cooked and

oven-dried foods showed that any soluble oxalates are always extracted into the cooking water (Hill and Rawate, 1982). Therefore, the resulting food would be of good nutritional quality.

1.5.3 Phenolics

Polyphenols are a group of compounds that have at least two hydroxyl groups substituted on the aromatic ring structure and occur commonly in many plants (Singleton, 1981; Butler, 1988). Polyphenols include compounds like safrole, coumarius, anthocyanins, flavanoids and tannins (Strumeyer and Malin, 1975; Singleton, 1981; Butler, 1988).Tannins are further sub-divided into the hydrolysable and the condensed, on the basis of their structure and reactivity towards hydrolysing agents like enzymes and dilute acids (Strumeyer and Malin, 1975; Ribereau-Gayon, 1972).

According to Butler (1988), relatively little is is known about plant polyphenols compared to other major components of plants because of the difficulty in isolating for characterization. Moreover, polyphenols are not directly involved in metabolism and are therefore considered as secondary metabolites.

Little work has been done on polyphenols in vegetables, especially when compared to the work done on the polyphenols in cereal crops like sorghum and legumes

especially beans. The factors that lead to synthesis of high levels of polyphenols by plants are likewise not very clear, but Butler (1988) reports that polyphenols are produced by plants as a defence mechanism against predators and as allelopathic compounds. The effect of N application on the synthesis of polyphenols has also not been reported.

Taste and palatability of many edible fruits and other plant products are dependent on the type and concentration of astringents present (Haslam, 1974). Astringency which is perceived as a burning taste on the tongue is attributed to the presence of tannins and other polyphenols in foods (Haslam, 1974; Strumeyer and Malin, 1975; Singleton, 1981). Astringency is believed to result from the interaction of natural polyphenols with salivary proteins and glycoproteins in the mouth (Goldstein and Swain, 1963, 1965).

The risk of serious toxicological effects to human by phenolics in foods seems vanishingly small. Phenolic derivatives like safrole and coumarins have, however, been reported to be carcinogenic (Singleton, 1981). Tannins are not only important for taste of foods (Singleton, 1981), but are also reported to react and precipitate with proteins in foods, thus decreasing digestibility and hence lowering protein quality (Hasham, 1974; Strumeyer and Malin, 1975; Hagerman and Bulter, 1978; Elias et al.,

1979). Utilization of some plant products like sorghum for food has been limited by the presence of high levels of tannins (Elias *el al.*, 1979; Butler, 1988). Tannins can also inhibit the enzyme trypsin that is involved in protein digestion, thereby rendering the protein unavailable for absorption in the gastro-intestinal tract (Elias et al., 1979).

While hydrolysable tannins are readily cleaved by enzymes as well as by dilute acid into sugars such as glucose and a phenocarboxylic acids like gallic acid (Strumeyer and Malin, 1975), condensed tannins are resistant to enzymatic degradation and upon acid treatment yield small amounts of anthocyanidins (Strumeyer and Malin, 1975; Elias et al., 1979 Butler, 1988). However, according to Bulter (1988), there is evidence that polyphenols would have much more severe antinutritional effects if it were not for specific proline-rich tannin-binding proteins present in the saliva of most animals (and probably in man).

MATERIALS AND METHODS

Seeds of the black nightshade were obtained from Mtotomwema's collections at Moi University, Eldoret. Two experiments were carried out between August, 1988 and March, 1989. The first experiment was carried out between August and November, 1988, and the second experiment between January and March, 1989.

2.1 Experimental Site

This study was carried out at the Field Station of the Faculty of Agriculture, Kabete Campus, University of Nairobi. The Field Station is at an altitude of 1940m above sea level, with an average rainfall of about 1000 mm per year, which is bimodal with peaks in April and November. The weather data recorded during the experimental period are shown in Table 1.

The soils at the experimental site is under nitosol unit according to FAO/UNESCO classification (FAO/UNESCO, 1974). These soils are deep and well drained and are also very resistant to soil erosion. They have a dark-brownish colour with a thick, acid top soil. The soils are called Kikuyu friable clay, derived from tertiary trachytic lava (Siderus, 1970).

Table 1. Mean monthly weather record at the Field Station, Kabete, between July, 1988 - April, 1989.

Month	Mean Radiation (MJM^{-2})	Mean Sunshine (hrs/day)	Temperature (^{o}C) Max	Min	Mean	Total Rainfall (mm/month)	Mean Evaporation (mm/day)	Mean % RH	Total Wind (Run/day)
1988									
July	15.58	3.3	20.7	11.6	16.1	18.7	2.5	77	48.2
Aug.	10.89	3.7	20.9	11.9	16.4	46.9	2.4	76	50.6
Sept.	13.15	4.6	22.6	12.1	17.3	27.1	2.9	71	67.9
Oct.	16.27	7.6	24.5	12.8	18.5	16.7	4.6	65	97.5
Nov.	15.07	6.9	22.1	13.5	17.8	105.3	3.7	73	112.2
Dec.	15.27	7.8	22.0	13.0	17.5	139.1	-[a]	72	137.2
1989									
Jan.	16.57	7.5	23.3	13.0	18.1	134.6	-	68	75.0
Feb.	18.92	9.4	23.9	12.3	18.1	45.1	-	57	110.3
Mar.	18.87	8.8	24.9	13.7	19.3	93.1	-	62	87.9
April	13.10	5.4	22.0	13.8	17.9	210.5	-	78	72.0

(-)[a] Data not recorded

Four soil samples were taken randomly from the main growing site at two depths, 0 - 15 cm and 15 - 30 cm just before transplanting. After air drying and grinding to pass through a 2 mm sieve, the samples were analysed for total nitrogen, cation exchange capacity (CEC), phosphorus, and pH by the methods described by Ahn (1975). The results are shown in Table 2.

2.2 Experimental Treatments and Design

Four rates of N (0, 5, 10 and 15g N per plant) in the form of Calcium Ammonium Nitrate (26% N) fertilizer formed the treatments. These rates were applied only once at two weeks after transplanting. The experiments were carried out in randomised complete block designs with three replicates. The size of a block was 8 m x 3 m whereas that of plots was 2 m x 3 m.

2.3 Cultural Practices

2.3.1 Raising seedlings

A fine tilled seedbed 3m x 1m was prepared. The seeds were sown in furrows about 2cm deep and 15cm apart. They were then thinly covered with top soil. The seedbed was watered immediately after planting and subsequently in the morning and evening of each day, using a watering can. Germination of seedlings occurred after 8 - 9 days.

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Table 2. Soil chemical characteristics before planting.

Nutrient/ soil reaction	First Experiment	Second Experiment
pH water	6.1 (0.21)[a]	6.5 (0.23)
CEC (Meq100g soil)	27.5 (2.71)	29.3 (2.98)
P (µg/100 g/soil)	25.6 (8.11)	18.8 (2.47)
Total N(µg/g soil)	2701 (186)	3002 (299)

[a]Mean (standard deviation), N = 2.

In order to harden the seedlings, the watering frequency was reduced to once a day during the last week before transplanting.

2.3.2 Preparation of plots and transplanting

The field was ploughed with a mould board plough and disc harrowed once. The plots were then marked out and a day before transplanting, the field was irrigated as there were no rains at the time.

The seedlings were transplanted when six weeks old and planted with Diammonium Phosphate (46% P_2O_5) at the rate of 2g per plant. Also 2g of furadan for the control of soil nematodes was thoroughly mixed with soil in each hole before planting. Only seedlings of uniform size were transplanted. The seedlings were planted with a spacing of 30cm between plants and within rows, giving a plant population of 111,111 per hectare. The plots were manually kept weed-free, throughout the experimental period.

2.3.3 Control of pests and diseases

Moles were controlled by trapping. Otherwise there were no other pests or disease incidences noticed during the experimental period.

2.3.4 Watering

Irrigation was carried out using overhead sprinklers to supplement the rains.

2.4 Evaluation of Growth Characteristics

Twenty plants per plot were randomly selected and tagged, then used for determination of plant height, number of leaves and branches per plant and yield (fresh weight) per unit area during each harvest at six, eight, ten and twelve weeks after seedling emergence.

2.4.1 Determination of plant height, leaves and branches per plant

Five plants per treatment had their heights measured in centimetres with a ruler during each harvest. The mean height per plant was then calculated.

The total number of leaves from the five plants per treatment was determined by counting during each harvest and the mean number of leaves per plant calculated.

The total number of branches from five plants per treatment were counted at each harvest, and the mean number of branches per plant calculated.

2.4.2 Determination of yield

Plants from an area of $4m^2$ on each plot were harvested by plucking the edible portions (soft branches stems, petioles and lamina). The edible portions were weighed to determine the fresh weight and expresed as tons per hectare. The samples from each plot were then placed in craft paper bags and brought to the laboratory for determination of dry matter.

