WILD VEGETABLES OF THE EASTERN CAPE OF SOUTH AFRICA: THE NUTRITIONAL VALUE AND DOMESTICATION OF *SOLANUM NIGRUM* L.

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DECLARATION

I, Callistus Bvenura, declare that this thesis, submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Ethnobotany in the Faculty of Science and Agriculture, is my original work; and that this work has not been submitted at any other University for the award of any degree.

I also declare that I am fully aware of the University of Fort Hare policy on plagiarism and have taken every precaution to comply with the regulations of the University.

Again, I declare that I am fully aware of the University of Fort Hare policy on research ethics and was cleared to conduct the Ethnobotanical survey in chapter 2 of this thesis.

Signed.....at the University of Fort Hare this.....day of the month of .....year 2014.

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ABSTRACT

Abstract

An ethnobotanical survey was conducted in Alice and Willowvale in the Nkonkobe and Mbashe municipalities of the Eastern Cape Province, South Africa to identify and document wild vegetables growing in the areas. The survey documented 22 vegetable species belonging to 12 different families. The Amaryllidaceae, Amaranthaceae, Apiaceae, Asteraceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cruciferae, Cucurbitaceaea, Euphorbiaceae, Tiliaceae, Solanaceae, Polygonaceae and Urticaceae were the families that were recorded. The species were *Tulbaghia violacea* Harv., *Amaranthus blitoides* S., *Amaranthus blitum* L., *Amaranthus hybridus* L., *Centella coriacea* Nannfd., *Bidens pilosa* L, *Cotula heterocarpa* DC., *Sonchus oleraceus* L., *Galinsoga parviflora* Cav., *Taraxacum officinale* Weber, *Hypochaeris radicata* L., *Stellaria media* L., *Chenopodium album* L., *Chenopodium murale* L., *Ipomoea batatas* L., *Sisymbrium thellungii* O. Schulz, *Cucurbita pepo* L., *Rumex crispus* L., *Acalypha virginica* L., *Nicandra physalodes* L., *Physalis peruviana* L., *Solanum nigrum* L., *Urtica urens* L. and *Corchorus olitorius* L. About 27 % of the wild vegetables were native to South Africa and about 45 % were also used as medicinal plants in the areas. Sun drying was the most common method of preserving the wild vegetables for the off season months. This study also revealed that, men and the younger generation knew less about wild vegetables than the women. The study also revealed a loss of knowledge of wild vegetables and their use by the rural dwellers who are more in favour of the exotic types such as spinach and cabbage.

During the survey, 14 wild vegetable leaves were collected and analysed for their nutritionally valuable minerals and vitamins. *Solanum nigrum*, *Tulbaghia violacea*, *Chenopodium album* and *Chenopodium murale* respectively had the highest concentrations of fibre, protein as well as lipid, phytate and ash. *Chenopodium murale* had the highest concentration of magnesium, potassium and phosphorus while *Physalis peruviana* had the highest concentration of iron and vitamin C. Copper was remarkably low in all the wild

vegetables. This study revealed the high nutritional value of wild vegetables growing naturally in the wild.

One of the most mentioned wild vegetables during the survey was *Solanum nigrum*. This wild vegetable was therefore selected for further study in cultivation and determination of nutritional value.

Seed drying technique, viability and germination tests were conducted to understand the behaviour of the seeds of *Solanum nigrum*. This was with the view to determining the optimum conditions required for their germination. Seed viability was 78, 72 and 70 % in air dried, freshly extracted and sun dried seeds respectively.

Air drying respectively produced 8, 0 and 16 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while no germination was recorded in the temperature treatment, however, 1, 3 and 7 days pre-chilling respectively produced 8, 28 and 4 % germination and needle pricking as well as sand paper scarification respectively produced 76 and 48 %. Freshly extracted seeds respectively produced 8, 16 and 8 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while 25, 35 and 45^{o}C temperature treatments respectively produced 43, 60 and 56 % germination, but, 1, 3 and 7 days pre-chilling respectively produced 8, 28 and 4 % germination and needle pricking as well as sand paper scarification respectively produced 72 and 64 %. Sun dried seeds respectively produced 12, 0 and 16 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while 25, 35 and 45^{o}C temperature treatments respectively produced 18, 12 and 20 % germination, however, 1, 3 and 7 days pre-chilling respectively produced 16, 36 and 12% germination and needle pricking as well as sand paper scarification respectively produced 40 and 44 %.

Subjecting air dried seed to 5N H_2SO_4 for 120 s produced the best chemical scarification result (64 %). Air dried, Freshly Extracted and Sun Dried seed controls respectively produced 12, 16 and 8 % germination. Pre-chilling and photoperiodism reduced germination and concentrated H_2SO_4 destroyed the seeds. Germination was hindered by physiological dormancy and this was overcome by temperature and scarification of the seed. Breaking of dormancy at 35^oC and dilute sulphuric acid indicated that temperature plays an important role in breaking dormancy of *Solanum nigrum* seeds.

Field and glasshouse trials were conducted to determine the effect of fertilisers on growth, physiological response, proximate and mineral compositions of *Solanum nigrum*. The experiments were laid out in a Randomised Complete Block Design (RCBD). The treatments were: Control (T1); 100 kg N/ha (T2); 8.13 t goat manure/ha (T3); 100 kg N/ha + 8.13 t goat manure/ ha (T4) and 50 kg N/ha + 4.07 t goat manure/ ha (T5). Plant height, total number of leaves formed, chlorophyll content, moisture content, root: shoot ratio, leaf area and stem diameter were measured using standard growth indicator methods. Chlorophyll content, moisture and leaf area were measured on the leaves and root to shoot ratio was determined using the whole shoot and the roots.

Different growth parameters responded differently to various fertiliser treatments, however, organic fertiliser alone did not affect the growth parameters in this study. The application of 100 kg N/ha increased stem diameter, moisture content and root: shoot ratio on the field and the root: shoot ratio as well as stem diameter in the glasshouse. Combining NPK (100 kg N/ha) and goat manure (8.13 t/ha) significantly improved chlorophyll content and number of leaves in the glasshouse and plant height, number of leaves and chlorophyll on the field. Leaf area in both the glasshouse and the field as well as plant height in the glasshouse were significantly increased by the application of 50 kg N/ ha + 4.07 t manure/ ha. Positive growth was recorded in both the glasshouse and the field.

Proximate values were high in both the glasshouse and field experiments thereby meeting the recommended daily human intakes. The application of 50 kg N/ ha + 4.07 t manure/ ha fertiliser increased both the lipid and protein concentration on the field as well as vitamin C and protein in the glasshouse while the effect of other treatments on ash, fibre and phytate contents varied.

Potassium, copper and iron were consistently high throughout the period of the trials while phosphorus, sodium, magnesium and zinc decreased with plant maturity. Calcium and manganese however increased with plant maturity. The mineral compositions of *Solanum nigrum* were generally higher than the recommended daily human intakes throughout the growth stages. In addition, the recommended mean intake of vegetables per day is between 150 and 300 g in children and adults. These results indicate that *Solanum nigrum* responds positively to fertilisers and that harvesting the leaves of this plant at any growth stage has the potential to supply the nutrients needed by the human body for growth and development.

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER ONE
General introduction

1.1 What are wild vegetables?

Wild vegetables are succulent plants or portions of plants which could be consumed as a side dish with a starchy staple (Siemonsma and Pilvek, 1994). They are either native to their habitats or were introduced and have become naturalised in those regions. They usually grow naturally in the wild. Their leaves, stems, flowers, roots and/or fruits are gathered from the wild, usually by women and/or children, cooked, baked or eaten raw depending on the plant species. Although wild vegetables are generally viewed as weeds in agricultural fields, in some regions, they occupy a prominent place in the local food system and are consequently a vital part of agricultural system and as such, they are allowed to grow on the fields along with agricultural crops (Mazhar et al., 2007; Modi et al., 2006). Common examples of wild vegetables are *Amaranthus* species, *Bidens pilosa* L., *Chenopodium album* L., *Corchorus olitorius* L., *Sonchus oleraceus* L., *Taraxacum officinale* Weber, *Urtica urens* L., and *Solanum nigrum* L. among others.

1.2 The role of wild vegetables in the human diet

Due to climate change, competing demands for the production of biofuels, natural resource constraints worldwide among other factors, it is estimated that the global demand for food is expected to increase by 60 % in the year 2050 (FAO, 2012). In addition, about 870 million people (12 % of the global population) are estimated to have been undernourished between 2010 and 2012. About 27 % of these were in Africa. Malnutrition occurs when the body does not get the right amount of the nutrients it needs to maintain healthy tissues and organ functions. Research has proven that vegetables could supply these demands. According to FAO/WHO (2011), about 1.7 million deaths worldwide are attributed to low fruit and vegetable consumption yet low fruit and vegetable intake is among the top 10 selected risk factors for global mortality. A recent study revealed that about 72.2 men and 66.7 % of

women in South Africa consume less than the minimum recommended levels of vegetables (Hall et al., 2009). Report has also shown that about 10.8 million annual child deaths globally arising from zinc, vitamin A and iron deficiency (Black, 2003).

Vegetables are a pivotal component of a healthy diet; providing vitamins, antioxidants, fibre, amino acids, minerals and other health promoting compounds for nutrition security (Ebert, 2013). Wild vegetables play an important role especially in the diet of poor marginalised rural communities whose diets are predominantly starchy. They are known to provide low cost food options because they are gathered from the wild or around homesteads where they grow naturally. Researchers agree that the main valuable attribute of wild vegetables besides their ease of access is their high nutritional composition (Nesamvuni et al., 2001; Steyn et al., 2001). For example, Ndlovu and Afolayan (2008) reported that *Corchorus olitorius* has higher magnesium content than cabbage. In fact, according to International Plant Genetic Resources Institute (2003), almost all wild vegetables are good sources of micronutrients including iron and calcium as well as vitamins A, B complex, C and E. For example *Amaranth* contains 57 times more vitamin A than green cabbage and about 13 times more iron and nearly 9 times the calcium. There is therefore no doubt that wild vegetables have the potential to supply the recommended daily intakes of nutrients and can play an important role in the human diet.

1.3 The status of wild vegetables in South Africa

Although wild vegetables are highly nutritious, their recognition and use have been declining in many parts of the world including South Africa. According to Slikkerveer (1995), pre-agricultural people, such as Neolithic hunter-gatherers and pastoralists, had access to over 1500 species of wild plants for food, but later, ancient civilisations used only about 500 vegetables. Much more recently, Wehmeyer and Rose (1983) identified more than 100 wild

plant species that were being used as wild vegetables in South Africa. Wehmeyer (1986) documented over 300 wild plant species growing in Namibia, Southern Zimbabwe and South Africa. Mertz et al. (2001) reported that wild vegetables made up between 35 and 59 % of total vegetable consumption in two Burkina Faso villages. In Tanzania, Flueret (1979) documented about 15 species of wild vegetables that were consumed 32 % of meals compared to 18 % of meals for cultivated vegetables. In southern Niger, wild vegetables reportedly comprise about 21 % of the diet. In Ethiopia, drought resistant wild vegetables were found to constitute the main item of every meal (Smith et al., 1996; Humphrey et al., 1993). Ogle and Grivetti (1985) found that 39 % of meals consumed in Swaziland, contained wild vegetables. Jaca and Kambizi (2010) recently documented 30 plant species that are consumed as wild vegetables in the Eastern Cape Province of South Africa. There is a general consensus among researchers that there is a decline in wild vegetable use and knowledge in South Africa due to westernisation of the African diet, rural to urban migration, the general perception that wild vegetables are poverty foods and foods meant for women and children only (Jansen van Rensburg et al., 2007). A renewed interest in the nutritional value of wild vegetables is helping to document their true nutritional value. However, knowing the true nutrional value of wild vegetables is not enough; there is a need to determine the optimum agronomic conditions needed for their cultivation in order to inform people and in an effort to domesticate them.

1.4 The use of fertilisers in crop (vegetable) production

The use of fertilisers as a regular farming practice has been the trend in the developing countries since the 1960s. The existence of a close relationship between fertiliser consumption levels and agricultural productivity has been established beyond doubt. The termination of the use of fertilisers in agricultural fields would no doubt lead to a progressive decline in yields. The use of mineral fertilisers has been linked to acidification of the soil and

the accumulation of toxic substances such as cadmium (Arisha and Bradisi, 1999). In general, the use of excess mineral fertilisers often culminates in their loss to the environment leading to environmental contamination. Although soil acidification can easily be corrected by liming, it may be difficult to reclaim a cadmium contaminated soil. However, extensive research has also proven that mineral fertilisers actually improve the agronomic properties of the soil by making the soil more friable and receptive to moisture, improving organic carbon, contributing to greater biomass production and consequently protecting the soil from erosion as well as decreasing soil bulk density and increasing soil porosity (Buol and Stokes, 1997; Suzuki, 1997; Haynes and Naidu, 1998). Organic fertilisers on the other hand are known to influence plant nutrient availability by increasing soil organic matter which consequently improves the soil's physical structure, water storage and cation exchange capacity. Furthermore, organic matter provides a source of carbon and energy for microbial activities and improves the availability of nutrients although they are slowly mineralised (Isherwood, 2000). Organic matter production may not be enough to provide the quantities of plant nutrients required by crops and as such, organic and mineral fertilisers should be viewed as complimentary rather than competitive soil amendments. In this study, both organic and inorganic fertilisers were applied to *Solanum nigrum* during trials.

1.5 *Solanum nigrum* L.

Solanum nigrum, commonly known as Black Nightshade belongs to the Solanaceae family and this family comprises many vegetables and fruits such as potatoes, tomatoes, paprika and chillies among others. *S. nigrum* is a short lived perennial shrub that is predominantly Eurasian in origin but is now found in many parts of the world and has become naturalised even in South Africa (Edmonds and Chweya, 1997). The species observed in the Eastern Cape Province of South Africa grows in the wild especially on cultivated land (Figure 1). It has either purplish or dull black juicy and soft berries that are 8-13 mm in diameter, globose

as well as glabrous. *Solanum nigrum* is a nutraceutical herb (Sarma and Sarma, 2011). Although primarily used as a vegetable in South Africa, a paste from its unripe berries is used to treat ring worms while the leaves are used to treat diarrhoea in children and dysentery among other uses (Husselman and Sizane, 2006). In addition, ripe berries are eaten raw or made into a jam. Green unripe berries contain the toxin, Solanine, a glycol-alkaloid found throughout the plant but having the highest concentrations in green unripe berries. When ripe the berries reportedly become the least toxic part of the plant and are sometimes without ill effects (Watt and Breyer-Brandwijk, 1962). This plant is gathered from the wild in the Eastern Cape and to the best of our knowledge, it is not yet cultivated in the Province.

1.6 The choice of *Solanum nigrum* for this study

An ethnobotanical survey conducted in Nkonkobe and Mbashe municipalities of the Eastern Cape to document wild vegetables growing in these areas revealed that *Solanum nigrum* was one of the most popular wild vegetables (Chapter 2). Various authors including Husselman and Sizane (2006) as well as Jaca and Kambizi (2010) have also mentioned the popularity of this wild vegetable. This plant is cultivated in other parts of the world for example Bolivia, Ethiopia, Nigeria, Papua Guinea, Peru and the Limpopo Province of South Africa (Edmonds and Chweya, 1997; Jansen van Rensburg et al., 2007). Although *Solanum nigrum* is recognised as a nutritionally rich wild vegetable, there is a lack of comprehensive agronomic information on its cultivation requirements and nutritional value. Researchers have shown very little interest in improving the crop and thus no commercial varieties have been released to date. Therefore, in this study, *Solanum nigrum* was cultivated with various levels of organic and/or inorganic fertilisers. This is with a view to determine the plant's growth, physiological and nutrient uptake response to these fertilisers with a mind to determine the best fertiliser option for its cultivation. This will hopefully encourage other researchers into

developing cultivars more suited to Eastern Cape and a subsequent development of commercial varieties.

Figure 1: *Solanum nigrum*: A = Leaves growing in the glasshouse. B = Stem, fruits and flowers growing in the glasshouse. C = Leaves and flowers growing on the field. D = Stem and fruits growing on the field.

In addition, wild vegetables are a known and documented source of vitamins and minerals hence the need to enhance agricultural opportunities especially for the rural poor, through diversification of their diets, and one such strategy would be to adopt wild vegetables in their mainstream diets, in an effort to combat the challenge of malnutrition.

1.7 Objectives of the study

The broad objective of this study was to investigate the optimum conditions of soil and seed pre-treatment that are necessary towards the domestication of *Solunum nigrum*. The wild vegetable was previously identified in the ethnobotanical survey as one of the most popular in the study area.

The specific objectives are to:

- Conduct an ethnobotanical survey to identify, document and collect wild vegetables consumed in Mbashe and Nkonkobe municipalities in the Eastern Cape Province of South Africa
- Investigate the nutrient contents of the wild vegetables collected from the survey
- Select the most mentioned wild vegetable from the ethnobotanical survey list for further study towards its domestication (From the survey, *Solanum nigrum* was chosen)
- Collect ripe and mature seeds of *Solanum nigrum* from the wild
- Carry out viability and germination tests on *Solanum nigrum* seeds
- Determine the best way of drying *Solanum nigrum* seeds for cultivation and preservation
- Determine the chemical and physical properties of the soil on which trials will be conducted
- Determine the chemical properties of the organic fertiliser (goat manure) to be used in the trials
- Investigate the effect of fertilisers on growth and physiological response of *Solanum nigrum* cultivated on the field

- Investigate the effect of fertilisers on growth and physiological response of *Solanum nigrum* cultivated in the glasshouse
- Investigate the effect of fertilisers on proximate composition of *Solanum nigrum* cultivated on the field
- Investigate the effect of fertilisers on mineral composition of *Solanum nigrum* cultivated in the glasshouse

1.8 The structure of this thesis

This thesis contains 8 chapters. Chapter 1 is the general introduction. Chapter 2 is the ethnobotanical survey of wild vegetables consumed in Alice and Willowvale municipalities of the Nkonkobe and Mbashe municipalities all in the Eastern Cape Province of South Africa. Chapter 3 contains the proximate and mineral compositions of 14 wild vegetables collected from the two municipalities during the ethnobotanical survey. Based on popularity, *Solanum nigrum* was selected for further study. Chapter 4 therefore contains viability and germination experiments on *Solanum nigrum* seeds. Chapter 5 contains the field and glasshouse trials to evaluate the various plant growth parameters of *Solanum nigrum* treated with organic and inorganic fertilisers. Chapter 6 is an evaluation of proximate uptake of *Solanum nigrum* in response to organic and inorganic fertilisers in the glasshouse and on the field. Chapter 7 is the glasshouse and field evaluations of mineral uptake of *Solanum nigrum* in response to various levels of organic and inorganic fertilisers. Chapter 8 gives a clearer picture of the conclusions and recommendations of the study.

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CHAPTER 2

ETHNOBOTANICAL SURVEY OF WILD VEGETABLES IN MBASHE AND NKONKOBE MUNICIPALITIES, EASTERN CAPE, SOUTH AFRICA

CHAPTER TWO

Ethnobotanical survey of wild vegetables in Mbashe and Nkonkobe Municipalities, Eastern Cape, South Africa

2.1 Introduction

The use of indigenous wild vegetables in South Africa has been well documented over the years in both science and history books. Fox and Norwood Young (1982) reported that the Xhosa tribe were already gatherers of wild plants for food, including wild vegetables by the time they settled in the south eastern part of the country in the 1650s. However, the use of wild vegetables rapidly declined due to the climatic conditions that were slowly becoming harsher on vegetation. According to Rose and Guillarmod (1974), indigenous wild foods played a major role in providing mineral; vitamin and protein supplement to diets low in essential proteins and sometimes provided the bulk of the food intake in the lean months before harvest or in the advent of a drought. In the Transkei for example, every housewife was expected to be able to distinguish the wild edible plants from the inedible species to supplement the diet of the family which would otherwise become carbohydrate monotonous unless there was meat. The introduction and cultivation of exotic vegetables like cabbage and spinach in gardens and commercial farms, coupled with persistent drought over the years, has dealt a major blow to the use and awareness of indigenous wild vegetables. People now prefer to cultivate exotic vegetables as science has over the years concentrated on breeding improved exotic varieties that are drought tolerant and disease resistant, while generally neglecting the indigenous wild vegetables. Concerns about the loss of knowledge of wild indigenous vegetables have been highlighted by Vorster and Van Rensburg (2005). Studies on nutritional contents of some wild vegetables were conducted in Kenya (Chweya, 1985), Nigeria (Achinewhu et al., 1995), Botswana (Madisa and Tshamekang, 1997), Zimbabwe (Mushita, 1997), the Mediterranean countries (Žnidarčič et al., 2011) and many other parts of the world. These studies revealed that some of the wild indigenous vegetables were nutritionally better than the exotic ones. Domesticating some of these vegetables may be ideal not only because they are cheaper to produce, but because of their high nutritional value. This

survey therefore aimed to identify and document some of the wild vegetables of the Eastern Cape Province in order to create awareness especially on those that people are beginning to neglect or forget with a mind to domesticate them.

2.2 Materials and Methods

2.2.1 The Study Site

The Eastern Cape Province is located in the south-east of South Africa, bordering the Free State and Lesotho in the north, KwaZulu-Natal in the north east, the Indian Ocean along its south and south eastern borders, and Western and Northern Cape in the west. The province encloses 169 580 km^2, constituting 13.9% of the total land area of the country making it in surface area, the second largest province but also ranked the second poorest in the country (SSA, 2003). The survey was conducted in Alice in the Nkonkobe and Willowvale (Fig 2) in the Mbashe Municipalities of the province. Mbashe municipality is located within 31° 39' 0" South, 28° 16' 0" East in the eastern corner of the Amathole district municipality. Mbashe also hosts the poorest (Elliotdale) and second poorest (Willowvale) magisterial districts in the country (SSA, 2000). Nkonkobe municipality which houses the University of Fort Hare where the researcher of this work is based is located within 32^o 47′ 0″ south, 26^o 50′ 0″ east. The area is bounded by the sea in the east and drier Karroo in the west. The Valley Bushveld and Eastern Province Thornveld make up the Flora of Alice and Willowvale respectively. The major ethnic group is the Xhosa with farming as their main occupation.

Fig 2: Map of South Africa showing Alice and Willowvale in the Eastern Cape

2.2.2 Collection of information

The information on wild vegetables was collected following the method described by Flyman and Afolayan (2006). Using a well structured questionnaire (Appendix 1), interviews were conducted with villagers, most especially older women who are generally thought to be the repositories of information related to wild vegetable types, their uses and preservation. General questions such as the local names of wild vegetables, how they are prepared and stored were asked. A total of 66 individuals (45 females and 21 males) of ages between 17 and 59 years were interviewed in Willowvale and Alice between May and June 2011. The mean age of the respondents was 34. Informant consensus was used to uncover the relative importance of each vegetable identified in the study area and this was executed according to

Phillips and Gentry (1993). The identification and nomenclature of the listed plants were based on the Flora of South Africa. The plants were initially identified by their vernacular names (Xhosa) and later validated at the University of Fort Hare herbarium. Voucher specimens were also prepared and deposited in the Griffin herbarium of the University (Table 2.1).

2.3 Results

During the survey, a total of 22 wild vegetable plants belonging to 12 different families (Table 2.1) were collected and identified. The Asteraceae, Amaranthaceae and Solanaceae families made up 27.27, 22.73 and 9.09 % of the total number of species reported in this study respectively while the remaining 10 families each make up 4.55 %. Interestingly, about 37 % of the vegetables from Mbashe were not known in Nkonkobe and 5 % of wild vegetables that were known in Nkonkobe were not known in Mbashe although they were found growing in both localities (Table 2.2). Furthermore, about 45 % of the wild vegetables were identified as possessing medicinal properties. This survey also revealed that 91 % of the wild vegetables in these areas are available in summer, 32 % in winter and 27 % in both summer and winter (Table 2.3). Surprisingly, about 80 % of the men interviewed indicated that they had never consumed wild vegetables.

From this survey, *Tulbaghia violacea*, *Centella coriacea*, *Cotula heterocarpa*, *Sonchus oleracea*, *Sisymbrium thellungii* and *Corchorus olitorius* were the only wild vegetable plants native to South Africa while the remaining were introduced from Europe, Asia, and/or America and have become naturalised in the region. Naturalised species (for example *Amaranthus species* and *Solanum nigrum*) were more popular (94-97 %) than the native species (6-36 %) (Table 2.2). Two wild vegetable plants, namely *Cucurbita pepo* and *Ipomoea batatas*, were being cultivated both in the gardens and on the fields during the rainy

season. These previously wild vegetables and introduced to Africa have been domesticated in many parts of the world including South Africa. In addition, berries from *Physalis peruviana* and *Solanum nigrum* were also consumed as fruits in both localities.

