EFFECT OF NITROGEN APPLICATION AND PLANT AGE ON EDIBLE LEAF YIELD AND QUALITY OF BLACK NIGHTSHADE (*Solanum nigrum L.*) PLANTS.

ARNOLD MATHEW OPIYO.

A THESIS SUBMITTED IN PARTIAL FULFILMENT

FOR THE DEGREE OF

MASTER OF SCIENCE

IN

HORTICULTURE

OF THE

UNIVERSITY OF NAIROBI

DEPARTMENT OF CROP SCIENCE.

Dedication

I dedicate this dissertation to my parents John and Cornelia who have had to endure the burden of seeing me through school.

It is also dedicated to my daughters Linda and Laurine, who have had to endure the anxiety of missing fatherly love at such early ages during the period of my study.

Declaration.

I Arnold Mathew Opiyo do hereby declare that the work contained in this dissertation is my original work; and which has not been presented for a degree in any other University.

30/08/2000

Arnold Mathew Opiyo. Date.

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

28/09/2000

Prof. J.K. Imungi. Date.

28/09/2000

Dr. S.I. Shibairo Date.

Acknowledgements.

I sincerely appreciate the enormous support from various sources which has resulted into the completion of this monograph. In particular I am extremely grateful to my supervisors, Prof. J.A. Chweya and Prof. J.K. Imungi for their diligent guidance and dedication to my success.

I am indebted to Egerton University (especially Prof. F.M. Itulya and Prof. C.A. Onyango) for the scholarship award. I am grateful to the Chairman Department of Horticulture - Egerton University (Dr. G.O. Tunya) for allowing me the use of Departmental facilities for my research. Caroline Miyunga (Dept. Of Hort.) provided secretarial services necessary to assemble the final document. Mr. Meso (Dept. Of Agronomy) provided technical advice and assistance in laboratory analysis.

This research would not have been successfully concluded without the assistance of Gerald Otieno. I am indeed grateful for his material and moral support throughout the period of my studies at Nairobi University. I will always be indebted to his magnanimity. Finally I would like to thank my daughters Linda and Laurine. Young as they are, they were always a source of inspiration and provided me with the tranquillity needed for such an undertaking.

CONTENTS **Page No.**

LIST OF TABLES.

Page No.

LIST OF FIGURES.

LIST OF APPENDICES.

Page No.

ABSTRACT:

Two experiments were carried out to investigate the effects of nitrogen application rates and plant age on the edible leaf yield and nutritive quality of black nightshade *(Solanum nigrum L.)* plants. The first experiment was between February and June; and the second between July and November 1995, at Egerton University Njoro, Kenya. Nitrogen rates were 0, 26, 52, 78 and 104 Kg N / ha; applied either as one, two or three split applications. The experiment had 15 treatments arranged in a split plot design, replicated thrice. Nitrogen rates constituted the main plots, while the application methods constituted the sub - plots. Seedlings were transplanted when 8 weeks old.

Edible shoots were harvested fortnightly starting from 8 weeks after transplanting, and continued up to 20 weeks after transplanting. Edible leaf yield increased with increasing nitrogen application rates. Increase in yield was not significant in season 1, but was significant in season 2. Nitrogen at a rate of 104 kg N / ha. gave the highest yield (15 t/ha.) in season 2, but it was not significantly different from application rate of 52 kg N / ha. (14.8 t/ha.) and 78 kg N / ha. (14.8 t/ha.). Leaf yield increased significantly with increasing plant age. Yield increased up to 14 weeks (2.4 t/ha.) and 18 weeks (2.7 t/ha.) after transplanting in season 1 and season 2 respectively; thereafter yields declined with successive harvests.

Harvested leaves were dried, and analyzed for oxalate and phenolic contents. Plant age significantly affected oxalate content of the leaves during both seasons. Oxalate

content decreased with increasing plant age from 611.0 mg/100g 8 weeks after transplanting to 480.7 mg/100g 20 weeks after transplanting, in season 1. In season 2 the lowest oxalate content of 652.7 mg/100mg was recorded during the first harvest, i.e. 8 weeks after transplanting. At the last harvest (20 weeks after transplanting) the oxalate content was 707.1 mg/100g. The highest oxalate content during season 2 was 938.5 mg/100g, recorded 12 weeks after transplanting. Plant age had no significant effect on phenolic content in season 1; but its effect in season 2 was significant. The lowest phenolic content in season 2 (1153 mg/100g) was observed 16 weeks after transplanting; while the highest content (1507 mg/100g) was recorded at 20 weeks after transplanting.

Tthis study shows that nitrogen application at 52 kg N/ha. applied in a single dose is the most economical. It is also evident that nitrogen fertilizer does not increase oxalate and phenolic contents in *Solanum nigrum* to harmful levels.

1. INTRODUCTION:

Solanum nigrum, commonly called Black nightshade, is one of the most popular traditional leafy vegetables in Kenya. All over the tropics, but most frequently in Africa, *Solanum nigrum* is used as a leaf vegetable. According to Oomen and Grubben (1978), it is normally gathered from the wild vegetation although it is cultivated in some areas.

Leafy vegetables are prepared for consumption in the tropics mainly by boiling only, after which they may be added to soups and stews to increase their taste. Leafy vegetables may be of high nutritional value. According to Chweya (1993b), these vegetables may supply much, if not most, of the populations' required vitamins (especially A, Bs and C), minerals, fibres, carbohydrates and proteins.

In Kenya, traditional green leafy vegetables, such as *Solanum nigrum, Corchorus olitorius, Gynandropsis gynandra and Crotolaria brevidens* constitute important items of diet for the common rural people. This is because they are cheap and readily available compared to cabbage, kales and spinach (Onyango, 1992). In most cases, they are either gathered from the wild or are semi - cultivated.

Usage of traditional vegetables in the tropics has greatly declined over the years. According to Fenwick *et al*., (1990), one of the major reasons for this decline is the belief that the bitter tasting species are poisonous. Despite that, indigenous vegetables are more adapted to local climatic conditions, and to pest and disease attacks than exotic vegetables which may be susceptible (Chweya, 1985). In Kenya, it is estimated that there could be up to 800 species of traditional food plants many of which are traditionally used as food (Maundu, 1993). Mtotomwema, (1987a), reported that 46 species have been taxonomically identified as edible wild leafy vegetables. Unfortunately very little systematic study of these potential crops has been done. Mtotomwema (1987b), also reported the possible presence of undesirable chemical principles in these potential crops. A neglect on the analysis of these indigenous leafy vegetables for the unknown principles may lead to consumption of toxic substances by populations that use the vegetables.

A large variety of indigenous vegetable crops are traditionally consumed in Western Kenya. According to Opole *et al.*, (1989), these vegetables dominate all the markets in urban and rural areas of Western Kenya, particularly Kisumu, Kakamega, Bungoma, Busia and Siaya districts. In the coastal towns of Tanzania, such as Tanga, Bagamoyo, Dar-es-Salaam, Lindi and Mtwara, wild leafy vegetables far exceed the cultivated ones in the market (Mtotomwema, 1987b).

Many traditional food species are known to possess high nutritional values and can be used to meet our nutritional needs (Ngugi, 1993). They are important sources of the mineral elements needed by the body; and are valuable sources of vitamins. However, while considering the nutritional value of a food plant, effects of anti-nutrients should also be borne in mind.

Analysis by Epenhuijsen (1974), have shown that most tropical greens are much richer than temperate types in proteins and vitamins; despite the fact that they (tropical vegetables) are the most neglected of all crop plants. However, Mtotomwema (1987b) cautions that these wild leafy vegetables apart from their known nutritional values, the sideline effects of the anti - nutrients from these products leave much to be desired. Although Kenyans have traditionally made some use of edible leaves of species growing wild or as weeds, little or no attention has been given to these plants as vegetables and have been neglected by agriculturists (Chweya, 1985).

Although some reports by various authors (Chweya, 1993b; Onyango, 1992; Omta and Fortuin, 1979 and Epenhuijsen, 1974) on yield of *Solanum nigrum* are available, no report is available on the optimum nitrogen application for best yields in *Solanum nigrum* or other indigenous leafy vegetables. So far, no information is available on the effect of split application of nitrogen on yields .It is evident that not much work has been done on factors affecting the yields of *Solanum nigrum*. Schmidt (1971), reported that the application of fertilizers together with other intensive management practices to indegenous tropical species would most likely increase production of leafy vegetables of high nutrient content.

Information and research on the production and quality of the indigenous leafy vegetables is limited. Although leafy vegetables are very rich in minerals, vitamin A and C, not much work has been done on their nutrient requirements (Mathai, 1978). Also hardly any information is available on the undesirable constituents (anti - nutrients) such as solanine, phenolic compounds and oxalate; which are responsible for the astringent taste of the leaves and which may sometimes lead to toxicity effects.

Despite *Solanum nigrum* being a popular vegetable, and in spite of its nutritive properties, very little study has been carried out on it. In order to increase and promote production of *Solanum nigrum* and other traditional leafy vegetables, more information is necessary regarding fertilizer requirements, as well as on the occurrence of anti-nutrients so as to safe - guard against any toxicity which might result from the consumption of these vegetables. Traditional foods moreover have been studied systematically for too short a time to avail sufficient number of literature in the area (Ferrando, 1981). Organized cultivation of *Solanum nigrum* has been hindered by the lack of information on its cultural requirements as well as the existence of anti - nutrients e.g. oxalates and phenolics.

The cost of production of traditional vegetables is lower than that of the exotic vegetables e.g. cabbage. This is because the traditional vegetables are well adapted to local conditions, and require less inputs. The promotion of these vegetables (traditional) could help overcame the problem of household food security; especially in the rural areas. Considering that these vegetables are also quite nutritious, their consumption needs to be encouraged.

With the foregoing in mind, the objectives of this study therefore were to show the effects of;

(i) different nitrogen rates on yield of edible leaves of Black Nightshade plants.

(ii) different nitrogen rates on the Phenolic and Oxalate concentrations in the edible leaves of Black Nightshade plants.

(iii) plant age on the Phenolic and Oxalate concentrations in the leaves of Black Nightshade plants.

Nitrogen is known to promote vegetative growth in plants. In *Solanum nigrum* the edible portion is the leaves. The application of nitrogen should therefore result in higher yields. The application of nitrogen fertilizer in splits should result in more efficient utilization of the nitrogen by plants; hence resulting into higher yields.

Oxalates and phenolics are anti - nutrients which are synthesized by many plants. If present in high concentrations in the plant, they may adversely affect the health of the consumer. The concentration of various compounds in plants may be affected by various factors including plant age. Should it be that the plant age affects the concentration of either oxalates and / or phenolics, in *Solanum nigrum*, then consumption can be at a stage when concentration of the anti - nutrient is at non - toxic levels.

2. LITERATURE REVIEW:

2:1. Background Information on Black Nightshade.

Black nightshade (*Solanum nigrum* L.) belongs to the family Solanaceae. According to Omta and Fortuin (1979), the vegetable type of *Solanum nigrum* L. is hexaploid. In West Java, Fortuin and Omta (1980), identified two types of Black nightshade during a survey; a large edible one and a small bitter one.

Epenhuijsen (1974), describes *Solanum nigrum* as a rather thin - growing plant with slender stems, often purplish and weak; generally falling and becoming semi - prostrate when the plant reaches a height of about 75cm. Leaves are carried more - or - less horizontally. Flower buds are continuously produced on the young shoots. The fruits are pendulous berries in clusters of 4 - 7.

According to Ivens (1971), Black Nightshade is a cosmopolitan weed, found in most tropical and temperate countries. It is widespread throughout East Africa from sea level to at least 2150m altitude. The centre of origin of this plant is West Africa, stretching from Senegal to Nigeria (Tindall, 1986). It is found between Latitude 54^0N and 45^0S, as a common weed in gardens, fields, waste areas and open forests (Holm, *et al.*, 1977).