During the final harvest, at twelve weeks after seedling emergence, edible portions were sampled at random from each plot and analysed for Vitamin A and C, total phenolics and oxalates.

2.5 Analytical Methods

These determinations were carried out on duplicate samples from the edible portions.

2.5.1 Determination of dry matter

Fifty grams of sample were placed in a craft paper bag and dried in an oven at $70^{o}C$ to constant weight. The dry weight was expressed as a percentage of the weight of the sample.

2.5.2 Determination of proximate composition

For determination of total ash, crude fat, crude protein and crude fibre, the dried material was

ground to pass through a 500 micron sieve.

2.5.2.1 Determination of total ash: Total ash was determined by AOAC methods (AOAC,1984). Two grams of sample were weighed in a porcelain ashing dish previously ignited, and cooled in a dessicator.

The dish was held in a muffle furnace at a temperature of 600°C overnight. It was then cooled to room temperature in a dessicator and weighed. The weight of residue was calculated as a percent of dry matter.

2.5.2 2 Determination of crude fat: Crude fat (ether extract) was determined by AOAC methods (AOAC,1984). Five grams of sample were weighed in a cellulose extraction thimble and extracted with petroleum spirit (40 - 60°C) in a Soxhlet extract for 12 hours. The ether extract which was contained in a 250 ml round-bottomed flask which had been previously dried, cooled and tared had the excess petroleum spirit evaporated away in a rotary vacuum evaporation and the residue dried at 80°C for 30 minutes. The flask was then cooled in a dessicator and weighed. The weight of the residue was calculated as percent of crude fat.

2.5.2.3 Determination of crude protein: Crude protein of all samples was determined by the micro-Kjeldahl method (AOAC, 1984). Percentage of total nitrogen was multiplied by 6.25 to convert it to protein.

A sample of 0.5 grams was weighed and placed in a Kjeldahl flask. Half a Kjeldahl tablet was added followed by 10 ml of concentrated sulphuric acid. The flask was heated on a Kjeldahl heating assembly at a low setting until frothing subsided and a clear solution remained. Heating was changed to high setting and the mixture digested further for two hours. After cooling to room temperature, the white residue was dissolved in distilled water. The solution was transfered to distillation apparatus and 4-5 drops of phenolpthalein indicator added. The solution was steam distilled after enough 40% sodium hydroxide solution was added to make the mixture sufficiently alkaline . The distillate from each sample was collected into an erlemeyer flask containing 20 ml of 2% boric acid and two drops of a mixed indicator containing bromocressol green and methyl red. Then the quantity of nitrogen in the distillate was determined by titration with 0.1 N hydrochloric acid.

2.5.2.4 Determination of crude fibre: Crude fibre was determined by AOAC methods of analysis (AOAC,1984). Two grams of sample were weighed into a 600 ml graduated beaker. A small amount of boiling distilled water and 25ml of 2.04N sulphuric acid added, then the volume adjusted to 200ml with boiling water and maintained close to this volume whilst boiling for 30 minutes on a hot plate. The contents of the beaker were filtered through a Buchner

funnel packed with glass wool and the residue washed three times with hot distilled water. The residue and the glass wool were transferred back into the beaker and boiled with 25ml of 1.78N potassium hydroxide in a constant volume of 200ml for another 30 minutes. The sample was then filtered as above. Finally, the residue was washed three times with small amounts of ethanol and transferred into a porcelain dish. The dish was later dried in an air oven at 105°C for 2 hours and cooled in a dessicator and weighed. The dish and the contents were then ignited at 550°C in a muffle furnace for 2 hours, cooled and weighed. The crude fibre content of sample was then calculated as a percentage on dry matter basis.

2.5.3 Determination of vitamin C

Vitamin C was determined as reduced ascorbic acid by the modified method of Barakat et al. (1955). Thirty grams of fresh material were blended with 90 ml of a 10% solution of trichloroacetic acid to a homogenous slurry. The slurry was filtered through Whatman No.41 filter paper. Five millilitres of the filtrate were mixed with 5 ml of a 4% solution of potassium iodide and 1 ml of 1% starch solution. The mixture was then titrated with a standard solution of N-bromosuccinimide to a faint violet/blue colour that persisted for at least 15 seconds. The amount of Vitamin C was calculated as milligrams per

100 grams of dry matter from the equation : Vitamin C = V.C x 176/178 mg, whereby V = volume of N - Bromosuccinimide (ml) and C = concentration of N Bromosuccinimide

2.5.4 Determination of vitamin A

Vitamin A was determined as ß-carotene by AOAC methods (AOAC,1984). Two grams of the fresh material were extracted completely by grinding in a mortar with portions of acetone. The combined extracts were pooled and made to 100 ml with acetone. Twenty five millilitres of the extract were evaporated to near dryness in a rotary vacuum evaporation. The residue was then dissolved in about 2 ml of petroleum spirit (40^{o}-60^{o}C) and eluted through a chromatography column packed with silica gel and activated with a mixture of petroleum spirit (40^{o}-60^{o} C),benzene and ethanol in the ratio of 100:10:7 (v/v), with petroleum ether as solvent, The first yellow fraction which represented the ß-carotene was collected and made to 25 ml with petroleum spirit and its absorbance determined at 440 nm on a Beckman model 25 spectrophotometer. The ß-carotene was calculated from a standard curve prepared from pure beta carotene solutions in petroleum spirit. Results were expressed as equivalent milligrams of ß-carotene per 100 grams dry matter.

2.5.5 Determination of specific minerals

The specific minerals, calcium, potassium, magnesium, iron, zinc and sodium were determined by AOAC method (AOAC,1984). One gram of ground dried sample was mixed with 10ml of Concentrated nitric acid in a Kjeldahl and allowed to stand overnight. The flask was then heated on the Kjeldahl apparatus until the production of nitrogen dioxide fumes stopped. The flasks were cooled to room temperature and 4 ml of a 70% hypochloric acid added. The samples were then, heated again slowly at a low temperature. The samples were transferred into 100 ml flasks and diluted to volume with distilled water and the addition of some 1% Lanthanum oxide (weight/volume) to prevent potential phosphorus interference in the determination of calcium and magnesium. The elements were analysed using a Perkin Elmer 2380 Atomic Aborption spectrophotometer equiped with an air-acetylene flame and a hollow cathode lamp. The spectrophotometer was operated under standard conditions using wavelengths and slit widths specified for each element. Standards were prepared by dilution of the stock solutions. The results were then expressed as milligrams per 100 grams dry matter.

2.5.6 Determination of nitrate-nitrogen

Nitrate-nitrogen was determined using the colorimetric method described by Cataldo et al. (1975) with a slight modification according to Chweya (1985). Ten milligrams of dry sample were suspended in 10 ml distilled

water in a 100ml beaker and incubated for 1 hour at 45°C. The mixture was filtered through Whatman No 41 filter paper. Of the filtrate, 0.2 ml were pipetted into 50 ml test tube and 0.8 ml of 5% (w/v) salicyclic acid in concentrated sulphuric acid were added and mixed thoroughly. The mixture was allowed to stand for 20 minutes at room temperature, after which 19ml of 2N sodium hydroxide were added. The mixture was cooled for 30 minutes then its absorbance read at 410 nm against a common blank (0.2 ml distilled water + 0.8 ml sulphuric acid + 19 ml 2N sodium hydroxide) on a Beckman model 25 spectrophotometer. Using a standard curve prepared from different concentrations of potassium nitrate, the nitrate-nitrogen was calculated as equivalent milligrams per 100 grams dry matter.

2.5.7 Determination of total phenolics

Total phenolics were determined by Folin-Denis method (Burns, 1963), with a slight modification where instead of dried material, fresh material was used. Five grams were ground on a mortar with 10ml distilled water, filtered through Whatman No.41 filter paper and the filtrate made to 500ml with distilled water. Two millilitres of the solution were mixed with 2ml Folin-Denis reagent and 5ml of a super-saturated sodium carbonate solution in a 100ml conical flask, and made to between 75-100ml with distilled water. After standing at

room temperature for at least 40 minutes, the absorbance was read at 725 nm on a Beckman Model 25 spectrophotometer. From a standard curve, the total phenolics contents were calculated as equivalent milligrams gallic acid per 100 grams dry matter.

2.5.8 Determination of oxalates

Oxalates were determined in the fresh leaves by the method described by Marshall et al. (1967). The oxalates were precipitated with calcium and their contents subsequently determined by titration with a standard potassium permanganate solution to a pink colour that persisted for at least 30 seconds. The oxalates were expressed as equivalent milligrams oxalic acid per 100 grams dry matter.