Table 2.1: Wild vegetables documented in the Mbashe and Nkonkobe Municipalities, Eastern Cape, South Africa

Scientific name	Vernacular name (isiXhosa)	Herbarium voucher	Part used and method of preparation
Amaryllidaceae family			
Tulbaghia violacea Harv.	Itswele lomlambo	BVE11/021	Leaves and bulbs are mixed with tomatoes in place of onion to make a soup or to cook with other vegetables
Amaranthaceae family			
Amaranthus blitoides S. Watson	Unomdlomboyi	BVE11/001	Fresh leaves are cooked with onion and tomatoes and served with pap, mashed pumpkins, rice or bread
Amaranthus blitum L.	Unomdlomboyi	BVE11/002	Shoots are cooked with onion and tomatoes and served with bread or pap
Amaranthus hybridus L.	Unomdlomboyi	BVE11/003	Shoots are cooked with onion and tomatoes and served with bread or pap
Chenopodium album L.	Imbikicane ebomvu	BVE11/007	Spices are added to boiled fresh leaves and eaten alone or served with rice, bread or pap
Chenopodium murale L.	Umfanuthenkqi	BVE11/008	Shoots are boiled in water and mixed with spinach or cabbage and served with any starchy food
Apiaceae family			
Centella coriacea Nannfd.	Unongotyozane	BVE11/005	Tender and fresh leaves are boiled in water and mixed with mealie rice or

			mealie meal to make into a slightly thick porridge. The leaves may also be eaten in raw form or in salads
Asteraceae family			
Bidens pilosa L.	Umhlabangubo	BVE11/004	Cut shoots are boiled in milk to reduce bitterness and spices added and served with any starchy food or eaten alone. Serving it alone is good especially for the sick
Cotula heterocarpa DC.	Unondlabiyele	BVE11/009	Young leaves and stems are boiled in water till soft and mealie rice or mealie meal added with more water to cook until it becomes a porridge
Galinsoga parviflora Cav.	Iindevu zomlungu	BVE11/011	Fresh and young leaves are cut and mixed with other wild vegetables, boiled and spices added and cooked until they are done. They are served alone or with pap or rice
Hypochaeris radicata L.	Unokrengezi	BVE11/012	Shoots and flowers are cooked with onion and tomatoes and served with any starchy food. Raw flowers may also be mixed with lettuce and eaten in salads
Sonchus oleraceus L.	Ihlaba	BVE11/018	Freshly cut leaves are cooked with fried onion and tomatoes until they are ready. They are either mixed with mealie rice or mealie meal or eaten lone. Fresh leaves can also be eaten raw as a salad or snack
Taraxacum officinale Weber	Irhaba	BVE11/020	Young and fresh leaves are boiled in water until tender. Oil and salt are added and served either alone or with any starchy food. Roots are also dried, baked, percolated and used as a substitute for coffee

Brassicaceae family			
Sisymbrium thellungii O. Schulz	Isqwashumbe	BVE11/016	Shoots are cut and mixed with onion and tomatoes and served with pap or mixed with mealie rice or mealie meal to make a slightly thick porridge
Caryophyllaceae family			
Stellaria media L.	Impontshane	BVE11/019	Young leaves, stems and flowers are boiled in water until they are cooked and onion, tomatoes and seasoning added and served with rice or bread
Convolvulaceae family			
Ipomoea batatas L.	Imbatata	BVE11/013	Tubers are boiled in water until they are soft and served with tea. Fresh leaves can also be cooked with onion and tomatoes and mixed with other vegetables such as cabbage and spinach and served with the tubers or mashed potatoes
Cucurbitaceaea family			
Cucurbita pepo L.	Imithwane	BVE11/ 010	Fresh leaves and young pumpkins are cut and boiled in water. Cooking oil, onions, tomatoes, cooked potatoes and powdered soup are added. This is served alone and is especially healthy to young children. May also be served with rice or pap

Malvaceae Family			
Corchorus olitorius L.	Imifino	BVE11/008	Fresh leaves are boiled in water with bicarbonate of soda, onion and tomatoes and seasoning added. These are cooked until they are slimy and served especially with pap. It's good for the sick who have difficulties swallowing food
Polygonaceae family			
Rumex crispus L.	Idolo Lenkonyane	BVE11/015	Young leaves are boiled in milk and mixed with mealie meal to make porridge and served. Seasoning may also be added to better the taste
Solanaceae family			
Physalis peruviana L.	Iguzu	BVE11/014	Shoots are boiled or fried with onion and tomatoes, seasoning added and served with bread, rice, pap or mealie rice. Berries are harvested especially by young boys when they turn orange and eaten uncooked
Solanum nigrum L.	Umsobo	BVE11/017	Cut fresh shoots are boiled together with spinach or cabbage, cooking oil and seasoning added and served with any starchy food. If cooked alone, milk is added to reduce bitterness. When the berries turn purple black, they are harvested and eaten fresh or used to make jam

Urticaceae family			
Urtica urens L.	Urhawu	BVE11/022	Fresh and cut leaves are fried with onion and tomatoes, cooked potatoes added together with seasoning. Served alone or with any starch food. Care needs to be taken when handing the leaves because the whole plant is very irritating to the skin

Table 2.2: Frequency of mention of wild vegetables per gender group in Mbashe (Willowvale) and Nkonkobe (Alice) Municipalities, Eastern Cape, South Africa

Wild vegetable	Females (%)	Males (%)	Alice	Willowvale
A. blitoides	97	45	×	×
A. blitum	97	45	×	×
A. hybridus	97	45	×	✓ ×
B. pilosa	9	3	✓ ×	✓ ×
C. coriacea	18	0		×
C. album	21	3	×	✓ ×
C. murale.	21	3	×	✓ ×
C. olitorius	9	0	×	
C. heterocarpa	6	0		×
C. pepo	39	21		×
G. parviflora	9	0	×	×
H. radicata	18	0		×
I. batatas	24	12		×
P. peruviana	9	3	×	×
R. crispus	12	0	✓	✓ ×
S. thellingii	18	0	✓ ×	✓ ×
S. nigrum	94	33	×	✓ ×
S. oleraceus	36	18	×	✓ ×
S. media	6	0		×

T. officinale	21	6	×			×	
T. violacea	27	0		✓			✓ ×
U. urens	21	9		✓	×		

× Indicates the area where the wild vegetable was identified

✓ Indicates the community that identified the wild vegetable's medicinal properties

NB: The percentages are based on total number of respondents from each gender group not all the respondents

Table 2.3: Medicinal uses and seasonal availability of wild vegetables in Mbashe and Nkonkobe Municipalities, Eastern Cape, South Africa

Scientific name	**Medicinal use**	**Summer**	**Winter**
A. blitoides	n/a		×
A. blitum	n/a		×
A. hybridus	Leaves used to treat chest		×
B. pilosa	Leaves used to treat stomach ailments and influenza		×
C. coriacea	n/a	×	×
C. album	Leaves treat diarrhoea , purify the blood and improve digestion		×
C. murale.	Leaves used to treat asthma, purify the blood and improve digestion		×
C. olitorius	n/a		
C. heterocarpa	n/a		×
C. pepo	n/a		×
G. parviflora	n/a		×
H. radicata	n/a	×	×
I. batatas	n/a		×
P. peruviana	n/a		×
R. crispus	Roots are ground and the juice is orally ingested to treat arthritis		×
S. thellingii	Aids in digestion	×	×

S. nigrum	Paste from unripe berries used to treat ringworm, leaves used to treat diarrhoea in children and dysentery		×
S. oleraceus	Juice from the leaves used to treat wounds and ulcers	×	×
S. media	n/a	×	
T. officinale	n/a	×	×
T. violacea	Treats sinus headaches, coughs and colds. Also used as an aphrodisiac, mosquito repellent in the house and planted around the home and/or houses to act as a snake repellent	×	×
U. urens	Leaves used to treats hypertension, diabetes and arthritis		×

2.4 Discussion

The findings of this work are favourably comparable to the previous works of others in the same province. Bhat and Rubuluza (2002) documented 36 wild plant species growing in the Eastern Cape Province while Jaca and Kambizi (2011) identified 30 in OR Tambo District Municipality, some of which are also being used for medicinal purposes. Earlier on, Wehmeyer and Rose (1983) had identified more than 100 species of wild plants that were being used as vegetables in South Africa. Preliminary investigations indicated that the Mbashe community is more natural resources dependent and actively involved in agricultural activities than Nkonkobe. According to Della et al. (2006), the closely related indigenous people are to their land, the higher the degree of usage of their natural plant resources. This is a possible explanation for the vast knowledge of indigenous plants possessed by the Mbashe community. As noted by Modi et al. (2006) and Vorster et al. (2007), this study confirmed that the younger generation had less knowledge of wild vegetables than the older members of the community. According to van Rensburg et al. (2007) this could be the result of urbanisation and the influence of urban life style on the rural African populations. Furthermore, some authors attribute this to the westernisation of the African diet, the wrong

perception that wild vegetables are poverty and drought foods. (Vorster et al., 2005; Vorster et al., 2007; Lewu and Mavengahama, 2010).

In addition, some people fear poisoning resulting from picking the wrong vegetable plant, hygiene related ailments as some community members defecate in the fields where the vegetable plants grow as well as a general lack of interest to learn from the older generations about wild vegetables (Husselman and Sizane, 1996). The high percentage of men (80 %) who indicated that they had never eaten wild vegetables because they are meant for women and children and that wild vegetables make men impotent is possibly a result of ego based misinformation from the older generations about the true value of the wild vegetables. Ego based misinformation is also revealed when some of the men indicate that they used to eat wild vegetables but as they grew older, they stopped because they did not want to be perceived by their peers as weak. However the interviewees who had interest in wild vegetables liked the wild vegetables' bitter taste and believed that they were healthy foods especially for vegetarians. In contrast, Łuczaj (2008) reported that in Poland, all members of the community participated in gathering wild vegetables, including men; and that children were the most important collectors. Furthermore, some species such as *Chenopodium* and *Urtica* were collected by older women because they were associated with old habits and poverty. In this current study, the reluctance of men to gather wild vegetables could reflect a hunter gatherer culture from which these communities partly emanated. In hunter gatherer societies, women and children gathered vegetables and fruits while men hunted (Endicott, 1999). A preliminary study indicated that men still occasionally engage in subsistence hunting for small animals such as rabbits and springbok in the forest to supplement their diet. However, the majority of men work in towns and send money to the women and children who reside in rural areas at the end of the month. The men therefore provide the majority of the family needs while the women provide the lesser needs for example, taking care of the

children and homestead as well as working in the garden to provide vegetables and this includes gathering vegetables from the wild. This study also revealed that the sun drying method of preserving wild vegetables was the most preferred (68 %) though the majority are consumed fresh. This observation was also made in Buffalo Municipality in the Eastern Cape by Husselman and Sizane (1996).

The popularity of naturalised species such as *Amaranthus* when compared to native species may be related to the wild vegetables' abundance in the fields after the first rains as volunteer weeds and preferred taste. *Amaranth* species are very popular and abundant throughout the world as both wild and domesticated vegetable and are cultivated in numerous parts of the world including South Africa (DAFF, 2010). While attention has been focused on such species as *Amaranth*, lesser focus has been directed towards the native wild vegetables and this has possibly led to their neglect as possible diet supplements.

The survey also revealed that the majority of the interviewees (70 %) were aware of the high nutrional content of the wild vegetables and the ability of the species to boost the immune system especially in HIV/AIDS patients. According to the WHO (2002), a poor diet exacerbates HIV/AIDS leading to an early death. Inclusion of the nutritionally rich wild vegetables in HIV/AIDS patients' diets may therefore improve their immune systems. The knowledge of both the nutritional and medicinal properties of wild vegetables may enhance their re-acceptance as important human diets.

2.5 Conclusion

The present study indicates that wild vegetables are not totally neglected by rural communities; however, their use has declined over the years. A total of 22 wild vegetable species were identified and cooking fresh leaves was the preferred mode of consumption. However, about 45 % of the wild vegetables were identified by the informants as possessing medicinal properties. About 18 % of the wild vegetables are native to South Africa and 82 %

were introduced from around the world. The majority of the respondents indicated that they use wild vegetable in their diets although it's the women and children who eat them; only 20 % of the men indicated that they eat the wild vegetables. Westernisation of the African diet, readily available conventional vegetables in supermarkets and among vegetable vendors, the lack of interest to learn, urban-rural linkages, backwardness and poverty attached stigma are some of the reasons for the gradual neglect of wild vegetables. There is an urgent need for the government and nongovernmental organisations to educate people on the nutritional benefits of eating wild vegetables and encourage their cultivation. Wild vegetables have high nutritional values and most of them also have important medicinal properties. The knowledge possessed by the older generations that are still alive needs to be passed down to the younger ones. When the older generations die without passing on vital information about these vegetables especially how to identify them and their many uses, their knowledge die with them as will culture and some traditions.

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CHAPTER 3

NUTRIENT COMPOSITIONS OF SOME WILD LEAFY VEGETABLES IN MBASHE AND NKONKOBE MUNICIPALITIES, EASTERN CAPE, SOUTH AFRICA

CHAPTER THREE

Nutrient compositions of some wild leafy vegetables in Mbashe and Nkonkobe Municipalities, Eastern Cape, South Africa

3.1 Introduction

Wild vegetables have been a part of the human diet since the beginning of time. Human consumption of these vegetables and the ability of the species to meet nutrient needs have been documented for a long time. It is estimated that, at least, one billion people globally still include wild foods in their diets (Aberoumand and Deokule, 2010). In South Africa, Wehmeyer and Rose (1983) identified more than 100 plant species that are being used as wild vegetables.

In many places of the developing world, there has been a low trend in the consumption of wild vegetables. This reduction in the consumption of wild vegetables has been attributed to seasonal availability and culture in Nigeria, while in South Africa, culture, taste and affordability have been cited as the major causes (WHO, 2003; Hart et al., 2005; Shackelton et al., 2009; Faber et al., 2010). Wild vegetables are usually consumed by the rural populace as supplements to their diet since the vegetables usually naturally grow on cultivated or fallow fields thereby making them easily accessible. However, the urban populace pay less attention to wild vegetables in favour of the conventional ones because of easy accessibility of the later. The use of herbicides, pesticides, as well as excessive cultivation of the fields has led to the decline in the availability of wild vegetables and subsequent decline in their knowledge (Odhav et al., 2007). Also, the perception, especially among young people, that such vegetables are foods for the poor, causes lack of interest in the cultivation and nutritional importance of these plants (Vorster et al., 2007).

Yet studies have revealed that wild vegetables have numerous beneficial nutritional values often better than the domesticated exotic breeds like spinach and cabbage (Odhav et al., 2007; Lewu and Mavengahama, 2010). The use of wild vegetables could be lost with time if their knowledge, especially on the identification, nutrient value, methods of preparation and preservation are not passed down to the younger generations and properly documented.

Therefore, this study was conducted to investigate the nutritional composition of some of the wild vegetables growing in the Eastern Cape Province of South Africa, in an effort to create an awareness of some important aspects of these neglected food plants.

3.2 Materials and methods

3.2.1 Plant collection and preparation

Fourteen, wild vegetables were collected fresh from the Mbashe and Nkonkobe Municipalities of the Eastern Cape between February and June 2011. This was the period when the wild vegetables were vegetatively growing in home gardens and fields. The plants were initially identified by their vernacular names (Xhosa) and later validated at the University of Fort Hare Herbarium. Young and tender shoots were plucked from the mother plant as practised locally, stored in khaki paper sampling bags and transported to the laboratory where they were washed thoroughly with distilled water. Leaves of the same plant from the two municipalities were combined to make a single composite sample, oven dried at 40^{o}C to a constant weight and homogenised using a 2 mm sieve Polymix (PX-MFC 90 D) electric grinder, after which they were sealed in polyethylene bags and stored in the refrigerator at 4^{o}C until needed for the various analysis.

3.2.2 Proximate analysis

Determination of ash content

About 5 g of the powdered plant leaf sample was weighed into a previously weighed crucible. This was incinerated in an E-Range muffle furnace with TOHO P4 programme at 550^{o}C for 12 h. The final weight of the sample was used to calculate the ash content as follows:

Ash content (%) = x 100 %

(Antia et al, 2006).

Determination of crude lipid

Crude lipid was determined as described by Antia et al. (2006). About 5 g of the powdered sample was measured into a 250 ml beaker, 100 ml of diethyl ether was added, covered with aluminium foil and shaken in an orbital shaker for 24 h. Filtration followed this process and the supernatant was decanted. Another 100 ml of diethyl ether was added to the residue and shaken for another 24 h. The residue obtained after filtration was the lipid free sample and was calculated as:

$$\text{Crude lipid} = \frac{\qquad\qquad\qquad\qquad\qquad\qquad}{\qquad\qquad\qquad\qquad\qquad\qquad} \times 100\ \%$$

Determination of crude fiber

The AOAC (1984) method was used in estimating the crude fibre. About 5 g of the powdered sample was weighed into a beaker and digested in 100 ml of 1.25 % sulphuric acid for 30 min. The acid digested sample was allowed to cool, and then filtered. The residue was collected into a beaker and further digested in 100 ml of 1.25 % sodium hydroxide. The sample was filtered and the residue dried in an oven at 100^{o}C to a constant weight. The dried residue was then incinerated in a muffle furnace for 24 h at 550^{o}C. The crude fiber was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat free samples (AOAC, 1984):

$$\%\ \text{fiber} = \frac{\qquad\qquad\qquad\qquad\qquad\qquad}{\qquad\qquad\qquad\qquad\qquad\qquad} \times 100\ \%$$

Determination of vitamin C

Preparation of iodine solution

The iodometric titration method was used to determine the vitamin C content. About 5 g of potassium iodide and 0.268 g potassium iodate were dissolved in 200 ml distilled water in a 400 ml beaker, followed by addition of 30 ml of 3 M sulphuric acid. The mixture was poured into a 500 ml graduated cylinder and then diluted to a final volume of 500 ml with distilled water.

Vitamin C standard solution was prepared by dissolving 0.250 g vitamin C in 100 ml water. This was made up to 250 ml with distilled water.

Standardisation of iodine with Vitamin C standard solution

About 25 ml of vitamin C standard solution was measured into a 125 ml Erlenmeyer flask, following which 10 drops of 1 % starch solution were added as the indicator. This was titrated against the acidified potassium iodide iodine solution until the end point (the first blue colour that showed after at least 20 s of swirling) was reached.

Vitamin C determination in the samples

About 5 g of fresh leaf samples were macerated in 20 ml of distilled water. The mixture was filtered and the filtrate collected in a 50 ml volumetric flask and made to the mark with distilled water. About 10 ml of the sample solution was transferred into an Erlenmeyer flask and 10 drops of 1 % starch added and titrated against the acidified potassium iodide solution.

Determination of phytate components

Phytate was determined according to the method of Wheeler and Ferrel (1971). A sample of finely ground plant material measuring 4 g was soaked in 100 ml of 2 % hydrochloric acid for 3 h and then filtered through Whatman No. 43 filter paper. About 25 ml of the filtrate was measured into a conical flask and 5 ml of 0.3 % ammonium thiocyanate solution was added

as indicator, followed by addition of 53.5 ml distilled water. This was titrated against a 1000 ppm standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The Phytate content was calculated from the iron determinations, assuming a 4:6 iron to Phytate molecular ratios and multiplied by a constant of 3.55 (Vijayakumari et al., 1996).

Determination of protein

About 0.5 g of finely ground vegetable samples was placed in dry, clean digestion tubes and 5 ml of the digestion mixture comprising 1 part $HCIO_4$ + 2 parts HNO_3 added. This mixture was digested at 230^{o}C on a digestion block for 70 min, allowed to cool down and made up to 100 ml volume with distilled water. The concentration of nitrogen was then determined using the Inductively Coupled Plasma - Optical Emission Spectrometer (ICP OES). Percentage crude protein was obtained by multiplying the nitrogen value by a factor of 6.25 (AGRILASA, 2008).

3.2.3 Mineral analysis

Macro-minerals (Phosphorus, Potassium, Calcium, sodium and Magnesium) and micro-minerals (Copper, Iron, Zinc and Manganese) were determined using the method described above for the determination of nitrogen.

3.2.4 Statistical analysis

Data of the nutrient concentrations of various wild vegetables were subjected to statistical analysis using MNITAB Release 12. A one way analysis of variance was used to compare the means of various nutrient concentrations among the wild vegetables. Means were segregated

using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

3.3 Results

3.3.1 Proximate composition

The proximate compositions of the wild vegetables gathered from Mbashe and Nkonkobe municipalities in the Eastern Cape Province of South Africa varied significantly ($p < 0.05$) among the vegetables (Table 3.1). Crude fibre ranged between 0.91 and 6.79 g/100g; crude protein between 0.47 and 1.53 g/100g; crude lipid between 0.14 and 3.83 g/100g; phytate between 1.09 and 8.92 g/100g and ash content was between 0.38 and 1.79 g/100g. *Tulbaghia violacea* had the highest protein and *Sonchus oleraceus* the highest lipid content. The lowest protein content was recorded in *Chenopodium murale* while the highest fibre and ash contents were recorded in *Solanum nigrum* and *Chenopodium murale* respectively. The lowest ash values were observed in *Solanum nigrum*.

3.3.2 Mineral composition

The mineral compositions of the 14 vegetables significantly differed among the vegetables as shown in Table 3.3. The mean concentration of nutrients in all the vegetables decreased in the order Vit C > K > Ca > Mg > Na > P > Fe > Zn > Mn > Cu. *Chenopodium murale* contained the highest K, Mg and P, while *Physalis peruviana* contained the highest concentration of Fe and Vit C. Cu was remarkably low in all the wild vegetables.

Table 3.1: Proximate compositions (g/100 g) of 14 vegetables in Mbashe and Nkonkobe Municipalities in the Eastern Cape Province of South Africa

Vegetable species	†Phytate	Crude protein	Crude lipid	Crude fibre	Ash
Bidens pilosa L.	2.4±6.51^{c}	1.19±0.03^{ab}	1.60±0.22^{d}	2.14±0.32^{c}	0.38±0.03^{b}
Centella coriacea Nannfd.	2.31±1.31^{a}	1.1±0.05^{ab}	0.52±0.02^{b}	1.09±0.03^{a}	1.74±0.05^{c}
Chenopodium album L.	8.92±0.38^{a}	0.48±0.01^{a}	1.15±0.19^{b}	1.68±0.02^{ac}	1.38±0.04^{d}
Chenopodium murale L.	3.07±4.30^{b}	0.47±0.01^{a}	3.50±0.03^{a}	1.35±0.01^{a}	1.79±0.02^{c}
Cotula heterocarpa DC.	2.23±0.57^{ab}	1.02±0.14^{ab}	0.84±0.05^{b}	1.01±0.02^{a}	1.44±0.01^{d}
Galinsoga parviflora Cav.	2.98±0.81^{c}	0.94±0.08^{ab}	2.51±0.46^{e}	1.66±0.29^{c}	0.45±0.03^{b}
Hypochaeris radicata L.	1.09±0.81^{a}	0.82±0.07^{ab}	0.14±0.03^{f}	1.00±0.01^{a}	0.80±0.16^{a}
Physalis peruviana L.	4.19±0.85^{ab}	1.02±0.14^{ab}	1.93±0.51^{de}	1.53±0.03^{ac}	1.39±0.24^{d}
Rumex obtusifolius L.	4.86±1.05^{a}	0.48±0.01^{a}	0.35±0.11^{c}	1.08±0.01^{a}	1.24±0.01^{d}
Solunum nigrum L.	2.34±0.50^{a}	0.56±0.02^{a}	0.58±0.02^{b}	6.79±0.01^{b}	0.39±0.02^{b}
Sonchus oleraceus L.	1.50±5.37^{d}	1.09±0.06^{ab}	3.83±0.04^{a}	1.11±0.02^{a}	0.77±0.05^{a}
Stellaria media L.	4.64±0.61^{b}	0.51±0.00^{a}	2.56±0.06^{e}	1.26±0.01^{a}	1.55±0.01^{d}
Tulbaghia violacea Harv.	1.55±2.14^{c}	1.53±0.13^{ab}	0.64±0.04^{b}	0.91±0.04^{a}	1.29±0.0.02^{d}
Urtica urens L.	2.51±0.90^{a}	0.87±0.13^{ab}	0.64±0.02^{b}	2.35±0.02^{c}	1.67±0.0.01^{cd}

Different letters down the same column represent significant differences at $p < 0.05$.

Table 3.2a: □ Recommended daily proximate intakes by life stage and gender (g/day)

	Protein	Lipid	Fibre
Boys	40-65	0.07-0.125	24-28
Girls	35-45	0.07-0.085	20-22
Women	46	0.09	25
Men	64	0.160	30

□Nutrient reference values for New Zealand and Australia (NHMRC, 2005).

Table 3.2b: □ Recommended daily mineral intakes by life stage and gender (mg/day)

	Boys	Girls	Women	Men
K	3000-3600	2500-2600	2800	3800
Mg	240-410	240-360	310-320	400-420
P	1250	1250	1000	1000
Na	400-920	400-920	460-920	460-920
Ca	1000-1300	1000-1300	1000-1300	1000
Cu	1.3-1.5	1.1	1.2	1.7
Fe	8-11	8-15	8-18	8
Mn	3-3.5	2.5-30	5	5.5
Zn	6-13	6-7	8	14
Vit C	40	40	45	45

□ Nutrient reference values for New Zealand and Australia (NHMRC, 2005).

Table 3.3: Mineral compositions (g/100 g) of 14 vegetables in Mbashe and Nkonkobe Municipalities in the Eastern Cape Province of South Africa

Vegetable species	Mineral Element									
	Ca	Mg	K	Na	P	Cu	Fe	Zn	Mn	Vitamin C
Bidens pilosa L.	14.70±3.48^{e}	3.46±1.51^{cb}	71.95±74.00^{cd}	0.28±1.99^{a}	2.57±0.67^{f}	0.01±0.01	0.87±0.53^{c}	0.28±2.73^{b}	0.13±0.06^{ab}	0.042±0.06^{a}
Centella coriacea Nannfd.	14.39±1.33^{e}	16.69±6.93^{f}	110.88±54.30^{fg}	2.54±0.98^{c}	4.29±0.11^{g}	0.01±0.00	0.26±0.19^{a}	0.15±3.76^{a}	0.15±0.04^{ab}	0.125±0.06^{a}
Chenopodium album L.	10.40±2.70^{cd}	2.69±0.20^{cb}	16.80±21.50^{a}	22.01±1.86^{g}	2.10±0.19^{a}	0.01±0.03	0.68±0.19^{b}	0.12±0.18^{a}	0.21±1.80^{b}	0.050±0.03^{a}
Chenopodium murale L.	7.27±4.66^{ab}	2.72±0.66^{b}	61.65±29.40^{c}	12.44±2.78^{e}	3.56±0.26^{e}	0.01±0.01	0.76±0.40^{bc}	0.16±0.31^{a}	0.07±0.03^{a}	0.058±0.06^{a}
Cotula heterocarpa DC.	11.34±4.26^{d}	22.79±19.64^{h}	125.97±44.20^{g}	5.37±2.14^{cd}	4.76±0.56^{i}	0.01±0.00	0.59±0.73^{a}	0.14±0.23^{a}	0.05±0.51^{a}	0.117±0.00^{b}
Galinsoga parviflora Cav.	24.77±3.41^{g}	4.04±1.51^{c}	53.31±114.00^{b}	0.38±1.13^{a}	4.51±0.74^{h}	0.01±0.01	0.53±0.07^{b}	0.14±0.30^{a}	0.13±0.08^{ab}	0.047±0.15^{d}
Hypochaeris radicata L.	9.14±6.80^{c}	4.10±0.17^{c}	52.00±11.10^{b}	19.54±6.00^{f}	2.57±0.55^{b}	0.01±0.02	0.42±0.15^{b}	0.28±0.22^{b}	0.07±0.06^{a}	0.048±0.02^{a}
Physalis peruviana L.	13.11±7.50^{e}	9.70±7.21^{e}	84.63±85.20^{cd}	0.25±2.82^{a}	2.89±0.91^{c}	0.02±0.01	2.60±2.29^{e}	0.34±0.22^{c}	0.10±0.49^{a}	0.225±0.03^{a}
Rumex obtusifolius L.	8.03±3.59^{b}	4.93±1.56^{c}	73.28±63.10^{c}	0.86±0.65^{a}	4.59±0.71^{h}	0.01±0.01	0.44±0.80^{ab}	0.14±0.29^{a}	0.06±0.85^{a}	0.108±0.10^{c}
Solunum nigrum L.	6.70±6.98^{a}	6.05±1.56^{d}	121.52±73.80^{g}	3.83±1.79^{c}	4.43±1.18^{g}	0.01±0.00	0.77±0.48^{cb}	0.30±3.91^{bc}	0.08±0.29^{a}	0.042±0.10^{a}
Sonchus oleraceus L.	16.98±4.59^{f}	3.72±1.46^{cb}	76.91±80.40^{d}	0.65±1.09^{ab}	3.88±0.70^{f}	0.01±0.06	0.43±0.66^{b}	0.12±0.50^{a}	0.07±0.02^{a}	0.014±0.04^{a}
Stellaria media L.	12.25±4.78^{de}	4.15±0.54^{c}	50.6±5.20^{b}	18.73±2.37^{f}	3.17±0.74^{d}	0.02±.03	0.56±0.59^{b}	0.14±0.39^{a}	0.04±0.41^{a}	0.058±0.15^{b}
Tulbaghia violacea Harv.	7.36±3.98^{ab}	1.54±0.80^{a}	80.44±38.70^{cd}	3.72±2.39^{c}	3.65±1.54^{e}	0.01±0.01	0.21±0.19^{a}	0.19±0.48^{a}	0.02±0.02^{a}	0.162±0.06^{b}
Urtica urens L.	34.84±3.65^{i}	4.25±1.58^{c}	66.99±12.90^{c}	0.41±1.20^{a}	4.68±0.56^{ih}	0.01±0.01	0.35±0.17^{ab}	0.16±0.47^{a}	0.10±0.04^{a}	0.045±0.02^{a}
Range	6.70-34.84	1.54-22.79	50.6-125.97	0.25-18.73	2.10-4.76	0.01-0.02	0.21-2.60	0.12-0.34	0.04-0.60	0.01-0.23

Different letters down the same column represent significant differences at $p < 0.05$

3.4 Discussion

3.4.1 Proximate composition

The proximate analysis revealed that all the 14 wild vegetables are rich in the various nutrients investigated but in varying proportions. Ash, protein, lipid and fibre contents in *Sonchus oleraceus* were lower than earlier reported by Jimoh et al. (2011). Odhav et al. (2007) investigated the proximate composition of 20 wild plants and reported higher values for protein (3 - 7 g/100 g) and ash (1.74-4.91 g/100 g), but lipid (0.2 - 2.7 g/100 g) and fibre (1.21 - 2.92 g/100 g) were within the range of the present study. *Sonchus oleraceus* and *Chenopodium murale* showed appreciable lipid contents in this study. Lyimo et al., (2003) reported a lipid range of between 0.1 and 1.0 g/100 g in 30 wild vegetables, but lower than observed in the current study. According to Antia et al. (2006); lipids increase the palatability of food by absorbing and retaining flavours. A diet providing 1 - 2 % of its calorific energy as fat is said to be sufficient for humans, as excess fat consumption is implicated in cardiovascular disorders such as atherosclerosis, cancer and aging (Sharma et al., 2012). The considerable amount of lipids in some of these vegetables in relation to the NHMRC (2005) values would therefore improve the palatability of the vegetables and reduce the risk of some diseases. Lyimo et al. (2003) reported a protein range between of 0.6 and 5.0 g/ 100 g in 30 wild vegetables, again this was higher than the current study with a range of between 0.47 and 1.53 g/100 g. Protein is essential for growth and repair of muscles, bones, skin, tendons, ligaments, hair and eyes amongst other tissues in humans. The protein values from this study indicate that the wild vegetables have the ability to supply a fraction of the Recommended Daily Intake (RDI) values (NHMRC, 2005). Children need to consume at least 150 g of vegetables per day to harness the required nutrients (NHMRC, 2003). For example; *Sonchus oleraceus, Tulbaghia violacea and Bidens pilosa* can supply 5.0, 5.5 and 7.1 % respectively

of children's RDI values. High ash content in plants is a reflection of high mineral content (Aberoumand and Deokule, 2010). The high ash content of *Chenopodium murale* compared to other vegetables in this study is an indication of high mineral value of the vegetable. High fibre diets have been linked with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, reduced risk of certain forms of cancer and an improved gastrointestinal function (Anderson et al., 1994). *Solanum nigrum, Urtica urens and Bidens pilosa* can supply about 67.9, 23.5 and 21.4 % of the required daily amount (Table 3.2a) in men if a quantity of about 300 g is consumed. The concentration of fibre would also presumably not lead to high fibre related conditions and diseases. Phytate is an antinutrient which has a strong ability to chelate multivalent metal ions especially Zn, Ca and Fe, leading to their poor bioavailability (Gupta et al., 2006). Although the presence of phytate could decrease mineral absorption in humans, this antinutrient is said to be heat labile (Akwaowa et al., 2000). It is therefore conceivable that the high phytate content in *Chenopodium album* (8.92 g/100 g) and *Physalis peruviana* (4.19 g/100 g) will be significantly reduced during cooking. Additionally, leafy vegetables are generally considered to be superior sources of mineral supplements and therefore would ideally lower the effect antinutrients would have on the availability of some nutrients (Odhav et al., 2007).