Black Nightshade is found in many parts of Kenya; and is known by various local names. These names include "Mnavu" (Giriama), "Ndulu" (Kamba), "Osuga" (Luo), "Lisutsa" (Luhya), "Managu" (Kikuyu), "Rinagu" (Kisii); (Kokwaro, 1976). The plant is not drought -resistant; and requires rich soil for good leaf production (Epenhuijsen, 1974). It is much relished by dwellers of large towns in Kenya. In Nairobi for example, loads of the vegetable are brought from Western Kenya (Maundu, 1990).

Solanum nigrum is a well - known household medicine. Several authors have reported on the medicinal properties of this plant. Kokwaro (1976), reported that in Kenya, the leaves are used to treat stomach ulcers, abdominal upsets, boils, swollen glands; whereas seeds are rubbed onto gums of children in case of crooked teeth development. In Europe, it is a remedy for convulsions as well as for treating headaches, ulcers and wounds (Watt, *et al.* 1962). In West Java, it is used against night blindness, tropical sprue and allergy of the skin (Omta and Fortuin, 1979). In Nepal, *Solanum nigrum* leaf poultice is applied to rheumatic joints, whereas extract of berries, leaves and stems are used for skin diseases (Joshi and Edington, 1990).

Solanum nigrum has a high nutritional value. In addition to being rich in most vitamins and minerals, it also has high contents of Beta - carotene and calcium

(Opole *et al.*, 1989). According to Opole *et al.*, (1989), *Solanum nigrum* shoots and flowers are eaten in combination with other plants, or added to soups.

2:2. Yields.

Several factors affect the yield of leafy vegetables. Work by Chweya (1993a) shows that *Solanum nigrum* yield increases with the increase in number of plants per m^2; with an optimum plant density of more than 33 plants / m^2 giving yields of 924g / m^2 (9240 kg / ha.) if harvesting is done fortnightly. He also found that fortnightly harvesting gave significantly higher yields than weekly harvesting. Similar results on harvesting frequency were reported by Onyango (1992). Different authors have reported varying yield potentials of the plant. Omta and Fortuin (1979) have reported a leaf yield of 2 tonnes / hectare which is higher than the production of high yield tomato varieties grown under the same conditions. Tindall, (1986) has reported a possible yield of 100g / plant, which works out to 11 tonnes / hectare. Epenhuijsen, (1974) reported yields of 6 - 8 kg / $5m^2$; (12 - 16 tonnes / hectare). Messiaen (1992) has reported a leaf yield of 20 tonnes / ha. in 60 days.

Best yields of *Solanum nigrum* are obtained during the rainy season, and in soils rich in humus with high levels of nitrogen and phosphorous. Studies by Onyango (1992) showed a general increase in fresh shoot weight (yield) as plant age increased, followed by a decrease in yield after the 7th week, depending on the frequency of harvesting. Amaranth studies by Norman and Sichone (1993), showed that fortnightly harvesting produced lower vegetable yield but of better quality than those harvested every three weeks. The fortnightly harvested vegetables were more leafy with less stems, and less flowering than those harvested every three weeks.

2:3. Nitrogen Application and Plant Growth.

Nitrogen is a nutrient required by plants for the synthesis of amino acids and the building blocks of proteins. Nitrogen promotes vegetative growth of plants through induced leaf production and increased surface area throughout growth (Chweya 1993b). Nitrogen tends to produce succulence, a quality of great importance in many vegetables (Thompson and Kelly, 1972). Plants normally contain 1 to 5% by weight of this nutrient. An adequate supply of nitrogen is associated with vigorous vegetative growth and a dark green colour; while an excess of this nutrient in relation to other nutrients can prolong the growing period and delay crop maturity; a deficiency is exhibited in form of a general chlorosis, especially in older leaves (Tisdale, *et al.*, 1985).

Most leafy vegetable crops have a high demand for nitrogen. As nitrogen is liable to be leached from the surface soil by heavy rain, dressings must be timed correctly to ensure that the crop has nitrogen when it needs it, and yet avoid wastage (Cooke, 1982). According to Webster and Wilson (1980), the practice of splitting dressings by giving some nitrogen at or soon after planting and more later has been found advantageous with some crops, both in reducing leaching and in ensuring the availability of needed nitrogen at critical stages of crop development.

Although studies have shown that in soils of low nitrogen content, a small rate of additional nitrogen fertilizer application commonly gives large yield increases, relatively very little fertilizer is currently used in the developing countries to obtain any substantial yield increase. This is because most small farmers cannot afford fertilizer (Webster and Wilson, 1980). Nitrogen promotes vegetative growth, which in a leafy vegetable gives yield to leaves (Chweya, 1992a). The application of nitrogen fertilizers to soils is one of the most important means by which crop yields can be increased (Noggle and Fritz, 1976). Murage (1990), reported that in *Solanum nigrum* increasing nitrogen application rates resulted in more leaves per plant , hence higher yields.

Nitrogen is prone to leaching from surface soil. Given the large demand for nitrogen by most leafy vegetables, and the economic cost of nitrogen application, the efficiency of nitrogen fertilizer use is important. It is important to apply adequate amount of the fertilizer at a time when it is most beneficial to the plant.

To avoid leaching, it may be beneficial to apply the fertilizer in splits. According to Mathai (1978), the general recommendation for leafy vegetables is to apply 80 - 100 kg N / ha. in splits.

2:4. Anti-Nutrients.

Some foodstuffs contain harmful or anti - nutritive substances naturally. The family Solanaceae, to which *Solanum nigrum* belongs, includes many alkaloid drug plants, such as Atropa, Hyoscyamus, Scopolia and Mandragora (Hawkes, 1990). According to Fenwick *et al.,* (1990) wild species of the genus *Solanum* are frequently very bitter and highly toxic, due to the presence of large amounts of glycoalkaloids. Ngugi (1993), reported that the presence of some anti - nutrients in human food from certain plants contribute to low absorption of other nutrients while some inhibit other physiological activities in the body. For example tannins have a protein - binding capacity and an ability to inhibit enzyme activities. The ability of plant polyphenols of relatively high molecular weight to cause coagulation of proteins is the basis for their use as tanning agents (Berk, 1983). According to Ivens (1971), *Solanum nigrum* toxicity appears to be variable. Unripe fruits are poisonous, sometimes only slightly, at times very much so, especially to children; and cattle have also been affected by grazing on the foliage.

Okutani and Sugiyama (1994), indicated that excessive intake of oxalate interferes with calcium adsorption in the digestive tract. Oxalates may cause possible blocking of the renal function by precipitation of insoluble oxalates, which may lead to death (Driver, 1983). Binding of calcium in vegetables having high oxalic acid content has also been reported by Oke (1968).

2:4:1. Phenolics.

Plants contain a large number of compounds which collectively are referred to as phenolics. Phenolics frequently occur in plants in the form of glycosides coupled with sugar molecules (Noggle and Fritz, 1976). According to Singleton (1981), phenolic substances are naturally present in mostly all plant materials; a few of which are potent toxins while others appear to have desirable physiological effects in diets or as drugs. One group of natural phenolic compounds, the "anthoxanthins", include bitter principles; while "tannins" (plant polyphenols) of relatively high molecular weight, are astringent components of many foods (Berk, 1983).

Phenolic compounds have an aromatic ring that contains various attached substituent groups, such as hydroxyl, carboxyl and methoxyl groups, and often other non - aromatic ring structures (Salisbury and Ross 1984). Although the functions of phenols in plants have been a continuous puzzle, it is clear that one function can be protection via toxicity (Singleton, 1981). Noggle and Fritz (1976), noticed that some of the simple phenols are powerful fungicidal and bactericidal agents, and it has been suggested that they protect the plant from invasion by fungi and bacteria.

Polyphenols occur commonly in many plants. They include safrole, coumarins, anthocyanins, flavanoids and tannins. Astringency is perceived as a burning taste on the tongue. It has been attributed to the presence of tannins and other related pholyphenols (Singleton, 1981; Strumeyer and Malin, 1975). Plant products invariably carry different quantities of phenolic compounds. Although most plant phenols are non - toxic, their potential as toxic compounds, need to be considered if many phenols are combined or excessively high amounts are consumed (Murthy 1989).

Varying reports have been recorded on the effect of nitrogen fertilizer on the synthesis of phenolics. Murage (1990), reported that the application of nitrogen fertilizer did not have effect on the synthesis of phenolics in *Solanum nigrum*. However, Mc.Clure (1979), reported that abundant nitrogen is generally inhibitory to phenolic accumulation.

Astringency which is one of the factors responsible for the low consumption of indigenous vegetables (including *Solanum nigrum*) may be due to the presence of polyphenols. The reduction in the concentration of these polyphenols in edible leaves of *Solanum nigrum*, and other traditional leafy vegetables, could result in increased consumption of these vegetables. Nitrogen fertilizer increases vegetative growth in plants. It is therefore likely that the use of nitrogen fertilizer would increase edible leaf yield in *Solanum nigrum.* It is hence important to establish how the use of nitrogen fertilizers affect the concentration of phenolics in edible leaves of *Solanum nigrum*.

2:4:2. Oxalates.

Oxalic acid is a dibasic acid that is widely distributed among vegetables (Ferrando, 1981). The simple organic acid oxalate accumulates in many plants, especially in the Polygonaceae, Chenopodiaceae and Oxalidaceae; where it occurs in the form of its soluble sodium and potassium salts and insoluble calcium salts or as acid oxalates (Driver 1983). Several studies carried out have reported the effect of oxalates on calcium metabolism. Ferrando (1981), reported that the ill - effects caused by the consumption of oxalic acid are of two kinds: First, it causes calcium deficiency in man, and second, it is toxic to the kidney at higher doses and causes serious lesions. It was observed by Driver (1983), that oxalates affect calcium metabolism possibly by blocking renal function by precipitation of insoluble oxalates in the kidneys. Analysis by Buck *et al.*, (1966) revealed the presence of oxalates in weeds that have caused Perirenal edema; which in swine has been attributed to the ingestion of plants such as Black nightshade. Studies by Oke (1968), showed that if vegetables have a high oxalic acid content, their calcium is not utilized by the organism, but is bound in the digestive tract as calcium oxalate, together with calcium from other foods.

According to Pingle and Ramasastri (1978), green leafy vegetables including *Solanum nigrum* are rich sources of several micro - nutrients such as calcium, iron, and beta - carotene (Table 1.). A number of the commonly consumed green leafy vegetables are however rich in oxalic acid, which reduces calcium availability (Pingle and Ramasastri 1978). Studies by Pingle and Ramasastri (1978), have also shown that oxalates present in the leaves renders the calcium from the rest of the diet unavailable. Libert and Franceschi (1987), cited by Okutani and Sugiyama in 1994, reported that excessive intake of oxalate interferes with calcium adsorption in the digestive tract; and that spinach's high oxalate level is the main problem associated with its use as food. Kitchen, *et al.*, (1964) have reported spinach to contain as much as 15% oxalate expressed as anhydrous oxalic acid on a dry weight basis. Marshall *et al.*, (1967), found that *Amaranthus retroflexus* contains levels of total oxalate up to 30% of dry weight in leaves.

Table 1: Composition of some traditional tropical leaf vegetables (per 100g edible portion).

SPECIES	DRY MATTER (g)	CALORIES KCal	PROTEIN (g)	FIBRE (g)	CALCIUM (mg)	IRON (mg)	CAROTENE (mg)	ASCORBIC (mg)
1.*Amaranthus spp.*	15.2	43	5.2	1.0	340	4.1	7.7	120
2.*Brassica chinensis*	5.8	17	1.7	0.7	100	2.6	2.3	55
3.*Corchorus olitorius*	13.9	43	5.6	1.7	270	7.7	7.9	55
4.*Hibiscus sabdariffa*	13.6	44	1.9	1.3	115	1.5	7.6	35
5.*Nasturtium officinale*	6.2	18	1.7	1.1	115	1.9	1.0	45
6.*Solanum nigrum*	**15.0**	**44**	**4.6**	**1.1**	**215**	**4.2**	**1.7**	**30**
7.*Tetragonia expansa*	8.5	22	2.8	0.8	180	3.8	3.5	25
8.*Vigna unguiculata*	11.6	34	4.2	1.7	110	4.7	2.4	35

Source: Oomen, and Grubben (1978).