2.5.9 Data analysis

The data obtained were subjected to analysis of variance (ANOVA) and the means separated by the least significant difference test (LSD) at 5% probability level as stipulated by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

3.1 Effect of Nitrogen on Growth and Yield of Solanum nigrum.

Nitrogen rates had significant effect on the leaf number per plant. Figure 1 shows that the number of leaves per plant increased with increasing rates of N. In both experiments, plants top-dressed with 5g N per plant had the highest number of leaves compared to those that were top-dressed with 10 or 15 g N per plant. Plants that were not top-dressed had significantly fewer leaves per plant than those that were top-dressed.

The increase in the number of leaves per plant with increasing N rates could be due to the fact that N induces leaf production throughout, growth amongst other factors (Hewitt and Smith, 1975). Chweya (1984) working with kale also found that application of N-fertilizer tended to increase the number of leaves per plant. Nitrogen has been reported to promote vegetative growth giving plants a green colour and succulency (Salisbury and Ross, 1984; Tisdale et al., 1985). .

The effect of N rates on plant height was significant. Plants top-dressed with N were significantly taller than those that were not top-dressed. Figure 2

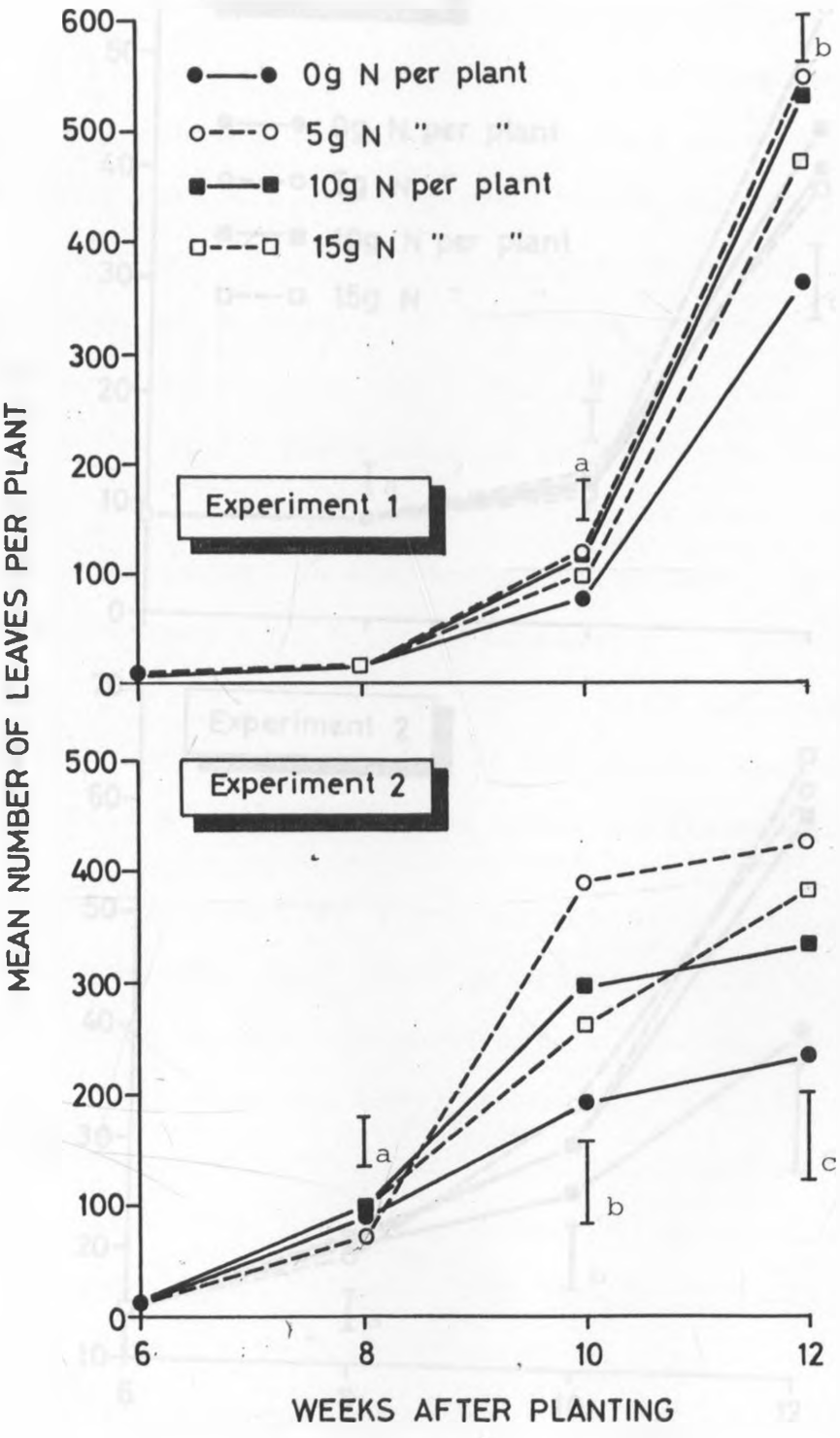

Fig. 1 : The effect of nitrogen rates on the number of leaves of Solanum nigrum.

^{abc}Least significant difference (LSD) bars

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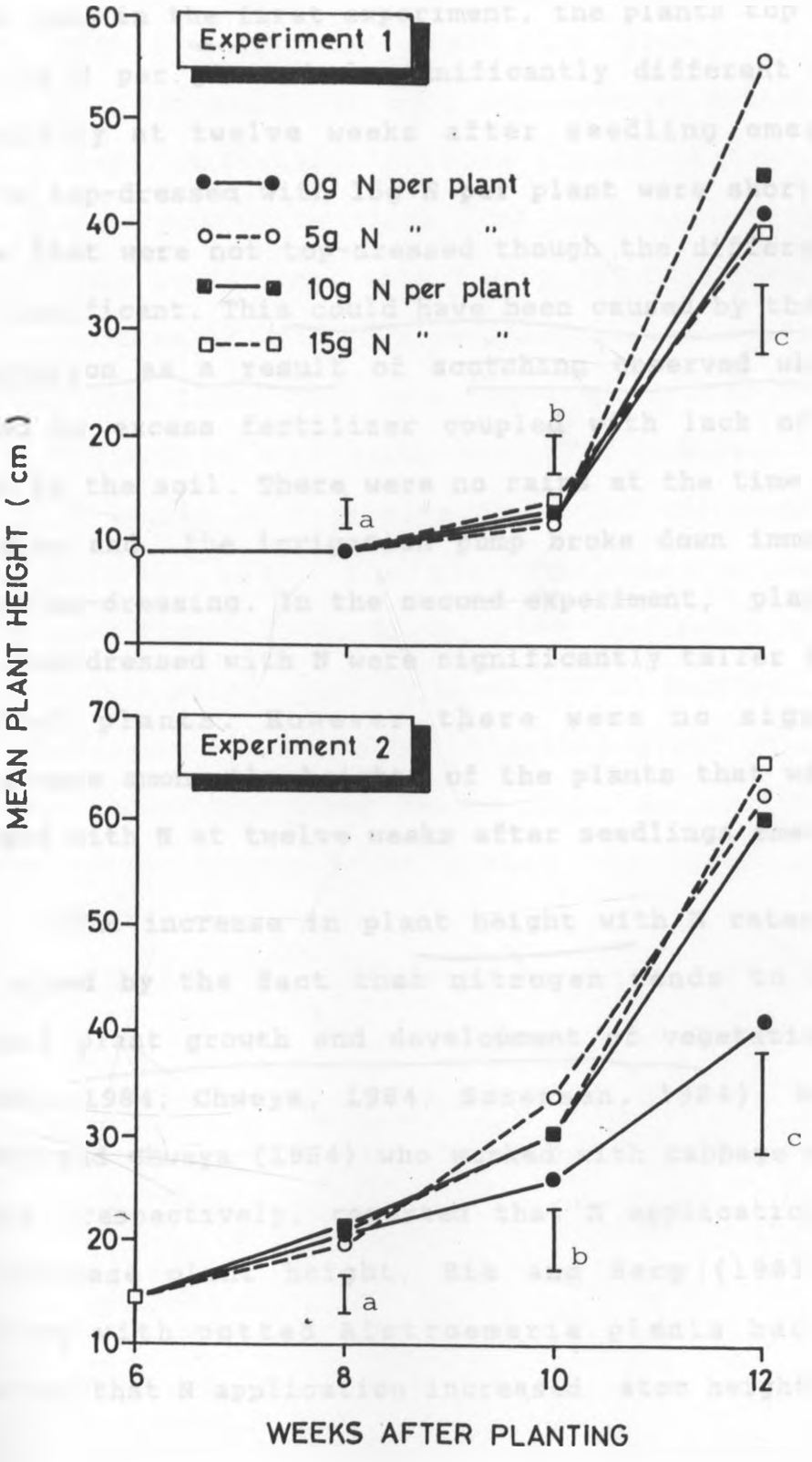

Fig. 2. : The effect of nitrogen rates on the height of Solanum nigrum L.