3.4.2 Mineral and vitamin C composition

Vitamin C was remarkably high in *Physalis peruviana* (0.225 g/100 g) which makes the vegetable a better source of the vitamin C compared to guava (0.188 g) and orange (0.07 g) fruits as well as some leafy vegetables such as broccoli (0.039 g) (Brand et al., 1982). The results of the current study are comparable with what was reported by Lyimo et al. (2003) in a study of 30 wild vegetables in Tanzania. These authors found that *Galinsoga parviflora*, *Bidens pilosa,* and *Solunum nigrum* respectively contained 0.054, 0.059 and 0.234 g/ 100 g

vitamin C. *Sonchus oleraceus* which had the lowest vitamin C concentration has the potential to supply about 31 % of vitamin C RDI while *Physalis peruviana* which had the highest concentration can supply about 500 % of the required vitamin C RDI in adults (Table 2.4). Vitamin C prevents tissue damage, aids in the recovery of several ailments and diseases including colds, cough, influenza, sores, wounds and skin diseases among others (Ogunlesi et al., 2010). According to Lopez and Martos (2004), vitamin C improves Fe availability, therefore reducing the risk of iron deficiency anaemia. Potassium was also remarkably high in the wild vegetables while Cu was low in the present study. In studies previously conducted by Bvenura and Afolayan (2012) in Nkonkobe Municipality, Cu was low in cabbage, spinach and carrot. The previous and current results possibly indicate the low levels of the mineral in the soil of the study area. Jimoh et al. (2011) and Kawada et al. (2002) reported slightly lower mineral values compared to the present study except for Mn, Cu and Zn, which were slightly higher. In Iran, lower levels of mineral nutrients were found in some wild vegetables as compared to the present study (Aberoumand and Deokule 2010). Research done in Akure, Nigeria indicated a slightly higher concentration of mineral nutrients compared to this study (Aletor et al., 2002). In KwaZulu Natal, South Africa, Odhav et al. (2007) found high levels of Ca, P and Mg with many wild vegetables exceeding 1000 mg/100 g. Variations in the nutrient compositions of edible plants are influenced by various factors including farming practices, prevailing environmental conditions including soil manipulation using organic and inorganic fertilisers and the age of the plants at harvest (Nordeide et al., 1996). In addition, some minerals such as Zn decrease with advancing plant age while other minerals such as Fe and Mn reportedly increase with increasing plant age (Tiffin, 1971). In this study, vegetables were collected from fields where the soil has been manipulated by addition of organic and mineral fertilisers and only young fresh plants were collected. These factors may have contributed to the amount of minerals in the vegetables. Findings of this study also indicated

that all vegetable samples had Na: K ratios of less than 1. Yang et al. (2011) linked a high Na: K ratio with increased risk of cardiovascular diseases leading to mortality. These authors further reported that a high Na intake and a low K intake are linked to high blood pressure. The consumption of these vegetables would therefore not only help lower blood pressure especially among the elderly but also boost their immune system. In sub-Saharan Africa, South Africa has the 4th highest number of people living with HIV/AIDS with an estimated 5.6 million (17.8 %) people infected. The Eastern Cape Province is the 6th most affected with 9 % of the population living with the virus (UN/AIDS, 2010). Researchers around the world have strongly linked HIV to nutrition; they view nutrition as a fundamental intervention in boosting the immune system of the infected in the early stages and ongoing treatment of the disease (Elbein, 1995; Tinnerello, 1998; Charles, 2009). Among other nutrients, people living with HIV are more susceptible to low levels of Zn and Fe in their blood (Barnett, 2006). As shown by the appreciable amounts of these minerals in this study, a wild vegetable inclusive diet for example comprising *T. violacea*, *S. oleraceus* and *S. nigrum* would presumably improve the nutrition of HIV/AIDS patients. The high levels of P observed in vegetables from this study may also increase the mineral's availability in human nutrition. Mn is an activator and constituent of several enzymes and occurs in very low quantities in humans though this mineral's importance cannot be overlooked (Medeiros and Wildman, 2000). The Mn content observed in this study, though low, may supplement its presence in the diet.

In humans, Mg is a critical co-factor in more than 300 enzymatic reactions in the body while in plants the most recognised role of the element is in photosynthesis, where the element must be incorporated into the chlorophyll molecule before chlorophyll is effective at gathering light for photosynthetic carbon reduction reactions (Schachter, 2012; Wilkinson et al., 1990). Therefore, the abundance of Mg in wild vegetables is essential in ensuring a healthy plant and the subsequent availability of other minerals and their supply in the human diet. In relation to

the RDI values (Table 3.2b) and the NMHRC (2003) recommended quantities for children, the mineral elements can be sufficiently supplied per 150 g cooked portion. However, one of the drawbacks with wild vegetables is their seasonal availability. These nutritionally rich foods are usually available during rainy season but this shortfall can be overcome by gathering in large quantities when available, drying and storing for off-season consumption. Furthermore, a more sustainable solution that may ensure a continuous fresh supply of these wild foods is to cultivate them in home gardens.

3.5 Conclusion

The results of this study reveal that the leaves of the 14 wild vegetables are rich in minerals. However, the vegetables differ in nutrient contents. However, *Sonchus oleraceus* which had the lowest vitamin C concentration has the potential to supply about 31 % of vitamin C RDI while *Physalis peruviana* which had the highest concentration can supply about 500 % of the required vitamin C RDI in adults per 300 g of serving. *Solanum nigrum, Urtica urens and Bidens pilosa* can supply about 67.9, 23.5 and 21.4 % of the required daily amount of fibre in men per 300 g serving. *Sonchus oleraceus, Tulbaghia violacea and Bidens pilosa* can supply 5.0, 5.5 and 7.1 % respectively of children's RDI values for protein per 150 g of serving.

When compared to other vegetables *Chenopodium murale* is a good source of Mg, K and P while *Physalis peruviana* is a good source of Cu and Fe. Furthermore, *Physalis peruviana* is a good source of Vit C as well as protein and *Solanum nigrum* is a good source of fibre. Mixing these vegetables when cooking them as is practised locally has the potential to meet the human requirements of nutrients for growth and development on a daily basis which in turn helps to overcome nutritional deficiency problems which are prevalent especially in poor rural areas. These vegetables are therefore recommended for consumption alone or in combination with other vegetables.

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CHAPTER 4

THE EFFECT OF LIGHT, TEMPERATURE, SCARIFICATION AND ACID ON GERMINATION OF *SOLANUM NIGRUM* SEEDS

CHAPTER FOUR

The effect of light, temperature, scarification and acid on germination of *Solanum nigrum* seeds

4.1 Introduction

In many parts of the world, *Solanum nigrum* plays an important economic role. The plant is considered a weed of over 37 crops in 61 countries around the world. It is a host to some insects, nematodes and disease organisms that attack crops and reduce grain quality with their juice if the berries erupt while harvesting grain crops such as beans and maize (Taab and Andersson, 2009; Ogg and Rogers, 1989; Holm et al., 1991; Ogg et al., 1981). In contrast, in many African countries including South Africa *Solanum nigrum* leaves are gathered from the wild, cooked alone or mixed with other wild vegetables and consumed as a side dish. Furthermore, in South Africa, ripe and mature berries are consumed as a fruit, or made into a jam (Husselman and Sizane, 2006). Medicinal properties of *Solanum nigrum* have also been reported (Chifundera, 1998; Bhat and Rubuluza, 2002; Khan and Khatoon, 2008; Moshi et al., 2009). *Solanum nigrum* usually grows naturally in the fields when crops such as maize are cultivated, and because it is regarded as wild, little attention is paid to the viability and germinability of its seeds. Seed viability is the capability of a seed to produce a normal seedling or to germinate under optimal conditions (Copeland and McDonald, 2001). Work done by various authors including Baskin and Baskin (2004) as well as Schmidt (2007) has suggested that when conditions that favour germination of a seed are not met, a viable seed may not necessarily germinate and may therefore be dormant. Roberts and Lockett (1978) reported that freshly harvested *Solanum nigrum* seeds germinate within a few weeks of separation from the berry and therefore such seeds possess little or no dormancy. However, Baskin and Baskin (1988) suggested that fresh seeds are conditionally dormant and therefore germinate under certain environmental conditions. High percentage germination has been widely reported in *Solanum nigrum* seeds after a period of storage at constant temperature in other parts of the world (Givelberg et al., 1984, Kremer and Lotz, 1998). In contrast, Roberts and Lockett (1978) reported alternating temperatures as the ideal temperatures for high

percentage germination of the species. Seed dormancy has also been linked to seasonal temperature variations, where cold stratification weakens seed dormancy in summer species leading to germination (Milberg and Andersson, 1998). Light has also been reported as a requirement for germination while some authors have reported germination in the dark (Roberts and Lockett, 1978, Baskin and Baskin, 1988). Understanding the germination requirements of *Solanum nigrum* is useful to develop a strategy to maximise its germination in an effort to bring this plant under cultivation especially in home gardens. This experiment was therefore carried out to determine the best conditions *Solanum nigrum* seeds need to be subjected to in order to break dormancy as well as determine the best way of extracting the seeds.

4.2 Materials and methods

4.2.1 Seed collection

The fruits of *Solanum nigrum* used in this experiment were harvested from wild but within Alice (located at 32^{o}47′ S, 26^{o}50′ E), in the Nkonkobe Municipality of the Eastern Cape. To determine the best method of extracting and drying the seeds, three methods were applied. In the first method, mature (purplish or dull black) fresh berries were harvested from the mother plant, the seeds separated from the pulp and washed in distilled water until they were clean (yellowish to brownish), allowed to dry for about an hour under ambient temperatures (19 and 25^{o}C) on a laboratory work bench and tests conducted on them. In the second method, berries were harvested, and sun dried for about 4 weeks before they were separated from the berry shells and trials conducted on them. In the third method, berries were harvested and air dried on the laboratory work bench in temperatures ranging between 19 and 25^{o}C for about 6 weeks, after which the seeds were separated from the berry shells. All tests were conducted in

three replicates of 50 seeds each and all seeds were surface sterilised using 1.0% sodium hypochlorite solution for 5 min and 95% ethanol for a further 5 min.

4.2.2 Viability test

Viability tests were conducted using the Tetrazolium Technique (Grabe, 1970). Following the method described by Peters (2000) for Solanaceae, the seeds were imbibed in water overnight between 20 and 25^{o}C; cut along the margin without damaging the embryo and soaked in colourless 0.1% solution of 2,3,5- triphenyltetrazolium chloride (TTC) whose pH was 6.61 for 24 h at a temperature between 20-25^{o}C in the dark. The seeds were removed from the TTC solution, washed in distilled water and soaked in 95% ethanol to permit direct observation of the embryo under the stereo microscope. Embryos of viable seeds appeared reddish in colour.

4.2.3 Germination experiments

Germination trials were conducted in 9 cm sterile Petri dishes lined with 2 Whatman No. 1 filter papers and moistened with sterile distilled water to ensure adequate water for the seeds. Treatments were arranged separately in randomised complete block designs (RCBD). Seed treatments included continuous light, continuous darkness, 16 h/8 h light/dark photoperiod, mechanical and chemical scarification and cold stratification (Kambizi et al., 2006).

4.2.3.1 Photoperiodism

The continuos light and 16 h/8 h light/dark photoperiods were used in separate Conviron growth chambers (Model E15; Controlled Environment Limited, Winnipeg, Manitoba, Canada). Petri dishes were covered in aluminium foil and placed in a dark room for the continuos darkness treatment. Safe green light was used to count total germination.

4.2.3.2 Pre-chilling and temperature

Seeds were placed in dry petri dishes and placed in the refrigerator set at 4^{o}C for 1, 3 and 7 days, after which germination was conducted on a laboratory bench. Another batch of seeds was placed in incubators set at 25, 35 and 45^{o}C for 40 days prior to the experiment, after which germination was conducted on a laboratory work bench at ambient temperatures (AOSA, 1998).

4.2.3.3 Mechanical scarification

The seedcoat was pricked with a needle at the radicle end or scratched with a 100 grit sand paper after which germination experiments were conducted on them on the laboratory bench at room temperature.

4.2.4 Chemical scarification

Seeds were separately soaked in concentrated sulphuric acid for 15 s, 30 s 60 s and 120 s. In the dilute acid treatment the seeds were soaked in 0.5 N, 1 N, 5 N and 10 N H_2SO_4 for 15 s, 30 s 60 s and 120 s according to the method of Lõpez-Granados and García-Torres (1996).

4.2.6 Moisture content

Moisture content on a dry basis was tested by weighing the seeds, drying them in an oven to a constant weight at 100^{o}C and using the final weight to calculate the percentage moisture content.

The seeds were examined on a daily basis and considered germinated when the radicle was visible.

4.2.7 Statistical analysis

Germination data from various treatments were separately subjected to statistical analysis using MNITAB Release 12. A one way analysis of variance was used to compare the means of each germination treatment. Means were segregated using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

4.3 Results

4.3.1 Seed attributes and viability

Seed viability treatment means of *Solanum nigrum* ranged between 70 and 78 %, but Air Dried (AD) seed was significantly more viable ($P < 0.05$) than Freshly Extracted (FE) and Sun Dried (SD) seed (Table 4.1). The method of extraction did not significantly affect the weight and moisture content of the seed.

Table 4.1: Viability, weight and moisture content of *Solanum nigrum* seed extracted using three different methods

	Air Dried	Freshly Extracted	Sun Dried
% Viability	78±3[a]	72±2[b]	70±3[b]
Weight (mg/seed)	1.01±0.00	1.05±0.01	1.03±0.01
Moisture content (%)	8.36±0.12	8.19±0.01	8.19±0.11

Values shown are mean ± S.D.
Means with different letters along the row represent significant differences at $p < 0.05$.

4.3.2 Seed germination

4.3.2.1 The effect of photoperiodism, temperature and mechanical scarification

The effect of photoperiodism, temperature and scarification of *Solanum nigrum* seeds produced variable results (Table 4.2). Germination was significantly higher ($p < 0.05$) in FE (16 %) and AD (16 %) as well as SD (16 %) seeds in the continuos darkens and 16 h/8 h light/ darkness treatments respectively. In the 25, 35 and 45^{o}C temperature treatments, germination was consistently and significantly higher in FE seeds (60 %) at 35^{o}C and 45^{o}C recorded the highest germination percentage (20 %) in SD seeds. In the pre-chilling treatment, germination was significantly higher in 3 day SD seeds (36 %) while there were no significant differences between AD and FE seeds (28 % each). In the scarification treatment, AD seeds produced the highest germination percentage (76 %) although this was not significantly higher than FE seeds (72 %). FE seeds had a significantly higher germination percentage (64 %) in the sandpaper treatment.

Needle pricking air dried seeds therefore produced the highest percentage germination in this work while photoperiodism produced the lowest results.

Table 4.2: Effect of photoperiodism, temperature, pre-chilling and mechanical scarification on seed germination of *Solanum nigrum*

		% Germination		
		Air Dried	Freshly Extracted	Sun Dried
Treatment				
Photoperiodism	Continuos light	8 ± 1^{b}	8 ± 1^{b}	12 ± 1^{a}
	Continuos darkness	0	16 ± 2^{a}	0
	16 h/8 h light/ darkness	16 ± 2^{a}	8 ± 1^{b}	16 ± 2^{a}
	Control	12 ± 3^{ab}	16 ± 3^{b}	8 ± 2^{a}
Temperature	$25^{o}C$	0	43 ± 2^{a}	18 ± 6^{b}
	$35^{o}C$	0	60 ± 1^{a}	12 ± 9^{b}
	$45^{o}C$	0	56 ± 2^{a}	20 ± 2^{b}
	Control	12 ± 3^{ab}	16 ± 3^{b}	8 ± 2^{a}
Pre-chilling	1 day	8 ± 1^{b}	8 ± 1^{b}	16 ± 2^{a}
	3 days	28 ± 1^{a}	28 ± 1^{a}	36 ± 1^{b}
	7days	4 ± 1^{a}	4 ± 1^{a}	12 ± 1^{b}
	Control	12 ± 3^{ab}	16 ± 3^{b}	8 ± 2^{a}
Scarification	Needle prick	76 ± 3^{a}	72 ± 1^{a}	40 ± 2^{b}
	Sand paper	48 ± 6^{b}	64 ± 4^{a}	44 ± 2^{b}
	Control	12 ± 3^{ab}	16 ± 3^{b}	8 ± 2^{a}

Different letters along the same row represent significant differences at $p < 0.05$
Values shown are mean ± S.D.

4.3.2.2 The effect of chemical scarification

Treating *Solanum nigrum* seeds extracted and dried using three different methods, with concentrated sulphuric acid destroyed the seeds. However, treating the seeds with various concentrations of sulphuric acid produced variable results. Exposure of seeds to 5 N H_2SO_4 for 120 s produced significantly high results (64 %) in air dried seeds (Fig 4.1). As the time of exposure to acid increased, the rate of germination also increased. Soaking the seed in 10

N H_2SO_4 for 60 and 120 s produced 20 % in freshly extracted seed (Fig 4.2). Exposure to 5 N H_2SO_4 for 15 s produced 20 % germination but as time was increased, the rate of germination decreased. Subjecting the seed to 10 N H_2SO_4 for 120 s produced 28 % germination in sun dried seeds (Fig 4.3). As the time of exposure increased, the rate of germination also increased. Exposure of air dried seed to 5 N H_2SO_4 for 120 s therefore produced the most favourable chemical scarification result in this work.

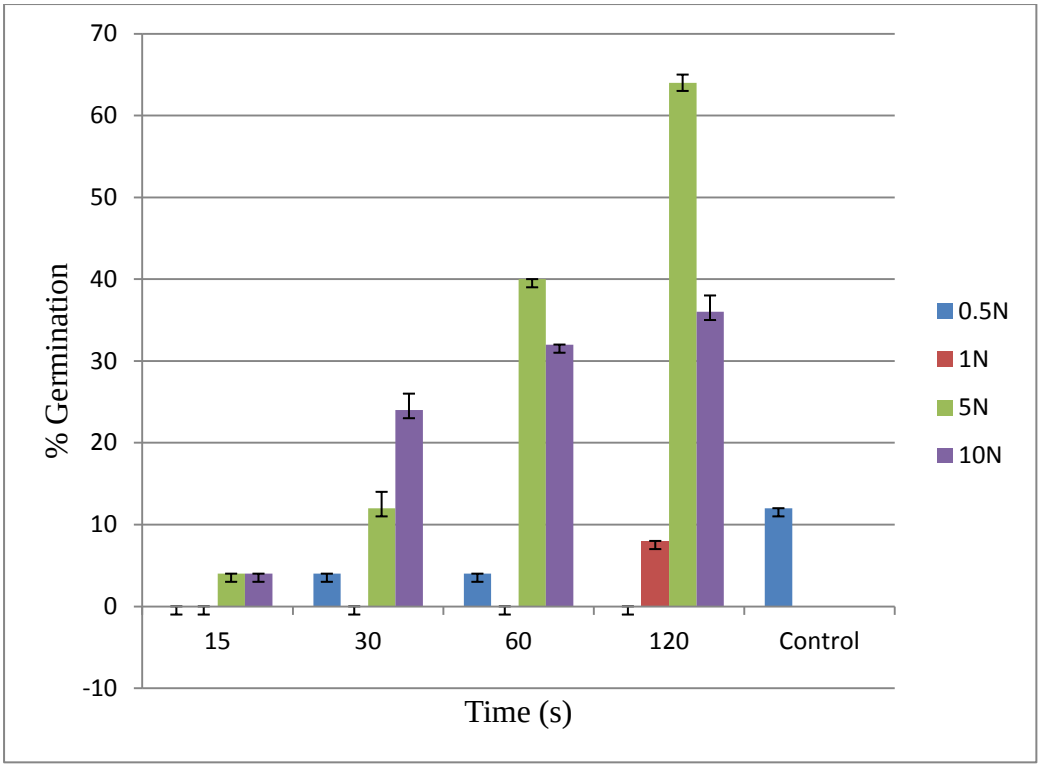

Fig 4.1: Germination of air dried *Solanum nigrum* seeds affected by sulphuric acid at different concentrations

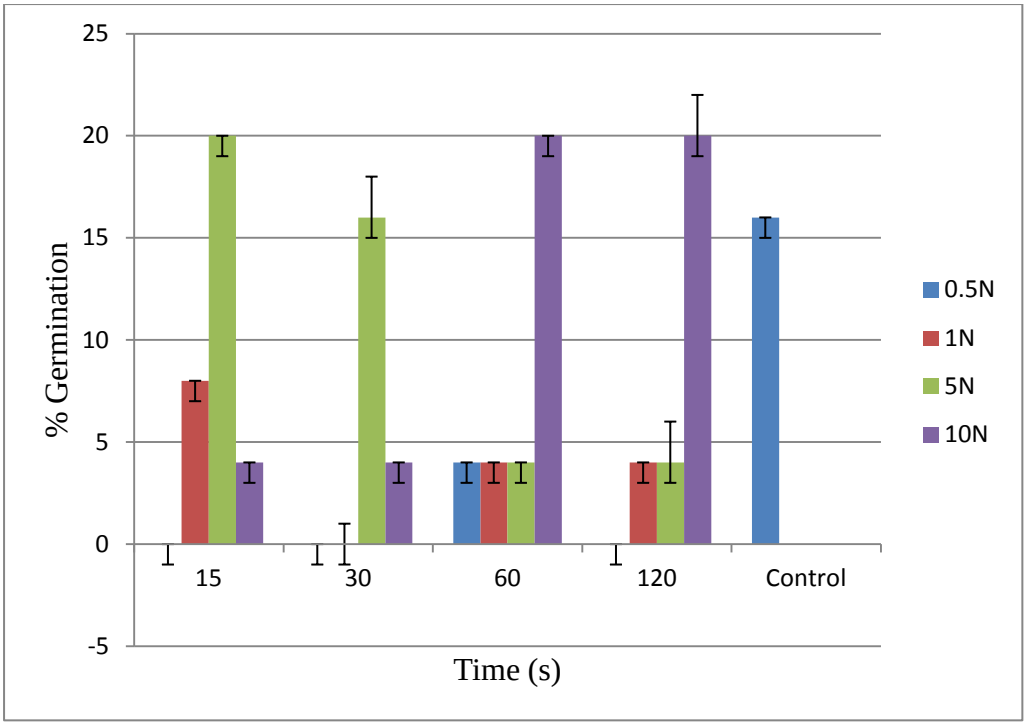

Fig 4.2: Germination of freshly extracted *Solanum nigrum* seeds affected by sulphuric acid at different concentrations

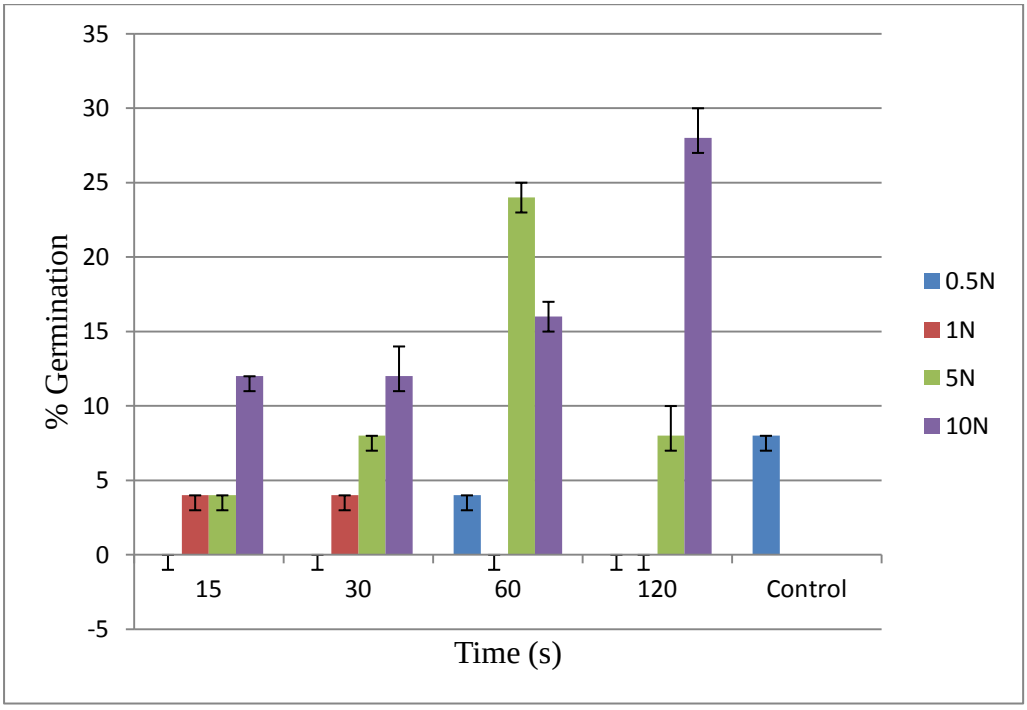

Fig 4.3: Germination of sun dried *Solanum nigrum* seeds affected by sulphuric acid at different concentrations

4.4 Discussion

4.4.1 Seed attributes and viability

A small change in seed moisture content can have a large effect on the storage life of seeds (Mane and Puri, 2013). Most orthodox seeds require moisture content of between 6 and 10% for them to retain viability after a long storage (Luna and Wilkinson, 2009). In this study the moisture content of *Solanum nigrum* seeds qualifies them for long term storage without reducing their viability. The current moisture content findings are within the range reported by Suthar et al. (2009). However, in contrast to the present study, Suthar et al. (2009) reported a higher viability value (95%) in freshly extracted *Solanum nigrum* seeds. In another study, Roberts and Lockett (1978) reported that *Solanum nigrum* seeds stored at room temperature between 1 and 9 years were 99, 100, 96, 98, 91, 73, 27, 2 and 0% viable. *Solanum nigrum* seeds are known to retain viability in the soil for long periods of time and consequently germinating and causing problems to crops (Edmonds and Chweya, 1997).

4.4.2 Germination trials

4.4.2.1 The effect of photoperiodism, pre-chilling, temperature and mechanical scarification

In this study, mechanical scarification produced the highest germination percentage. Air dried needle pricked seeds produced the best results although this was not significantly higher ($p < 0.05$) than sand paper scarification. This study differs slightly with that of Suthar et al. (2009) who reported that the sand paper treatment produced the best results in their work. As proposed by Emongor et al. (2004), mechanical scarification presumably ruptured the seedcoat, allowing moisture to permeate the seed tissues and causing physiological changes within the seed leading to the subsequent germination of the embryo. Moreover, it is reported

that in the field, the seeds undergo natural scarification by microbial degradation of the seedcoat, soil temperature fluctuations and tillage implements (Lopez-Granados and Garcia-Torres, 1996).