Oxalates occur in many leafy vegetables. The oxalic acid content in these leafy vegetables is high; e.g. 200g leaves of Amaranth contain almost 3g oxalates, including 1g in the soluble form (Messiaen, 1992). Messiaen (1992), further reported that in *Solanum nigrum* var. guineense plants contain low contents of pure oxalic acid, or else the oxalic acid is in the combined form as oxalate. Oxalates also occur in *Brassica oleracea* and *Spinacia oleracea* (Carlsson, 1983).

Carlsson (1983), attributed the synthesis of high levels of oxalates in plants to genetic and environmental factors. He also reported that increase in nitrogen fertilizer rates decreased the oxalate content in leafy vegetables. Similar findings have been reported in *Solanum nigrum* by Murage (1990) and Mwafusi (1992).

As much as leafy vegetables are rich in minerals and vitamins, the presence of high levels of oxalates may be a drawback in their increased consumption. There is limited information regarding nitrogen effect on oxalate concentration. No information however is available on how split application of nitrogen would affect the oxalate content in the edible leaves of leafy vegetables, (including *Solanum nigrum*).

2:5. Plant Age and Leaf Yield and Quality.

Studies on *Solanum nigrum* by Onyango (1992), showed that fortnightly harvesting of shoots increased fresh shoot weight with each subsequent harvest until the 7th week, after which yields declined. Older leaves of *Gynandropsis gynandra*, are more bitter than the younger leaves (Waithaka and Chweya, 1991). The fact that concentration of toxic constituents can vary with the stage of growth and growing conditions of the plant, has also been reported by Cooper and Johnson (1988). Okutani and Sugiyama (1994), while carrying out studies on spinach, found that the higher the leaf position from the plant base, the lower the oxalate concentration. Hirooka and Sugiyama (1992), correlated the decrease in oxalate concentration between harvests to the increase in leaf fresh weight between the first and second harvests.

According to Kameno *et al.*, (1991), oxalate concentration increased with increasing plant age in Spinach. While studying five tropical leafy vegetables, including *Solanum aethiopicum* and *Solanum macrocarpon*, Taylor *et al.*, (1983), reported that with increasing age, the P, Na, Zn and Mn contents of the vegetables increased significantly; while that of Fe and Cu decreased.

3. MATERIALS AND METHODS:

For this study two trials were carried out. The first trial was carried out between February and June, and the second one between July and November 1995.

3:1.Experimental Site.

The study was conducted at Egerton University, Njoro; located on co-ordinates 0^0 22' South and 35^0 55' East, at an altitude of 2225m above sea level. The two trials were located at the Horticultural Demonstration Field (Field 3). On the basis of the FAO - UNESCO (1974) classification, the soil in the field is deep, moderately well drained, with slightly acidic top soil. The texture of top soil ranges from clay loam to silty clay loam, and colour varies from dark brown to brown. Texture of sub - soil is dominantly silty clay loam, but colour varies form dark reddish brown to dark yellowish brown (Kinyanjui, 1979).

Soil analysis was carried out in order to ascertain the nitrogen status of the soil, as well as pH. Random soil samples were taken using a soil auger. There were two sampling depths, i.e. 0 -15cm and 15 - 30cm. The samples of the different depths were tested separately. The samples were air - dried by spreading shallowly in a shallow tray, in a well - ventilated part of the laboratory. Soil lumps were gently crushed. The soil was then sieved through a 2mm sieve. A representative sample of the sieved soil was retained by coning and quartering, for analysis. The soil was then analysed for soil pH using the Electrometric method; total nitrogen by Kjedhal method and organic carbon by Walkley - Black method (Page *et al*). The soil test results for the two seasons are given in Table 2.

The rainfall pattern is bimodal, with the long rains falling between March and June and the short rains between October and December. The average annual rainfall is 1000mm per annum. The mean monthly maximum and minimum temperatures range between 26^0 and 8^0C respectively. The rainfall and temperature conditions at the site during the study period are shown in Appendix 1.

Table 2. Soil chemical properties at the experimental site.

	SEASON 1		SEASON 2	
NUTRIENT/REACTION	TOP SOIL	SUB SOIL	TOP SOIL	SUB SOIL
pH WATER	6.32	6.25	6.31	6.36
pH ACID	5.18	5.09	5.34	5.36
TOTAL NITROGEN %	0.16%	0.17%	0.19%	0.21%
CARBON %	1.50%	1.53%	2.07%	2.01%

3:2. Planting Materials.

The seeds for this study were obtained from the University of Nairobi - Crop Science Department. They were raised in a nursery; thereafter the seedlings were transplanted when eight weeks old.

3:3. Treatments.

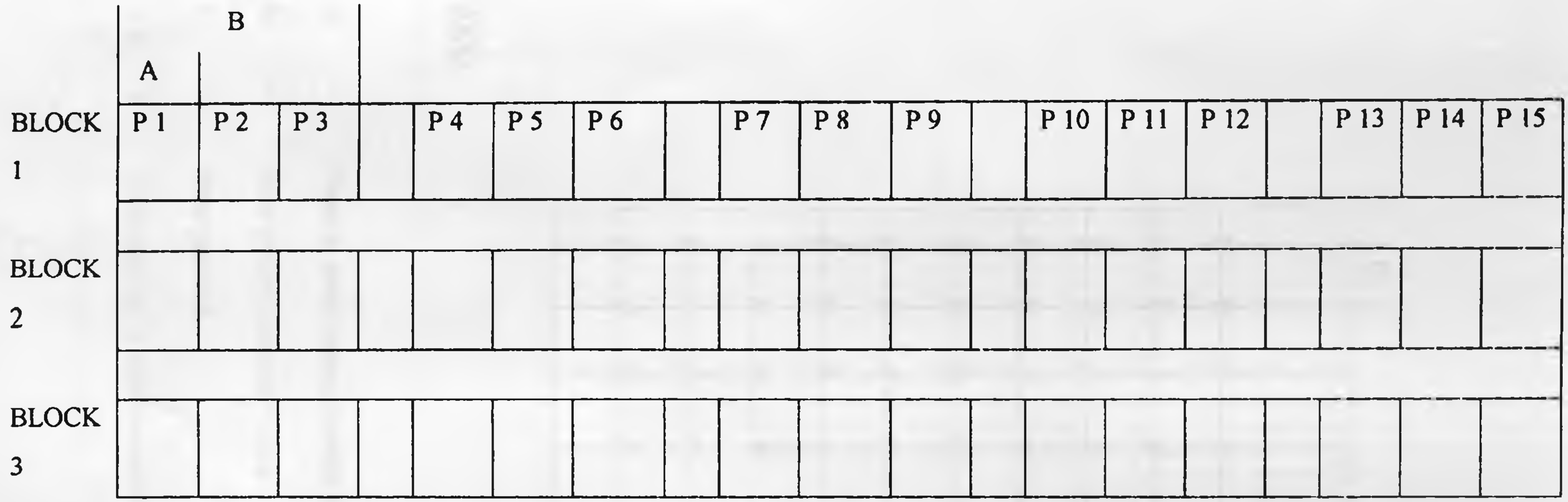
Five nitrogen rates were applied in this study (0, 26, 52, 78 and 104 kg N per hectare). Calcium Ammonium Nitrate fertilizer (26% N) was used as the source of nitrogen. To achieve the desired nitrogen rates, the fertilizer was applied at a rate of 0, 63, 126, 189 and 252 g of C.A.N / plot for each respective nitrogen treatment; which is equivalent to 0, 100, 200, 300 and 400 kg C.A.N / ha. For each of the nitrogen rates three application methods were used; single, two and three split applications.

3:4. Experimental Design.

The experiment consisted of 15 treatments (including the control) arranged in a split - plot design, and replicated thrice. Whereas the different nitrogen levels constituted the main plots, the application methods constituted the sub-plots. The field layout showing the experimental design is illustrated in Fig. 1. Each experimental plot measured 3.0m. x 2.1m. (i.e. 6.3m^2). Plants were spaced at a distance of 30cm. x 30cm. (i.e. 30cm between rows and 30 cm between plants within a row (Fig.2). Each experimental plot had seven rows, each having ten plants; giving a plant population of 70 plants per plot (i.e. 111,111 plants per hectare).

3:5. Cultural Operations:

During the two seasons, the land was ploughed and harrowed once using a tractor. After which breaking of clods was done using a hoe. The first nitrogen application was top - dressed three weeks after transplanting. Subsequent top dressings were at intervals of three weeks. After each top dressing the plots were raked so as to incorporate the fertilizer into the soil. Triple superphosphate was applied at transplanting at a rate of 2g per planting hole, equivalent to 140g per plot or 222.22 kg / ha.



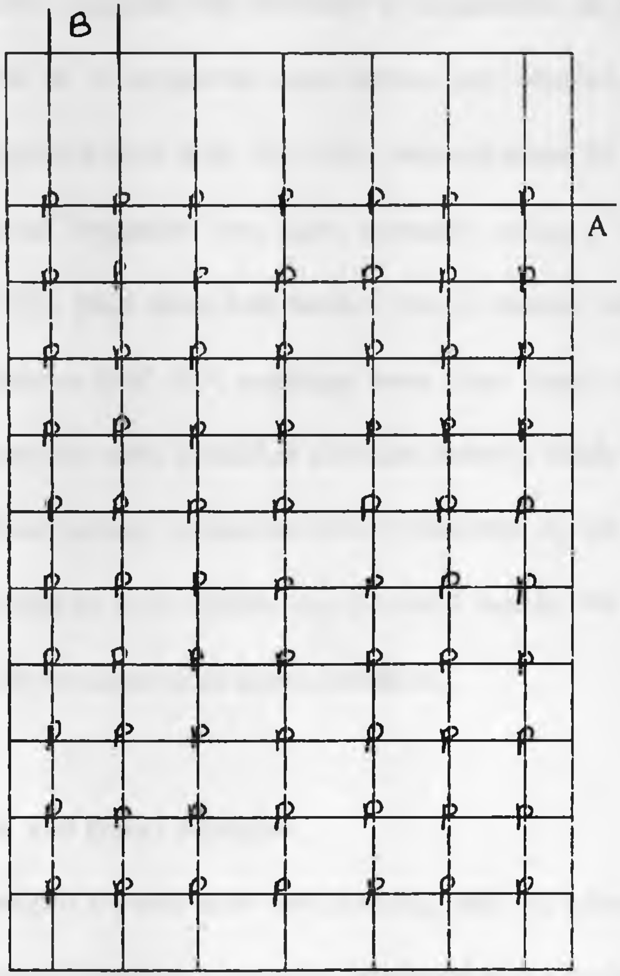
KEY:

A = Sub-plot constituted by nitrogen application method)

B = Main plot constituted by nitrogen levels)

P = Sub-plot

Fig 1: Experimental design field layout.



KEY:

A	=	Plant to plant spacing (within row) = 30cm
B	=	Row to Row spacing = 30cm
P	=	Plant position.

Fig: 2. Plant spacing in experimental sub-plot

During both seasons, irrigation was necessary to supplement the rainfall. In the first season a total of 10 irrigations were carried out, whereas in the second season only 5 irrigations were done. Each plot received about 20 litres of water per each irrigation. Irrigation was done manually using a watering can. Throughout the trials, plots were kept weed - free by manual weeding using a hoe. In each season a total of 4 weedings were done. Insect pests recorded during the two seasons were whiteflies (*Bemisia tabaci*), black aphids (*Aphis fabae*) and ladybird beetles (*Epilachna hirta*). However, at no time was any attack serious enough so as to warrant any chemical control. No diseases were recorded during the two seasons of experimentation.