^{abc}Least significant difference (LSD) bars

shows that in the first experiment, the plants top-dressed with 5g N per plant had significantly different heights especially at twelve weeks after seedling emergence. Plants top-dressed with 15g N per plant were shorter than those that were not top-dressed though the difference was not significant. This could have been caused by the growth retardation as a result of scotching observed which was caused by excess fertilizer coupled with lack of enough water in the soil. There were no rains at the time of top-dressing and the irrigation pump broke down immediately after top-dressing. In the second experiment, plants that were top-dressed with N were significantly taller than the control plants. However there were no significant difference among the heights of the plants that were top-dressed with N at twelve weeks after seedlings emergence.

The increase in plant height with N rates can be explained by the fact that nitrogen tends to improve overall plant growth and development of vegetative parts (Brady, 1984; Chweya, 1984; Sorensen, 1984). Sorensen (1984) and Chweya (1984) who worked with cabbage and kale plants, respectively, reported that N application tended to increase plant height. Bik and Berg (1981) while working with potted Alstroemeria plants had ealier reported that N application increased stem heights.

Figure 3 shows the effect of N rates on the number of branches per plant. In both experiments, branching was significantly increased in those plants that were top-dressed as compared to those that were not. Differences among plants top-dressed with N were not significant, but, plants top-dressed with 5g N per plant tended to have more branches than those that were top-dressed with more than 5g N per plant in both experiments. It has been reported that high levels of N promote growth of additional plant tissues, such that, the plant becomes excessively vegetative (Black, 1968; Brady, 1984; Salisbury and Ross, 1984; Tisdale *et al.*, 1985). Black (1968) further reported that deficiency of N tended to retard plant growth such that the leaves and stems became thin, and the number of lateral stems became fewer giving the plant a sparse appearance.

Effect of N on the fresh weight of the edible portion; per hectare was significant. As Figure 4 shows, fresh weight increased with increasing rates of N. Plants that were not top-dressed with N had significantly less fresh weight than those that were top-dressed. However, plants top-dressed with more than 5g N per plant did not show significant increase in fresh weight. The best results were from those plants top-dressed with 5g N per plant.These gave an average of 44.5 tons fresh weight per hectare for both experiments.

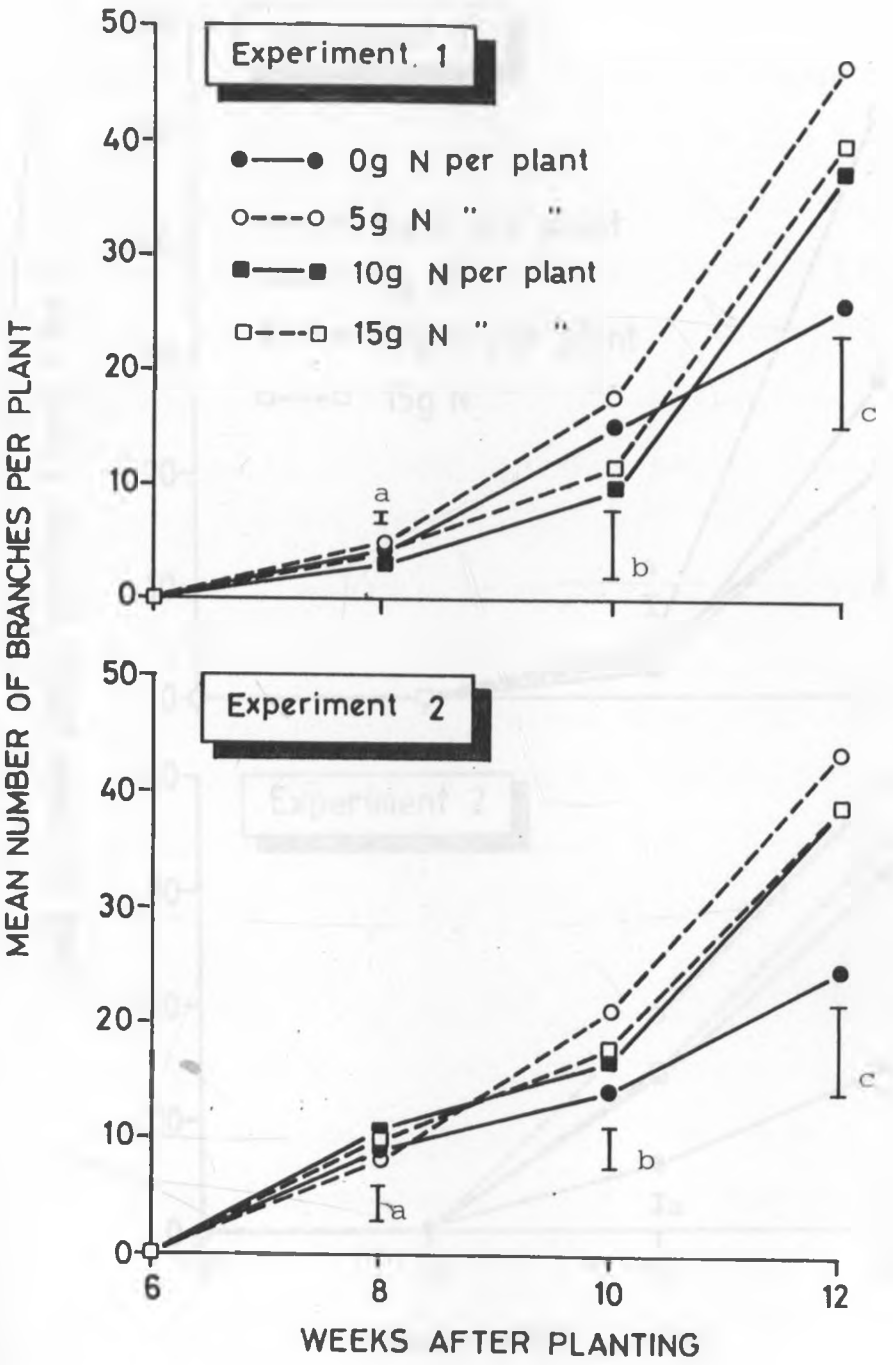

Fig. 3. : The effect of nitrogen rates on the number of branches of Solanum nigrum L. per plant.