Although there was an increase in germination due to cold stratification in 3 days pre-chilled seed, further exposure to the cold (7 days) led to lower germination than what was recorded in 1 day pre-chilled seeds and far lower than 3 days. It is evident that continued exposure to the cold increases seed germination until a certain point, after which any further prolonged exposure to the cold will be lethal to the seeds leading to a reduction in germination. Roberts and Locket (1978) reported poor germination in freshly harvested *Solanum nigrum* seeds in temperatures ranging between 4 and 30^{o}C, however, the same seeds germinated well at alternating temperatures. Although cold stratification has been reported to enhance germination in many species (Baskin et al., 2001), it has also been reported to be lethal to some seeds (Ren and Tao, 2004; Lewu et al., 2010).

Solanum nigrum seed germination increased in seed that was exposed to 25, 35 and 45^{o}C temperature regimes although the percentage germination was lower than the viability test. Germination was highest in freshly harvested seed in both treatments but lower in air dried seeds. Previous studies by Bithell et al. (2002) reported high levels of germination in *Solanum nigrum* seeds that had been exposed to constant temperatures of between 10 and 25^{o}C prior to the experiment, however, the experiment was performed at alternating temperatures of 20/30^{o}C. Roberts and Boddrell (1983) reported increased germination in seeds that were exposed to temperatures between 27 and 32^{o}C while Zhou et al. (2005) reported temperatures between 27 and 33^{o}C to increase germination. Work done by Suthar et al. (2009) indicated low germination as compared to this study after exposing the seed to temperatures between 5 and 60^{o}C for 5 days. Temperature is one of the most important environmental factors controlling the timing of germination. According to Baskin and Baskin

(1988), non-dormant seeds have specific temperature for germination and non-dormant seeds of some species are induced into dormancy by certain temperatures. In relation to physical, physiological and morphophysiological dormancy, temperature plays a critical role in overcoming dormancy (Nikolaeva, 1977). In this work, the exposure of seeds to high temperatures presumably slowly brought the seed out of physiological dormancy (after-ripening) and led to germination although the results were lower than the viability tests.

Light has been reported by various workers including Baskin and Baskin (1988), Givelberg et al. (1984) as well as Roberts and Lockett (1978) as a requirement for germination. In this study, exposure to continuous light, 16 h/8 h light/darkness and continuous darkness alone did not lead to high germination percentage. Germination also occurred in seed that was exposed to continuous darkness and this was not very different from the control. According to Baskin and Baskin (1988), some seeds may gain the ability to germinate in the dark, although they will best germinate if exposed to light.

4.4.2.2 The effect of chemical scarification

In contrast to findings by Suthar et al. (2009), exposing *Solanum nigrum* seeds to concentrated H_2SO_4 did not lead to germination but instead had detrimental effects on them. *Solanum nigrum* seeds were too small to withstand the corrosive nature of concentrated sulphuric acid. The use of concentrated sulphuric acid may be useful in seeds with a harder seed coat. In a work done by Darris (2010), exposure of seeds of the grass *Danthonia californica* to concentrated H_2SO_4 produced similar results to this current study. However, dilute H_2SO_4 produced variable results. In comparison, exposing *Solanum nigrum* seeds to 0.5 N and 1 N H_2SO_4 did not produce significant germination difference from the control. These results are not very different from what was reported by Lopez-Granados and Garcia-Torres (1996). Exposure of seeds of the weed, *Crenate Broomrape* to low concentrations of H_2SO_4 for a short time increased germination while on the other hand, exposure of the seeds

to high concentrations of the acid suppressed germination. In another study, acid treatment of seeds belonging to the tree species, *Tamarindus indica* (Muhammad and Amusa, 2003) in 98% H_2SO_4 for 30 min produced favourable results. As proposed by Gealy et al. (1985), it is inferable that exposure of seeds to acid reduces the thickness of the pericarp and its coverage of the seed which often hinders radicle penetration; however, small generally soft coated seeds may not survive high concentrations of acid while the larger and hard coated may be ideal for the treatment.

4.5 Conclusion

Wild *Solanum nigrum* seeds from the Eastern Cape are viable and as indicated by their moisture content which was 8.36, 8.19 and 8.19 % in AD, FE and SD seeds respectively. The seeds can potentially be stored at room temperature for long periods of time, sometimes up to 10 years, for future use without reducing their viability. Comparing the germination results with the TTC test, it is conceivable that although the seeds were sufficiently viable, germination was hindered by physiological dormancy. Physiological dormancy was however successfully overcome by temperature, mechanical and chemical scarification. More specifically, air drying the seeds respectively produced 8, 0 and 16 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while no germination was recorded in the temperature treatment, however, 1, 3 and 7 days pre-chilling respectively produced 8, 28 and 4 % germination and needle pricking as well as sand paper scarification respectively produced 76 and 48 %. Freshly extracted seeds respectively produced 8, 16 and 8 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while 25, 35 and 45^{o}C temperature treatments respectively produced 43, 60 and 56 % germination, but, 1, 3 and 7 days pre-chilling respectively produced 8, 28 and 4 % germination and needle pricking as well as sand paper scarification

respectively produced 72 and 64 %. Sun dried seeds respectively produced 12, 0 and 16 % germination in continuos light, continuos darkness and 16 h/ 8 h light/ darkness treatments, while 25, 35 and 45^{o}C temperature treatments respectively produced 18, 12 and 20 % germination, however, 1, 3 and 7 days pre-chilling respectively produced 16, 36 and 12% germination and needle pricking as well as sand paper scarification respectively produced 40 and 44 %. Subjecting air dried seed to 5N H_2SO_4 for 120 s produced the best chemical scarification result (64 %). Air dried, Freshly Extracted and Sun Dried seed controls respectively produced 12, 16 and 8 % germination. Temperature clearly plays an important role in breaking the dormancy of *Solanum nigrum* as shown by the increase in germination in the acid treatment. In the soil, scarification imposed by micro-organisms and temperature probably breaks physiological dormancy leading to germination. Needle prick scarification produced the best result in this work although this was not significantly different from sand paper scarification; however, for practical purposes, at subsistence small scale farming, sand paper scarification or leaving the seeds to dry at high temperatures for a long time would be ideal. Although acid produced some favourable results, this method is not practicable especially in the rural areas where the plant is intended for cultivation. Air drying was the best method of drying seed.

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CHAPTER 5

EFFECT OF FERTILISERS ON GROWTH AND PHYSIOLOGICAL RESPONSE OF *SOLANUM NIGRUM*

CHAPTER FIVE

Effect of fertilisers on growth and physiological response of *Solanum nigrum*

5.1 Introduction

Poor soil fertility is one of the limiting factors to crop production. According to Mandiringana et al. (2005), the soils of the Eastern Cape generally contain low amounts of nitrogen and phosphorus while micronutrients such as sulphur, manganese, zinc, copper and boron are abundant. These authors cited low soil organic matter, low geological reserves of phosphorus, potassium and calcium. Continuos cultivation of lands without adequate nutrient replenishment is one of the major causes of the low nutrient status of these soils. There is therefore a need to boost the nutrient capacity of the Eastern Cape soils with fertilisers to achieve maximum crop yields. The enhancement of soil nutrient capacity with organic fertiliser increases the soil organic matter content that in turn improves the soil's physical properties (Sanchez et al., 1989). Colloids have been reported to hold cations like calcium, potassium and magnesium in exchangeable forms so that they become available to the plant and are not leached by water (Brady and Weil, 1999). Organic matter also decreases soil surface crusting thereby reducing displacement of soil particles by raindrops and therefore increases the infiltration capacity and hydraulic activities of the soil (Cross and Fischbach, 1972; Mazurak et al., 1975; Epstein et al., 1992). Nitrogen, phosphorus, potassium and micro-nutrients are constituents of organic fertilisers from which they are slowly released through mineralisation and are therefore made available to the plant. However, quality of organic fertiliser plays a critical role in their mineralisation. Inorganic fertilisers are often applied to vegetables for higher yields, but the over use of mineral fertilisers may cause environmental problem (Arisha and Bardisi, 1999; Stewart et al., 2005). However, inorganic fertilisers are considered a major source of plant nutrients (Adediran et al., 2004; Naeem et al., 2006). Information on cultivation of wild vegetables in home gardens and on the field is very rare in South Africa. Wild vegetables usually grow as volunteer weeds from previous year's mature seeds that drop to the soil while harvesting conventional crops and are thus

gathered for food during the farming season. Although *Solanum nigrum* is popular in the Eastern Cape, it has not been adopted for cultivation. The present investigation was therefore carried out in both the field and glasshouse to study the effect of different doses of organic and inorganic fertilisers on the growth and the physiological response of *Solanum nigrum* for successful domestication and cultivation of the plant.

5.2 Materials and methods

5.2.1 The Experimental site

The experiment was conducted in the glasshouse and on the field at University of Fort Hare, Alice campus, South Africa between September and December 2012. The campus falls under 32^{o} 47′ S and 26^{o} 50′ E and 535 m a.s.l. and is within a semi arid ecological zone with an average annual rainfall of approximately 575 mm in summer; mean daily temperatures of 22.5^{o}C during the day and 18.8^{o}C at night while during the winter the temperature is about 13.6^{o}C during the day and less than 10.3^{o}C at night (Marais and Brutsch, 1994). According to the South African system of soil classification, the soils are deep alluvial of the Oakleaf form (Oa) and belong to the Jozini series and texturally sandy loam (Soil Working Group, 1991). According to the soil map of the world, the soils are Eutric fluvisols (Fle) (FAO-UNESCO- ISRIC, 1988). The properties of the soil, used for this experiment are shown in Table 5a.

5.2.2 Agronomic practices

Ripe and mature *Solanum nigrum* berries were harvested between the 3rd and 26th of April 2012 from the wild in Alice. The seeds were separated from the pulp, washed in distilled water and dried at room temperature on the laboratory bench for 2 h and kept in sealed bottles

until further use. For the field trial, the extracted seeds were planted in cavity trays in the glasshouse and later transplanted to the field when they were about 6 weeks old and with at least 6 true leaves and about 10 – 15 cm tall. For the glasshouse trial, the seeds were planted in cavity trays in the glasshouse and transplanted into the prepared polythene bags containing 5 kg of soil when they were about 6 weeks old. The soil used in the glasshouse was obtained from the field where the field trial was conducted so that the properties of the soils for the two experiments would be similar or almost the same. The organic fertiliser (goat manure) used in this experiment was obtained from the University of Fort Hare animal farm while the inorganic fertilisers (NPK [2:3:4] and Limestone Ammonium Nitrate [LAN]) were purchased from Umtiza Farmers Co-operative a local agricultural implements and inputs dealer. The properties of the organic fertiliser used for the experiment are shown in Table 5a.

Table 5a: The chemical properties of the experimental soil (Upper 0-30 cm depth) and organic fertiliser

	Soil	Organic fertiliser
pH(KCI)	6.54	7.17
Bulk density (g cm^{-3})	1.20	-
EC (μS/cm)	162.05	10.75
CEC_{sum} (meq/ 100g)	12.10	-
Available P (mg kg^{-1})	71	8 500
Exchangeable K (mg kg^{-1})	406	26 000
Exchangeable Ca (mg kg^{-1})	1653	29 700
Exchangeable Mg (mg kg^{-1})	335	9 900
Exchangeable acidity (cmol/L)	0.06	-
Total cations (cmol/L)	12.10	-
Saturated acid (%)	0	-
Zn (mg kg^{-1})	10.2	172
Mn (mg kg^{-1})	17	582
Cu (mg kg^{-1})	5.7	54
Organic C (mg kg^{-1})	10000	-
N (mg kg^{-1})	1400	24 800
Clay (%)	17	-
Na (mg kg^{-1})	-	1 564
Fe (mg kg^{-1})	-	12 439
Al (mg kg^{-1})	-	5 335

5.2.3 Experimental design

The experiments were laid out in a Randomised Complete Block Design (RCBD) with five treatments and five replicates. The treatments were:

1. Control (T1)
2. Inorganic fertiliser: 100 kg N/ha (T2);
3. Organic fertiliser: 8.13 t /ha (T3);
4. 100 kg N/ha + 8.13 t manure /ha (T4);
5. 50 kg N/ ha + 4.07 t manure/ ha (T5).

Nitrogen was supplied in the form of NPK and LAN fertilisers. The organic fertiliser (goat manure) and NPK were applied at transplanting and LAN fertiliser applied 4 weeks after transplanting. These fertilisers were applied in the top 5-7 cm of soil depth by mixing with a spade in plots measuring 3 m × 2 m on the field. In the glasshouse, the experiment was laid out as described in the field except that treatments were replicated 5 times with 10 experimental units. Each replicate consisted of one *Solanum nigrum* plant in polythene bag containing 5 kg of soil.

5.2.4 Data collection

For growth parameters, 10 plants per treatment were randomly selected, uprooted and tagged and the following parameters were measured:

5.2.4.1 Plant height and number of leaves

A meter rule was used to measure the shortest distance between the upper boundary of the main photosynthetic tissues on the plant and the ground level (Cornelissen et al., 2003). The plant height was measured before the plants were uprooted. Leaves formed were physically counted from each plant and the average of the 10 plants determined.

5.2.4.2 Stem diameter

Stem diameter was measured about 2.5 cm above ground level using a vernier calliper (US EPA, 2001).

5.2.4.3 Leaf area

Leaf area was determined by the non destructive length × width method (Saxena and Singh, 1965) using the relation: LA = 0.75 (length × width), where 0.75 is a constant.

5.2.4.4 Chlorophyll

The non-destructive approach method was used to determine total chlorophyll in fresh leaves from the base or apex of the plant using a spectrophotometer (Konica Minolta, SPAD -502 PLUS).

5.2.4.5 Moisture

The method of Osborne and Voogt (1978) was used for moisture determination. About 2 g of plant samples were dried to a constant weight in an oven at 110^{o}C in clean and dry porcelain crucibles. Using the final and initial weight of the samples, the percentage content was determined.

5.2.4.6 Root: Shoot ratio

Roots were separated from the whole plant and dried in the oven at 40^{o}C to a constant weight. The ratio was determined as the dry weight of the roots to the dry weight of the shoot (Harris, 1992).

The experiment was terminated in the 9th and 12th week for the glasshouse and field experiments respectively and this was when all the berries on the plant were mature and ripe.

5.2.5 Statistical analysis

Data collected were subjected to statistical analysis using MNITAB Release 12. A one way analysis of variance was used to compare the means of various growth parameters among the treatments and a two way analysis of variance was used to determine the interaction between plant age (weeks after transplanting) and treatment on particular growth parameters. Means were segregated using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

5.3 Results

5.3.1 Plant height and number of leaves

a) Field

The effects of fertilisers on height and number of leaves of *Solanum nigrum* cultivated on the field are shown in Table 5.1a and Figure 5.1b respectively. Although there were no significant differences ($p < 0.05$) among the treatment means, plant height increased with plant age from 8.97 to 118 cm from the time of transplanting until the termination of the experiment. Similarly, the number of leaves increased with increasing plant maturity from 6 – 1881 leaves but there were significant differences among the treatment means. The height treatment mean for the duration of the trial was highest in T4 (64.86 cm) and lowest in the control (57.60 cm) and similarly, the total number of leaves was highest in T4 (888) and lowest in the control (642). Statistical analysis showed an interaction between plant age and the fertiliser treatment on height. Regression analysis with plant height as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 95.5 % indicating that plant age had a significant effect on plant height. There was no interaction between plant age and treatment on the total number of leaves.

Table 5.1 a: Effect of organic and inorganic fertilisers on plant height (cm) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	8.97±0.11	14.30±2.34	24.00±1.00	38.33±3.92	49.00±4.00	57.00±3.61	69.67±7.09	83.00±11.00	88.00±8.72	100.00±11.14	101.33±21.94
T2	8.97±0.11	14.33±2.72	23.00 ±2.05	40.00±1.73	44.67±5.77	56.00±5.20	78.67±4.16	95.67±6.03	102.33±15.31	104.67±3.51	107.33±6.81
T3	8.97±0.11	15.37±0.64	23.67±1.53	39.33±0.58	51.33±2.08	56.00±5.20	70.33±11.06	84.67±20.55	96.67±1.53	97.33±2.08	101.00±4.58
T4	8.97±0.11	16.53±3.18	25.33±1.53	41.67±1.53	54.67±2.08	61.67±3.06	80.00±6.00	96.00±1.73	107.00±8.54	109.33±12.50	112.33±10.07
T5	8.97±0.11	14.90±2.13	24.33±2.31	38.67±2.08	49.00±4.58	59.00±5.57	74.67±5.13	97.33±14.01	110.67±14.19	113.33±22.19	118.00±26.29

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.1 b: Effect of organic and inorganic fertilisers on leaf number of *Solanum nigrum* L. cultivated on the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	6±1.03	41±7.37	112±15.28^{b}	135±7.57^{d}	311±10.21^{bc}	316±22.85^{d}	634±27.00^{c}	1209±10.10^{c}	1248±14.60^{d}	1486±6.50^{e}	1568±9.30^{e}
T2	6±1.03	63±13.58	103±19.55^{b}	116±7.02^{d}	329±22.61^{b}	377±25.03^{c}	901±5.50^{b}	1231±21.90^{c}	1545±38.10^{b}	1668±19.60^{b}	1767±15.70^{b}
T3	6±1.03	55±6.56	135±10.02^{ab}	244±28.16^{b}	273±23.01^{c}	478±14.64^{b}	678±20.00^{c}	1027±18.30^{d}	1471±16.30^{c}	1538±20.40^{d}	1594±6.60^{d}
T4	6±1.03	47±10.02	158±21.55^{a}	294±18.56^{a}	476±22.01^{a}	587±10.54^{a}	982±6.60^{a}	1668±19.00^{a}	1793±6.90^{a}	1875±20.60^{a}	1881±22.00^{a}
T5	6±1.03	50±6.56	131±22.11^{ab}	197±11.72^{c}	292±20.03^{bc}	495±11.50^{b}	876±24.10^{b}	14.39±21.10^{b}	15.62±10.40^{b}	1572±16.50^{c}	1613±13.10^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$

5.3.1 Plant height and number of leaves

b) Glasshouse

In the glasshouse, there were significant differences among the treatment means on plant height and total number of leaves (Table 5.2a and 5.2b). Plant height and leaf number increased with increasing plant maturity. The height treatment means for the duration of the trial were highest in T5 (54.33 cm) and lowest in the control (34.41 cm) while the total number of leaves were highest in T4 (174) and lowest in the control (101). Analysis showed an interaction between plant age and fertiliser treatment on plant height and number of leaves. Regression analysis with plant height and number of leaves as the dependant variables and time (plant age) as the regressor showed a coefficient of determination (R^2) of 97 and 96 % respectively indicating that plant age had a significant effect on plant height and number of leaves.

Table 5.2a: Effect of organic and inorganic fertilisers on plant height (cm) of *Solanum nigrum* L. cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	8.97±0.11	21.33±2.07^{a}	35.33±2.08^{ab}	36.33±1.37^{a}	41.00±4.73^{a}	43.33±2.25^{a}	44.00±3.37^{a}	45.00±6.26^{a}
T2	8.97±0.11	25.10±3.81^{b}	40.67±5.03^{a}	44.00±0.89^{b}	70.00±3.23^{b}	76.67±1.37^{b}	77.67±1.86^{b}	88.00±10.16^{b}
T3	8.97±0.11	20.33±0.52^{a}	30.67±2.08^{b}	44.00±3.23^{b}	46.33±1.03^{a}	51.33±6.28^{c}	54.00±6.99^{c}	67.17±0.93^{c}
T4	8.97±0.11	22.33±0.93^{ab}	40.00±1.00^{a}	49.00±0.89^{c}	66.67±6.09^{b}	75.33±2.73^{b}	77.00±1.79^{b}	80.33±4.13^{b}
T5	8.97±0.11	23.67±1.37^{ab}	41.00±2.65^{a}	49.67±3.14^{c}	64.667±1.37^{b}	75.33±1.03^{b}	81.00±3.10^{b}	90.33±0.52^{b}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.2b: Effect of organic and inorganic fertilisers on leaf number of *Solanum nigrum* L. cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	6.00±1.03	82±11.04^{a}	89±3.61	110±26.39^{ab}	115±4.98^{a}	126±2.25^{a}	131±3.39^{a}	145±19.62^{a}
T2	6.00±1.03	74±16.50^{ab}	86±49.07	90±6.26^{a}	190±20.83^{b}	259±17.12^{b}	349±3.39^{b}	361±5.75^{b}
T3	6.00±1.03	62±8.31^{b}	80±18.60	99±10.55^{a}	103±18.85^{a}	107±8.20^{a}	124±9.96^{a}	133±3.39^{a}
T4	6.00±1.03	66±9.40^{ab}	86±16.57	124±7.17^{b}	229±46.66^{b}	255±52.23^{b}	300±19.72^{c}	327±24.57^{c}
T5	6.00±1.03	53±8.53^{b}	71±12.94	99±14.72^{a}	188±55.92^{b}	236±16.27^{b}	245±38.40^{d}	253±19.78^{d}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.3.2 Stem diameter

a) Field

The effects of fertiliser on stem diameter on the field are shown in Table 5.3a. The treatment means were significantly different ($p < 0.05$) in the 4^{th}, 5^{th} and 12^{th} week and increased from the time of transplanting (1.27 mm) to the 12^{th} week (12.28 mm). The treatment means for the trial period were highest in T2 (8.10 mm) and lowest in the control (7.38 mm). Analysis of variance showed that there was no interaction between plant age and fertiliser treatment on stem diameter.

b) Glasshouse

In the glasshouse, treatment means were significantly different except in the 4^{th} week. Stem diameter increased exponentially in all the treatments between the time of transplanting and the 4^{th} week after which it continued to increase steadily until the 9^{th} week (Table 5.3b). The means for the trial period were highest in T2 (5.83 mm) and lowest in T3 (4.46 mm). However, statistical analysis showed an interaction between plant age and the fertiliser treatment on stem diameter. Regression analysis with stem diameter as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 81 % indicating that plant age had a significant effect on stem diameter. The field stem diameter values were higher than those of the glasshouse.

Table 5.3a: Effect of organic and inorganic fertilisers on stem diameter (mm) of *Solanum nigrum* L. cultivated in the field

	Age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	1.27±0.14	4.01±1.44	4.79±2.12^{b}	6.67±2.62^{b}	7.03±3.53	7.82±3.07	8.58±4.28	9.52±5.38	9.79±7.08	10.63±6.05	11.04±6.43^{b}
T2	1.27±0.14	3.87±1.49	6.41±2.45^{a}	7.66±2.36^{a}	7.81±3.76	8.88±5.08	9.56±4.26	9.79±5.66	10.51±7.33	11.06±5.64	12.28±7.69^{a}
T3	1.27±0.14	4.29±1.57	5.53±2.21^{ab}	6.55±2.02^{b}	7.33±3.55	7.79±3.85	8.56±5.91	8.95±5.38	10.21±6.71	11.03±6.87	12.16±7.25^{a}
T4	1.27±0.14	4.49±1.79	6.29±1.85^{a}	7.51±2.35^{ab}	7.98±4.07	8.91±4.75	9.01±4.50	10.22±5.84	10.29±7.22	10.70±4.43	11.08±6.79^{ab}
T5	1.27±0.14	4.30±1.41	5.98±1.54^{ab}	7.08±2.41^{ab}	8.54±4.15	8.72±4.51	8.84±5.24	9.59±6.16	10.20±7.16	11.09±7.07	11.91±7.19^{b}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.3b: Effect of organic and inorganic fertilisers on stem diameter (mm) of *Solanum nigrum* L. cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	1.27±0.14	4.49±0.50^{a}	4.76±0.12^{a}	4.85±0.27^{a}	4.96±0.45^{a}	5.00±0.27^{a}	5.20±0.22^{a}	5.80±0.41^{a}
T2	1.27±0.14	5.59±0.34^{b}	6.12±0.54^{b}	6.38±0.45^{b}	6.69±0.14^{b}	6.86±0.51^{b}	6.95±0.72^{b}	7.35±0.06^{d}
T3	1.27±0.14	4.50±0.12^{a}	4.64±0.18^{a}	4.75±0.13^{a}	4.82±0.22^{a}	5.18±0.08^{a}	5.24±0.52^{a}	5.29±0.13^{c}
T4	1.27±0.14	5.20±0.35^{b}	5.49±0.18^{c}	5.53±0.26^{c}	6.23±0.36^{b}	6.41±0.26^{b}	6.51±0.24^{b}	6.89±0.31^{b}
T5	1.27±0.14	4.92±0.29^{ab}	5.11±0.60^{ac}	5.51±0.24^{c}	6.67±0.39^{b}	6.74±0.36^{b}	6.91±0.15^{b}	7.19±0.34^{b}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.3.3 Leaf area

a) Field

Treatment means were significantly different ($p < 0.05$) and varied between 2.37 cm^2 at the time of transplanting and 74.14 cm^2 in the 7th week in T5 (Table 5.4a). The highest mean was observed at T5 followed by T2 while the least was in T3. However, treatment means for the trial period were highest in T5 (58.2 cm^2) and lowest in T3 (42.1 cm^2). Statistical analysis showed an interaction between plant age and the fertiliser treatment on leaf area. Regression analysis with leaf area as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 28 % indicating that plant age had a minimal effect on leaf area.

b) Glasshouse

In the glasshouse, leaf area increased until the 7th week in all the treatments and started to decrease (Table 5.4b). The treatment means were significantly different ($p < 0.05$) and ranged between 2.44 cm^2 from the time of transplanting and 92.98 cm^2 in T5. The means of the treatments for the trial period were highest in T5 (90.6 cm^2) and lowest in the control (48.8 cm^2). Statistical analysis showed an interaction between plant age and the fertiliser treatment on leaf area. Regression analysis with leaf area as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 72 % indicating that plant age had a significant effect on leaf area. Treatment means were higher in the glasshouse than on the field.

Table 5.4a: Effect of organic and inorganic fertilisers on leaf area (cm^2) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	2.37±1.82	22.67±5.72^{b}	35.43±5.96	37.85±6.90^{b}	38.12±11.44^{b}	39.46±11.68^{b}	43.00±11.62^{b}	34.95±8.32	34.48±9.11	32.98±5.73^{b}	31.47±7.87^{b}
T2	2.37±1.82	22.05±8.01^{b}	33.85±6.21	54.92±8.15^{a}	64.65±6.55^{a}	62.30±6.27^{a}	55.09±17.70^{a}	51.87±12.44	44.03±9.23	38.98±8.87^{a}	25.23±4.07^{b}
T3	2.37±1.82	22.50±10.76^{b}	32.83±8.23	33.33±5.68^{b}	34.17±7.40^{b}	35.02±6.62^{b}	47.76±6.06^{b}	36.50±16.73	35.02±7.23	35.02±10.09^{b}	32.91±9.63^{b}
T4	2.37±1.82	30.35±8.68^{a}	39.99±7.52	43.83±5.87^{b}	53.57±6.92^{ab}	54.63±16.90^{ab}	51.89±10.93^{b}	50.66±11.82	44.77±8.94	40.78±8.81^{a}	28.64±5.76^{b}
T5	2.37±1.82	18.63±9.28^{b}	33.58±4.02	56.71±4.29^{a}	56.79±13.22^{a}	74.14±17.78^{a}	74.14±9.59^{a}	52.76±4.20	49.92±6.01	35.72±5.65^{b}	34.89±12.99^{a}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$

Table 5.4b: Effect of organic and inorganic fertilisers on leaf area (cm^2) of *Solanum nigrum* L. leaves cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	2.44±0.33	30.74±7.32^{a}	36.85±5.80^{a}	40.79±2.92^{a}	51.79±6.57^{a}	53.55±5.67^{a}	39.35±3.88^{a}	37.43±5.66^{a}
T2	2.44±0.33	62.26±8.79^{b}	62.73±8.36^{b}	68.53±8.04^{b}	70.61±3.45^{b}	78.83±13.89^{b}	70.52±4.88^{b}	68.22±9.88^{b}
T3	2.44±0.33	28.51±14.45^{a}	49.24±21.15^{ab}	50.34±18.52^{ab}	54.91±6.42^{ab}	56.85±8.72^{a}	45.29±8.01^{a}	41.50±21.26^{a}
T4	2.44±0.33	43.88±1.33^{a}	54.13±5.82^{ab}	59.77±5.05^{b}	62.30±11.49^{ab}	71.90±8.61^{b}	64.31±6.17^{b}	62.68±4.17^{b}
T5	2.44±0.33	64.54±10.70^{b}	65.22±10.93^{b}	66.24±7.52^{b}	73.71±14.32^{b}	92.98±7.93^{b}	90.02±25.33^{b}	88.48±10.90^{c}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.3.4 Chlorophyll

a) Field

There were no significant differences ($p < 0.05$) among the treatment means throughout the trial in chlorophyll content. The chlorophyll increased from the time of planting to the 4th week, after which it varied (Table 5.5a). The treatment means ranged between 23.11 and 60.34 SPAD values at the time of transplanting and the 7th week in T2. T4 had the highest treatment means (49.87 SPAD values) followed by T2 (48.94 SPAD values) and the least was T1 (48.05 SPAD values). Statistical analysis showed an interaction between plant age and the fertiliser treatment on chlorophyll content. Regression analysis with chlorophyll content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 49.8 % indicating that plant age had a minimal effect on chlorophyll content.

b) Glasshouse

In the glasshouse, means were significantly different from the 4th week to the 6th week and ranged between 23.11 at the time of transplanting and 50.82 SPAD values in T4 (Table 5.5b). The means followed the same pattern with the field experiment with the highest mean at T4 followed by T2 and the least at T1. Statistical analysis showed an interaction between plant age and the fertiliser treatment on chlorophyll content. Regression analysis with chlorophyll content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 34.6 % suggesting that plant age had a minimal effect on chlorophyll content. The field plants contained more chlorophyll than those cultivated in the glasshouse.