3:6. Observations and Data Collection.

Harvesting commenced 8 weeks after transplanting, with the subsequent harvests occurring at fortnightly intervals. Harvesting continued over a period of 14 weeks. For quality determination, samples harvested at 8, 12, 16 and 20 weeks plant age were analysed for oxalate and phenolic content. From each harvest, fresh material of 150g were obtained from each plot, and taken to the laboratory for the determination of phenolics and oxalates. 100g of each of the fresh samples were dried at 60^{0} C for 48 hours in an oven and then ground using a pestle and mortar. The ground samples were then stored in paper bags at room temperature during the period of analysis.

3:6:1. Yield determination.

At each harvest, edible shoots measuring between 5 and 10cm in length were picked from the whole plant. The shoots were weighed, and the edible shoot yield computed in tonnes per hectare.

3:6:2. Determination of Oxalates.

Oxalates were determined in dried leaf samples. This was done using the method described by Marshall *et al.*, (1967). The oxalates were precipitated with calcium, after which their contents were determined by titration with a standard potassium permanganate solution. 100mg sample was extracted with 30mls of 0.5m Hcl. This was placed in a water bath at 100^0C for 30 minutes.

The mixture was cooled and thoroughly shaken, after which it was filtered through Whatman No.1 filter paper. Thereafter 8M NH_4OH was added to adjust the pH to 8. Filtrate acidity was further adjusted to pH 5 by addition of 6N CH_3COOH. Duplicate samples of 10ml (of the filtrate) were precipitated with 0.4ml of 5% $CaCl_2$, and left overnight to settle. The samples were then centrifuged at 3000 rpm for 15 minutes and the supernatant discarded. The sample was rinsed twice with 2ml of 0.35M H_2SO_4. This was then titrated with 0.05N K_2MnO_4 at 60^0C to a violet / pink colour (from colourless) that persisted for about 15 seconds. The reaction between oxalic acid and potassium permanganate proceeds too slowly at room temperature to give a sharp end point during titration; hence the need to heat the solution (no indicator was used).

For soluble oxalates, instead of using 0.5 M Hcl, 30ml distilled water was used for extraction. The rest of the procedures were as in the determination of total oxalates. The results so obtained were expressed as milligrams of oxalic acid in 100g of the dry leaf samples.

3:6:3. Determination of total phenolics.

Total phenolics was determined on dry leaf samples by the PRUSSIAN BLUE method as described by Price and Butler (1977). This method measures the blue complex formed due to oxidation of phenolic rings via Fe^{+3}, and the colour obtained is read at 720nm. The colour read should be yellow, green and deep blue depending on the concentration of phenolic components in the material.

A quantity of 200mg of the sample was extracted with 100ml methanol - Hcl, and the contents refluxed for 2 hours, then allowed to cool. The extract was filtered through Whatman No. 1 filter paper and the volume made up to 100ml with Methanol - Hcl after a few washings.

An aliquot of 0.1ml of the extract was then added to 30ml of glass distilled water. To this, 3ml a mixture of 0.05N Ferric Chloride and 0.1N hydrochloric acid was added and the reaction allowed to proceed for 3 minutes. Another 3ml of 0.008M Potassium ferricyanide was then added.

After 20 minutes, the absorbance was read at 720nm on a Spectronic 20 Spectrophotometer within an interval of 1 minute. The absorbance obtained for 0.1ml was calculated on per gram basis and expressed as $A_{720g^{-1}}$. The phenolic content was expressed as mg tannic acid equivalent / 100 g. Sample.

3:7. Data Analysis.

All the data collected were subjected to Analysis of Variance (ANOVA) using methods described by Steel and Torrie (1981), and means separated using Least Significant Difference (L.S.D).

4. RESULTS:

4:1. LEAF YIELDS.

4:1:1. Effect of nitrogen rates on edible leaf yield.

Nitrogen application generally increased the yield of edible leaves (Table 3). During season 1, the increase in yield was not significant, but in season 2, the increase was significant (P = 0.05). Nitrogen rates at 52, 78 and 104 kg/ha. gave significantly higher yields than at 26 kg/ha. and the control (0 kg / ha.). However, no significant differences were observed in yields between the rates 52, 78 or 104 kg/ha. (Table 3). The highest yield(15 t/ha.) was recorded at the highest nitrogen rate i.e. 104 kg/ha. (Fig. 3.); whereas at both 52 and 78 kg N / ha., the yield recorded was 14.8 t/ha at each rate (Table 3).

During both seasons of experimentation, yields generally increased with increasing nitrogen application rates. In both seasons the yields obtained at a rate of 52 kg / ha. were only slightly lower than those obtained at 104 kg N / ha. (Fig. 3). In season 2 yield at 52 kg N / ha was equal to yield at 78 kg N / ha. But in season 1 nitrogen at a rate of 52 kg / ha gave slightly higher yields than nitrogen at a rate of 78 kg N / ha.

Table 3: Effect of nitrogen rates on the edible leaf yield of *Solanum nigrum.*

NITROGEN (Kg/ha)	SEASON 1 YIELD (t/ha)	SEASON 2 YIELD (t/ha)
0	9.1[a]	12.2[b]
26	10.2[a]	12.2[b]
52	11.0[a]	14.8[a]
78	10.7[a]	14.8[a]
104	11.1[a]	15.0[a]

Means followed by the same letter down the column are not significantly different (LSD:P=0.05)

Coefficient of Variation (CV) :

Season 1 = 16.11%.

Season 2 = 17.02%.

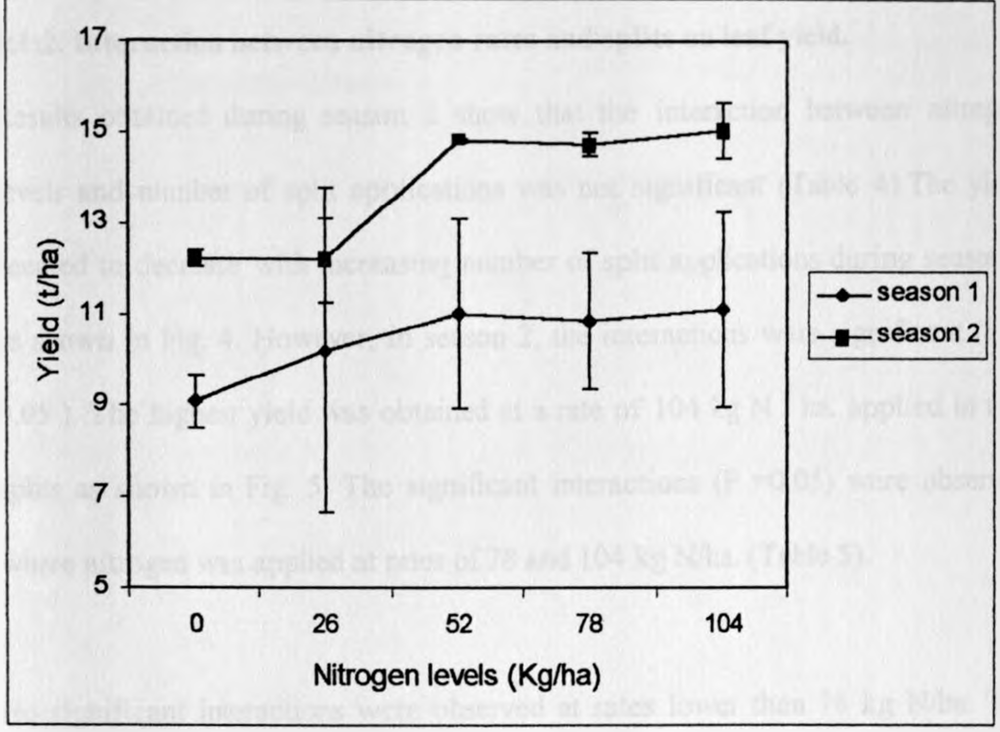


Fig. 3 : Effect of nitrogen on leaf yield.

4:1:2. Interaction between nitrogen rates and splits on leaf yield.

Results obtained during season 1 show that the interaction between nitrogen levels and number of split applications was not significant (Table 4).The yield seemed to decrease with increasing number of split applications during season 1 as shown in Fig. 4. However, in season 2, the interactions were significant (P = 0.05). The highest yield was obtained at a rate of 104 kg N / ha. applied in two splits as shown in Fig. 5. The significant interactions (P =0.05) were observed where nitrogen was applied at rates of 78 and 104 kg N/ha. (Table 5).

No significant interactions were observed at rates lower than 78 kg N/ha. The highest yields were realized when nitrogen was applied at a rate of 104 kg/ha in two splits.

Table 4: Effect of interaction between nitrogen levels and application method on edible leaf yield of *Solanum nigrum*.

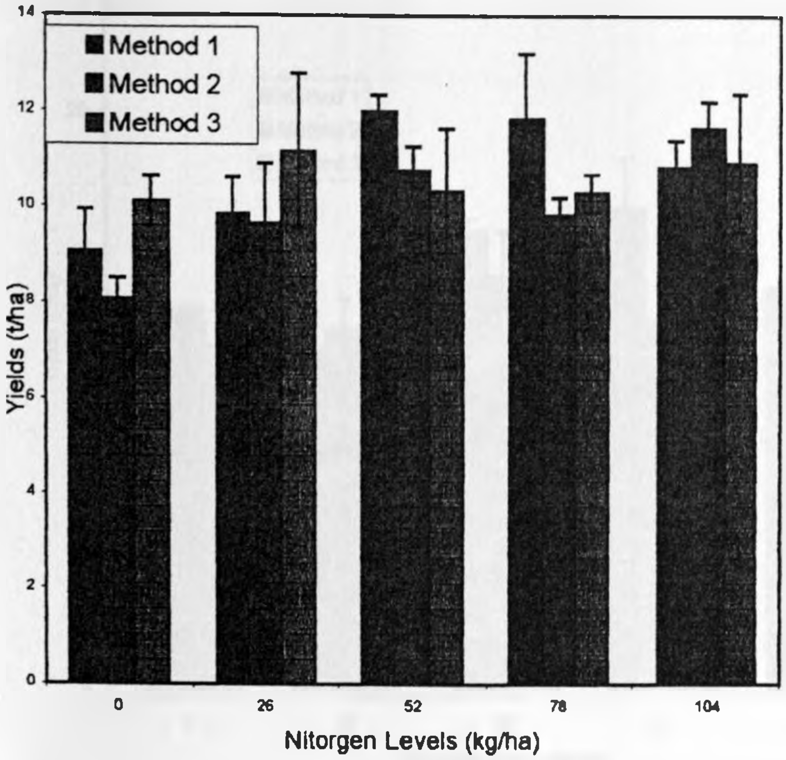
(SEASON 1)

NO OF SPLIT APPLICATIONS	NITROGEN RATES (Kgs/ha) & YIELDS t/ha				
	0	26	52	78	104
1.	9.1[a]	9.8[a]	12.0[a]	11.8[a]	10.8[a]
2.	8.1[a]	9.7[a]	10.8[a]	9.8[a]	11.6[a]
3.	10.1[a]	11.1[a]	10.3[a]	10.3[a]	10.9[a]

Means followed by the same letter down the column are not significantly different (LSD: $P = 0.05$).