^{abc}Least significnat difference (LSD) bars

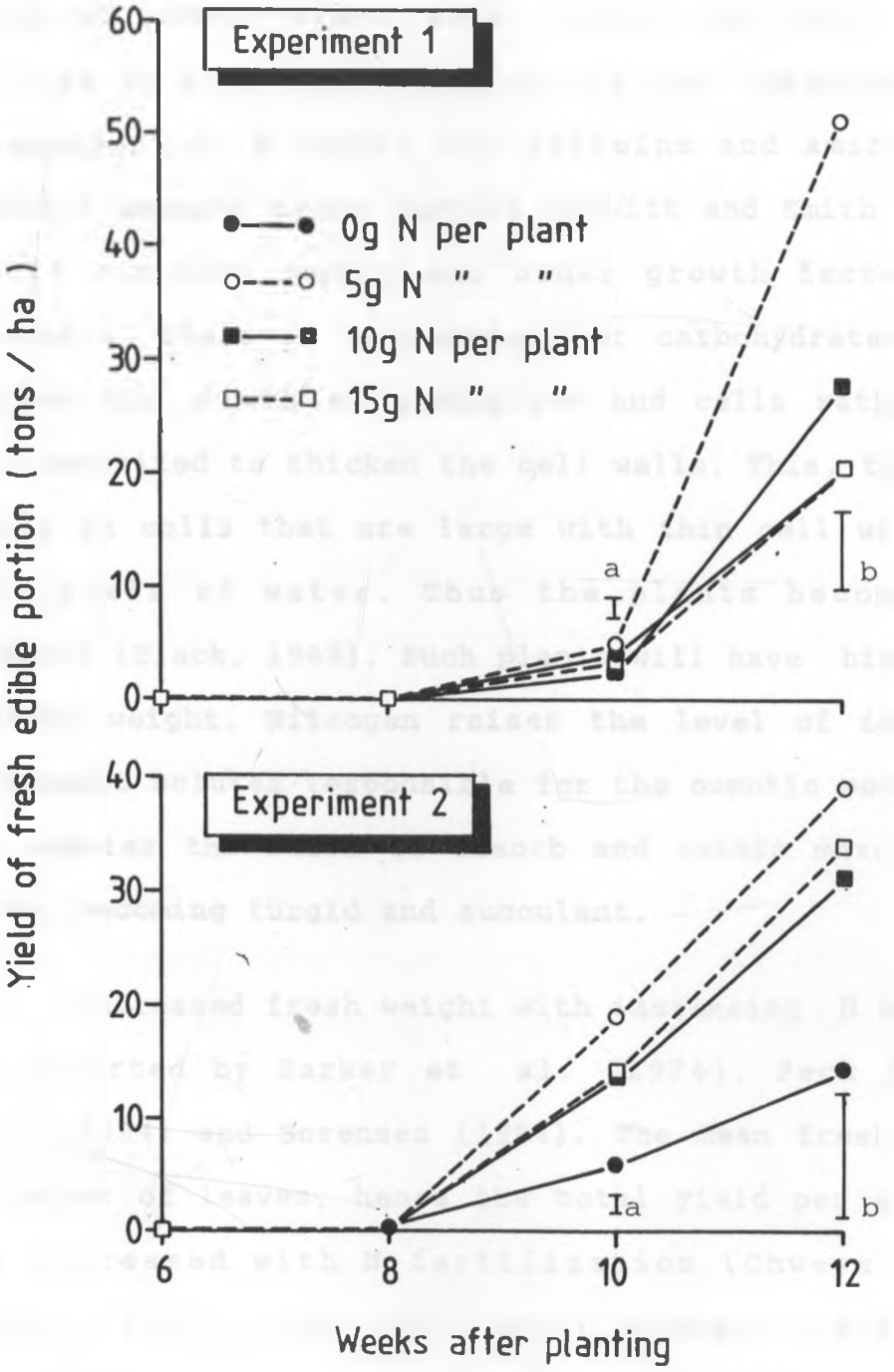

Fig. 4. The effect of nitrogen rates on the yield of S. nigrum.

ab Least significant difference (LSD) bars

Nitrogen promotes vegetative growth through induced leaf production and increased leaf surface area throughout growth (Black, 1968; Hewitt and Smith, 1975). Leaf size is primarily determined by cell expansion which is dependent on N supply for proteins and amino acid synthesis amongst other factors (Hewitt and Smith, 1975). Also if nitrogen supply and other growth factors are favourable, there is a tendency for carbohydrates to be utilized for growth of protoplasm and cells rather than being deposited to thicken the cell walls. This, therefore results in cells that are large with thin cell walls and high levels of water. Thus the plants become very succulent (Black, 1968). Such plants will have high level of fresh weight. Nitrogen raises the level of inorganic and organic solutes responsible for the osmotic potential. This enables the cells to absorb and retain more water, thereby becoming turgid and succulent.

Increased fresh weight with increasing N has also been reported by Barker et al. (1974), Peck (1974), Chweya (1984) and Sorensen (1984). The mean fresh weight and number of leaves, hence the total yield per plant in kale increased with N fertilization (Chweya, 1984; Kanampiu, 1987). Peck (1974) and Sorensen (1984), both working with cabbage, and Barker et al. (1974) who worked with spinach reported significant increases in total yields with increased N supply. Mengel (1979) attributed

increase in fresh weight due to N application to improved water status of plants.

3.2 Effect of Nitrogen on the Chemical Composition

The proximate chemical composition is shown in Table 3. The effect of nitrogen rates on the percentage dry matter was significant. Plants that were not top-dressed with N had significantly higher dry matter contents in both experiments than those plants that were top-dressed. Increased N application tended to lower the dry matter content. The dry matter contents were within the ranges previously reported (FAO, 1968; Oke, 1968; Sreeramalu, 1982).

The decline in dry matter with increased N agrees with the finding of Peck (1974) and Sorensen (1984). Sorensen (1984) reported that in cabbage, dry matter contents dropped from 11.8% at 0 kg N/ha to 10.2% at 600 kg N/ha. It has been reported that N enhances the succulency of plant tissues thus lowering the dry matter content (Black, 1968).

Top-dressing with N did not significantly affect the total ash content in the first experiment Table 3 shows that in the second experiment, plants from the control plots had significantly lower levels of total ash than those plants that were top-dressed. However, among the plants that were top-dressed with N, the differences

Table 3: Effect of nitrogen rates on proximate chemical composition of Solanum nigrum L. leaves (g/100g dry matter)[a].

Nitrogen rates (g N/plant)	First experiment					Second experiment				
	Dry % matter	Total ash	Crude fat	Crude protein	Crude fibre	Dry % matter	Total ash	Crude fat	Crude protein	Crude fibre
0	12.33^{d}	16.72^{b}	4.99^{b}	16.04^{b}	18.51^{c}	11.99^{d}	16.52^{b}	6.26^{d}	18.73^{b}	20.32^{b}
	(0.34)	(1.34)	(0.12)	(0.69)	(1.71)	(0.72)	(0.76)	(0.34)	(0.66)	(0.10)
5	9.17^{b}	18.51^{b}	6.81^{d}	30.80^{c}	16.25^{bc}	8.37^{c}	21.96^{c}	5.08^{b}	28.75^{c}	20.90^{b}
	(0.23)	(1.30)	(0.08)	(0.32)	(0.56)	(1.01)	(0.16)	(0.40)	(0.47)	(1.02)
10	10.06^{c}	17.13^{b}	6.51^{c}	32.04^{d}	14.94^{b}	8.37^{c}	22.73^{c}	5.21^{bc}	29.47^{c}	20.78^{b}
	(0.30)	(1.37)	(0.56)	(0.40)	(1.05)	(1.09)	(0.40)	(0.18)	(0.69)	(1.01)
15	10.06^{c}	16.59^{b}	6.53^{cd}	34.42^{e}	14.55^{b}	8.17^{b}	21.94^{c}	5.68^{c}	31.10^{d}	19.09^{b}
	(0.13)	(0.77)	(0.50)	(0.29)	(0.27)	(0.07)	(0.60)	(0.17)	(0.25)	(0.28)

[a]Mean (standard deviation), N = 3.

bcde Means in the same column followed by the same superscript are not significantly different by protected LSD test ($P \leq 0.05$).

Table 3: Effect of nitrogen rates on proximate chemical composition of Solanum nigrum L. leaves (g/100g dry matter)[a].

Nitrogen rates (g N/plant)	First experiment: Dry % matter	Total ash	Crude fat	Crude protein	Crude fibre	Second experiment: Dry % matter	Total ash	Crude fat	Crude protein	Crude fibre
0	12.33^{d}	16.72^{b}	4.99^{b}	16.04^{b}	18.51^{c}	11.99^{d}	16.52^{b}	6.26^{d}	18.73^{b}	20.32^{b}
	(0.34)	(1.34)	(0.12)	(0.69)	(1.71)	(0.72)	(0.76)	(0.34)	(0.66)	(0.10)
5	9.17^{b}	18.51^{b}	6.81^{d}	30.80^{c}	16.25^{bc}	8.37^{c}	21.96^{c}	5.08^{b}	28.75^{c}	20.90^{b}
	(0.23)	(1.30)	(0.08)	(0.32)	(0.56)	(1.01)	(0.16)	(0.40)	(0.47)	(1.02)
10	10.06^{c}	17.13^{b}	6.51^{c}	32.04^{d}	14.94^{b}	8.37^{c}	22.73^{c}	5.21^{bc}	29.47^{c}	20.78^{b}
	(0.30)	(1.37)	(0.56)	(0.40)	(1.05)	(1.09)	(0.40)	(0.18)	(0.69)	(1.01)
15	10.06^{c}	16.59^{b}	6.53^{cd}	34.42^{e}	14.55^{b}	8.17^{b}	21.94^{c}	5.68^{c}	31.10^{d}	19.09^{b}
	(0.13)	(0.77)	(0.50)	(0.29)	(0.27)	(0.07)	(0.60)	(0.17)	(0.25)	(0.28)

[a]Mean (standard deviation), N = 3.

bcde Means in the same column followed by the same superscript are not significantly different by protected LSD test ($P \leq 0.05$).

were not significant, though there was a tendency for the total ash content to decrease with increased N application. The contents of total ash in the present study were much higher than those so far reported for *S. nigrum* and related species of leafy vegetables (Oke, 1968; Ifon and Bassir, 1979). Oke (1968) reported a range of 12.5 -18.0%, while Ifon and Bassir (1979) reported a value of 18.6% total ash for *S. nigrum*. The present study gives a range of 16.52% - 22.73% total ash. . Nitrogen application did not affect the levels of total ash in plants significantly probably because plant mineral uptake depends on the level of minerals in the soil (Mengel, 1979). It is possible, however, that levels of specific minerals were affected by N application.