Table 5.5a: Effect of organic and inorganic fertilisers on chlorophyll (SPAD units) content of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	23.11±2.28	42.25±5.25	51.42±3.39	50.29±4.26	47.66±3.69	58.05±2.53	49.88±3.57	49.40±2.85	52.63±2.67	55.30±3.03	48.34±3.64
T2	23.11±2.28	42.39±5.16	55.48±3.11	49.82±1.45	49.78±2.87	56.96±2.64	52.53±1.83	54.11±4.14	56.05±2.80	51.29±2.71	46.79±3.64
T3	23.11±2.28	42.33±3.95	52.21±3.01	46.46±2.90	48.11±2.70	55.70±1.97	51.44±2.15	52.40±2.61	52.19±2.91	49.98±1.95	50.56±3.83
T4	23.11±2.28	49.59±2.42	51.23±3.76	50.43±3.76	47.56±2.80	60.34±2.76	52.96±2.91	52.33±2.42	55.38±2.66	51.85±2.91	53.78±2.47
T5	23.11±2.28	43.88±6.38	50.60±4.11	48.11±1.77	48.27±2.55	54.34±3.91	49.73±3.20	51.43±2.45	56.40±2.66	50.54±2.41	52.70±2.74

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.5b: Effect of organic and inorganic fertilisers chlorophyll content (SPAD units) of *Solanum nigrum* L. leaves cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	23.11±2.28	39.74±0.15	38.01±1.06^{ab}	39.78±3.68^{a}	38.28±3.86^{a}	32.82±3.49^{a}	24.02±1.80^{a}	23.24±1.74^{a}
T2	23.11±2.28	39.35±1.55	41.93±3.15^{a}	47.91±1.07^{b}	46.16±1.87^{ab}	37.22±3.11^{ab}	44.15±3.07^{b}	49.49±2.06^{b}
T3	23.11±2.28	39.17±2.42	35.55±0.87^{b}	36.87±0.65^{a}	38.44±3.14^{a}	38.04±1.53^{ab}	32.18±4.06^{c}	25.49±2.22^{a}
T4	23.11±2.28	38.84±3.19	40.36±0.88^{a}	48.44±2.57^{b}	47.82±2.84^{b}	40.58±5.42^{b}	40.09±4.87^{bc}	47.25±0.60^{b}
T5	23.11±2.28	36.54±1.52	40.58±0.55^{a}	45.69±2.69^{b}	40.28±1.00^{a}	43.21±1.56^{b}	41.98±1.22^{b}	46.38±3.71^{b}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.3.5 Moisture

a) Field

The moisture treatment means were significantly different ($p < 0.05$) and were consistently high throughout the trial, ranging between 80.59 in the 7th week and 91.68 % in the 4th week in T3 (Table 5.6a). The means of the treatments were highest in T2 and followed by T5 and least in T1. Statistical analysis showed an interaction between plant age and the fertiliser treatment on moisture content. Regression analysis with moisture content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 39.1 % indicating that plant age had a minimal effect on moisture content.

b) Glasshouse

In the glasshouse, treatments differed significantly and were consistently high throughout the experiment ranging between 75.16 and 92.08 % in the 7th and 3rd week in T1 and T2 respectively (Table 5.6b). The means of the treatments were highest in T5 followed by T2 and least in T1. Statistical analysis showed an interaction between plant age and the fertiliser treatment on moisture content. Regression analysis with moisture content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 31 % indicating that plant age had a minimal effect on moisture content.

Table 5.6a: Effect of organic and inorganic fertilisers on moisture content (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	88.52±0.34	86.22±0.03^{c}	90.65±0.02^{b}	88.01±0.01^{c}	89.23±0.04^{c}	80.79±0.02^{c}	87.03±0.06^{c}	82.80±0.80^{b}	85.18±0.05^{c}	84.21±1.97^{b}	83.56±1.06
T2	88.52±0.34	86.08±0.04^{c}	90.45±0.03^{c}	89.37±0.02^{b}	90.55±0.26^{a}	83.78±0.01^{b}	88.85±0.05^{a}	85.71±0.10^{a}	89.11±0.06^{a}	83.83±0.02^{b}	86.44±1.02
T3	88.52±0.34	87.20±0.07^{a}	91.68±0.04^{a}	88.82±0.04^{a}	90.07±0.04^{b}	80.59±0.04^{c}	87.72±0.02^{b}	83.06±0.06^{b}	83.10±1.69^{d}	84.66±2.13^{b}	83.75±0.01
T4	88.52±0.34	84.34±0.04^{d}	90.54±0.04^{b}	88.55±0.2^{a}	89.45±0.02^{c}	83.42±0.03^{b}	85.22±0.10^{d}	85.11±0.01^{a}	87.24±.012^{b}	83.24±0.17^{b}	84.37±0.07
T5	88.52±0.34	86.96±0.04^{b}	90.65±0.04^{b}	89.10±0.2^{cb}	85.27±0.03^{d}	89.66±0.07^{a}	81.99±0.09^{e}	84.95±1.00^{a}	87.44±0.11^{b}	87.75±0.02^{a}	84.06±0.06

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.6b: Effect of organic and inorganic fertilisers on moisture content (%) of *Solanum nigrum* L. leaves cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	88.52±0.34	98.25±0.05^{a}	82.62±0.96^{a}	78.75±0.13^{a}	79.02±0.04^{a}	75.16±0.07^{a}	80.05±0.04^{a}	80.32±0.02^{a}
T2	88.52±0.34	92.08±0.04^{b}	86.78±0.09^{b}	83.20±0.18^{b}	85.34±0.09^{b}	86.10±0.10^{b}	86.36±0.11^{b}	86.75±0.02^{b}
T3	88.52±0.34	90.50±0.45^{c}	83.47±0.23^{a}	80.32±0.03^{c}	80.89±0.09^{c}	83.54±0.04^{c}	83.95±0.02^{c}	85.55±0.04^{c}
T4	88.52±0.34	91.29±0.90^{d}	86.32±2.70^{b}	81.85±0.12^{d}	85.10±0.13^{b}	86.54±0.04^{b}	86.72±0.04^{b}	87.39±0.09^{d}
T5	88.52±0.34	89.55±0.09^{e}	88.07±0.06^{b}	85.75±0.11^{e}	85.09±0.03^{b}	86.55±0.09^{b}	86.62±0.09^{b}	86.47±0.06^{b}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.3.6 Root: shoot ratio

a) Field

The root to shoot ratio on the field decreased exponentially between the time of transplanting and the 5th week and began to vary (Table 5.7a). The treatment means significantly differed ($p < 0.05$) from the 9th to the 12th week. They respectively ranged between 0.05 and 0.28 in the 10th week and time of planting in T2. The treatment means were highest in T1 and least in T2. Statistical analysis showed an interaction between plant age and the fertiliser treatment on the root to shoot ratio. Regression analysis with the root to shoot ratio as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 56.5 % indicating that plant age had a fairly significant effect on the root to shoot ratio.

b) Glasshouse

In the glasshouse, the root: shoot ratio decreased exponentially between the time of transplanting and the 3rd week and began to vary there after (Table 5.7b). There were significant differences in treatment means except in the 4th and 5th week and ranged between 0.08 and 0.28 in the 3rd week and time of planting respectively. The means were highest at T3 and least at T2 and T5. Statistical analysis showed an interaction between plant age and the fertiliser treatment on the root to shoot ratio. Regression analysis with the root to shoot ratio as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 23.3 % indicating that plant age had a minimal effect on the root to shoot ratio. It was observed that the glasshouse trial possessed higher root: shoot ratios than the field trial.

Table 5.7a: Effect of organic and inorganic fertilisers on Root: Shoot ratio of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	0.28±0.01	0.14±0.03	0.14±0.02	0.12±0.02	0.12±0.01	0.13±0.03	0.10±0.01^{b}	0.14±0.02^{a}	0.12±0.01^{b}	0.13±0.01^{a}	0.09±0.01^{a}
T2	0.28±0.01	0.13±0.03	0.10±0.04	0.09±0.03	0.09±0.01	0.11±0.01	0.09±0.02^{b}	0.07±0.01^{c}	0.05±0.01^{d}	0.09±0.01^{a}	0.05±0.01^{b}
T3	0.28±0.01	0.12±0.02	0.11±0.03	0.10±0.01	0.11±0.02	0.13±0.03	0.10±0.01^{b}	0.11±0.01^{b}	0.09±0.01^{c}	0.08±0.01^{b}	0.06±0.01^{a}
T4	0.28±0.01	0.13±0.03	0.11±0.03	0.10±0.01	0.10±0.04	0.10±0.01	0.06±0.01^{a}	0.13±0.02^{ab}	0.15±0.01^{a}	0.10±0.02^{b}	0.06±0.01^{b}
T5	0.28±0.01	0.11±0.03	0.12±0.03	0.11±0.02	0.10±0.01	0.12±0.01	0.11±0.01^{b}	0.12±0.02^{ab}	0.09±0.01^{c}	0.12±0.01^{b}	0.07±0.02^{ab}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 5.7b: Effect of organic and inorganic fertilisers on Root: shoot ratio of *Solanum nigrum* L. leaves cultivated in the greenhouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	0.28±0.01	0.19±0.04^{a}	0.12±0.01	0.17±0.03	0.22±0.02^{a}	0.22±0.02^{a}	0.22±0.01^{a}	0.25±0.05^{a}
T2	0.28±0.01	0.08±0.02^{b}	0.11±0.02	0.13±0.02	0.12±0.02^{b}	0.15±0.02^{b}	0.11±0.02^{b}	0.12±0.02^{b}
T3	0.28±0.01	0.16±0.02^{a}	0.15±0.02	0.20±0.03	0.19±0.03^{a}	0.21±0.01^{a}	0.19±0.02^{a}	0.21±0.02^{a}
T4	0.28±0.01	0.12±0.02^{b}	0.12±0.04	0.17±0.03	0.18±0.02^{a}	0.16±0.02^{b}	0.12±0.04^{b}	0.14±0.02^{b}
T5	0.28±0.01	0.09±0.01^{b}	0.10±0.05	0.16±0.03	0.14±0.02^{ab}	0.13±0.02^{b}	0.12±0.04^{b}	0.12±0.02^{b}

0 represents data collected at the time of transplanting
Values shown are mean ± SD
Means with different letters down the same column represent significant differences at $p < 0.05$.

5.4 Discussion

5.4.1 Plant height and number of leaves

Millspaugh (1974) reported a height of between 30.48 and 60.96 cm while Edmonds and Chweya (1997) reported 70 cm in naturally growing *Solanum nigrum*. In this study, a maximum height of 118 cm was reported on the field and 90.33 cm in the glasshouse. Sair et al. (2007) reported that 100 kg N/ ha produced the best height in a tea plant experiment and this is similar to our observations in this study where, 100 kg N/ha + 8.13 t/ha produced the maximum height on the field. However, in the glasshouse, the best result was obtained from 50 kg N/ha + 4.07 t goat manure/ha. In the work of Aliyu (2000) a mixture of farm yard manure and nitrogen produced the tallest *Capsicum annuum* (pepper) plants. Adeoye et al. (2011) found organic fertilisers to affect not only plant height but all growth parameters in their study. In this study, 100 kg N/ha + 8.13 t goat manure/ha produced the highest average number of leaves in the glasshouse while 100 kg N/ha was the best on the field. According to Moorby and Besford (1983), nitrogen fertilisation promotes vegetative growth in plants. This was also observed in this study where nitrogen that was supplied in the form of organic and inorganic fertilisers as well as soil moisture that was kept at field capacity throughout the trial presumably ensured vigorous vegetative growth of the plants. As the height of the plants increased, more internodes were formed, leading to an increase in the total number of leaves formed.

5.4.2 Stem diameter

Organic fertiliser increased the stem diameter of *Solanum nigrum*. This observation is in line with the report of Gharib et al. (2008) who reported that there was an increase in stem diameter of *Majorana hortensis* when compost and bio-fertiliser was applied to the plant.

Ondieki et al. (2011) reported a smaller diameter (6.64 mm) in *Solanum scabrum* as compared to the highest diameter value (7.35 mm) reported in the current study. Stems function to support and elevate leaves, flowers as well as fruits, transport fluids between the roots and the shoots, store nutrients and produce new living tissue (Raven et al., 1981). This study showed that application of 100 kg N/ ha fertiliser produced the maximum diameter values obtained.

5.4.3 Leaf area

Leaf area measurement is vital in monitoring growth and vigour in plants, which in turn has an effect on photosynthesis, transpiration, rain interception and other physiological processes that link vegetation to climate (Gobron, 2009). Leaf area measurement is critical in understanding the water and nutrient use, as well as the growth and yield potential of the crop (Pandey and Singh, 2011). The life cycle of a plant usually involves the early phase of increasing photosynthetic rates while the leaf expands, a mature phase where such rates peak and a senescence phase where they decline (Gepstein, 1988). It has been observed that during the senescence phase nutrients are transported from the senescent leaves into young leaves and seeds (Hua et al., 2012). In this study, it is suggested that senescence began to set in the 7^{th} week in both experiments and this led to a reduction in leaf area. Early flowering and the consequent fruit formation and ripening possibly caused a decline in leaf expansion while the minerals were channelled towards the fruits and the newly developing leaves. By the 7^{th} week, the older bottom leaves were turning light green or yellowish possibly indicating nitrogen remobilisation. Application of 50 kg N/ ha + 4.07 t manure/ ha produced the highest values in both experiments.

5.4.4 Chlorophyll

Chlorophyll of the plants on the field is slightly higher than the one observed in the glasshouse. This observation agrees with the work of Prehl (2010) who reported a similar observation on *Solanum nigrum* leaves. Results of the current study are within the range in the glasshouse and slightly higher on the field experiments as compared to those reported by Prehl (2010). He reported between 30 and 40 SPAD units in young *Solanum nigrum* leaves while the current study observed between 23.11 and 50.82 SPAD units in the glasshouse and 23.11 and 60.34 SPAD units on the field. Loh et al. (2002) reported up to 60 SPAD units in *Ficus benjamina* and *Populus deltoides***.** Masinde et al. (2009) reported higher maximum values than the current study (between 40 and 70 SPAD units in *Solanum villosum*). Different methods of chlorophyll determination used by different researchers may produce different values. To measure the relative chlorophyll concentration in the leaves, the spectrophotometer used in this work (SPAD 502 PLUS) measures the absorbance of the leaf in two wavelengths (400-500 nm and 600-700 nm) and the values are therefore given in SPAD values (SPAD-502 Plus manual, 2009). An increase in SPAD values indicates an increase in chlorophyll content while values above 30 indicate good chlorophyll concentration. The high SPAD values in this experiment therefore indicate the good health and nutritional status of cultivated *Solanum nigrum*. Chlorophyll content is known to increase in proportion to the amount of nitrogen present in the leaf since the majority of leaf nitrogen is contained in chlorophyll molecules (Netto et al., 2005). This study therefore further indicates a high nitrogen content which in turn is an indication of high protein concentration. The application of 100 kg N/ha + 8.13 t manure /ha produced the highest values in both experiments.

5.4.5 Moisture

Moisture content values of between 75.16 and 92.08 % were recorded in this study. This is similar to the observations made by Akubugwo et al. (2007) , Sarma and Sarma (2011) and Oduse et al. (2012) who reported moisture content values of 84.70, 78.00 and 88.47 % respectively in *Solanum nigrum* leaves. Similarly, Srianta et al. (2012) reported 82.55 and 87.14 % in *Paederia foetida* and *Erechtites hieracifolia* respectively. Other similar reports on vegetables include that of Ng et al. (2012) who reported a range between 92.6 and 96.8 % in 6 wild vegetables while Funke (2011) reported 90.35 % in *amaranth* leaves. Plants need water to maintain cell turgor pressure which is essential for physiological processes such as cell enlargement, gas exchange in the leaves, transport in the phloem and various transport processes across membranes (Dainty, 1976). Furthermore, turgor pressure also contributes to the rigidity and mechanical stability of non lignified plant tissues. All the treatments in this study indicate sufficient water in the leaves for plant growth. Furthermore, the field trial indicates that 100 kg N/ha produced the highest moisture content values while 50 kg N/ ha + 4.07 t manure/ ha was best in the glasshouse.

5.4.6 Root: shoot ratio

Various groundnut genotypes produced root: shoot ratios ranging between 0.04 and 0.32 in work conducted by Jagana et al. (2012) and these values are similar to what is reported in the current study. Ramteke and Shirgave (2012) reported higher ratios compared to this present work (0.50 - 0.84) in *Trigonella foenum graecum* treated with urea, DAP and biomass. Nahar and Gretzmacher (2011) as well as Muthomi and Musyimi (2009) reported that root: shoot ratio of some tomato cultivars and African nightshades respectively increased in response to water stress. The root to shoot ratio increased in the glasshouse and this may be due to nutrient stress. According to Dixon (2006), reduction in nutrient supply increases root to

shoot ratio thereby compensating for loss in root foraging capacities. Therefore the root to shoot ratio in the glasshouse possibly increased in response to nutrient stress since soil moisture was kept at field capacity throughout the experiment. The relatively low root: shoot ratio values reported in this work are a good indicator of the favourable nutrient and moisture supply conditions the plants were subjected to during growth, as well as the plant's favourable health status. The application of 100 kg N/ha generally produced the best root: shoot ratio values.

5.5 Conclusion

This study provides useful information on the cultivation of *Solanum nigrum* at a small scale level. The response of this plant to various levels of fertilisers has thus been documented. More specifically, the ability of inorganic fertiliser (NPK) applied at a rate of 100 kg N/ha to increase stem diameter, moisture content and root: shoot ratio on the field. The application of 100 kg N/ha further increased root: shoot ratio and stem diameter in the glasshouse. Combining NPK (100 kg N/ha) and goat manure (8.13 t/ha) significantly improved chlorophyll content and number of leaves in the glasshouse while plant height, number of leaves and chlorophyll were significantly increased on the field. Leaf area in both the glasshouse and the field as well as plant height in the glasshouse were significantly boosted by the application of 50 kg N/ ha + 4.07 t manure/ ha. More specifically, plant height, number of leaves and stem diameter increased from the time of transplanting to termination of the experiment. However, 100 kg N/ha + 8.13 t/ha produced the plant maximum height and number of leaves on the field and this was 118cm and 1892 leaves while the maximum plant height in the glasshouse was 90.33cm from application of 50 kg N/ha + 4.07 t/ha while 100 kg N/ha produced the highest number of leaves in the glasshouse and this was 361.On both the field and glasshouse, the maximum stem diameter was observed in 100 kg N/ha treated

plots and these were 12.28mm and 5.83mm respectively. Leaf area increased exponentially in 50 kg N/ha + 4.07 t/ha amended plots and began to decrease after 7 weeks in both the field and glasshouse and the highest means observed were 58.2 cm^2 and 92.98 cm^2 respectively. Chlorophyll content was highest in the 7^{th} and 6^{th} weeks after transplanting on the field and glasshouse and was respectively 60.34 and 50.82 SPAD values. Also, the concentration increased from the time of transplanting to the 4^{th} week in both trials after which it varied. The highest moisture content value (91.68 %) on the field was observed in the 4^{th} week in 8.13 t/ha amended treatment while in the glasshouse, a maximum of 92.08 % was observed in the 3^{rd} week after transplanting. The moisture content remained high and consistent throughout the trial. The root: shoot ratio decreased exponentially between the time of transplanting and the 3^{rd} week on the field and continued to decrease but began to vary from the 3^{rd} week in the glasshouse. The 10^{th} week recorded the least value (0.05) on the field and 0.08 was reported in the glasshouse and these were both reported from the 100 kg N/ha treatment. Thus, different growth parameters responded differently to various fertiliser treatments, however, organic fertiliser alone did not positively affect the growth parameters in this study. The glasshouse control exhibited signs of nutrient stress as evidenced by slow growth (plant height) as opposed to other treatments and the field trial. The results of the application of organic manure may not be immediately visible due to the slow process of mineralisation of nutrients from organic manure source. However, the application of both of organic and inorganic fertilisers may be a good option to poor resource, rural farmers who may not be able to afford fertilisers from dealers but who can make use of manure from their livestock kraals that are often situated at the back of their homes.

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CHAPTER 6

EFFECT OF FERTILISERS ON PROXIMATE COMPOSITION OF *SOLANUM NIGRUM* CULTIVATED ON THE FIELD AND GLASSHOUSE

CHAPTER SIX
Effect of fertilisers on proximate composition of *Solanum nigrum* cultivated on the field and glasshouse

6.1 Introduction

Vegetable plants are one of the sources of mineral nutrients for man and the plants obtain some of their mineral nutrients from soils. The mineral compositions and quality of vegetables is dependent on the interactions of many factors which include genetic makeup, climatic conditions, stage of maturity, soil and type of fertiliser used for cultivation, harvesting, handling, storage and processing of the vegetable plant (Asenjo, 1962). Minerals are of critical importance in the human diet as they maintain certain physicochemical processes which are essential to life (Soetan et al., 2010). There is an increasing interest among consumers who are demanding to know the nutritional quality and safety of the food they consume. It has been observed that the nutrient composition of vegetable leaves generally declines with maturity, the leaf protein content declines and more indigestible structural carbohydrates are formed (Raymond, 1969; Baranga, 1983). There is no doubt that the use of fertilisers boosts soil fertility; but, their overuse has enormous adverse effects on the soil, plants and animals that consume the plants (Haynes and Naidu, 1998; Arisha and Bradisi, 1999). Apart from improving soil nutrient capacity, organic manure modifies soil physical conditions by improving the water holding capacity, aeration, drainage and friability (Chaudhary and Narwal, 2005). In addition, organic manure helps crops by decreasing the bioavailability of mineral salts and toxic substances (Imoro et al., 2012). According to Mandiringana et al. (2005), soils of the Eastern Cape where this present study was carried out are generally low in macronutrients N and P, while micronutrients such as B, Cu, S, Mn and Zn are abundant. Therefore, soils in this area need to be boosted with fertilisers rich in N and P in order to improve their productivity.

Although *Solanum nigrum* is known for its nutraceutical properties (Sarma and Sarma, 2011), the plant also contains Solanine, a toxic substance whose concentration is high in green

unripe berries (Cooper and Johnson, 1984). However, the toxin declines as the berries mature and ripen (Watt and Breyer-Brandwijk, 1962). Despite the significance and importance of this plant both nutritionally and medicinally; there are no reports available in literature on its cultivation in Eastern Cape home gardens. The purpose of this study was therefore to investigate the effects of organic and/or inorganic fertilisers on the proximate composition of the leaves of *Solanum nigrum* at various stages of its growth; the best fertiliser option for cultivation of *Solanum nigrum* and the optimum harvesting time for *Solanum nigrum* leaves based on the proximate composition of the leaves.

6.2 Materials and methods

The experimental site, agronomic practices and experimental design were as described in Chapter 5.

6.2.1 Data collection

The third youngest fully expanded leaves (Jones et al., 1971) were collected from the shoots by uprooting the whole plant; washed in distilled water to remove sediments and other impurities before drying the samples in a dust free, forced-draft oven at 40^{o}C to a constant weight. The samples were then ground to a powder using a mortar and pestle and passed through a 2 mm sieve. The samples were kept in val bottles and stored in a refrigerator at 4^{o}C till when needed. However, vitamin C was determined from green freshly harvested plant samples. The first data were collected on the day of transplanting and 3 weeks after transplanting, data were collected on a weekly basis. The experiment was terminated in the 9th week for the glasshouse experiment and 12th week for the field experiment when all the berries on the plant were mature and ripe.

6.2.2 Proximate analysis

The ash, lipid, protein, fibre, vitamin C and phytate were determined as described in chapter 3.

6.2.3 Statistical analysis

Data of the proximate concentrations of various treatments were subjected to statistical analysis using MNITAB Release 12. A one way analysis of variance was used to compare the means of various proximate parameters among the treatments and a two way analysis of variance used to determine the interaction between plant age (weeks after transplanting) and treatment on proximate concentration in the plant. Means were segregated using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

6.3 Results

6.3.1 Ash

a) Field

The effect of fertilisers on ash content differed significantly ($p < 0.05$) and varied between 6.77 and 8.95 % on the field (Table 6.1a) and 7.44 and 9.45 % in the glass house (Table 6.1b). Treatment means for the duration of the trial were highest in T2 (8.23 %) and lowest in T4 (8.06 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on the ash content. Regression analysis with the ash content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 71.6 % indicating that plant age had a significant effect on the ash content.

b) Glasshouse

In the glasshouse, the treatment means for the trial period were highest in T3 (8.63 %) and lowest in T5 (8.30 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on the ash content. Regression analysis with the ash content as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 47.6 % indicating that plant age had a minimal effect on the ash content. The ash content increased from the time of transplanting to the 4^{th} week after which it generally remained consistently high throughout the trial and the highest values were recorded in the 4^{th} week in both the glasshouse and field.

Table 6.1a: Effect of organic and inorganic fertilisers on ash (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	7.44±0.38	7.37±1.92^{a}	7.28±3.46^{a}	8.09±0.48^{a}	8.33±0.36^{a}	8.19±0.02^{a}	8.39±0.14^{a}	8.38±0.15^{a}	8.37±0.08^{a}	8.60±0.21^{a}	8.43±0.17^{a}
T2	7.44±0.38	8.15±0.63^{b}	8.95±0.88^{b}	8.04±0.44^{a}	8.31±0.41^{a}	8.31±0.03^{a}	8.32±0.03^{b}	8.45±0.07^{b}	8.38±0.04^{a}	8.68±0.35^{b}	8.13±0.45^{b}
T3	7.44±0.38	7.41±0.55^{a}	7.38±0.72^{a}	8.07±0.22^{a}	8.34±0.07^{ab}	8.30±0.08^{a}	8.42±0.20^{c}	8.42±0.02^{c}	8.44±0.12^{b}	8.46±0.29^{c}	8.44±0.24^{a}
T4	7.44±0.38	7.73±0.95^{c}	8.03±1.51^{c}	8.14±0.45^{ab}	8.32±0.15^{a}	7.13±9.53^{ab}	8.35±0.08^{d}	8.29±0.12^{d}	8.37±0.09^{a}	8.56±0.14^{a}	8.53±0.90^{c}
T5	7.44±0.38	8.07±3.43^{b}	8.71±6.48^{b}	8.16±0.26^{b}	8.39±0.35^{b}	6.77±13.12^{b}	8.29±0.04^{e}	8.44±0.12^{b}	8.42±0.21^{c}	8.58±0.23^{a}	8.57±0.50^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.1b: Effect of organic and inorganic fertilisers on ash (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	7.44±0.38	8.24±1.19^{a}	9.04±1.99	8.99±0.57^{a}	9.15±0.62^{a}	9.13±0.24^{a}	9.05±0.19^{a}	8.81±0.74^{a}
T2	7.44±0.38	8.28±2.67^{a}	9.13±4.96	8.83±0.20^{b}	8.71±0.27^{b}	8.68±0.57^{b}	8.51±0.85^{b}	8.67±0.44^{b}
T3	7.44±0.38	8.44±2.22^{b}	9.45±4.05	9.05±0.39^{c}	8.83±0.12^{c}	8.84±0.83^{c}	8.95±0.55^{c}	8.78±0.34^{a}
T4	7.44±0.38	8.33±3.67^{ab}	9.25±6.95	8.90±0.28^{d}	8.74±0.76^{b}	8.70±0.13^{b}	8.51±0.61^{b}	8.57±0.04^{c}
T5	7.44±0.38	8.74±2.21^{c}	8.74±4.05	8.57±0.30^{e}	8.640±0.15^{d}	8.46±0.46^{d}	8.44±0.22^{b}	8.42±0.36^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

6.3.2 Fibre

a) Field

Fibre increased exponentially on the field from the time of transplanting to the 4th week and varied to the 11th week after which it decreased in the 12th week (Table 6.2a). The treatment means were significantly different ($p < 0.05$) in the 3rd, 4th, 5th, 6th, 11th and 12th weeks. The treatment means for the duration of the trial were highest in T1 (18.91 %) and least in T5 (17.98 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on fibre. Regression analysis with fibre as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 45.3 % indicating that plant age had a minimal effect on fibre.

b) Glasshouse

In the glasshouse, fibre content increased exponentially from the time of transplanting to the 4th week and varied, but decreased in the 9th week (Table 6.2b). The treatment means were significantly different throughout the trial except in the 6th and 7th weeks. The treatment means for the duration of the trial were highest in T1 (16.56 %) and lowest in T5 (13.57 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on fibre. Regression analysis with fibre as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 3 % indicating that plant age had a very minimal effect on fibre. Fibre content was highest in the 11th and 4th week on the field and glasshouse respectively. These results further reveal that *Solanum nigrum* cultivated on the field had higher fibre content than that cultivated in the glasshouse.