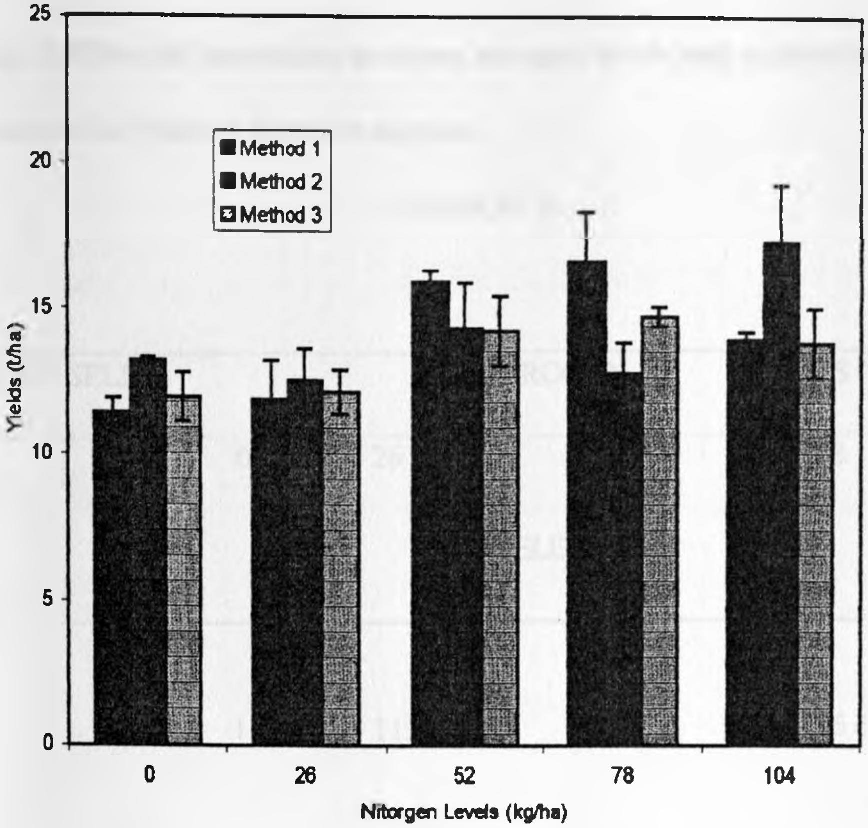
Coefficient of Variation (CV):

CV = 10.19



Key:
Method 1 : Single application
Method 2 : Double split application
Method 3 : Triple split application

Fig. 4 : Effect of nitrogen level and application method on leaf yield (season 1).



Key:
Method 1 : Single application
Method 2 : Double split applications
Method 3 : Triple split applications

Fig. 5 : Effect of nitrogen level and application method on leaf yield (season 2).

Table 5:Effect of interaction between nitrogen levels and application method on edible leaf yield of *Solanum nigrum*.

(SEASON 2)

NO. OF SPLIT	NITROGEN RATES (Kg/ha)				
	0	26	52	78	104
	(YIELDS t/ha)				
1	11.4^{a}	11.9^{a}	15.9^{a}	16.6^{a}	14.4
2	13.2^{a}	12.5^{a}	14.3^{a}	12.8^{b}	17.3
3	12.0^{a}	12.2^{a}	14.2^{a}	14.9^{ab}	13.8

Means followed by the same letter down the column are not significantly different (LSD: P=0.05).

Coefficient of Variation (CV) :

CV = 12.93%.

4:1:3. Effect of Plant Age on leaf yield.

Plant age starting from transplanting significantly (P = 0.05) affected edible leaf yield in both seasons of experimentation (Table 6). In season 1, except at the 2nd harvest, when there was yield reduction (from 1.5 to 1.2 t/ha.), yields increased with each successive harvest to a maximum of 2.4 t/ha. at the fourth harvest. From the fifth harvest, yield decreased again (to 1.5 t/ha.), and continued decreasing with each successive harvest. The lowest yield (0.9 t/ha) was recorded at the seventh harvest i.e. 20 weeks after transplanting (Fig. 6).

In season 1, the highest yield was recorded during the fourth harvest at a plant age of 14 weeks. In season 2, as was the case in season 1, there was a reduction in yield during the second harvest from 1.6 t/ha. at the first harvest to 1.0 t/ha. Thereafter yields increased with each successive harvest until the sixth harvest; then slightly declined during the seventh harvest to 2.6 t/ha. from the 2.7 t/ha recorded during the sixth harvest as shown in Fig. 6.

During both seasons, there were yield peaks during the fourth harvest, i.e. at a plant age of 14 weeks. Season 2 had a second yield peak at 18 weeks of plant age (Fig. 6).

Table 6:Effect of plant age on edible leaf yield of *Solanum nigrum.*

PLANT AGE (WEEKS)	SEASON 1 YIELD (t/ha)	SEASON 2 (YIELD (t/ha)
8	1.5^{b}	1.6^{c}
10	1.2^{c}	1.0^{e}
12	1.6^{b}	1.4^{d}
14	2.4^{a}	2.3^{d}
16	1.5^{b}	2.3^{b}
18	1.2^{c}	2.7^{a}
20	0.9^{d}	2.6^{a}
Total (Leaf yield)	10.3	13.9

Means followed by the same letter down the column are not significantly different

(LSD: P=0.05).

Coefficient of Variation (CV) :

Season 1 = 32.10%.

Season 2 = 34.04%.

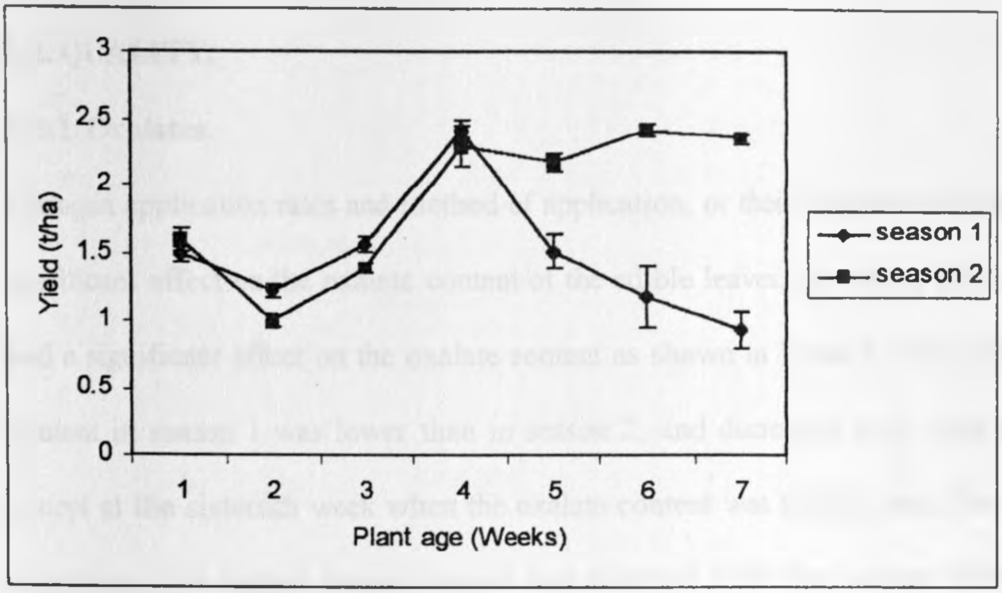


Fig. 6 : Effect of plant age on leaf yield.

4:2. QUALITY.

4:2:1. Oxalates.

Nitrogen application rates and method of application, or their interactions had no significant effect on the oxalate content of the edible leaves. However, plant age had a significant effect on the oxalate content as shown in Table 7. The oxalate content in season 1 was lower than in season 2; and decreased with plant age, except at the sixteenth week when the oxalate content was slightly high, but not significant. The highest oxalate content was recorded at the first harvest whereas the lowest was at the seventh harvest.

Unlike season 1, oxalate content in season 2 at 12 weeks plant age increased with plant age. The lowest oxalate content was at the first harvest and highest at the second harvest. The last harvest however had a significantly (P = 0.05) lower oxalate content than the second and third harvests (Table 7). The trend of oxalate content in leaves with plant age is shown in Fig. 7. In season 1 the oxalate content in leaves was significantly lower than in season 2; with conspicuous declines at twelve and twenty weeks of plant age. In season 2 the oxalate content steadily increased from the eighth to twelfth week; thereafter decreased drastically.

Table 7: Effect of plant age on the oxalate content of edible leaves of *Solanum nigrum*

PLANT AGE (WEEKS)	SEASON1 (mg/100g)	SEASON 2 (mg/100g)
8	611.0[a]	652.7[b]
12	511.9[ab]	938.5[a]
16	591.9[ab]	895.1[a]
20	480.7[c]	707.1[b]

Means followed by the same letter down the column are not significantly different (LSD: P = 0.05).

Coefficient of Variation (CV) :

Season 1 = 15.67%.

Season 2 = 17.74%.

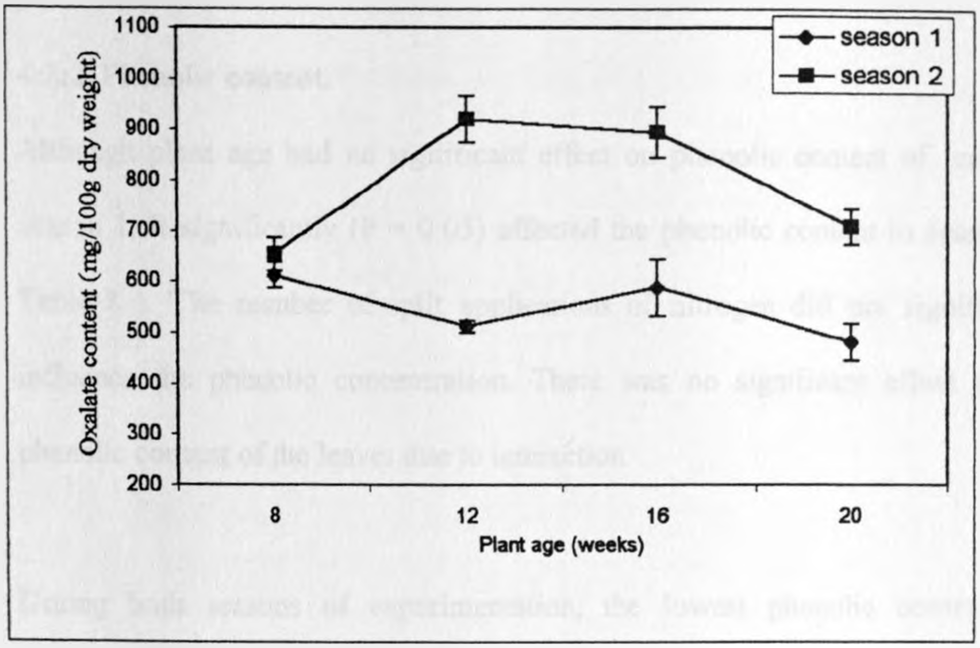


Fig. 7: Effects of plant age on oxalate content.

4:2:2. Phenolic content.

Although plant age had no significant effect on phenolic content of leaves in season 1, it significantly (P = 0.05) affected the phenolic content in season 2 (Table 8). The number of split applications of nitrogen did not significantly influence the phenolic concentration. There was no significant effect on the phenolic content of the leaves due to interaction.

During both seasons of experimentation, the lowest phenolic content was recorded during the5th harvest (16 weeks of plant age). In season 1 the highest phenolic content was recorded at the first harvest (8 weeks of plant age), after which there was a steady decline in the phenolic content of leaves. However, there was an increase in phenolic content at 20 weeks plant age (Table 8). In season 2, after an initial increase in phenolic content at 12 weeks plant age, there was a decrease at 16 weeks followed by another increase at 20 weeks plant age. This trend is shown in Fig. 8. The phenolic content of leaves in season 2 was significantly higher than in season 1.

Table 8:Effect of plant age on the phenolic content of edible leaves of *Solanum nigrum*

PLANT AGE (WEEKS)	SEASON 1 (mg/100g)	SEASON 2 (mg/100g)
8	938.6[a]	1327[b]
12	909.7[a]	1455[ab]
16	797.6[a]	1153[c]
20	903.1[a]	1507[a]

Means followed by the same letter down the column are not significantly different (LSD: P=0.05).

<u>Coefficient of Variation (CV):</u>

Season 1 = 12.60 %.

Season 2 = 12.35 %.