Table 3 shows the influence of nitrogen rates on the crude fat. As the N rate increased crude fat significantly increased. Crude fat was significantly higher in plants that had been top-dressed with N than in those plants that were not top-dressed. In both experiments, the plants that were top-dressed with 5g N per plant had the highest levels of crude fat.

The levels of crude fat were within the ranges so far reported for *S. nigrum* and other leafy vegetables commonly consumed in Africa (Oke, 1968; Ifon and Bossir, 1979; Sreeramalu, 1982; Imungi and Potter, 1983). Sreeramalu (1982) working with *S. nigrum* and Ifon and

Bassir (1979) who worked with other *Solanum spp* found a range of between 4.4% and 8.1% crude fat, while Imungi and Potter (1983) reported a value of 2.6% fat on dry matter basis in cowpeas leaves. Crude fat which includes all lipids, chlorophyll, carotenes and all other fat soluble material tends to be enhanced by N application (Mengel, 1979). Nitrogen fertilization coupled with higher light intensity increases the production of chloroplasts which contain thylakoid membranes responsible for fatty acids synthesis (Mengel, 1979).

Nitrogen application had significant effects on the crude protein as shown in Table 3. Levels of crude protein increased with increasing rates of N. Plants top-dressed with N had significantly more crude protein than those from the control plots. The crude protein levels compared well with the ranges previously reported for other leafy vegetables grown in Africa (Imbamba, 1973; Ifon and Bassir, 1979; Sreeramalu, 1982). Imbamba (1973) who worked with some local Kenyan leafy vegetables found a leaf protein content of between 20-30% on dry matter basis, with *S. nigrum* having an average value of 29.3%.

Nitrogen is a major component of amino acids and proteins, hence adequate supply enhances protein synthesis (Mengel, 1979). Plants grown in soils deficient in N will have a limited amount of proteins (Hehl and Mengel, 1972; Blanny and Chapman, 1974). When N supply to a plant is

not limited, more photosynthates are preferentially used for protein than for carbohydrate synthesis (Mengel, 1979).

The crude fibre decreased significantly with N rates in the first experiment. Table 3 shows that the crude fibre contents of the plants top-dressed with N were significantly higher than for those plants that were not top-dressed. However, the differences in crude fibre contents among the top-dressed plants were not significant. In the second experiment there were no significant differences among the crude fibre contents of the treatments but there was a general tendency for the crude fibre content to decrease with increasing N rates, with the plants treated with 15 g N per plant having the lowest crude fibre content. The crude fibre contents were within the ranges reported previously for *S. nigrum*, other *Solanum* spp and related leafy vegetables (Oke, 1968; Ifon and Bassir, 1979; Sreeramalu, 1982). *Solanum nigrum* has been reported to have an average crude fibre content of 8.3% (Sreeramalu, 1982), while Ifon and Bassir (1979) reported a range of 8.5 - 20.9% crude fibre on dry weight basis for other common leafy vegetables consumed in Africa.

The decline in crude fibre content with increased N rates can probably be attributed to the fact that plants tended to use more of their photosynthates on protein

rather than carbohydrate synthesis.

It has been reported that if the supply of N and other growth factors are favourable, plants tend to utilize more carbohydrates for the formation of protoplasm than for thickening of cell walls (Black, 1968). Young plant tissues will tend to use more photosynthates for protein synthesis than for carbohydrates sythensis (Mengel, 1979). However, old plant tissues are rich in carbohydrates especially cellulose, lignins and pectins (Hehl and Mengel, 1972).

3.3 Effect of Nitrogen on the Contents of Vitamins and Minerals

3.3.1 Carotene

Nitrogen rates had a significant effect on the ß-carotene content of leaves. Table 4 shows that the levels of ß-carotene tended to increase with increasing N rates. Plants that were not top-dressed with N had significantly lower content of ß-carotene than those that were top-dressed. In the first experiment, plants top-dressed with 10g N per plant had lower levels of ß-carotene than those either top-dressed with 5 g N or 15g N per plant. In the second experiment, plants top-dressed with more than 10g N per plant showed a marked decline in the ß-carotene contents.

The levels of ß-carotene obtained were within the ranges reported for most tropical leafy vegetables (Oome

Table 4: The effect of nitrogen on the contents of vitamins and minerals in *Solanum nigrum* leaves (per 100g solids)[a]

	First Experiment								Second Experiment							
Nitrogen rates(g) per plant	B-carotene (mg)	Ascorbic (mg)	K (g)	Ca (g)	Mg (mg)	Fe (mg)	Na (mg)	Zn (mg)	B-carotene (mg)	Ascorbic (mg)	K (g)	Ca (g)	Mg (mg)	Fe (mg)	Na (mg)	Zn (mg)
0	163^{b}	191^{b}	5.19^{b}	2.26^{b}	351^{bc}	56.8^{b}	47^{b}	6.30^{b}	24^{b}	884^{bc}	4.85^{b}	1.08^{b}	395	33.7^{b}	47^{b}	7.10^{b}
	(4.99)	(19.16)	(0.07)	(0.13)	(8.73)	(22.64)	(5.44)	(0.33)	(5.44)	(55.90)	(0.02)	(0.00)	(11.90)	(5.19)	(5.66)	(0.41)
5	103^{c}	435^{d}	6.49^{c}	2.48^{d}	390^{d}	24.9^{b}	47^{b}	6.70^{b}	88^{c}	1123^{d}	6.88^{c}	1.50^{d}	405^{b}	34.7^{b}	43^{b}	6.5^{b}
	(19.47)	(29.96)	(0.32)	(0.08)	(15.94)	(5.47)	(9.43)	(0.37)	(16.35)	(47.86)	(0.18)	(0.04)	(6.60)	(1.44)	(9.39)	(0.90)
10	93^{bc}	301^{d}	5.73^{b}	2.44^{cd}	372^{cd}	42.6^{bc}	52^{b}	7.30^{b}	99^{c}	889^{c}	7.06^{c}	1.34^{c}	394^{b}	54.2^{b}	36^{b}	7.4^{b}
	(2.41)	(76.46)	(0.27)	(0.01)	(5.32)	(5.07)	(6.34)	(0.66)	(8.73)	(68.67)	(0.17)	(0.06)	(3.74)	(7.64)	(4.97)	(0.62)
15	110^{c}	246^{c}	5.68^{b}	2.09^{b}	324^{b}	60.2^{c}	47^{b}	6.7^{b}	78^{c}	748^{b}	6.87^{b}	1.36^{c}	388^{b}	54.7^{b}	40^{b}	6.3^{b}
	(16.53)	(5.22)	(0.05)	(0.03)	(14.71)	(9.18)	(1.89)	(0.64)	(12.84)	(14.77)	(0.19)	(0.10)	(2.36)	(23.53)	(4.71)	(0.67)

[a]Mean (standard deviation) where N=3.

bcd Means in the same column followed by the same supescript are not significantly different by protected LSD test ($p < 0.05$).

and Grubben, 1977; Gomez, 1981, 1982). However, the levels of ß-carotene in *S. nigrum* were higher than the levels reported in kale, collards or spinach (Gomez, 1981, 1982). Gomez (1981) reported a carotene content of 72 mg per 100 g dry weight in *S. nigrum* compared to that of 60 mg/100 dry weight in kale. In the present study, the level of ß-carotene on average was 82 mg/100 g dry weight. Increase in N rates tended to increase the ß-carotene contents probably because N is required for the formation of chloroplasts, which are rich in ß-carotene (Mengel, 1979). Habben (1973) and Fritz and Habben (1973) working with carrots and spinach respectively, reported that increased N application raised the levels of vitamin A in the vegetables.

3.3.2 Ascorbic acid

Table 4 shows that the levels of ascorbic acid significantly decreased with increase in N rates. Plants not top-dressed with N had significantly lower levels of ascorbic acid than those that were top-dressed. Top dressing plants with more than 5g N per plant significantly reduced the levels of ascorbic acid in both experiments. However, the mean ascorbic acid contents were much lower during the first than during the second experiment. During the first experiment, the samples had to be stored in the freezer for a couple of weeks to await analysis as the chemical reagents for analysis were not

available at the time of harvesting. This prolonged storage could have been ineffective in completely conserving the vitamin in the vegetables (Ottosson, 1979). In brocolli and lettuce, it has been reported that a 50% loss of ascorbic acid can occur within 7 days when stored at 2°C (Ottosson, 1979).