Table 6.2 a: Effect of organic and inorganic fertilisers on fibre (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	9.86±2.76	16.12±1.63^{b}	18.14±1.90^{a}	19.28±2.39^{ab}	21.75±3.26^{b}	23.96±5.30	22.37±29.18	21.45±0.41	21.00±1.51	24.80±1.18^{a}	14.05±5.61^{a}
T2	9.86±2.76	14.00±2.33^{a}	22.38±0.49^{b}	21.52±0.93^{a}	18.47±6.72^{ab}	20.37±1.56	19.35±16.58	20.32±2.04	20.87±1.33	23.32±4.37^{ab}	17.82±0.65^{cb}
T3	9.86±2.76	14.16±1.58^{a}	18.46±0.40^{a}	20.50±1.87^{ab}	21.69±0.49^{b}	21.40±0.91	18.30±1.22	20.56±1.04	20.27±2.66	21.17±0.65^{ab}	19.85±0.02^{cb}
T4	9.86±2.76	14.97±1.81^{ab}	20.08±0.84^{ab}	18.82±1.23^{b}	18.87±4.82^{ab}	23.64±1.10	22.93±4.97	16.50±2.16a	18.84±0.39	21.41±5.33^{ab}	17.33±1.84^{c}
T5	9.86±2.76	15.82±3.19^{b}	21.77±3.62^{b}	19.22±2.82^{ab}	16.48±0.27^{a}	20.51±6.83	21.48±2.19	20.96±0.04	15.10±1.69a	19.59±3.29^{b}	21.11±2.57^{b}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.2b: Effect of organic and inorganic fertilisers on fibre (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	9.86±2.76	15.11±2.14^{a}	20.37±1.51^{a}	18.18±1.73^{ab}	15.51±2.29	18.10±4.00	20.98±1.13^{a}	15.96±1.37^{a}
T2	9.86±2.76	16.14±1.75^{b}	21.42±0.74^{ab}	19.53±0.51^{a}	19.53±0.59	12.31±4.80	11.96±2.21^{b}	11.99±0.56^{b}
T3	9.86±2.76	16.27±2.31^{c}	22.67±1.86^{b}	17.73±0.96^{b}	15.93±0.07	17.17±10.26	18.85±0.71^{c}	13.27±0.24^{b}
T4	9.86±2.76	16.17±1.45^{c}	22.49±0.13^{b}	19.06±0.91^{ab}	13.90±2.91	16.71±1.44	11.46±2.94^{a}	9.90±2.37^{c}
T5	9.86±2.76	14.46±1.84^{d}	19.06±0.92^{c}	16.18±1.04^{c}	16.18±2.29	14.87±3.14	10.71±0.24^{a}	9.97±0.69^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.2c: □ Recommended daily fibre intakes by life stage and gender

	Boys	Girls	Women	Men
Concentration (g/day)	24-28	20-22	25	30

□ Nutrient reference values for New Zealand and Australia (NHMRC, 2005).

6.3.3 Crude lipid

a) Field

The lipid content differed significantly ($p < 0.05$) among the treatments throughout the trial in both the field (Table 6.3a) and the glasshouse (Table 6.3b). Lipid content increased on the field from the time of transplanting until the 4th week and decreased in the 5th week after which it increased until the 11th week. The treatment means for the duration of the trial were highest in T5 (3.87 %) and least in T2 (3.42 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on lipids. Regression analysis with lipids as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 43.2 % indicating that plant age had a minimal effect on lipids.

b) Glasshouse

In the glasshouse, lipids decreased from the time of transplanting until the 4th and 5th week and increased exponentially, reaching a peak in the 6th week after which they decreased variably. The treatment means for the duration of the trial were highest in T4 (3.51 %) and least in T1 (1.89 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on lipids. Regression analysis with lipids as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 19.8 % indicating that plant age had a minimal effect on lipids. The lipid content was therefore at its

peak in the 11th and 6th weeks on the field and glasshouse respectively. Comparing the lipid content in the glasshouse and the field, the results of this experiment show that lipid content was higher on the field than in the glasshouse.

Table 6.3a: Effect of organic and inorganic fertilisers on crude lipid (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	2.44±0.01	3.17±0.40^{a}	3.89±0.78^{ab}	2.51±0.65^{ab}	1.13±0.45	3.02±0.06^{ab}	1.72±0.89^{a}	3.26±0.47^{a}	5.13±1.08^{a}	6.03±0.50^{a}	6.76±0.10^{a}
T2	2.44±0.01	2.47±0.59^{b}	2.49±1.16^{a}	3.21±0.65^{a}	1.10±0.15	2.97±0.17^{ab}	3.08±0.71^{b}	3.49±0.16^{ab}	3.95±0.12^{b}	5.28±0.97^{a}	6.13±0.59^{ab}
T3	2.44±0.01	3.90±1.17^{a}	5.35±2.32^{b}	2.07±0.47^{b}	1.07±0.05	2.82±0.30^{a}	3.07±0.37^{b}	3.22±0.43^{a}	5.37±0.01^{a}	5.68±0.45^{a}	5.17±0.21^{b}
T4	2.44±0.01	2.92±0.38^{b}	3.40±0.75^{ab}	3.00±0.54^{a}	0.98±0.13	3.15±0.10^{b}	2.08±0.30^{a}	3.69±0.38^{ab}	3.98±0.49^{b}	7.58±0.15^{b}	6.40±0.68^{ab}
T5	2.44±0.01	3.34±0.20^{ab}	4.23±0.39^{ab}	1.85±0.55^{b}	1.09±0.11	3.49±0.19^{c}	3.34±0.33^{b}	3.92±0.04^{b}	5.30±0.28^{a}	7.74±1.19^{b}	5.69±0.48^{b}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.3b: Effect of organic and inorganic fertilisers on crude lipid (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	2.44±0.01	1.74±0.20^{a}	1.03±0.38^{ab}	1.19±0.41^{a}	1.93±0.24^{b}	2.75±0.16^{b}	2.28±0.26^{a}	1.15±0.32^{c}
T2	2.44±0.01	1.49±0.17^{b}	0.54±0.33^{a}	2.38±0.05^{b}	4.64±0.43^{a}	3.99±0.03^{a}	4.47±0.03^{b}	2.92±0.21^{a}
T3	2.44±0.01	2.26±0.90^{c}	2.08±1.79^{ab}	1.82±0.51^{c}	2.90±0.40^{b}	2.98±0.01^{b}	2.29±0.41^{a}	1.52±0.12^{c}
T4	2.44±0.01	2.79±1.32^{c}	3.13±2.63^{b}	3.06±0.03^{d}	5.52±1.50^{a}	4.14±0.20^{a}	4.10±0.32^{c}	3.32±0.24^{b}
T5	2.44±0.01	1.83±0.03^{a}	1.22±0.05^{ab}	2.32±0.08^{b}	3.40±0.32^{b}	3.18±0.22^{b}	3.60±0.07^{d}	2.34±0.40^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.3c: □ Recommended daily lipid intakes by life stage and gender

	Boys	Girls	Women	Men
Concentration (g/day)	0.07-0.125	0.07-0.085	0.09	0.160

□ Nutrient reference values for New Zealand and Australia (NHMRC, 2005).

6.3.4 Vitamin C

a) Field

Vitamin C increased on the field from the time of transplanting to the 9th week when the highest value (250 mg/ 100g) was recorded in T2 and exponentially decreased in the 10th week and remained constant until the 12th week (Table 6.4a). The lowest values were recorded in week 12 in all the treatments. Significant differences ($p < 0.05$) among the treatments were noted in week 7, 8, 10 and 11. The treatment means for the duration of the trial were however highest in T4 (118 mg/ 100g) and lowest in T1 (97 mg/ 100g). Statistical analysis showed an interaction between plant age and the fertiliser treatment on vitamin C. Regression analysis with vitamin C as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 0.4 % indicating that plant age had a very minimal effect on vitamin C.

b) Glasshouse

In the glasshouse, the effect of fertiliser treatments on vitamin C were not significant in week 6 alone (Table 6.4b). Vitamin C increased to a maximum of 187 mg/ 100g (T2) in the 6^{th} week and decreased exponentially in the 7^{th} week and continued to decrease until the concentration was below the limit of detection in week 9. The treatment means for the duration of the glasshouse trial were highest in T5 (79 mg/ 100g) and lowest in T1 (52 mg/ 100g). Statistical analysis showed an interaction between plant age and the fertiliser treatment on vitamin C. Regression analysis with vitamin C as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 3 % indicating that plant age had a very minimal effect on vitamin C. The lowest value recorded in the final week of observation in the glasshouse was below the limit of detection and yet the lowest value reported on the field was 30 mg/ 100g. Also, vitamin C content was higher on the field than the glasshouse.

Table 6.4a: Effect of organic and inorganic fertilisers on vitamin C (mg/ 100g) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	50±0.10	60±0.14	70±0.17	127±0.25	103±0.50	100±0.26^{a}	167±0.49^{ab}	200±0.20	067±0.29^{ab}	47±0.12	30±0.06^{a}
T2	50±0.10	69±0.21	87±0.31	97±0.29	117±0.15	183±0.47^{b}	217±0.29^{ab}	250±0.35	043±0.10^{a}	42±0.12^{ab}	39±0.17
T3	50±0.10	61±0.08	73±0.06	103±0.23	110±0.26	120±0.00^{ab}	240±0.26^{a}	237±0.23	067±0.29^{ab}	57±0.12^{b}	33±0.12
T4	50±0.10	70±0.13	117±0.15	117±0.21	130±0.35	100±0.10^{a}	223±0.25^{ab}	220±0.26	100±0.00^{b}	73±0.12^{b}	60±0.17
T5	50±0.10	67±0.18	83±0.25	100±0.00	167±0.23	117±0.29^{a}	163±0.23^{b}	213±0.31	83±0.21^{b}	60±0.10^{b}	50±0.20

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.4b: Effect of organic and inorganic fertilisers on vitamin C (mg/ 100g) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	50±0.10	53±.10^{a}	55±0.00^{a}	80±0.35^{a}	170±0.26	27±0.06^{a}	13±0.06^{a}	nd
T2	50±0.10	104±0.25^{b}	157±0.40^{b}	103±0.12^{a}	187±0.15	60±0.10^{b}	30±0.00^{b}	nd
T3	50±0.10	55±0.14^{a}	60±0.17^{a}	97±0.06^{a}	167±0.51	33±0.06^{a}	30±0.10^{b}	nd
T4	50±0.10	94±0.18^{b}	137±0.25^{b}	117±0.06^{ab}	163±0.15	40±0.10^{ab}	10±0.00^{a}	nd
T5	50±0.10	89±0.21^{ab}	127±0.32^{ab}	153±0.06^{b}	177±0.15	70±0.10^{b}	30±0.00^{b}	nd

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

6.3.5 Protein

a) Field

Protein concentration differed significantly ($p < 0.05$) among the treatments from the 4th to the 12th week on the field (Table 6.5a). The concentration increased exponentially from the time of transplanting until the 3rd week after which it varied, and began to decrease in the 7th week until the 12th week. The treatment mean for the duration of the trial were highest in T5 (37.50 %) and lowest in T3 (34.39 %), however, the highest concentration was recorded in week 6 in T2. Statistical analysis showed an interaction between plant age and the fertiliser treatment on protein. Regression analysis with protein as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 43.6 % indicating that plant age had a very minimal effect on protein.

b) Glasshouse

In the glasshouse, the treatment means differed significantly throughout the trial and T1 and T3 gradually decreased in protein content from the time of transplanting to week 9 (Table 6.5b). However, T2, T4 and T5 decreased but variably. The treatment means for the duration of the trial were highest in T5 (35.53 %) and lowest in T1 (19.28 %). However, the peak concentration was recorded in week 3 in T2. Statistical analysis showed an interaction between plant age and the fertiliser treatment on protein. Regression analysis with protein as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 76.9 % indicating that plant age had a significant effect on protein. The lowest protein content values were recorded in the control (T1) throughout the trial. The field trial produced higher treatment means as compared to the glasshouse trial. The maximum value reported in the field (42.81 %) was higher than the value reported in the glasshouse (39.64 %).

Table 6.5a: Effect of organic and/or inorganic fertilisers on protein (%) of *Solanum nigrum* cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	34.50±0.22	42.62±0.11	39.75±0.17^{a}	40.43±0.39^{a}	38.76±0.28^{a}	38.56±0.11^{a}	35.88±0.11^{a}	34.31±0.51^{a}	38.82±0.11^{a}	33.00±0.22^{a}	29.56±0.17^{a}
T2	34.50±0.22	42.63±0.11	38.82±0.11^{b}	42.81±0.28^{b}	41.73±0.64^{b}	41.94±0.05^{b}	38.25±0.34^{b}	37.00±0.11^{b}	34.31±0.51^{b}	27.44±0.50^{b}	24.49±0.11^{b}
T3	34.50±0.22	41.18±0.56^{a}	39.75±0.34^{a}	39.50±0.22^{c}	38.87±0.61^{a}	34.57±017^{c}	31.36±0.19^{c}	33.32±0.17^{c}	28.44±0.28^{c}	29.13±0.17^{c}	27.63±0.22^{c}
T4	34.50±0.22	42.56±0.11	40.58±0.56^{c}	41.74±0.22^{d}	41.44±0.17^{b}	40.19±0.17^{d}	34.68±0.28^{d}	36.32±0.06^{d}	33.56±0.39^{d}	28.49±0.17^{d}	28.75±0.28^{d}
T5	34.50±0.22	42.38±0.45	42.01±0.11^{d}	41.93±0.56^{d}	42.45±0.50^{b}	40.20±0.09^{d}	37.95±0.39^{b}	35.56±0.51^{e}	34.21±0.30^{b}	29.13±0.34^{c}	32.02±0.15^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.5b: Effect of organic and inorganic fertilisers on protein (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	34.50±0.22	28.69±0.28^{a}	19.24±0.22^{a}	18.43±0.28^{a}	15.18±0.17^{a}	12.94±0.39^{a}	14.51±0.11^{a}	10.74±0.11^{a}
T2	34.50±0.22	39.64±0.22^{b}	39.37±0.84^{b}	27.25±0.17^{b}	32.37±0.22^{b}	31.43±0.17^{b}	30.62±0.28^{b}	30.75±0.11^{b}
T3	34.50±0.22	31.57±0.11^{c}	19.70±0.28^{a}	19.43±0.11^{c}	21.05±0.28^{c}	16.56±0.28^{c}	16.69±0.39^{c}	12.95±0.39^{c}
T4	34.50±0.22	36.37±0.11^{d}	30.20±0.17^{c}	26.51±0.11^{b}	32.51±0.11^{b}	31.56±0.28^{b}	29.32±0.51^{b}	25.44±0.22^{d}
T5	34.50±0.22	39.26±0.22^{b}	34.18±0.11^{d}	32.82±0.06^{d}	36.26±0.11^{d}	36.25±2.24^{d}	38.01±0.45^{d}	32.99±0.22^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.5c: □ Recommended daily protein intakes by life stage and gender

	Boys	Girls	Women	Men
Concentration (g/day)	40-65	35-45	46	64

□Nutrient reference values for New Zealand and Australia (NHMRC, 2005).

6.3.6 Phytate

a) Field

The concentration of phytate in *Solanum nigrum* cultivated on the field ranged between 1.13 and 3.43 % from the time of transplanting to the termination of the experiment (Table 6.6a). The values were significantly different ($p < 0.05$) among the treatments from the 5^{th} to the 12^{th} week. The concentration decreased from the time of transplanting to the 7^{th} week and increased until the 11^{th} week after which it decreased in the 12^{th} week. The treatment means for the duration of the trial were highest in T5 (2.43 %) and lowest in T3 (1.99 %). The concentration was however lowest and therefore favourable in week 12 (1.17 %) in T2. Statistical analysis showed an interaction between plant age and the fertiliser treatment phytate. Regression analysis with phytate as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 1.1 % indicating that plant age had a very minimal effect on phytate.

b) Glasshouse

In the glasshouse, significant differences among the treatment means were recorded between the 4^{th} and 9^{th} weeks (Table 6.6b). The concentration increased exponentially between the 4^{th} and 5^{th} week after which it decreased. The treatment means for the duration of the trial were highest in T5 (4.58 %) and least in T1 (2.38 %). The lowest and therefore most favourable value was however recorded in T1 (1.67 %) in week 9. Statistical analysis showed an interaction between plant age and the fertiliser treatment phytate. Regression analysis with phytate as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 28.9 % indicating that plant age had a minimal effect on phytate. The glasshouse experiment recorded higher values than the field.

Table 6.6a: Effect of organic and inorganic fertilisers on phytate (mg/ 100g) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	2.40±0.50	2.19±.66	1.97±0.81	1.80±0.15^{ab}	1.33±0.14^{a}	1.43±0.19^{a}	2.67±0.42^{a}	3.00±0.45^{a}	3.43±0.27^{a}	3.30±0.56^{a}	1.63±0.23^{a}
T2	2.40±0.50	2.47±0.34	2.50±0.18	1.70±0.59^{ab}	1.67±0.31a	1.33±0.26^{a}	2.50±0.00^{a}	2.00±0.00^{b}	2.83±0.52^{ab}	2.23±0.14^{b}	1.17±0.14^{b}
T3	2.40±0.50	2.56±0.71	2.67±0.92	1.37±0.14^{a}	1.50±0.32^{a}	1.13±0.23^{a}	1.67±0.05^{b}	2.20±0.18^{b}	2.33±0.49^{b}	2.63±0.45^{ab}	1.83±0.52^{a}
T4	2.40±0.50	2.21±0.30	1.97±0.10	2.00±0.27^{ab}	2.47±0.05^{b}	2.10±0.24^{b}	3.27±0.34^{c}	3.03±0.10^{a}	2.67±0.34^{ab}	2.70±0.59^{ab}	1.67±0.19^{a}
T5	2.40±0.50	2.46±0.28	2.47±0.05	2.27±0.83^{b}	2.33±0.26^{b}	1.87±0.21^{b}	2.73±0.29^{a}	2.93±0.40^{a}	3.03±0.75^{ab}	2.93±0.40^{ab}	1.60±0.15^{ab}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 6.6b: Effect of organic and inorganic fertilisers on phytate (mg/ 100g) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	2.40±0.50	2.60±0.00	1.87±0.21^{b}	3.50±0.36^{a}	2.67±0.40^{a}	2.67±0.14^{a}	1.70±0.09^{a}	1.67±0.14^{a}
T2	2.40±0.50	2.57±0.05	2.83±0.26^{a}	5.60±0.09^{b}	4.50±0.77^{b}	5.80±0.24^{b}	4.30±1.03^{b}	3.80±0.18^{b}
T3	2.40±0.50	2.60±0.00	1.93±0.10^{b}	4.00±0.45^{c}	3.83±0.52^{b}	3.83±0.42^{c}	3.30±0.73^{c}	2.17±0.10^{c}
T4	2.40±0.50	2.63±0.05	2.30±0.31^{ab}	5.50±0.09^{b}	3.90±0.15^{b}	4.60±0.47^{d}	4.83±0.19^{b}	3.60±0.15^{b}
T5	2.40±0.50	2.67±0.10	2.63±0.10^{a}	6.40±0.09^{d}	6.20±0.71^{c}	5.73±0.23^{b}	5.90±0.18^{d}	4.67±0.19^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

6.4 Discussion

6.4.1 Ash

Ash ranged between 6.77-8.95 % on the field and 7.44-9.45 % in the glasshouse. The maximum value of ash in *Solanum nigrum* cultivated in both the glasshouse (9.45 %) and the field (8.95 %) is higher than 4.43, 3.93 and 7.8 % that was reported by Oduse et al. (2012), Sathya Meonah et al. (2012) and Sarma and Sarma (2011) respectively, but lower than 10.18 % that was reported by Akubugwo et al. (2007a) in *Solanum nigrum* leaves obtained from the wild. Comparing with other wild vegetables, Jimoh et al. (2011) reported higher values in *Sonchus asper* (18.75%) and *Sonchus oleraceus* (14.25) while Ogbemudia et al. (2013) reported 6.55 and 5.20 % in *Ipomoea batatas* and *Lecointea ovalifolia* respectively. Cakilcioglu and Khatun (2011) reported a range between 4 and 7.50 % in 16 wild vegetables while work conducted on spinach cultivated in the garden in New Zealand revealed an ash content of 2.43% (McLaughlin, 1929). Ash content is a measure of the nutritional value of food whilst mineral content is a measure of specific inorganic components present within food such as Ca, Mn and Mg among others (Pomeranz and Meloan, 1994). The ash composition of fresh foods rarely exceeds 5% although some processed foods can have ash contents as high as 12 % (Chaudhary and Verma, 2011). The results of this study indicate on a preliminary standpoint the rich nutritional composition of *Solanum nigrum*, cultivated both in the glasshouse and the field. However, the field appears to reveal more favourable results than the glasshouse.

6.4.2 Fibre

The fibre reported in this present work is higher than what was reported by Akubugwo et al. (2007a), Lola (2009) as well as Oduse et al. (2012), who reported 6.81, 2.56 and 1.13 % fibre in *Solanum nigrum* leaves. In other wild vegetables Jimoh et al. (2011) reported 18.33 and

15.25 % in *S asper* and *S oleraceus* respectively while Aberoumand (2009) reported 18.50 % in *Asparagus officinalis*. In addition, Antia (2006) reported 7.20 % fibre in *Ipomoea batatas* whereas Ndlovu and Afolayan (2008) reported 20.30 % in *Corchorus olitorius*. Although there was a varied increase in fibre content in our trial, Ghadaki et al. (1975) as well as Oduntan and Olaleye (2012) reported that maturity had an increasing effect on fibre content of plants and this is slightly different from the present study where there was an initial increase but later varied response. In human anatomy and physiology, fibre is important in reducing the risk for cardiovascular diseases, weight management, gastrointestinal disorders including speeding up of excretion of waste and toxins from the body as well as cancer prevention (Anderson et al., 2009). The American Dietetic Association (ADA, 2002) recommends consumption of 20-35 g dietary fibre per day while the National Health and Medical Research Council of Australia (NHMRC, 2005) recommends between 14 and 30 g/day. According to Food and Drug Administration (FDA, 2005), a good source of fibre must have between 10-15 % of the Adequate Intake (AI). The substantial amount of fibre in *Solanum nigrum* from both the glasshouse and field in this experiment indicate the potential of the vegetable to contribute positively to the AI and proper functioning of the human body especially the normal functioning of the digestive system. In addition, this study indicates that in order to extract the highest concentration of fibre from *Solanum nigrum* cultivated under field conditions, the plant should be harvested in the final stages of its life cycle, while the early stages of its cycle would be ideal under glasshouse conditions. This is when the fibre content is at its peak.

6.4.3 Crude lipid

Due to the complex nature of lipids, in this work, crude lipid refers to and includes among others, triglycerides, diglycerides, monoglycerides, phospholipids, steroids, free fatty acids,

and fat soluble vitamins. In this study, exposure of *Solanum nigrum* to the variable weather elements in the field boosted its lipid content as compared to the glasshouse. The maximum lipid value in both the glasshouse and field trial of this present trial is however, higher than what was reported by Akubugwo et al. (2007a), Lola (2009), Sarma and Sarma (2011), Oduse et al. (2012) and these authors reported 4.60, 0.96, 2.59, and 0.50 % crude lipid in uncultivated *Solanum nigrum* leaves harvested from the wild respectively. The mineral compositions and quality of vegetables is dependent on the interactions of many factors which include genetic makeup, climatic conditions, stage of maturity, soil and type of fertiliser used for cultivation, harvesting, handling, storage and processing of the vegetable plants (Asenjo, 1962). These factors may possibly have led to differences in lipid concentrations reported in the present study as compared to other authors. In other wild vegetables, Adeniyi et al. (2012) reported a range between 2.57 and 5.07 % while Jimoh et al. (2011) reported 7.75% and Aberoumand (2009) reported 3.44 %. Lipids are essential in the human diet because they are the body's most concentrated form of energy constituting about 37 kJ/g and they also aid in the absorption of fat-soluble vitamins A, D, E and K and other fat soluble biologically-active components (NHMRC, 2005). The results of this study indicate that *Solanum nigrum* contains a favourable amount of lipids to meet the AI values in children consume 150 g or adults consume 300 g per day. Consumers around the world are also increasingly demanding food that has reduced total fat, saturated fat and cholesterol for improving health and this plant would preferably be an important option. In order to acquire the maximum concentration of lipids from *Solanum nigrum* cultivated on the field, the plant should be harvested in the 11^{th} week while the 6^{th} week would be ideal in the glasshouse due to the high lipid concentrations obtained during these times in the present study.

6.4.4 Vitamin C

The maximum vitamin C value (250 mg/100g) reported in this study is higher than what was reported by Thenmozhi et al. (2011) who reported a range between 103 and 180.6 mg/ 100g in *Solanum nigrum*. Akubugwo et al. (2007a) reported 35.18 mg/ 100g vitamin C in *Solanum nigrum* leaves harvested in the wild. Although Lyimo et al. (2003) reported a high vitamin C value (234.5 mg/ 100g) in *Solanum nigrum*; this was lower than the maximum value observed in this study. In other wild vegetables, Gupta et al. (2005) reported a higher value (295 mg/ 100g) of vitamin C in *Delonix elata* leaves than in the current study. Akubugwo et al. (2007b) reported that *Amaranthus hybridus* leaves contained 25.40 mg/ 100g vitamin C and this is lower than the results of this current study. Vitamin C is the most important vitamin in fruits and vegetables for human nutrition. In this trial the addition of Nitrogen fertiliser increased the vitamin C content as compared to organic fertiliser alone. Nitrogen is known to increase plant foliage and enhance plant growth, however, higher than recommended rates have been shown to reduce vitamin C content in cauliflower (Lisiewska and Kmiecik, 1996). A report by Klein and Perry (1982) proposed that climatic conditions including light and temperature have a strong influence on the chemical composition of horticultural crops while Lee and Kader (2000), further proposed that plants exposed to more sunlight contain more vitamin C than those that are less exposed to sunlight. This might explain the high variations between the concentrations of vitamin C on the field and in the glasshouse. Results from this study indicate that harvesting *Solanum nigrum* with the intention of harnessing vitamin C would be ideal 9 weeks after transplanting under field conditions and 6 weeks in the glasshouse. This is when the vitamin is at its peak, after which its content declines exponentially especially in the glasshouse.