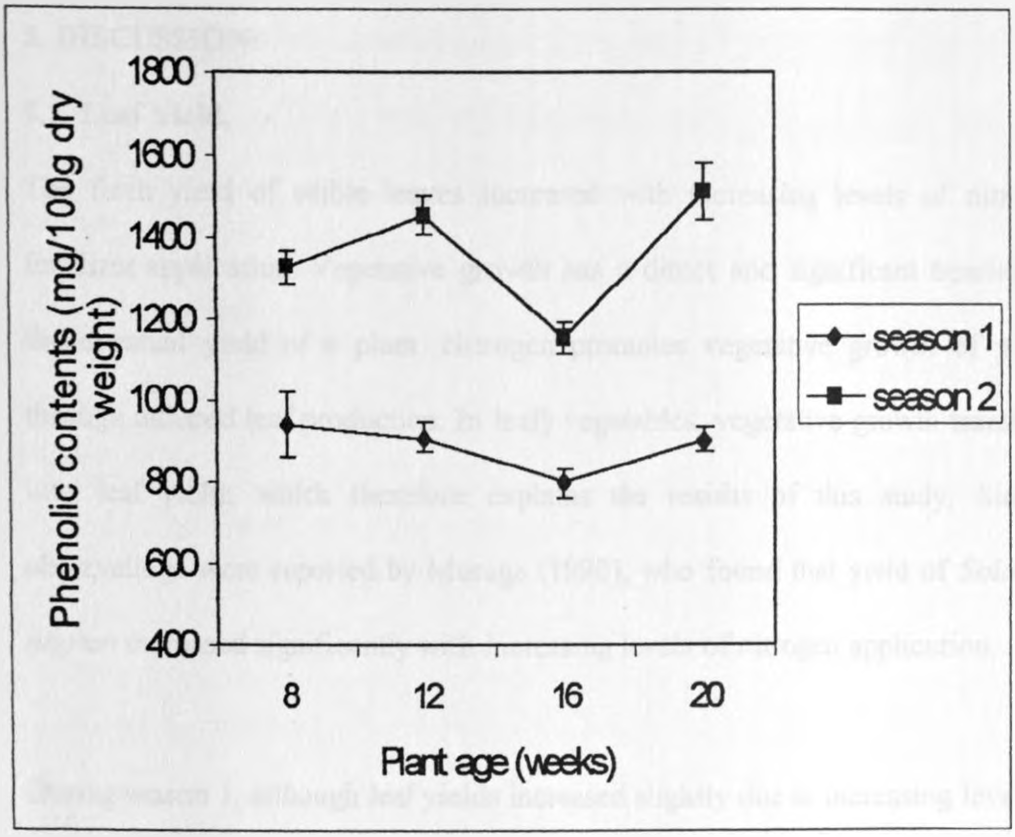


Fig. 8 : Effect of plant age on leaf phenolic content.

5. DISCUSSION:

5.1. Leaf Yield.

The fresh yield of edible leaves increased with increasing levels of nitrogen fertilizer application. Vegetative growth has a direct and significant bearing on the eventual yield of a plant. Nitrogen promotes vegetative growth of plants through induced leaf production. In leafy vegetables, vegetative growth translates into leaf yield; which therefore explains the results of this study. Similar observations were reported by Murage (1990), who found that yield of *Solanum nigrum* increased significantly with increasing levels of nitrogen application.

During season 1, although leaf yields increased slightly due to increasing levels of nitrogen application, the increase was not significant. During this season, there were periods of dry spells. This explains the low yields in season I compared to season 2. If drought conditions prevail during the growing season, inadequate soil water may restrict crop uptake of nitrogen (Webster and Wilson, 1980). Too little moisture has a limiting effect on plant response to applied fertilizer. According to Edmond *et al*. (1975), under conditions of water deficit plant growth is slow and yields are low. This possibly explains the lack of significance in yield increase during season 1 and also in the different nitrogen rates. Chahira (1982), reported that during the short rains of 1978, there was no significant differences between the effects of different levels of C.A.N on leaf yield of Kale.

Yields realized in season 2 were higher than in season 1 probably because of more uptake of nitrogen as a result of adequate moisture content in the soil. The moisture availability in the 2nd season was better than in season 1, especially with respect to time of topdressing (Appendix 1). Davies *et al.* (1975), reported that in situations of adequate moisture availability the soil supplies considerable amounts of nitrogen, which is readily absorbed by the roots. Applied nitrogen in situations of adequate moisture is readily dissolved and easily absorbed by plant roots. This possibly explains the yield differences in the two seasons.

A general recommendation is that in leafy vegetables nitrogen should be applied at a rate of 80 - 100 kg N/ha in splits; to help reduce leaching losses (Mathai 1978). Murage (1990), reported an optimum nitrogen level of 5g N/plant for *Solanum nigrum* equivalent to 556 kg N/ha. Based on 5g N/plant, a farmer would be expected to apply 2135 kg C.A.N per hectare. Webster and Wilson (1980) reported that relatively little fertilizer is currently used in the developing countries because most small farmers cannot afford it. Considering that *Solanum nigrum* is grown or gathered by peasants, they cannot afford to apply 2135 kg C.A.N per hectare. In this study the highest level of nitrogen applied per plant was 0.936g, which is one - fifth of the optimum recommended by Murage (1990).

Chahira (1982), reported that the number of split applications of nitrogen had no significant effect on the yield of kales. But according to Cooke (1982), extra yields are obtained by splitting fertilizer doses. In this study the number of split applications of nitrogen had no significant effect on the yield of edible leavers of *Solanum nigrum*.

It was observed that single dose application tended to give better yields in season 1, while in season 2 double dose application tended to give better yields. Frequent applications of nitrogen are most effective where no appreciable soil moisture deficit ccurs (Archer, 1985). This possibly explains why double dose application appeared to be better during season 2, while single dose appeared to be better in season 1.

The time interval between splits during this study was three weeks. According to Chahira (1982), if the interval between splits is small, lack of response to split application may occur. Three weeks between split application is the recommendation for kales. This interval for *Solanum nigrum*, might have been too short. Therefore a specific interval for *Solanum nigrum* needs to be established..

Although the interaction between nitrogen levels and application method was not significant during season 1, it significantly affected the yield during season 2.

Significant interactions were at nitrogen rates 78 and 104 kg N/ha. It is possible that at less than 78kg N/ha., the amount of nitrogen is too low such that it is all utilzed by the plant before it is leached.

Plant age significantly (P = 0.05) affected edible leaf yield during both seasons of experimentation. At the second harvest, there was a reduction in yield in comparison to the first harvest. This was possibly due to shock on the plant as a result of defoliation (in the first harvest). Thereafter there was an increase in yield during subsequent harvests. Similar observation were made by Onyango (1992), who reported that fresh shoot weight in *Solanum nigrum* increased with each subsequent harvest until the seventh week, after which yields declined. Aworh, *et al* (1980), also reported that shoot fresh weight in spinach increased with increasing plant age, particularly when nitrogen fertilizer is applied. Mnzava and Masam (1985) observed that in *Amaranthus cruentus* (L.), the edible leaf yield showed a characteristic decline in the second harvest, then an increase during susequent harvests and finally a decline as the plants approached flowering.

Observations made during this study indicate that provided moisture is not limiting, economic yields can be realized beyond 20 weeks after transplanting. Similar observations were made by Onyango (1996, personal communication), at the Kenya Agricultural Research Institute

(K.A.R.I) in Kisii. This means that yields higher than 18t/ha. per season or 36t/ha per year can be attained.

5:2. Oxalate Content.

The results of this study are in conformity with reports by Murage *et al.* (1996), that nitrogen application had little effect on concentrations of oxalates in the leaves of *Solanum nigrum.* However, varying observations have been reported by other authors. Carlsson (1983), found that increasing nitrogen fertilizer rate decreased the oxalate contents in leafy vegetables; similar observations were reported by Murage (1990) and Mwafusi (1992) in *Solanum nigrum.* However, in those studies, nitrogen was applied in a single dose while in this study application of nitrogen was done in splits and harvesting done fortnightly. Considering that oxalate content decreased with plant age and that younger leaves are likely to have higher concentrations of oxalates than older ones, this could have influenced the effect of nitrogen on oxalate concentration in this study.

The effect of split applications of nitrogen had no significant effect on the oxalate content during both seasons. However, during both seasons, there were slight increases in oxalate content with increasing number of split applications of nitrogen. Very little information exists on this subject.

Plant age significantly affected the oxalate content. The oxalates content decreased with increasing plant age. After initial rise in oxalate concentration, there was a decrease with each successive harvest. Various authors have reported similar observations. Oke (1966) reported that in *Celosia argentea*, oxalic acid content initially rose, but dropped to safe levels towards the end. The same author (Oke 1968), also reported that oxalic acid content of some Nigerian vegetables was variable with no clearly defined pattern of change. In spinach oxalate concentration in plant tops decreased gradually with plant age (Okutani and Sugiyama, 1992). Adipala and Mugerwa (1993) also reported that oxalic acid content in *Amaranthus hybridus* decreased with plant age. In sweet and sour carambola, Joseph and Mendonca (1989), found that the oxalic acid levels decreased as the fruit matured.

Forbes and Watson (1992), reported that quite often a plant is most toxic of anti-nutrients when very young or when regenerating after cutting, while Marderosian *et al.* (1980) reported that oxalate levels increased with increasing maturity in Amaranth. In this study harvesting was done at fortnightly intervals. Considering that a plant may be most toxic when very young, or when growing again after cutting, it is possible that oxalate content of harvested leaves (from a single plant) may fluctuate depending on the amount of new growth after a harvest.

The type of nitrogen applied may influence the oxalate content of leafy vegetables. Roughan and Warington (1976) found that use of urea or ammonium resulted in small but significant decreases in oxalate concentrations of foliage of *Setaria sphacelata* (cv. Kazungula) compared to use of nitrate.

Oxalic acid is a terminal product of metabolism in plants. It has been observed that the most frquent pathway of oxalate synthesis seems to be from glycolate via glyoxylate to oxalate (Zindler-Frank, 1976). Metzler (1977), reported that glycolate is produced from phosphoglycolate; which (glycolate) through the glycolate - glyoxylate shuttle (in which oxygen molecule is removed), forms glyoxylate in the peroxisomes. The glyoxylate is then converted to glycine and oxalate.

In the biosynthesis of oxalate, nitrogen is not involved. This is possibly the reason why application of nitrogen during this study had no significant effect on the oxalate content of edible shoots of *Solanum nigrum*. In season 2, the oxalate content was generally higher than in season 1. In season 2 the moisture availability was better than in season 1, which could have resulted in higher synthesis of oxalate.

5:3. Phenolic Content.

According to Mc.Clure (1979), abundant nitrogen is generally inhibitory to phenolic accumulation, hence phenolic concentration would be expected to decrease with increasing nitrogen rates. Murage (1990) reported that nitrogen fertilizer rates had no significant effect on the synthesis of phenolics. Reports by Murage *et al*. (1996) also confirmed that nitrogen application had little effect on phenolic compounds in *Solanum nigrum*. The above observations are in agreement with those made during this study. Increasing nitrogen application rates had no significant effect on the phenolic contents of *Solanum nigrum* leaves. The phenolic content tended to decrease with increasing nitrogen levels.

In season 2 the general phenolic content at all stages of harvest was higher than in season 1. During season 2, there was a better rainfall distribution than in season 1. Since the other treatments during the two seasons were similar, it could be that the better moisture availability in the 2nd season, resulted into higher synthesis of phenolics. Sakharova and Novikova (1981) found that in *Bergenia crassifolia*, the levels of polyphenols were highest in years with moderate or prolific rainfall.

Phenolics are diverse in their chemical structure. They have an aromatic ring with attached hydroxyl, methoxyl and carboxyl groups. There is no simple pathway for biosynthesis of phenolics and, both anabolic and catabolic processes appear to participate in their formation (Garard, 1976). Matsuki (1996), reported that higher plant polyphenols are formed from shikamate via the shikimic acid pathway. He also reported that phenolics are not end products that accumulate unchanged. They are thought to be part of a dynamic equilibrium in which even when produced in quantity, there is continual synthesis, turnover, and degradation.