The ascorbic acid contents were comparable with the level of 144 mg/100 g reported by Gomez (1982). Other local leafy vegetables were also shown to have comparable values of the vitamin (Gomez. 1982). The ascorbic acid contents tended to decrease with increasing nitrogen rates. The primary precursor in the synthesis of ascorbic acid is glucose (Mengel, 1979). Therefore, any events leading to reduced carbohydrate synthesis will result in the reduction of the synthesis of the vitamin. When the nitrogen supply is high, more photosynthates are used for protein than for carbohydrate synthesis (Werner, 1957). This could lead to a decline in the amount of ascorbic acid synthesis as was observed in spinach and cabbage by Fritz and Habben (1973), and Sorensen (1984) respectively.

3.3.3 Minerals

Table 4 gives the levels of minerals for varying rates of nitrogen. Potassium and calcium contents varied significantly with the levels of N fertilizer in both experiments. Plants treated with N had higher levels of potassium and calcium than the control.

However, top-dressing plants with more than 5g N per plant tended to reduce the levels of both minerals in the leaves. In the first experiment, magnesium contents were affected by N-application with plants top-dressed with 5g N per plant giving the highest values that were significantly different from values for the rest of the treatments. In the second experiment there was no significant effect of N rates on the levels of magnesium. Sodium, iron and zinc levels were not significantly affected by N-application.

The levels of calcium and iron were much higher than those previously reported (Epenhuijsen, 1974; Gomez, 1982). *Solanum nigrum* has been reported to have 12 mg iron and 291mg calcium per 100g fresh weight (Gomez, 1982). In the present study, the levels were found to range between 2.09 to 2.48g for calcium and 21.9 to 60.2mg for iron per 100g dry weight, respectively. The vegetable is therefore, a very good source of the minerals potassium, magnesium and zinc compared to other leafy vegetables consumed in Africa (Ifon and Bassir, 1980). The low levels of sodium in the vegetable is a dietetic advantage in the wake of an increasing concern over high levels of the element in foods.

Although the vegetable is a good source of minerals, their availability could be impaired adversely

by the presence of oxalates and cynanogenic substances which are capable of complexing with minerals like calcium and iron to form insoluble complexes (Ifon and Bassir, 1980).

3.4 Effect of Nitrogen Rates on Nitrate Accumulation in the Leaves

Effect of N rates on nitrate-nitrogen (NO_3-N) accumulation in the leaves was significant. Table 5 shows that the NO_3-N content of the leaves increased with increasing rates of Nitrogen. In both experiments, plants that were not top-dressed with N had significantly lower NO_3-N levels than those top-dressed. In the first experiment, NO_3-N accumulation increased with increasing N rates, though the increase was not significant. However, in the second experiment, differences between NO_3-N accumulation in the leaves of all treatments were significantly different from each other, except between plants treated with 10 g N and 15 g N per plant.

The accumulation of NO_3-N in plant tissues is not static. It depends on the difference between the amount absorbed and that which is assimilated. At high rates of N more nitrates may be taken up than can be assimilated, resulting in NO_3-N accumulation.

Table 5. Effect of nitrogen rates on nitrate-nitrogen accumulation in leaves of *Solanum nigrum* L.

Nitrogen rates (gN per plant)	Mg Nitrate-nitrogen per 100g dry matter[a]	
	First experiment	Second experiment
0	382.3 (190.5)[b]	225.7 (65.2)[b]
5	2500.0 (123.6)[c]	2456.3 (263.9)[c]
10	2550.0 (324.1)[c]	2920.0 (185.4)[d]
15	2544.0 (472.9)[c]	3120.3 (150.5)[d]

[a]Mean (stand deviation), N = 3

[bcd] Means in the same column followed by the same superscript are not significantly different by protected LSD test ($P \leq 0.05$)

The increase of NO_3-N accumulation in *S. nigrum* due to increased N-application has been reported for other leafy vegetables (Pimpini et al., 1973; Aworh et al., 1980; Kunsch, 1983; Carlsson, 1983; Chweya, 1986; Kanampiu, 1987). Pimpini et al. (1973) working with spinach, red beets, cabbage and cauliflower found that increased N rates caused increase in NO_3-N accumulation in all the vegetables. Carlsson (1983) working with Amaranthus spp. *S.nigrum*, *Chenopodium album* and *Brassica oleracea* var. *acephala*, and Chweya (1986) and Kanampiu (1987) working with kale and collards all noted that increasing N rates enhanced NO_3-N accumulation.

Nitrates are toxic to humans. High levels in dietaries should therefore be avoided. The fatal adult dose is 15 to 70 mg NO_3-N per kg body weight (Lee, 1970). Therefore,from the present study, an adult of 70 kg body weight would need to consume about 0.42 - 1.96 kg of the S. nigrum leaves at ago in order to ingest a fatal dose if all the nitrate was available to the body. It is most unlikely that an adult consumes such an amount of leaves at ago, and, therefore, acute toxicity is unlikely.

3.5 Effect of Nitrogen Rates on Oxalate Contents

The oxalate contents were influenced by N rates significantly. Table 6 shows that in the first experiment,

Table 6. Effect of nitrogen rates on oxalate contents of *Solanum nigrum* L.

Nitrogen rates (gN per plant)	Mg oxalic acid per 100g dry matter [a]			
	First experiment		Second experiment	
0	1281.3	(3.5)[c]	1603.3	(191.2)[b]
5	1265.3	(5.5)[c]	1400.7	(258.4)[b]
10	1208.0	(14.5)[b]	1498.3	(121.9)[b]
15	1108.0	(19.9)[b]	1450.7	(257.7)[b]

[a]Mean (stand deviation), N = 3

[bc]Means in same column followed by the same superscript are not significantly different by protected LSD test ($P \leq 0.05$).

the oxalate contents varied significantly with N rates. However, in the second experiment, there were no significant differences in the levels of oxalate between the plants from control plots and those that were top-dressed with nitrogen. In both experiments, the oxalate contents tended to decrease with increasing N rates. Plants not top-dressed with N produced leaves with higher levels of oxalate than those that were top-dressed. The mean oxalate contents of the leaves from the first experiment were slightly lower than those from the second experiment.

The oxalate contents were within the values reported for *S. nigrum* and other related vegetables (Buck *et al.*, 1966; Oke, 1968; Pingle and Ramasastri, 1978b). Buck *et al.* (1966) working with *Amaranthus retroflexus*, *Chenopodium album* and *S. nigrum* reported an oxalate content of between 12.00% and 30.75% oxalic acid on dry weight basis. Oke (1968) reported a value of 5% oxalic acid in *S. inacum* and *S. macrocarpon*, while Pingle and Ramasastri (1978b) found a value of 1402 mg oxalic acid/100 dry weight in *Amaranthus spp.*. In the present study, the levels of oxalate varied between the two seasons.

The decrease in oxalate contents of the leaves with increased N rates could be due to the competition between proteins and oxalate for the photosynthates that provide the carbon-skeleton for their synthesis.

Probably when N supply is optimal, more protein than oxalate synthesis is favoured (Carlsson, 1983; Abd-El-Hadi et al., 1985). In spinach the total oxalate content in leaves was reported to decrease in mature plants as N rates increased from 0 to 150 mg N per kg of soil (Abd-El-Hadi et al., 1985).

High concentrations of oxalate may be of great nutritional disadvantage to both man and animals. High levels of oxalate in the diet could interfere with calcium absorption, resulting in calcium deficiency even in calcium adequate dietaries. However, as Tables 4 and 6 show, the levels of calcium greatly exceeds that of the oxalates in *S. nigrum*.

3.6. Effect of Nitrogen Rates on Phenolic Content

Nitrogen rates had a significant effect on the total phenolics contents in the leaves. Table 7 shows that in the first experiment, the total phenolics contents of the leaves from plants not top-dressed with N were significantly lower than those from plants that were top-dressed with either 5g N or 10 g N per plant. However, in the second experiment, there was a tendency for the phenolics content to decrease with increase in N rates, though the decline was not significant. This implies that N does not influence the synthesis of phenolics.

Table 7. Effect of nitrogen rates on phenolic contents [a] of *Solanum nigrum* L.

Nitrogen rates (g N per plant)	Phenolic (as equivalent mg gallic acid/100g dry matter)	
	First experiment	Second experiment
0	888 (184.7)[b]	865 (156.7)[b]
5	1027 (88.7)[bc]	718 (198.5)[b]
10	1010 (160.1)[d]	817 (84.7)[b]
15	910 (195.6)[b]	830 (97.9)[b]

[a]Mean (standard deviation), N = 3

[bc]Means in the same column followed by the same superscript are not significantly different by protected LSD test ($P \leq 0.05$).

Phenolics are synthesised by plants as secondary metabolites as they are not directly involved in metabolic pathways for growth and reproduction (Butler, 1988).