6.4.5 Protein

The maximum percentage protein value reported in this study is (42.81 %) higher than what was reported by Sarma and Sarma (2011), Akubugwo et al. (2007a) as well as Lyimo et al. (2003). These authors respectively reported 5.2, 24.90 and 1.0 % protein in *Solanum nigrum* leaves gathered from the wild. From the current study, it is clear that cultivated *Solanum nigrum* possesses more favourable protein content compared to that harvested from the wild and other wild vegetables as reported by other authors. For example, Akubugwo et al. (2007b) reported 17.92 % in *Amaranthus hybridus* while Jimoh et al. (2011) reported 13.25 and 7.0 % protein in *Sonchus asper* and *Sonchus oleraceus* respectively. In another study Achinewhu et al. (1995), reported 43.1 % protein in *Cola milenii* and this was higher than the maximum value of the present study. The importance of a protein rich diet especially in children cannot be over emphasised. Protein is essential for growth, development and repair of body tissues. However, proteins are deficient in most people in developing countries and poor communities (Aletor et al., 2000). Given the high protein content of cultivated *Solanum nigrum* as indicated in this current study, the intake of this vegetable could be expected to contribute a large proportion of the mineral requirement in the body. Furthermore, the results of this study indicate that in order to obtain the highest concentration of protein in cultivated *Solanum nigrum*, the plant should be harvested about 3 weeks after transplanting since the protein content gradually declines afterwards.

6.4.6 Phytate

The phytate content of *Solanum nigrum* reported in this work is not at variance with what was reported by Akubugwo et al. (2007a; 2008), who reported 0.82 and 0.52-0.95 mg/ 100g respectively in processed and unprocessed *Solanum nigrum* leaves gathered from the wild, however, the maximum value (6.40 mg/100g) reported in the present study is higher than

these authors' values. Sarma and Sarma (2011) conducted work on the seed of *Solanum nigrum* and found its phytate content low (0.13 mg/ 100g). Work conducted in other wild vegetables also indicated low values but not very different from our findings. Jimoh et al. (2011) reported 5.16 and 5.12 mg/ 100g in *Sonchus asper* and *Sonchus oleraceus* leaves gathered from the wild and this was within the range of our findings. Gupta et al. (2005) reported values ranging between 0.92 and 13.06 mg/ 100g in 14 wild vegetables. Phytic acid is the principal storage form of phosphorus in many plant tissues especially the bran portion of grains and other seeds and as such, the majority of research has been conducted on grain and not on leafy vegetables (Nagel, 2010). Phytate decreases the bioavailability of critical nutrients such as Zn, Fe, Ca and Mg because of its high binding affinities to minerals (Lang et al., 2007). Although phytic acid is known as an antinutrient, some beneficial effects have also been reported such as its antioxidant and anticarcinogenic properties (Jenab and Thompson, 2002). The phytate values reported in this study are below the maximum permissible limit (25 mg/ 100g) for phytate containing foods (Nagel, 2010). In addition, to being low in *Solanum nigrum*, phytate content in vegetables is reduced during the process of cooking (Akwaowa et al., 2000). Since phytic acid is reduced during the process of cooking, it is conceivable that what is consumed by humans in *Solanum nigrum* might not be the amount reported in this work and therefore the risk of mineral deficiencies resulting from a high intake of this acid, such as rickets and osteoporosis are further reduced (Wills et al., 1972). Furthermore, the results of this study reveal that the concentration of phytate in cultivated *Solanum nigrum* is lowest in the early stages and the end of the plant's life cycle and this would be the ideal time to harvest it.

6.5 Conclusion

Results of this study indicate that the field trial performed better than the glasshouse experiment in all the proximate constituents under investigation. More specifically, Ash ranged between 6.77-8.95 % on the field and 7.44-9.45 % in the glasshouse and was consistently high throughout the both trials. Fibre ranged between 9.86-24.80 % on the field and 9.86-22.67 % in the glasshouse. Lipid ranged between 0.98-7.74 % on the field and 0.54-5.52 % in the glasshouse. Vitamin C ranged 30-250 mg/ 100g on the field and levels below the limit of detection and 187 mg/ 100g in the glasshouse. Protein ranged between 24.49-42.63 % on the field and 10.74-39.64 % in the glasshouse. Phytate ranged between 1.17-3.43 mg/ 100g on the field and 1.67-6.40 mg/ 100g in the glasshouse. However, the universal optimum harvesting time and the applicable fertiliser for all the proximate constituents could not be determined as this would depend on the proximate constituent of particular interest. More specifically, 50 kg N/ ha + 4.07 t manure/ ha fertiliser increased both the lipid and protein concentration on the field as well as vitamin C and protein in the glasshouse and therefore, this fertiliser would be ideal for these proximate constituents. Furthermore, there is an indication of high nutritional content of cultivated *Solanum nigrum* as compared to other wild vegetables. Given *Solanum nigrum*'*s* high proximate constituents especially protein and vitamin C, there is little or no doubt that once cultivated, this plant may be used in enhancing the nutritional value of low nitrogen and vitamin C foods such as maize and rice which form the main staple food of the South African diet. The consumption of *Solanum nigrum* could be expected to contribute a large proportion of the nutritional requirements in the body.

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CHAPTER 7

EFFECT OF FERTILISERS ON MINERAL COMPOSITION OF *SOLANUM NIGRUM* CULTIVATED ON THE FIELD AND GLASSHOUSE

CHAPTER SEVEN

Effect of fertilisers on mineral composition of *Solanum nigrum* cultivated on the field and glasshouse

7.1 Introduction

Plants play an important role in the life of man, not only because of their rich nutritional value but also for the treatment and prevention of chronic diseases. Most plant foods are currently based on a limited number of crops and they comprise about 103 plant species which contribute 90% of national per capita supplies of food plants (Prescott-Allen and Prescott-Allen, 1990). Although wild vegetables were an important part of traditional agricultural systems their consumption has over the years declined in South Africa due to their association with poverty and poverty foods, degree of urbanisation, modernisation of agriculture, distance to fresh produce markets and season of the year, among other factors (Flyman and Afolayan, 2007; Jansen van Rensberg et al., 2007). According to Modi et al. (2006), the poor utilisation of wild vegetables may be associated with a lack of knowledge about how to access quantities that can satisfy daily human food requirements. Wild vegetables usually grow as volunteer plants alongside conventional crops in agricultural fields during the planting season and are mostly viewed as weeds that must be removed usually by mechanical or chemical means. Nevertheless, in many parts of the world, including South Africa, the use of wild vegetables is not negligible (Misra et al., 2008). The rich nutritional value of wild vegetables has been documented by many authors (Edmonds and Chweya, 1997; Flyman and Afolayan, 2007; Odhav et al., 2007; Lewu and Mavengahama, 2010). However, some wild vegetable species are already under cultivation in South Africa, for example some *Amaranth species* and *Brassica rapa* in Limpopo and Mpumalanga Provinces (Jansen van Rensberg et al., 2007). However, agronomic data required to cultivate them is scanty. *Solanum nigrum* is one of the popular wild vegetables consumed by the rural populace of the Eastern Cape and usually gathered from the wild for food. To the best of our knowledge, there are no reports of its cultivation in the Eastern Cape. The aim of this study was to examine the mineral concentration of the leaves of *Solanum*

nigrum at different stages of growth when cultivated under different concentrations of fertiliser with a view to determine the best fertiliser option for the cultivation of the wild vegetable in the Eastern Cape in South Africa.

7.2 Materials and methods

The experimental site, agronomic practices and experimental design were as described in Chapter 3.

7.2.1 Data collection

The data were collected as described in chapter 6.

7.2.2 Mineral analysis

The concentration of K, Mg, P, Na, Ca, Cu, Fe, Mn and Zn were determined as described in chapter 3.

7.2.3 Statistical analysis

Data of the nutrient concentrations of various treatments were subjected to statistical analysis using MNITAB Release 12. A one way analysis of variance was used to compare the means of various nutrient concentrations among the treatments and a two way analysis of variance used to determine the interaction between plant age (weeks after transplanting) and treatment on nutrient accumulation in the plant. Means were segregated using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

7.3 Results

7.3.1 Potassium (K)

a) Field

Potassium decreased on the field from the time of transplanting to the final week of observation (Table 7.1a). The treatment means significantly differed ($p < 0.05$) and ranged between 3.88 and 6.98 %. The means for the duration of the trial were highest in T5 (5.25 %) and lowest in T3 (4.97 %), however, the mineral was at its peak in the 4^{th} week in T2 (6.98 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on potassium. Regression analysis with potassium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 63.6 % indicating that plant age had a significant effect on potassium.

g) Glasshouse

In the glasshouse, potassium showed a variable trend from the time of transplanting to the final week of observation but was at its peak in the final week of observation in T5 and ranged between 3.11 and 6.98 % (Table 7.1 b). Treatment means differed significantly throughout the trial and the treatment means for the duration of the trial were highest in T5 (6.20 %) and least in T1 (4.08 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on potassium. Regression analysis with potassium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 83.6 % indicating that plant age had a significantly strong effect on potassium. Comparing the field and glasshouse trials, higher values were recorded in T1 – T3 on the field whereas higher values were recorded in T3 and T4 in the glasshouse.

Table 7.1a: Effect of organic and inorganic fertilisers on K (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	5.65±0.02	5.00±0.05^{a}	5.81±0.01^{a}	5.00±0.05^{a}	4.91±0.01^{a}	5.23±0.03^{a}	4.82±0.02^{a}	4.51±0.01^{a}	4.75±0.05^{a}	4.41±0.02^{a}	4.80±0.02^{a}
T2	5.65±0.02	5.04±0.04^{a}	6.98±0.02^{b}	4.43±0.03^{b}	5.40±0.02^{b}	5.26±0.03^{a}	5.77±0.02^{b}	5.15±0.05^{b}	4.78±0.02^{a}	4.37±0.02^{a}	3.88±0.04^{b}
T3	5.65±0.02	4.81±0.02^{b}	5.69±0.03^{a}	5.28±0.04^{c}	5.03±0.03^{c}	4.57±0.05^{b}	4.71±0.01^{c}	4.77±0.02^{c}	4.47±0.02^{b}	4.72±0.02^{b}	4.99±0.01^{c}
T4	5.65±0.02	5.01±0.01^{a}	5.18±0.06^{c}	5.08±0.04^{a}	5.63±0.03^{d}	5.07±0.02^{c}	5.18±0.06^{d}	5.18±0.06^{b}	5.08±0.08^{c}	4.14±0.07^{c}	5.24±0.02^{d}
T5	5.65±0.02	5.62±0.02^{c}	4.87±0.02^{d}	5.05±0.05^{a}	5.45±0.05^{b}	5.66±0.03^{d}	5.43±0.03^{e}	5.36±0.02^{d}	4.96±0.02^{c}	4.57±0.04^{d}	5.11±0.02^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.1b: Effect of organic and inorganic fertilisers on K (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	5.65±0.02	4.55±0.02^{a}	4.28±0.02^{a}	4.53±0.03^{a}	3.29±0.01^{a}	3.24±0.02^{a}	3.97±0.02^{a}	3.11±0.02^{a}
T2	5.65±0.02	5.55±0.04^{b}	6.32±0.02^{b}	4.64±0.04^{a}	5.57±0.05^{b}	5.45±0.02^{b}	4.50±0.04^{b}	4.74±0.02^{b}
T3	5.65±0.02	5.26±0.03^{b}	4.00±0.02^{a}	4.24±0.04^{b}	4.86±0.03^{c}	4.11±0.02^{c}	4.10±0.03^{a}	3.73±0.03^{c}
T4	5.65±0.02	5.30±0.02^{b}	5.20±0.04^{d}	4.86±0.03^{c}	5.22±0.02^{d}	5.20±0.02^{b}	5.79±0.02^{c}	5.50±0.04^{d}
T5	5.65±0.02	6.93±0.03^{c}	6.10±0.03^{b}	5.97±0.04^{d}	5.87±0.02^{e}	6.58±0.04^{d}	5.54±0.04^{d}	6.98±0.04^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.2 Magnesium (Mg)

a) Field

Magnesium increased from the time of transplanting to the 4th week after which it decreased and increased again in the 10th week and declined between the 11th and 12th weeks and ranged between 0.48 and 0.82 % (Table 7.2a). The least and highest values of magnesium (0.48 and 0.82 %) were respectively recorded in the 12th and 10th weeks. Treatment means were significantly different ($p < 0.05$) throughout the trial and the means for the duration of the trial were highest in T1 (0.72 %) and least in T2 (0.66 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on magnesium. Regression analysis with magnesium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 53.10 % indicating that plant age had a fairly significant effect on magnesium.

b) Glasshouse

In the glasshouse, the concentration of magnesium ranged between 0.37 and 0.93 % and generally decreased from the time of transplanting to the 9th week (Table 7.2b). The treatment means differed significantly throughout the trial and the means for the duration of the trial were highest in T2 (0.69 %) and lowest in T1 (0.49 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on magnesium. Regression analysis with magnesium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 77.6 % indicating that plant age had a significant effect on magnesium.

Table 7.2a: Effect of organic and inorganic fertilisers on Mg (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	0.73±0.03	0.76±0.02	0.74±0.04	0.70±0.02	0.67±0.01^{a}	0.69±0.02	0.72±0.03^{a}	0.67±0.02^{a}	0.82±0.04^{a}	0.73±0.03^{a}	0.69±0.03^{a}
T2	0.73±0.03	0.76±0.03	0.75±0.02	0.64±0.04^{a}	0.63±0.03^{b}	0.68±0.02	0.63±0.02^{b}	0.60±0.05^{b}	0.76±0.03^{b}	0.62±0.02^{b}	0.51±0.02^{b}
T3	0.73±0.03	0.80±0.04	0.74±0.04	0.73±0.03	0.74±0.02^{c}	0.72±0.03	0.58±0.02^{c}	0.65±0.04^{ab}	0.68±0.02^{c}	0.67±0.01^{c}	0.48±0.02^{b}
T4	0.73±0.03	0.75±0.04	0.72±0.02	0.72±0.02	0.68±0.02^{a}	0.67±0.04	0.64±0.04^{b}	0.64±0.02^{ab}	0.69±0.04^{c}	0.67±0.03^{c}	0.54±0.04^{b}
T5	0.73±0.03	0.77±0.02	0.70±0.05	0.71±0.01	0.72±0.03^{c}	0.70±0.04	0.66±0.03^{b}	0.62±0.02^{ab}	0.65±0.02^{c}	0.69±0.04^{c}	0.67±0.01^{a}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.2b: Effect of organic and inorganic fertilisers on Mg (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	0.73±0.03	0.71±0.02[a]	0.54±0.04[a]	0.53±0.01[a]	0.35±0.02[a]	031±0.01[a]	0.38±0.02[a]	0.37±0.02[a]
T2	0.73±0.03	0.81±0.02[b]	0.93±0.03[b]	0.60±0.02[b]	0.64±0.02[b]	0.51±0.02[b]	0.74±0.02[b]	0.59±0.03[b]
T3	0.73±0.03	0.68±0.02[a]	0.50±0.02[a]	0.53±0.03[a]	0.54±0.04[c]	0.41±0.02[c]	0.49±0.04[c]	0.47±0.03[c]
T4	0.73±0.03	0.81±0.01[b]	0.69±0.03[c]	0.59±0.04[b]	0.57±0.01[c]	0.51±0.04[b]	0.52±0.02[c]	0.52±0.02[d]
T5	0.73±0.03	0.79±0.04[b]	0.70±0.02[c]	0.63±0.03[b]	0.57±0.02[c]	0.49±0.04[b]	0.51±0.02[c]	0.47±0.03[c]

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.3 Phosphorus (P)

a) Field

Phosphorus accumulation decreased as *Solanum nigrum* matured and was highest in the 5th week and lowest in the 12th week and ranged between 0.29 and 0.77 % (Table 7.3a). The treatment means differed significantly ($p < 0.05$) throughout the trial and the means for the duration of the trial were highest in T2 (0.62 %) and lowest in T4 (0.56 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on phosphorus. Regression analysis with phosphorus as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 81.3 % indicating that plant age had a very significant effect on phosphorus.

b) Glasshouse

The glasshouse treatments differed significantly and showed a decreasing trend from the time of transplanting to the 9th week and ranged between 0.23 and 0.80 % (Table 7.3b). The means for the duration of the trial were highest in T5 (0.63 %) and lowest in T1 (0.40 %). These were lower than those recorded on the field with the exception of T5. The treatment means ranged between 0.23 and 0.80 % in the 7th and 3rd weeks respectively while analysis of variance showed that there was a significant interaction between the age of the plant and the treatment. Statistical analysis showed an interaction between plant age and the fertiliser treatment on phosphorus. Regression analysis with phosphorus as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 92.6 % indicating that plant age had a very significant effect on phosphorus.

Table 7.3a: Effect of organic and inorganic fertilisers on P (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	0.77±0.02	0.68±0.04^{a}	0.62±0.02^{a}	0.74±0.02^{ab}	0.62±0.02^{a}	0.63±0.01^{a}	0.61±0.01^{a}	0.56±0.05	0.58±0.02^{a}	0.51±0.02^{a}	0.33±0.03
T2	0.77±0.02	0.69±0.03^{a}	0.61±0.01^{a}	0.75±0.04^{a}	0.71±0.02^{b}	0.72±0.04^{b}	0.64±0.04^{a}	0.57±0.01	0.43±0.03^{b}	0.47±0.02^{b}	0.50±0.02^{a}
T3	0.77±0.02	0.62±0.02^{b}	0.58±0.02^{b}	0.71±0.01^{b}	0.64±0.02^{a}	0.59±0.01^{c}	0.57±0.02^{b}	0.55±0.04	0.56±0.03^{a}	0.39±0.01^{c}	0.32±0.02
T4	0.77±0.02	0.64±0.04^{ab}	0.61±0.03^{ab}	0.74±0.01^{ab}	0.71±0.03^{b}	0.64±0.01^{a}	0.53±0.03^{b}	0.51±0.01	0.37±0.02^{c}	0.32±0.02^{d}	0.29±0.03
T5	0.77±0.02	0.69±0.03^{a}	0.68±0.03^{c}	0.76±0.03^{a}	0.72±0.05^{b}	0.65±0.05^{a}	0.58±0.02^{ab}	0.55±0.05	0.39±0.02^{bc}	0.42±0.04^{c}	0.31±0.03

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.3b: Effect of organic and inorganic fertilisers on P (%) of *Solanum nigrum* L. cultivated in the glasshouse Sodium

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	0.77±0.02	0.60±0.02^{a}	0.44±0.02^{a}	0.39±0.04^{a}	0.27±0.04^{a}	0.23±0.03^{a}	0.28±0.02^{a}	0.24±0.02^{a}
T2	0.77±0.02	0.66±0.03^{b}	0.66±0.03^{b}	0.49±0.04^{b}	0.46±0.03^{b}	0.38±0.02^{b}	0.34±0.02^{b}	0.41±0.02^{b}
T3	0.77±0.02	0.62±0.02^{ab}	0.48±0.04^{a}	0.53±0.03^{b}	0.50±0.02^{b}	0.44±0.04^{bc}	0.43±0.03^{c}	0.48±0.02^{b}
T4	0.77±0.02	0.55±0.04^{c}	0.54±0.02^{c}	0.49±0.04^{b}	0.37±0.02^{c}	0.34±0.04^{bc}	0.38±0.02^{b}	0.30±0.05^{d}
T5	0.77±0.02	0.80±0.02^{d}	0.70±0.04^{d}	0.66±0.03^{c}	0.58±0.02^{d}	0.57±0.02^{d}	0.35±0.04^{b}	0.61±0.02^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.4 Sodium (Na)

a) Field

The concentration of sodium in *Solanum nigrum* cultivated on the field exponentially decreased between the time of transplanting and the 3rd week and varied but was lowest in the 12th week but ranged between 187 and 1535 mg/kg (Table 7.4a). The treatment means differed significantly ($p < 0.05$) and ranged between 187 and 1534.5 mg/kg in the 12th week and time of transplanting respectively. Means for the duration of the trial were highest in T5 (721.7 mg/kg) and least in T4 (635.3 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on sodium. Regression analysis with sodium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 68.8 % indicating that plant age had a significant effect on sodium.

b) Glasshouse

In the glasshouse, sodium generally decreased and the highest value was recorded at the time of transplanting and the lowest in the 9th week but ranged between 749 and 1535 mg/kg (Table 7.4b). The means for the duration of the trial were highest in T4 (1287.2 mg/kg) and lowest in T3 (1071.2 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on sodium. Regression analysis with sodium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 34.6 % indicating that plant age had a minimal effect on sodium. The glasshouse treatment showed an increase between 51 and 66 %.

Table 7.4a: Effect of organic and inorganic fertilisers on Na (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	1535±0.36	596±0.36^{a}	700±0.22^{a}	966±0.45^{a}	510±4.47^{a}	726±0.63^{a}	607±0.89^{a}	331±0.36^{a}	684±0.18^{a}	686±0.18^{a}	187±0.18^{a}
T2	1535±0.36	760±0.18^{b}	640±0.18^{b}	427±0.18^{b}	680±0.45^{b}	621±0.89^{b}	706±0.94^{b}	440±0.18^{b}	757±0.27^{b}	561±0.90^{b}	224±0.18^{b}
T3	1535±0.36	724±0.09^{c}	674±0.27^{c}	947±0.09^{a}	538±0.09^{c}	446±0.18^{c}	422±0.45^{c}	489±0.36^{c}	672±0.27^{a}	447±0.18^{c}	231±0.18^{b}
T4	1535±0.36	670±0.05^{d}	677±0.18^{cd}	530±0.07^{c}	602±0.18^{d}	559±0.36^{d}	544±0.45^{d}	694±0.89^{d}	513±0.90^{c}	387±0.18^{d}	279±0.36^{c}
T5	1535±0.36	888±0.18^{e}	680±0.27^{d}	864±0.27^{d}	638±0.23^{e}	812±0.90^{e}	557±0.54^{d}	582±0.18^{e}	589±0.27^{d}	503±0.18^{e}	293±0.27^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.4b: Effect of organic and inorganic fertilisers on Na (mg/kg) of *Solanum nigrum* L. cultivated in the glasshouse Sodium

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	1535±0.36	1164±0.31^{a}	1049±8.94^{a}	1113±0.09^{a}	828±0.23^{a}	1068±0.36^{a}	1075±0.45^{ac}	1137±0.31^{a}
T2	1535±0.36	1345±0.81^{b}	1248±0.45^{b}	968±0.18^{b}	1149±0.18^{b}	1006±0.36^{b}	2400±0.09^{b}	1003±0.27^{b}
T3	1535±0.36	1034±0.89^{c}	901±0.49^{c}	848±0.18^{c}	1371±0.23^{c}	778±0.18^{bc}	1041±0.18^{a}	1059±0.36^{c}
T4	1535±0.36	1457±0.18^{d}	1302±0.18^{d}	1001±0.18^{d}	1838±0.18^{d}	1327±0.18^{d}	1176±0.18^{c}	1059±0.36^{c}
T5	1535±0.36	1340±0.27^{b}	1023±0.09^{a}	1115±0.18^{a}	1030±0.27^{e}	974±0.18^{b}	1120±0.45^{c}	749±0.18^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.5 Calcium (Ca)

a) Field

On the field, calcium increased exponentially between the time of transplanting and the 3rd week and remained constant until the 11th week and exponentially increased again in the 12th week (Table 7.5a). The means of the treatments differed significantly ($p < 0.05$) and ranged between 1.39 and 3.98 % at the time of transplanting and 12th week respectively. The means for the duration of the trial were highest in T1 (2.54 %) and lowest in T2 (2.37 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on calcium. Regression analysis with calcium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 58.2 % indicating that plant age had a fairly significant effect on calcium.

b) Glasshouse

The glasshouse means ranged between 1.23 and 3.72 % in the 4th and 9th weeks respectively (Table 7.5b). The means for the duration of the trial were lower than those from the field and were highest in T2 (2.02 %) and lowest in T5 (1.57 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on calcium. Regression analysis with calcium as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 12.7 % indicating that plant age had a minimum effect on calcium.

Table 7.5a: Effect of organic and inorganic fertilisers on Ca (%) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	1.39±0.02	2.57±0.02[a]	2.27±0.02[a]	2.25±0.04[a]	2.61±0.01[a]	2.92±0.02[a]	2.78±0.02[a]	2.81±0.01[a]	2.53±0.03[a]	2.40±0.02[a]	3.45±0.04[a]
T2	1.39±0.02	2.43±0.02[b]	2.15±0.02[b]	2.18±0.02[b]	2.18±0.02[b]	2.54±0.04[b]	2.41±0.01[b]	2.19±0.01[b]	2.62±0.02[b]	2.03±0.03[b]	3.98±0.02[b]
T3	1.39±0.02	2.62±0.02[c]	2.27±0.02[a]	2.15±0.04[b]	2.84±0.02[c]	2.83±0.03[c]	2.49±0.02[c]	2.52±0.02[c]	2.94±0.04[c]	2.45±0.02[c]	2.92±0.04[c]
T4	1.39±0.02	2.49±0.01[d]	2.36±0.04[c]	2.60±0.02[c]	2.65±0.04[a]	2.94±0.02[d]	2.23±0.03[d]	2.94±0.02[d]	2.49±0.02[d]	2.81±0.01[d]	2.90±0.02[c]
T5	1.39±0.02	2.44±0.02[b]	2.12±0.05[b]	2.41±0.01[d]	2.73±0.01[d]	2.77±0.01[e]	2.89±0.01[e]	2.36±0.02[e]	2.42±0.02[e]	2.26±0.02[e]	3.09±0.06[d]

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.5b: Effect of organic and inorganic fertilisers on Ca (%) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	1.39±0.02	1.83±0.03^{a}	1.39±0.01^{a}	1.31±0.01^{a}	1.24±0.02^{a}	1.59±0.04^{a}	2.01±0.01^{a}	3.72±0.02^{a}
T2	1.39±0.02	1.95±0.04^{b}	2.14±0.02^{b}	1.39±0.02^{b}	1.77±0.02^{b}	1.84±0.02^{b}	3.11±0.01^{b}	2.59±0.02^{b}
T3	1.39±0.02	1.70±0.02^{c}	1.23±0.03^{c}	1.32±0.02^{a}	1.65±0.04^{c}	1.81±0.01^{b}	2.17±0.01^{c}	3.30±0.02^{c}
T4	1.39±0.02	1.89±0.02^{d}	1.63±0.03^{d}	1.49±0.02^{c}	1.68±0.02^{c}	2.04±0.04^{c}	2.17±0.02^{c}	2.38±0.02^{b}
T5	1.39±0.02	1.66±0.02^{c}	1.63±0.12^{d}	1.39±0.02^{b}	1.48±0.04^{d}	1.54±0.02^{d}	1.92±0.02^{a}	1.57±0.01^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.6 Copper (Cu)

a) Field

Copper exponentially increased between the time of transplanting and the 3rd week on the field, after which it varied but was highest in the 11th week in T3 (Table 7.6a). Means of the treatments significantly differed ($p < 0.05$) and ranged between 8.10 and 23.50 mg/kg. The means for the trial were highest in T1 (16.2 mg/kg) and lowest in T4 (15.5 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on copper. Regression analysis with copper as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 25.7 % indicating that plant age had a minimum effect on copper.

b) Glasshouse

In the glasshouse, copper exponentially increased between the time of transplanting and the 3rd week after which it decreased (Table 7.6b). Treatment means significantly differed and ranged between 5.30 and 21.40 mg/kg. The means for the trial were highest in T3 (13.6 mg/kg) and least in T1 (9.50 %). Statistical analysis showed an interaction between plant age and the fertiliser treatment on copper. Regression analysis with copper as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 12.7 % indicating that plant age had a minimum effect on copper.