Since phenolics do not have nitrogen in their strcture, this possibly explains why nitrogen did not significantly affec the phenolic content in this study. Moreover in the shikimic acid pathway (from which polyphenols are formed), nitrogen is not involved. Considering that phenolics are not end products, and that there is continous synthesis, turnover, and degradation, their content in the plant is likely to fluctuate over time. This explains the results of this study in which there was no clear trend on the phenolic content of edible shoots with increasing plant age.

6. CONCLUSION:

The results obtained during this study indicate that increasing nitrogen fertilizer rates will most likely increase the edible leaf yield of *Solanum nigrum*. However, since there was no significant difference in yield at rates of 52, 78, and 104kg N/ha., it is more economical to apply nitrogen at a rate of 52kgs per hectare in one split rather than applying 78 or 104kg N/ha.

After the first harvest, plants seem to suffer from some shock resulting into decreased yields at the second harvest. This is possibly due to defoliation. To reduce the impact of shock, it is advisable to carry out light harvesting during the first harvest and possibly during the second harvest. Where more than one harvest is to be taken, sufficient foliage should be left on the the plant to enable the plant satisfy its photosynthetic requirements.

Observations made during season 2 show that provided moisture is not a limiting factor, economic yields can be realized beyond 20weeks after transplanting.

Within nitrogen rates of between 26 and 104Kg N/ha.there is no fear of oxalates or phenolic concentrations rising to harmful levels in the edible shoots of *Solanum nigrum*. Since oxalate content reduces with plant age, harvesting can be delayed, by which time the oxalate content of the leaves will have reduced.

Although phenolics may be a cause of astringency in leafy vegetables, use of nitrogen fertilizers can be encouraged without fear of increasing astringency in the case of *Solanum nigrum*.

7. RECOMMENDATIONS FOR FURTHER RESEARCH:

The results of this study are not conclusive, but rather, should serve as a basis for further research.

Only the effects of nitrogen were studied. Effects of phosphorous and potassium, and how they interact with those of nitrogen to yields and anti-nutrients should be studied.

Solanine is a glycoalkaloid found in plants of the solanaceae family (to which *Solanum nigrum* belongs). Glycoalkaloids are potentially toxic compounds. Considering the undesireable effects of solanine, studies are necessary to establish its prominence and synthesis in *Solanum nigrum*.

There are indications that economical yields may be realized beyond 20wks after transplanting. The period of economical yield, as well as the yield potential of *Solanum nigrum* needs to be investigated.

This study considered plant age, but not leaf age. Whether the harvesting frequency may have a significant effect on the anti-nutrients should be studied.

The study was only carried out at Njoro (Egerton University). It is neccessary to study the climatic (temperature and rainfall) effects on yield and anti - nutrients of *Solanum nigrum.* This should be done under different ecolgical regions.

REFERENCES:

Adipala, E. and J. S. Mugerwa. (1993). Effects of N, P and K. fertilizers on the dry matter, yield and chemical composition of *Amaranthus hybridus sub-species hybridus* (Green Head). *E. Afr. Agr. For. J.* 59: (2): 131-136.

Archer, J. (1985). Crop Nutrition and Fertilizer Use. Farming press Ltd. Ipswich, Suffolk. pp. 44.

Aworh, O.C., J.R. Hicks., P.L. Minotti and C.Y. Lee. (1980). Effects of plant age and nutrition fertilization on nitrate accumulation and post-harvest nitrate accumulation in fresh spinach. *J. Amer. Soc. Hort. Sci.* 105 (1): 18-20.

Berk, Z. (1983). Introduction to the Biochemistry of Foods. Elsevier science publishing Co. Inc. New York. pp. 235.

Buck, W.B., K.S. Preston., M. Abel and V.L. Marshall. (1966). Perirenal edema in swine: A disease caused by common weeds. *J. Amer. Vet. Med. Assoc.* 148: 1525-1531.

Carlsson, R. (1983). Nitrate and oxalate content in leaf and stems of wild and cultivated leafy vegetables. Leaf nutrient content production as a means of detoxification. In: Research in Food Science and Nutrition vol. 1. Proceedings of the 6th International Congress of Food Science and Technology; Dublin.

Chahira, P.W. (1982). The influence of spacing, leaf picking frequency, initial time of first harvest, levels and splits of nitrogen on leaf yield of Kales (*Brassica oleracea var. acephala*. D.C.). M Sc. Thesis, University of Nairobi, Kenya.

Chweya, J.A. (1985). Identification and nutritional importance of indigenous green leafy vegetables in Kenya. *Acta Hort.* 153: 99-108.

Chweya, J.A. (1993a). Agronomic studies on indigenous vegetables in Kenya. In: Proceedings of the Indigenous Food Plants workshop; April 14 - 16 1993. (Edt. Maundu, P.M., C.H.S. Kabuye and J.A. Chweya). Kenya.

Chweya, J.A. (1993a). Effect of nitrogen application and deflowering on leaf yield of *Gynandropsis gynandra*. In: Agronomy Progress Report ; Kengo Indigenous Vegetable Project. Kenya.

Cooke, G.W. (1982). Fertilizing for Maximum Yield. 3rd. Ed. Granada publishing Ltd. London.

Cooper, M.R. and A.W. Johnson. (1988). Poisonous Plants and Fungi. An illustrated guide; Min. of Agr. and Fisheries. CAB.

Davies, D.B., D.J. Eagle and J.B. Finney. (1975). Soil Management. 2nd. Ed. Farming press Ltd. Ipswich, Suffolk. pp.31.

Driver, G.A. (1983). Chemical substances in plants toxic to animals. In: Handbook of Naturally Occurring Food Toxicants. (Edt. Rechcigil, M.). CRC. Press. Florida. pp.231.

Edmond, J.B., T.L. Senn., F.S. Andrews and R.G. Halfacre. (1975). Fundamentals of Horticulture. Tata Mc.Graw Hill. India.

Epenhuijsen, C.W. Van. (1974). Growing Native Vegetables in Nigeria. F.A.O. Rome. Italy.pp. 86 - 88.

Fenwick, G.R., C.L. Curl., N.M. Griffiths., R.K. Heaney and K.R. Price. (1990). Bitter principles in food plants. In: Developments in Food Science 25. Bitterness in Foods and Beverages. (Edt. Rouseff, R.L.). Elsevier science publishers B.V. Amsterdam. pp. 205 - 241.

Ferrando, R. (1981). Traditional and Non-traditional Foods. F.A.O. Rome, Italy. pp 3 - 4, and 32 - 33.

Forbes, J.C. and R.D. Watson. (1992). Plants in Agriculture. Cambridge University Press. Great Britain. pp. 334.

Fortuin, F.T.J.M. and S.W.P. Omta. (1980). Growth analysis and shade experiment with *Solanum nigrum* L.; the black nightshade, a leaf and fruit vegetable in West Java.
Neth. J. Agr. Sci. 28: 199 - 210.

Garard, D. (1976). Introductory Food Chemistry. AVI Publishing company, INC. Connecticut.

Hawkes, J.G. (1990). The Potato - Evolution, Biodiversity and Genetic Resources. Belhaven press. London. pp. 2 - 3.

Hirooka, M. and N. Sugiyama. (1992). Effect of growth rates on oxalate concentrations in spinach leaves. *J. Jap. Soc. Hort. Sci.* 61: (3). 575 - 579.

Holm, L. G., D. L. Plucket, J. V. Pancho and J. P. Herberger. (1977). The World's Worst Weeds. Biology and Distribution. University press of Hawaii, Honolulu.

Ivens, G.W. (1971). East African Weeds and Their Control. Oxford University press. Nairobi. Rev. Ed. pp. 218 - 220.

Joseph, J. and G. Mendonca. (1989). Oxalic acid content of Carambola (*Averrhoa carambola L.*) and Bilimbi (*Averhoa bilimbi L.*). *Proc. Interam.Soc. Trop. Hort.* 33: 117 - 120.

Joshi, A.R. and J.M. Edington. (1990). The use of medicinal plants by two village communities in the central development region of Nepal. *Economic Botany* 44: (1). 71-83.

Kameno, T., T. Kinoshita., M. Kusuhara and M. Noguchi. (1991). The effects of cultural conditions and cultivars on the changes in the contents of components related to the quality of spinach. *Hort. Abstr.* 61: 3663.

Kinyanjui, H.C.K. (1979). Detailed survey of Tatton farm - Egerton College (Njoro). Detailed Soil Survey Report No. D14, Min. of Agr. Nat. Agric. Labs. - Kenya soil survey.

Kitchen, J.W., E.E. Burns and B.A. Perry. (1964). Calcium oxalate content of spinach (*Spinacia oleracea*). *Proc. Amer. Soc. Hort. Sci.* 84: 441 - 445.

Kokwaro, J.O. (1976). Medicinal Plants of East Africa. East African Litreature Bureau, Kenya. pp 207.

Marderosian, A.D., J. Beutler and W. Pfender. (1980). Nitrate and oxalate content of vegetable amaranth. *Abstr. Trop. Agr.* 7: (4). 35440.

Marshall, V.L., W.B. Buck and G.L. Bell. (1967). Pigweed (*Amaranthus retroflexus*).: An oxalate - containing plant. *Amer. J. Vet. Res.* 28: (124). 888 - 889.

Mathai, P.J. (1978). Amaranthus, a neglected vegetable. *Indian Farming* 28: (1). 29 - 32.

Masuki, M. (1996). Regulation of Plant Phenolic Synthesis: from Biochemistry to Ecology and Evolution. Aust. J. Bot. 44: 613 - 634.

Maundu, P.M. (1990). *Solanum nigrum.* Indigenous Food Plants Program Newsletter Issue 5.

Maundu, P.M. (1993). Important indigenous food plants (IFPS) of Kenya. In: Proceedings of the Indigenous Food Plants workshop. April 14 - 16 1993. Kenya.

Mc.Clure, J.W. (1979). The physiology of phenolic compounds in plants. In: Recent Advances in Phytochemistry Vol. 12 - Biochemistry of Plant Phenolics. (Edt. Swain, T., J.B. Harbone and C.F. Van. Sumere). Plenum press. New York and London.

Messiaen, C-M. (1992). The Tropical Vegetable Garden. Macmillan press Ltd. London. pp.357 - 372.

Metzler, D.E. (1977). Biochemistry - The Chemical Reactions of Living Cells. Academic press Inc Ltd. London. pp. 768.

Mnzava, N.A. and A.M. Masam. (1985). Regeneration Potential, Leaf and Seed Yield of Vegetable Amaranth, (Amaranthus cruentus (L.), as a Function of Initial Topping Heights. *Acta Hort*. 153: 151 - 160.

Mtotomwema, K. (1987a). I.F.S. seminar on : Vegetable Crops Hortcultural Techniques in Wild Leafy Vegetables with Special Reference to *Solanum nigrum* L Tunis, Tunisia.

Mtotomwema, K. (1987b). The nutritional potential of wild leaf vegetables in East Africa. In: 4th. Symposium on the Chemistry of Tropical Natural Products.

Murage, E.N. (1990). The effects of nitrogen rates on the growth, leaf yield and nutritive quality of the black nightshade. M.Sc. Thesis. University of Nairobi, Kenya.

Murage, E.N. , J.A. Chweya and J.K. Imungi. (1996). Changes in leaf yield and nutritive quality of Black nightshade (*Solanum nigrum.*) as influenced by nitrogen application. *Ecol. of Food and Nutr.* 35: (2) 149 - 157.

Murthy, V.S. (1989). Food toxins. In: Development in Food Science 25. (Edt.Rouseff, R). Elsevier science publishers, B.V. Amsterdam. pp. 328 - 349.