Some naturally occuring phenolics if present in the diet can render dietary protein unavailable, while some like the coumarins and safrole can be potent toxins (Singleton, 1981). However, the risk of serious toxic effects from phenolics commonly present naturally in normal foods is vanishingly small (Singleton, 1981; Butler, 1988).

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH WORK

4.1 Conclusions

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

Increasing N rates, increased significantly the number of leaves, branches and height per plant and also the fresh weight. However, the increments were not significant for plants top-dressed with higher rates of N than 5g per plant. Since *Solanum nigrum* is valued in terms of both weight of edible portions and number of leaves, I would recommend to farmers from areas with soils and climatic conditions similar to those of Kabete that it would be uneconomical to apply more than 5g N per plant.

Whereas fresh weight of plants top-dressed with N increased significantly the dry matter and the crude fibre contents were significantly decreased. Top-dressing plants with N did not significantly affect the total ash content. The levels of crude fat and protein increased significantly as the N rates increased from 5 to 15g N per plant. Therefore, top-dressing *S. nigrum* with 5g of N per plant in areas and of similar soil and climate like Kabete, would give reasonable growth, yield and help to enhance the nutrient chemical composition of leaves.

While the levels of ascorbic acid in leaves tended to decrease significantly with increased N, the level

of ß-carotene increased significantly. Plants top-dressed with 5g N per plant gave the best levels of minerals, potassium, calcium and magnesium. However, the level of iron, sodium and zinc were not significantly influenced by N application. Therefore, plants top-dressed with 5g N per plant would ideally provide good amounts of pro-vitamin A and ascorbic acid as well as minerals like potassium, calcium, zinc, iron and magnesium.

The level of yield and nutrients in *S. nigrum* were improved by top-dressing plants with N,whereas Nitrate-nitrogen accumulation significantly increased with N rates from 5 to 15g N per plant.Oxalates and Phenolics contents were not influenced significantly by top-dressing with N. Hence, it appears that application of 5g N per plant would be a better rate for farmers growing *S. nigrum*.

4.2 Suggestions for Further Research Work

In the present study, top-dressing *S. nigrum* L. with more than 5g N per plant did not give significant increase in growth and yield whereas NO_3-N accumulation increased significantly. However, it is not clear how growth, yield and NO_3-N accumulation would change between 0-5g N per plant at a spacing of 30 x 30cm. Thus, there is a need for further investigation to that effect.

The effect of N application on the contents of oxalates and phenolics in this study was not very clear therefore, further research work would help to establish a clearer pattern. Since S. nigrum is consumed by humans when cooked, it would be interesting to investigate the effect of cooking on the concentration of various nutrients and anti-nutrients like NO_3-N, oxalates and phenolics in the edible portion.

From the present, N application was only applied once at only a spacing of 30 x 30cm and harvesting was only done once at twelve weeks after planting. Thus, there is a need to carry out further research work on the effect of split application of N using other sources of N like Ammonium sulphate and at other spacings other than 30 x 30cm on the growth, yield and nutrient of S. nigrum.

Similarly, time of harvesting should be varied over the growth period in order to investigate how the level of nutrients and anti-nutrients vary with plant age. In the present study only S. nigrum L was investigated and at one ecological zone, thus it would be interesting to try out the species S. pseudonigrum and S. eldoretti and at various ecological zones in order to see the effect of N application on growth, yield and nutritive quality.

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Appendix 1: Analysis of variance (ANOVA) for the effect of nitrogen rates on growth and yield of *Solanum nigrum* plants.

Time after planting in weeks	Source of variation	df	Mean Sum of Squares							
			First Experiment				Second experiment			
			Total no. of leaves	Plant height	Total No. of branches	Fresh weight	Total No. of leaves	Plant height	Total No. of branches	Fresh weight
8	Blocks (B)	2	1.75	0.97	0.08	0.00041	175.83	2.21	2.58	0.0012
	Nitrogen rates (NR	3	0.56	0.13	0.97	0.0018	376.53	3.09	4.67	0.00031
	Error (E)	6	2.31	1.97	0.64	0.0037	531.09	2.08	2.25	0.00075
10	Blocks (B)	3	20.59	0.86	0.58	0.54	2451.09	26.42	1.58	0.95
	Nitrogen rates (NR)	3	1563.89*	21.04*	30.56	3.36	20875.22**	37.97	30.00*	84.14**
	Error (E)	6	315.14	3.55	10.14	0.84	1451.64	10.13	3.25	0.61
12	Blocks (B)	2	2720.25*	2.03	25.09	13.65	5210.60	85.37	2.54	28.68
	Nitrogen rates (NR)	3	19549.67**	159.06**	215.22**	629.41**	20538.34**	366.82**	191.64**	335.38**
	Error	6	456.58	10.35	16.31	15.64	1739.91	24.95	14.47	32.55

*, ** = F-ratio significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. The rest are not significant (n.s.).

Appendix 2: ANOVA for the effect of nitrogen rates on promimate chemical composition of Solanum nigrum plants.

Source of	df	Mean sum of squares									
		First experiment					Second experiment				
		Dry matter (%)	Total ash	Crude fat	Crude protein	Crude fibre	Dry matter (%)	Total ash	Crude fat	Crude protein	Crude fibre
Replication (R)	2	$0.08^{n.s}$	$0.21^{n.s}$	$0.11^{n.s}$	$0.24^{n.s}$	$0.84^{n.s}$	$0.36^{n.s}$	$0.01^{n.s}$	0.32*	$0.07^{n.s}$	$1.13^{n.s}$
Nitrogen Rate (NR)	3	4.59**	$2,31^{n.s}$	2.04*	207.97*	9.57*	10.23*	24.63**	0.87**	94.33**	$2.47^{n.s}$
Error (E)	6	0.11	2.78	0.26	0.33	1.92	1.24	0.56	0.06	0.57	1.10

*, ** = F-ratio significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. The rest are not significant (n.s)

Appendix 3: ANOVA for the effect of nitrogen rates on the level of vitamins and minerals of *Solanum nigrum* plants (First experiment).

Source of variation	df	Mean sum of squares							
		β-carotene	Ascorbic acid	K	Ca	Mg	Fe	Na	Zn
Replications (R)	2	25.50	550.35	9605.63	10066.75	231.73	21065.55	117.00	25.08
Nitrogen rates (NR)	3	1286.67	32736.23**	872555.55**	95108.22**	2436.96**	77475.34	16.33	52.31
Error (E)	6	326.17	3364.55	87378.18	8742.31	210.31	25599.23	42.00	45.64

*, ** = F-ratio significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. The rest are not significant (n.s)

Appendix 4: ANOVA for the effect of Nitrogen rates on the level of vitamins and minerals of Solanum nigrum plants (Second experiment).

Source of variation	df	Mean sum of squares							
		β-carotene	Ascorbic acid	K	Ca	Mg	Fe	Na	Zn
Replication (R)	2	221.00	1045.50	13619.25	10459.00	101.33	30618.25	12.25	20.34
Nitrogen rates (NR)	3	3300.67**	72570.33**	3308311.30**	91215.00	138.30	39917.67	67.22	77.00
Error (E)	6	185.33	4826.00	44621.08	3904.53	68.56	21849.25	79.47	83.33

*, ** = F-ratio significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. The rest are not significant (n.s)..

Appendix 5: ANOVA for the effect of Nitrogen rates on Nitrate-Nitrogen Accumulation in *Solanum nigrum* plants.

Source of variation	df	Mean sum of squares	
		First Experiment	Second Experiment
Blocks	2	144907.63	61993.13
Nitrogen rates	3	3465141.40**	5327604.30**
Error	6	78432.63	22969.31

*, ** = F-ratio significant at P $\leq$ 0.05 and $\leq$ 0.01 respectively. The rest are not significant (n.s)

Appendix 6: ANOVA for the effect of Nitrogen rates on Oxalate content of Solanum nigrum plants.

Source of variation	df	Mean sum of squares	
		First Experiment	Second Experiment
Blocks	2	29433.17 n.s	32352.13 n.s
Nitrogen rates	3	20435.04 n.s	22429.11 n.s
Error	6	48847.66	50739.40

n.s. = F-ratio not significant at $P \leq 0.05$ and $P \leq 0.01$.

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Appendix 7: ANOVA for the effect of Nitrogen rates on Phenolic content of Solanum nigrum plants.

Source of variation	df	Mean of squares	
		First Experiment	Second Experiment
Blocks	2	$24773.75^{n.s}$	$44968.60^{n.s}$
Nitrogen rates	3	$241339.44^{n.s}$	$15899.68^{n.s}$
Error	6	61900.86	11904.91

n.s = F-ratio not significant at $P \leq 0.05$ and $P \leq 0.01$.