Table 7.6a: Effect of organic and inorganic fertilisers on Cu (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	7.20±0.18	17.20±0.18^{a}	15.70±0.18^{a}	18.20±0.18^{a}	16.90±0.36	12.40±0.18^{a}	16.80±0.36^{a}	19.80±0.18^{a}	17.40±0.18^{a}	18.70±0.18^{a}	18.00±0.18^{a}
T2	7.20±0.18	16.60±0.18^{b}	16.10±0.09^{b}	17.80±0.18^{b}	14.50±0.18^{a}	14.30±0.27^{b}	12.70±0.18^{b}	17.40±0.36^{b}	19.50±0.18^{b}	16.40±0.18^{b}	21.50±0.18^{b}
T3	7.20±0.18	16.70±0.09^{b}	21.60±0.27^{c}	15.60±0.27^{c}	16.60±.027	16.60±0.27^{c}	12.70±0.18^{b}	15.60±0.27^{c}	20.60±0.27^{c}	23.50±0.18^{c}	8.10±0.18^{c}
T4	7.20±0.18	18.80±0.36^{c}	17.90±0.09^{d}	17.10±0.09^{d}	16.90±0.09	15.40±0.18^{d}	14.40±0.18^{c}	19.00±0.09^{d}	17.70±0.18^{a}	15.00±0.09^{d}	10.93±0.23^{d}
T5	7.20±0.18	17.00±0.18^{ab}	17.60±0.27^{d}	16.10±0.09^{e}	16.80±0.18	15.00±0.09^{e}	16.50±0.45^{a}	17.70±0.27^{e}	19.50±0.09^{b}	15.60±0.27^{e}	14.10±0.09^{e}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.6b: Effect of organic and inorganic fertilisers on Cu (mg/kg) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	7.20±0.18	17.60±0.27^{a}	13.70±0.18^{a}	12.80±0.18^{a}	6.90±0.09^{a}	5.80±0.18^{a}	6.10±0.18^{a}	5.60±0.27^{a}
T2	7.20±0.18	16.20±0.18^{b}	17.90±0.18^{b}	12.50±0.18^{a}	9.00±0.18^{b}	5.50±0.18^{a}	6.20±0.18^{a}	5.50±0.18^{a}
T3	7.20±0.18	20.40±0.36^{c}	15.10±0.09^{c}	15.50±0.18^{b}	15.40±0.18^{c}	12.70±0.18^{b}	10.90±0.09^{b}	11.50±0.27^{b}
T4	7.20±0.18	18.20±0.18^{d}	14.00±0.18^{a}	11.50±0.45^{c}	7.60±0.18^{a}	6.20±0.18^{a}	7.00±0.18^{c}	5.30±0.27^{a}
T5	7.20±0.18	19.70±0.18^{e}	21.40±0.18^{d}	17.00±0.27^{d}	13.10±0.09^{d}	11.30±0.27^{c}	7.30±0.18^{c}	9.20±0.18^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.7 Iron (Fe)

a) Field

Iron differed significantly ($p < 0.05$) and ranged between 213 and 766 mg/kg at the time of transplanting and the 5th week respectively (Table 7.7a). The means for the trial were highest in T3 (528 mg/kg) and lowest in T5 (440 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on iron. Regression analysis with iron as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 37.7 % indicating that plant age had a minimum effect on iron.

b) **Glasshouse**

Results of the glasshouse experiment were lower than those of the field and ranged between 178 and 523 mg/kg (Table 7.7b). The means for the duration of the trial were highest in T1 (526 mg/kg) and lowest in T5 (239 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on iron. Regression analysis with iron as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 0.9 % indicating that plant age had a very minimum effect on iron.

Table 7.7a: Effect of organic and inorganic fertilisers on Fe (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	213±2.68	426±2.68^{a}	348±3.58^{a}	591±0.89^{a}	562±1.79^{a}	475±4.47^{a}	439±2.25^{a}	629±1.79^{a}	408±3.58^{a}	570±1.79^{a}	435±4.47^{a}
T2	213±2.68	624±3.58^{b}	246±1.79^{b}	766±2.68^{b}	403±2.68^{b}	480±1.79^{a}	374±1.79^{b}	521±0.89^{b}	483±2.68^{b}	540±1.79^{b}	750±1.79^{b}
T3	213±2.68	514±1.79^{c}	300±1.79^{c}	587±1.79^{a}	678±1.79^{c}	669±3.58^{b}	548±1.79^{c}	556±2.68^{c}	667±1.79^{c}	677±1.79^{c}	403±2.68^{a}
T4	213±2.68	371±0.89^{d}	406±2.68^{d}	617±1.79^{c}	395±4.47^{b}	575±4.47^{c}	671±0.89^{d}	396±2.68^{d}	567±1.79^{d}	578±1.79^{a}	375±4.47^{c}
T5	213±2.68	305±4.47^{e}	499±1.37^{e}	655±4.47^{d}	366±2.68^{d}	499±0.89^{d}	414±1.79^{e}	501±0.89^{b}	466±2.68^{b}	567±1.37^{a}	356±3.58^{c}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.7b: Effect of organic and inorganic fertilisers on Fe (mg/kg) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	213±2.68	430±1.79^{a}	299±4.03^{a}	337±6.26^{a}	246±1.79^{a}	325±4.47^{a}	327±1.79^{a}	433±2.68^{a}
T2	213±2.68	320±1.79^{b}	323±2.68^{b}	212±5.37^{b}	255±4.47^{b}	254±1.79^{b}	302±1.79^{b}	256±2.68^{b}
T3	213±2.68	447±1.79^{a}	340±1.79^{b}	265±4.47^{c}	296±2.68^{c}	363±2.68^{c}	269±4.03^{c}	304±3.58^{c}
T4	213±2.68	260±1.79^{c}	523±2.68^{c}	178±3.58^{d}	195±4.47^{d}	239±1.79^{d}	224±1.79^{d}	258±7.16^{b}
T5	213±2.68	217±1.79^{d}	432±1.79^{d}	191±0.89^{d}	179±3.14^{e}	230±3.58^{d}	209±8.05^{e}	239±0.89^{b}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.8 Manganese (Mn)

a) Field

Manganese concentration increased as the plant the plant matured and ranged between 85 and 222 mg/kg (Table 7.8a). The means for the trial were highest in T2 (123 mg/kg) and lowest in T1 (104 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on manganese. Regression analysis with manganese as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 71.1 % indicating that plant age had a significant effect on manganese.

b) Glasshouse

In the glasshouse trial means also differed significantly ($p < 0.05$) among the treatments and ranged between 64 and 215 mg/kg (Table 7.8b). The means for the trial were highest in T5 (144 mg/kg) and lowest in T1 (83 mg/kg) and this was lower than what was reported on field trial except for T5 which was higher in the glasshouse. Statistical analysis showed an interaction between plant age and the fertiliser treatment on manganese. Regression analysis with manganese as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 65.8 % indicating that plant age had a significant effect on manganese.

Table 7.8a: Effect of organic and inorganic fertilisers on Mn (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	88.67±3.14	101±0.89^{a}	89±3.14^{a}	90±3.58^{a}	95±2.68^{a}	108±7.16^{a}	102±1.79^{a}	85±4.47^{a}	110±4.47^{a}	137±2.68^{a}	143±2.68^{a}
T2	88.67±3.14	116±4.47^{b}	87±3.58^{a}	121±2.68^{b}	110±2.68^{b}	139±4.03^{b}	153±2.68^{b}	114±6.26^{b}	156±2.68^{b}	129±4.03^{b}	134±1.79^{b}
T3	88.67±3.14	109±4.93^{c}	86±2.68^{a}	90±4.47^{a}	95±1.79^{a}	124±1.79^{c}	89±4.03^{c}	86±2.68^{a}	114±6.26^{a}	132±1.79^{b}	222±0.89^{c}
T4	88.67±3.14	103±1.37^{d}	104±3.58^{b}	104±0.52^{c}	101±0.89^{c}	126±2.68^{c}	132±1.79^{d}	127±6.26^{c}	159±4.03^{b}	121±0.89^{c}	154±3.58^{d}
T5	88.67±3.14	106±1.79^{cd}	112±5.37^{c}	108±2.68^{c}	92±1.79^{a}	131±0.89^{d}	136±5.37^{d}	111±1.79^{b}	127±1.79^{c}	149±4.03^{d}	154±2.68^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.8b: Effect of organic and inorganic fertilisers on Mn (mg/kg) of *Solanum nigrum* L. cultivated in the glasshouse

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	89±3.14	88±3.58b^{c}	78±3.58^{a}	73±0.89^{a}	64±1.79^{a}	79±4.03^{a}	79±4.47^{a}	114±6.26^{a}
T2	89±3.14	83±2.68^{c}	108±3.58^{b}	88±1.79^{b}	103±2.25^{b}	101±0.89^{b}	128±3.58^{bc}	148±1.79^{b}
T3	89±3.14	94±1.79^{b}	79±4.03^{a}	90±4.47^{b}	118±2.68^{c}	125±1.79^{c}	125±4.47^{c}	167±6.26^{c}
T4	89±3.14	86±2.68^{bc}	98±3.58^{c}	78±3.58^{a}	90±4.47^{d}	102±1.79^{b}	138±5.37^{b}	147±1.79^{b}
T5	89±3.14	117±13.42^{a}	153±2.68^{d}	119±4.47^{c}	160±4.47^{e}	164±3.58^{d}	134±3.58^{b}	215±1.79^{d}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

7.3.9 Zinc (Zn)

a) Field

Zinc exponentially increased on the field between the time of transplanting and decreased until week 12 (Table 7.9a). The means of the treatments significantly differed ($p < 0.05$) and ranged between 33 and 78 mg/kg. The means for the trial were highest in T5 (62 mg/kg) and least in T1 (59 mg/kg). Statistical analysis showed an interaction between plant age and the fertiliser treatment on zinc. Regression analysis with zinc as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 59.4 % indicating that plant age had a fairly significant effect on zinc.

b) Glasshouse

In the glasshouse, zinc also increased between the time of transplanting and the 4^{th} week after which it decreased (Table 7.9b). The treatment means significantly differed and ranged between 16 and 74 mg/kg. The means for the trial were highest in T2 (49 mg/kg) and least in T5 (37 mg/kg) and these values were lower than those reported on the field. Statistical analysis showed an interaction between plant age and the fertiliser treatment on zinc. Regression analysis with zinc as the dependant variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 81.7 % indicating that plant age had a very significant effect on zinc.

Table 7.9a: Effect of organic and inorganic fertilisers on Zn (mg/kg) of *Solanum nigrum* L. cultivated in the field

	Plant age (Weeks after transplanting)										
	0	3	4	5	6	7	8	9	10	11	12
T1	60±3.00	78±1.67	68±8.37	66±5.86	62±2.51^{a}	67±3.35	60±4.67^{ab}	56±3.45	43±3.35^{a}	39±4.18^{ab}	42±6.69
T2	60±3.00	76±1.53	72±4.00	66±2.00	66±3.00^{ab}	69±10.00	59±4.00^{ab}	53±5.00	59±4.00^{b}	37±1.00^{a}	43±3.00
T3	60±3.00	75±5.00	74±2.00	64±4.00	71±1.53^{b}	59±4.00	56±3.00^{ab}	56±2.00	47±2.00^{a}	47±2.00^{b}	33±3.00
T4	60±3.00	78±4.00	71±2.52	65±5.00	70±5.00^{b}	70±6.00	52±4.00^{b}	53±3.00	49±9.00^{ab}	45±6.00^{ab}	37±2.00
T5	60±3.00	77±2.00	73±3.00	67±6.51	73±3.00^{b}	67±5.00	63±3.00^{a}	61±2.00	57±4.00^{b}	41±3.00^{ab}	39±3.51

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.9b: Effect of organic and inorganic fertilisers on Zn (mg/kg) of *Solanum nigrum* L. cultivated in the glasshouse Sodium

	Plant age (Weeks after transplanting)							
	0	3	4	5	6	7	8	9
T1	60±2.68	59±7.15^{a}	48±3.58^{a}	46±5.37	26±4.93^{a}	16±5.37^{a}	23±4.00^{a}	19±2.68^{a}
T2	60±2.68	72±1.78^{b}	74±3.58^{b}	46±2.68	46±2.68^{c}	32±3.58^{a}	32±4.00^{ab}	33±2.68^{b}
T3	60±2.68	59±8.05^{a}	38±3.58^{c}	37±3.58^{a}	34±3.58^{b}	21±1.79^{b}	23±3.00^{a}	24±3.58^{c}
T4	60±2.68	68±0.52^{b}	53±2.68^{a}	50±4.47	41±3.14^{bc}	33±2.68^{b}	34±4.00^{b}	34±3.58^{b}
T5	60±2.68	63±2.68^{ab}	60±4.47^{d}	46±2.25	39±4.03^{b}	29±3.57^{b}	32±3.51^{b}	31±1.79^{b}

0 indicates readings taken at the time of transplanting
Values shown are mean ± S.D.
Means with different letters down the same column represent significant differences at $p < 0.05$.

Table 7.10: □ Recommended daily mineral intakes by life stage and gender (mg/day)

	Boys	Girls (9-18 yr)	Women	Men
K	3000-3600	2500-2600	2800	3800
Mg	240-410	240-360	310-320	400-420
P	1250	1250	1000	1000
Na	400-920	400-920	460-920	460-920
Ca	1000-1300	1000-1300	1000-1300	1000
Cu	1.3-1.5	1.1	1.2	1.7
Fe	8-11	8-15	8-18	8
Mn	3-3.5	2.5-30	5	5.5
Zn	6-13	6-7	8	14

□ Nutrient reference values for New Zealand and Australia (NHMRC, 2005)

7.4 Discussion

7.4.1 Potassium (K)

The results of this study are comparable with those of Flyman and Afolayan (2008). In their report, potassium concentration decreased with maturity in *Vigna unguiculata* while in *Momordica balsamina*, it increased up to the 3rd week and started to decrease in a glasshouse experiment. The values obtained by these authors are however lower than was obtained in this work. Because of the decrease in potassium content with plant maturity on the field, it would be ideal to harvest *Solanum nigrum* in its early stages of growth and 50 kg N/ ha + 4.07 t manure/ ha would be the most favourable fertiliser if the mineral of interest is potassium. However, the results of this study indicate that there is more than sufficient potassium in cultivated *Solanum nigrum* to supply human daily requirements according to the New Zealand and Australian standards (Table 7.10). It was also observed that harvesting the

leaves of the plant at any age for consumption will potentially ensure an adequate supply of the mineral to the body per day.

7.4.2 Magnesium (Mg)

Results of this study show appreciable amounts of magnesium in both the field and glasshouse treatments. This agrees with the work of Flyman and Afolayan (2008). They reported a range of 0.38 and 0.90 % in *Vigna unguiculata* and 0.35 and 1.45 (%) in *Momordica. balsamina* leaves cultivated in the glasshouse. Also in India, Khader and Rama (2003) reported very high values of magnesium between 22.33 and 80.33 % in 6 wild vegetables in trails conducted on the field. High accumulation of magnesium in the leaves of *Solanum nigrum* at all stages of growth have the potential to supply human diet with recommended daily intake values according to the New Zealand and Australian standards (Table 7.10).

7.4.3 Phosphorus (P)

Concentrations of phosphorus in the leaves of *Solanum nigrum* observed in this study are similar to those reported by Flyman and Afolayan (2008). They reported a range between 0.19 and 0.51 % in *Vigna unguiculata* and 0.15 and 0.53 (%) in *Momordica balsamina* leaves cultivated in the glasshouse while is this study, the range was between 0.23 and 0.80 %. The findings in this study are also in agreement with a field trial conducted by Khader and Rama (2003) who reported a decrease in phosphorus concentration in some wild vegetables with advancing age. Although total amount of phosphorus in the soil may be high, it is often immobile and up to 80 % is available in organic form and this is not readily available to the plant for uptake (Schachtman et al., 1998). This may be responsible for its low concentrations

in *Solanum nigrum*. In addition, phosphorus is known to be most abundant in actively growing tissues of young plants and this possibly explains the high concentration of the element in the first stages of growth. The results of this study indicate that harvesting the plant at its early growth stages would be suitable for phosphorus requirements, however, these results further show that cultivated *Solanum nigrum* has the potential to supply the daily recommended concentrations of phosphorus at all stages of its growth according to the New Zealand and Australian standards for mineral intake (Table 7.10).

7.4.4 Sodium (Na)

The trend for the accumulation of sodium in *Solanum nigrum* leaves in the present trial is in contrast with the trend reported for *Vigna unguiculata* leaves cultivated in the glasshouse (Flyman and Afolayan, 2008). These authors reported that sodium concentration in *Vigna unguiculata* increased up to 4 weeks after transplanting, after which it started to decrease. The current results from the field study were also lower than field results reported by Mahala et al. (2012). These authors observed an increase in sodium concentration with plant maturity and this is at variance with the current findings. It has been observed that the extent to which sodium is taken up by plants varies with species and is influenced by other minerals present in the soil especially potassium and nitrogen. Due to the chemical similarity between potassium and sodium, competition for common absorption sites in the roots may lead most plant species to readily absorb potassium instead of sodium though the concentration of sodium may be high in the soil (Smith et al., 1980). Observation in this study showed that *Solanum nigrum* readily absorbs potassium rather than sodium. The K/Na ratio in the glasshouse ranged between 43.91 and 59.62 while in the field it was between 72.74 and 80.75, indicating the natrophobic nature of the plant. In human physiology, sodium is the principal electrolyte and plays an important role in maintaining the ionic balance of body

tissues and fluids, its osmotic characteristics are utilised in the bloodstream for regulating osmotic pressure within the cells and body fluids where it protects against excessive water loss (Harrison, 1991). The findings of this work indicate that sodium accumulation in the leaves of *Solanum nigrum* may not be sufficient to take care of recommended daily intake of sodium according to the New Zealand and Australian standards for mineral intake (Table 7.10). However, harvesting the leaves during the early stages of growth may be ideal for sodium requirements, because sodium content is at its peak during this time. Better still, table salt can serve as alternative source.

7.4.5 Calcium (Ca)

The results of calcium concentration are in line with the field observation of Khader and Rama (2003). However, it was at variance with what was reported by Flyman and Afolayan (2008) as well as Mahala et al. (2012) in the glasshouse and field respectively. Calcium increased from the early stages to the final stages of growth in the present study. This might be due to the immobile nature of the mineral and its failure to translocate from older parts of the plant to the growing and flowering parts as is the tendency of minerals in plants (Loneragen, 1968). The accumulation of calcium in more mature plants than the younger plants was also reported by Loneragen and Snowball (1969). The results of this study showed that the leaves of *Solanum nigrum* from both the field and glasshouse studies accumulate sufficient calcium that could supplement human diet according to the New Zealand and Australian standards (Table 7.10). This study further reveals that the best time to harvest *Solanum nigrum* leaves for calcium requirements is in the final stages of the plant's growth life cycle. Comparing values reported by Odhav et al. (2007) for wild uncultivated *Solanum nigrum* and the values reported in this present trial, the cultivated *Solanum nigrum* conclusively possesses higher nutritional concentration than the wild uncultivated species.

7.4.6 Copper (Cu)

The field experiment showed higher concentrations of copper than the glasshouse experiment. According to Shorrocks and Alloway (1988), the availability of copper for uptake by plants is determined by soil pH and organic matter content. The higher concentration of copper on the field may be due to high organic matter concentrations as compared to the glasshouse. The observation here is slightly different from what was reported on the field by both Morillo et al. (1997) and Atta et al. (2010). The results of this study indicate that *Solanum nigrum* has the potential to provide more copper than the needed daily recommended values according to New Zealand and Australian standards (Table 7.10) at any growth phase of the plant. However, under field conditions, *Solanum nigrum* may best be harvested in the final stages of growth while under glasshouse conditions, the early growth stages would be ideal.

7.4.7 Iron (Fe)

Iron concentration was observed to be variable on the field but slightly decreased as the plant aged in the glasshouse experiment. The observation here is different from the field report of Morillo et al. (1997). In the same vein, Flyman and Afolayan (2008) observed variations in iron concentration in *Momordica balsamina* and *Vigna unguiculata* cultivated in the glasshouse. According to Graham and Stangoulis (2003), solubility of iron is very low particularly in the presence of moderate oxygen and high soil pH. The high organic matter content on the field and its continuos release of minerals to the soil may have contributed to the differences reported in comparison with the glasshouse in the current study. Notwithstanding the variations in concentration between the field and the glasshouse, the concentration of iron in *Solanum nigrum* remained more than sufficient to supply the required human daily average intake across all age groups and gender according to the New Zealand

and Australian standards (Table 7.10). However, harvesting the plant for maximum iron content would be best during the middle stages of the plant's growth cycle when iron will be at its peak. Furthermore, the range reported in this study is higher than what Odhav et al. (2007) reported in wild uncultivated *Solanum nigrum* leaves gathered directly from the wild. Cultivated *Solanum nigrum* therefore has the potential to supplement a starch diet in poor rural communities at all stages of growth in view of the fact that iron is one of the most prevalent forms of micronutrient malnutrition in the world (FAO, 2004).

7.4.8 Manganese (Mn)

Statistical analysis shows that there is a relationship between the age of the plant and manganese concentration. As the plant matured in age, the concentration of manganese also increased. This observation is in line with the glasshouse report of Flyman and Afolayan (2008) and field report of Atta et al. (2010) but at variance with the field report of Morillo et al. (1997). The continuous increase of manganese in *Solanum nigrum* leaves in the present study is an indication of the continuous release of the mineral from the soil for uptake by the plant. According to McGrath et al. (1994), during the summer season, the relatively high decomposition rate of organic matter releases manganese in the soil solution for possible uptake by plants and this is a possible reason why concentrations were higher on the field than in the glasshouse. Once taken up into plant tissue, manganese becomes immobile and this is possibly why the mineral continued to increase in the leaves (McGrath et al., 1994). Instead of being reassigned to the reproductive parts of the plant, the element remained immobile in the leaves of *Solanum nigrum*. The results of this work indicate the ability of *Solanum nigrum* to provide manganese needed to supply the required human daily intake according to New Zealand and Australian standards (Table 7.10) at all stages of the plant's

growth. However, results of this study indicate that in order to harness the maximum amount of the mineral, leaves of mature plants should be harvested.

7.4.9 Zinc (Zn)

Zinc was found to decrease as the plant matured. This observation is in line with the glasshouse report by Flyman and Afolayan (2008). However, Atta et al. (2010) reported up and down variations in zinc concentration in *Hibiscus sabdariffa* as it grew older while Morillo et al. (1997) observed no change in zinc concentration in *Andropogon gayanus* both from field trials. According to Kabata-Pendias (2001), about 75 % of total zinc taken up by plants is stored in the shoots of young plants whereas about 20-30 % occurs in the shoots of old plants. This phenomenon is a possible explanation to the decline in zinc concentration with advancing plant maturity in the present study. Furthermore, Kabata-Pendias (2001) proposed that the roots often contain more zinc than the shoots. The minimum value of zinc reported in the present study (19 mg/kg in the glasshouse) can potentially supply only 51.8% of the daily recommended human intake values of the mineral in pregnant women according to the New Zealand and Australian standards (Table 7.10), however, the maximum value reported (78 mg/kg in the field) can potentially supply 213 % of the recommended daily human intake in women if they consume 300 g of the vegetable per day. Although the values reported in this trial declined with increasing plant maturity, the concentrations have the potential to supply the daily recommended human intake of the mineral across all age groups and gender up to the 12^{th} week on the field while in the glasshouse, the supply is adequate across all age groups and gender up to the 5^{th} week when it begins to vary. The optimum time for harvesting *Solanum nigrum* for zinc would therefore be during early stages of its growth.

7.5 Conclusion

On the field, the coefficients of determination decreased in the order (%): P (81.3) > Mn (71.1) > Na (68.8) > K (63.6) > Zn (59.4) > Ca (58.2) > Mg (53.1) > Fe (37.7) > Cu (25.7), indicating that plant maturity on the field significantly affected the uptake of P while the least effect was on Cu. Throughout the trial, 50 kg N/ ha + 4.07 T manure/ ha increased, compared to other treatments, the uptake of Zn, K and Na; while the control increased the uptake of Mg, Ca and Cu. Also, 8.13 t /ha increased the uptake of Fe. Zinc (78 mg/kg), K (6.98%) and Na (1535 mg/ kg) were at their maximum in the $12^{th}$and 4^{th} weeks and at the time of transplanting respectively but Mg (0.82%), Ca (3.98%), Cu (21.60 mg/kg) were at their maximum in the 10^{th}, 12^{th} and 11^{th} weeks of transplanting respectively. Also, P (0.77%) was at its maximum at the time of transplanting while Mn (159 mg/kg) and Fe (766 mg/kg) were at their maximum concentration in the 10^{th} and 5^{th} weeks respectively.

In the glasshouse, the coefficients of determination decreased in the order (%): P (92.6) > K (83.6) > Zn (81.7) > Mg (72.6) > Mn (65.8) > Na (34.6) > Ca (12.7) > Cu (12.7) > Fe (0.9). Comparing with other treatments for the duration of the trial, 50 kg N/ ha + 4.07 t manure/ ha increased the uptake of K, P and Mn, while 100 kg N/ha increased the uptake of Mg, Ca and Zn. Also, 8.13 T /ha increased the uptake of Cu, the control of Fe and 100 kg N/ha + 8.13 t manure /ha the uptake of Na. In the 9^{th} of observation, K (6.98%), Ca (3.72 %), Cu (21.40 mg/kg), Mn (215mg/kg) and Zn (74 mg/kg) were at their maximum concentration. P was at its maximum concentration in the 3^{rd} week while Mg, Fe and Na were at their maximum in the 4^{th} and 5^{th} weeks and time of transplanting respectively.

These values represent the potential fertiliser applications for specific minerals and optimum times of harvesting. This study further revealed that *Solanum nigrum* is a highly nutritious wild vegetable that can be cultivated and incorporated into human diet of the rural communities whose diets are mainly starch based. The best fertiliser to apply and best time

to harvest the plant leaves for food may be a challenge as different minerals respond differently to different fertiliser options as has been shown, therefore it is critical to recommend the best fertiliser and time of harvesting based on specific nutritional interventions. It was also observed that there was a significant interaction between the plant age and treatment on the accumulation of all the minerals. In general, the nutrient values recorded indicate the ability of the wild vegetable to supply the molarity of recommended daily mineral intakes of both the macro and micro minerals at all stages of the plant's growth in children and adults if they consume at least 150 and 300 g of the vegetable respectively.

7.6 References

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CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Chapter Eight

8.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

From this study, it is concluded that:

- Wild vegetables are not completely neglected, however their use has declined
- Wild vegetables growing naturally in the wild have high nutritional values, however, compositions of particular minerals may vary, for example, *Solanum nigrum* is high in fibre while *Chenopodium murale* is high in Mg, K and P and *Physalis peruviana* a good source of Cu and Fe
- Mixing wild vegetables during their preparation may be a good way of harnessing the majority of nutrients required by the human body for growth and development
- Some of the wild vegetables are used as medicinal plants, therefore making them more valuable
- *Solanum nigrum* seed is highly viable
- Physiological dormancy hinders the germination of *Solanum nigrum*
- Scarification can successfully overcome dormancy in *Solanum nigrum* seed
- Temperature clearly plays an important role in breaking the dormancy of *Solanum nigrum* as shown by increased germination due to exposure to $25 – 45^{o}C$ temperature and acid
- In the soil, scarification imposed by micro-organisms and temperature probably breaks physiological dormancy leading to germination
- Air drying ripe, mature berries is the best way of drying *Solanum nigrum* seed for cultivation
- *Solanum nigrum* responds well to fertiliser amendments
- Results of the application of organic manure may not be immediately visible due to the slow process of mineralisation

- The uptake of nutrients on the field is higher than in the glasshouse, possibly due to unlimited supply of organic matter on the field as well as a larger surface area for root growth and expansion and therefore unlimited soil nutrient uptake
- Cultivated *Solanum nigrum* yields better results than the species growing naturally in the wild
- Cultivated *Solanum nigrum* has the potential to supply the recommended daily proximate and mineral intakes in all age groups and at all stages of the plant's growth if children consume 150 g and adults 300 g of the wild vegetable
- This study further revealed that *Solanum nigrum* can be successfully cultivated in small home gardens, however, there is need for the government and nongovernmental organisations to implement programmes to conscientise people on the nutritional benefits of consuming wild vegetables, encourage their cultivation and also create seed banks for seed preservation

Appendix 1

Ethnobotanical survey of wild vegetables from the Eastern Cape, South Africa

Questionnaire:

Informants' consent for the participation in the study:

I, Zodwa Dyushu hereby give my full consent and conscious to participate in this study and declare that to the best of my knowledge the information that I have provided are true, accurate and complete.

Date 20 May 2011 (Signature/Thumb impression of Informant) Z Dyushu

Informants' details:

Name Zodwa Dyushu

Gender Female

Age 55

Occupation Cleaner

Location/Residence Alice

Data about wild vegetable plant and its use:

Plant (Local name) Urhawu

Habit (Tree/ ✓Herb/ Shrub/Climber/.....)

Plant part used leaves

Mode of preparation cut fresh leaves, fry with onion and tomatoes, mix with potatoes and seasoning

Availability (✓Summer/Autumn/Winter/Spring)

Availability in the wild (✓easy/ difficulty/ very difficult)

Method of collection Pluck fresh shoots with hands (take note of spikey stems)

Method of preservation Sun drying

Medicinal properties YES

Name of disease(s) treated Arthritis, diabetes, hypertension

Other uses (if any) N/A

Remarks:

Plant identified as Urtica urens L. (Botanical name and family)
Urticaceae family