Mwafusi, C.N. (1992). Effects of propagation method and deflowering on vegetative growth, leaf yield, phenolic and glycoalkaloid content of three black nightshade types used as vegetable in Kenya. M.Sc. Thesis University of Nairobi, Kenya.

Ngugi, G.W. (1993). Nutritional analysis of indigenous food plants. In: Proceedings of The Indigenous Food Plants workshop. April 14-16 1993. (Edt. Maundu, P.M., C.H.S. Kabuye. and J.A.Chweya.).Kenya.

Noggle, G.R. and G.J. Fritz. (1976). Introductory Plant Physiology. Prentice Hall. Hall. pp. 241 - 245., and 480 - 482.

Norman, J.C. and F.K. Sichone. (1993). Vegetable yield and quality of amaranth as influenced by species and harvesting frequency. *UNISWA. J. Agr.* 2:

Oke, O.L. (1966). Chemical studies on some Nigerian vegetables. *Trop. Sci.* 8: 128 - 132.

Oke, O.L. (1968). Chemical changes in some Nigerian vegetables during growth. *Exp. Agr.* 4: 345 - 349.

Okutani, I. and N. Sugiyama. (1992). Oxalate concentration in spinach leaves during ontogenesis. *HortScience. Abstr.* 27: (6). 642.

Okutani, I. and N. Sugiyama. (1994). Relationship between oxalate concentration and leaf position in various spinach cultivars. *Hortscience.* 29 (9): 1019 - 1021.

Omta, S.W. and F.T. Fortuin. (1979). Vegetables for The Humid Tropics. 4: 6 - 9.

Omta, S.W. and F.T. Fortuin. (1980). The black nightshade, as a vegetable in the tropics, with special reference to West Java. Review Articles. Institute of Ecology, Pajajaram University, Bandung, Indonesia and Dept. of Plant physiology, State University of Groningen, The Netherlands. pp. 6 - 8.

Onyango, M.A. (1992). Effects of plant density and harvesting frequency and age on nutritive quality of four variants of *Solanum spp.* M.Sc. Thesis. University of Nairobi, Kenya.

Oomen, H.A.P.C. and G.H.J. Grubben. (1978). Tropical Leaf Vegetables in Human Nutrition. Comm. 69. Dept. of Agr. Res. Koninklijk Instituut Voor de Tropen. Amsterdam. pp. 36 - 37, and 81 - 83.

Opole, M., J.A. Chweya and J.K. Imungi. (1989). A report on field and laboratory research. Indigenous Vegetable Crops (1988-1989), H28: KENGO, Kenya.

Page, A. L. , R.H. Miller and D.R. Keeney., Edts. (1982). Methods of Soil Analysis. Part 2 - Chemical and Microbiological Properties. 2 nd. Edition. American Society of Agronomy, Inc. Soil Science Society of America, Inc.

Pingle, U. and B.V. Ramasastri. (1978). Absorption of calcium from a leafy vegetable rich in oxalates. *Brit. J. Nutr.* 39: 119 - 125.

Price, M.L. and L.G. Butler. (1977). Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. *J. Agri. Food Chem.* 25: (6). 1268 - 1273.

Roughan, P.G. and J.J. Warrington. (1976). Effect of nitrogen source on oxalate accumulation in *Setaria sphacelata* (cv. Kazungula). *J. Sc. of Food and Agric.* 27: (3) 281 - 286.

Sakharova, N.A. and L. B. Navikova. (1981). Changes in the content of active substances in the herbage of *Bergenia crassifolia* in the Kuznetskii Alatau. *Hort. Abstr.* 51: 9517.

Salisbury, F.B. and C.W. Ross. (1984). Plant Physiology. 3rd. Ed. CBS publishers and distributors, India. pp. 278.

Schmidt, D.R. (1971). Comparative yields and composition of eight tropical leafy vegetables grown at two soil fertility levels. *Agron. J.* 63: 546 - 550.

Singleton, V.L. (1981). Naturally occurring food toxicants: phenolic substances of plant origin common in foods. *Adv. in Food Res.* 27: 149 - 221.

Steel, R.G.D. and J.H. Torrie. (1981). Principles and Procedures of Statistics. A Biometrical Approach. 2nd. Ed. Mc.Graw - Hill Book Co. New York.

Strumeyer, D.N. and M.J. Malin. (1975). Condensed tannins in grain sorghums. Isolation, fractionation and characterisation. *J. Agri. Food Chem.* 23: 909 - 914.

Taylor, O-O. A., B.L. Fetuga and V.A. Oyenuga. (1983). Accumulation of mineral elements in five tropical leafy vegetables as influenced by nitrogen fertilization and age. *Scientia Hort.* 18: 313 - 322.

Thompson, H.C. and W.C. Kelly. (1972). Vegetable Crops 5th. Ed. T.M.H. Ed. India. pp. 13 - 16, and 52.

Tindall, H.D. (1986). Vegetables in The Tropics. 1st Ed. Macmillan Educ. Ltd. pp. 370 - 371.

Tisdale, S.L., W.L. Nelson and J.D. Beaton. (1985). Soil Fertility and Fertilizers. 4th. Ed. Macmillan publishing Co. New York. pp. 59 - 62.

Waithaka, K. And J.A Chweya. (1991). *Gynandropsis gynandra.* A Tropical Leaf Vegetable. Its Cultivation and Utilization. F.A.O. Rome, Italy.

Watt, J.M. and M.G. Breyer-Brandwijk. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd. Ed. E and S Livingstone Ltd. Edinburgh.

Webster, C.C. and P.N. Wilson. (1980). Agriculture in The Tropics. 2nd. Ed. Longmans group Ltd. London. pp. 68 - 71, and 218 - 221.

Zindler-Frank, E. (1976). Oxalate Biosynthesis in Relation to Photosynthetic Pathway and Plant Productivity - a Survey. *Z. Pflanzenphysiol. Bd.* 80. S. 1 - 13.

APPENDIX 1

WEATHER DATA AT EGERTON UNIVERSITY NJORO (TRIAL SITE)

SEASON 1

PERIOD (1995)	RAINFALL (MM)	TEMPERATURE ^0C MAX.	TEMPERATURE ^0C MIN.	TEMPERATUR ^0C MEAN
JANUARY	02.10	25.60	10.00	17.80
FEBRUARY	60.90	25.30	09.60	17.45
MARCH	80.00	24.30	10.30	17.30
APRIL	91.60	24.90	10.90	17.90
MAY	102.40	22.80	10.80	16.80
JUNE	106.00	24.00	11.30	17.65

SEASON 2

PERIOD (1995)	RAINFALL (MM)	TEMPERATURE ^0C MAX.	TEMPERATURE ^0C MIN.	TEMPERATURI ^0C MEAN
JULY	78.40	21.20	09.80	15.50
AUGUST	50.80	23.40	10.80	17.10
SEPTEMBER	131.70	23.00	09.70	16.35
OCTOBER	128.40	22.20	10.30	16.25
NOVEMBER	48.80	23.00	10.50	16.75
DECEMBER	47.50	23.20	08.80	16.00

APPENDIX 2

ANALYSIS OF VARIANCE TABLE.
EFFECT OF NITROGEN LEVELS, APPLICATION METHOD AND PLANT AGE ON EDIBLE LEAF YIELD OF *Solanum nigrum*
(SEASON 1)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	2	20237.619	10118.810	0.5434NS	
Factor A (N levels)	4	93056.032	23264.008	1.2493NS	0.3643
Error	8	148975.873	18621.984		
Factor B (method)	2	16739.048	8369.524	1.5285NS	0.2194
AB	8	67831.587	8478.948	1.5485NS	0.1426
Factor C (plant age)	6	1618643.016	269773.836	49.2676**	0.0000
AC	24	95700.635	3987.526		
BC	12	23439.841	1953.320		
ABC	48	69328.413	1444.342		
Error	200	1095136.508	5475.683		
Total	314	3249088.571			

* Significant at 5% probability level

** Significant at 1% probability level

NS - Non-Significant at 5% probability level

APPENDIX 3
ANALYSIS OF VARIANCE TABLE
EFFECT OF NITROGEN LEVELS, APPLICATION METHOD AND PLANT AGE ON EDIBLE LEAF YIELD OF *Solanum nigrum* (SEASON 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	2	2154.762	1077.381	0.0925NS	
Factor A (N-Levels)	4	293213.968	73303.492	6.2963*	0.0136
Error	8	93138.889	11642.361		
Factor B (method)	2	12462.857	6231.429	0.6690	
AB	8	199135.556	24891.944	2.6723**	0.0083
Factor C (Plant Age)	6	3141819.683	52363.614	56.2162**	0.0000
AC	24	168654.921	7027.288	0.7544NS	
BC	12	27072.698	2256.058	0.2422NS	
ABC	48	170345.556	3548.866	0.3810NS	
Error	200	1862939.683	9314.698		
Total	314	5970938.571			

* Significant at 5% probability level

** Significant at 1% probability level

NS-Non-Significantat5% probability level

APPENDIX 4

ANALYSIS OF VARIANCE TABLE
EFFECT OF NITROGEN LEVELS, APPLICATION METHOD AND PLANT AGE ON OXALATE CONTENT OF EDIBLE LEAF YIELD OF *Solanum nigrum*
(SEASON 1)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	2	96191.657	48095.829	0.6293^{NS}	
Factor A (N-levels)	4	232791.145	58197.786	0.7615^{NS}	
Error	8	611409.846	76426.231		
Factor B (method)	2	92063.670	46031.835	0.8549^{Ns}	
AB	8	393117.380	49139.672	0.9126^{NS}	
Factor C (Plant Age)	3	527408.720	175802.907	3.2651*	.0241
AC	12	591919.644	49326.637	0.9161^{NS}	
BC	6	278772.368	46462.061	0.8629^{NS}	
ABC	24	1511639.073	62984.961	1.1698^{NS}	0.2856
Error	110	5922764.495	53843.314		
Total	179	10258077.998			

* Significant at 5% probability level

NS-Non-Significant at 5% probability level

APPENDIX 5

ANALYSIS OF VARIANCE TABLE

EFFECT OF NITROGEN LEVELS, APPLICATION METHOD AND PLANT AGE ON OXALATE CONTENT OF EDIBLE LEAF YIELD OF *Solanum nigrum*

(SEASON 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	2	180045.767	90022.883	1.6349 NS	0.2539
Factor A (N-levels)	4	72956.868	18239.217	0.3312 NS	
Error	8	440511.343	55063.918		
Factor B (method)	2	74757.306	37378.653	0.6710 NS	
AB	8	115342.711	14417.839	0.2588 NS	
Factor C (Plant Age)	3	2634428.709	878142.903	15.7634**	0.0000
AC	12				
BC	6				
ABC	24	1225706.158	51071.090	0.9168 NS	
Error	110	6127833.876	55707.581		
Total	179	12064134.807			

* Significant at 5% probability level

**Significant at 1% probability level

NS Non - Significant at 5% probability level.

APPENDIX 6
ANALYSIS OF VARIANCE TABLE
EFFECT OF NITROGEN LEVELS, APPLICATION METHOD AND PLANT AGE ON PHENOLIC CONTENT OF EDIBLE LEAF YIELD OF *Solanum nigrum*
(SEASON 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	2	197640.000	98820.000	0.2571NS	0.2539
Factor A (N-Levels)	4	420720.222	105180.056	0.2736NS	
Error	8	3075307.111	384413.389		
Factor B (method)	2	30004.133	15002.067	0.1014NS	
AB	8	949826.311	118728.289	0.8023NS	
Factor C (Plant Age)	3	3347855.400	1115951.800	7.5413**	0.0000
AC	12	1259503.600	104958.633	0.7093NS	
BC	6	185470.000	30911.677	0.2089NS	
ABC	24	3219181.333	134132.556	0.9064NS	0.2856
Error	110	16277564.889	147977.863		
Total	179	28963073.000			

* Significant at 5% probability level

**Significant at 1% probability level

NS - Non-Significant at 5% probability level