

**EFFECTS OF WATER STRESS ON ANTIOXIDANT ACTIVITY AND
PHENOLIC CONTENTS OF AFRICAN NIGHTSHADES AND THEIR
DISTRIBUTION IN KISII AND SIAYA COUNTIES OF KENYA**

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DECLARATION

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

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DEDICATION

To my late dad Zachary Odhiambo and mum Redemptor Nduku, and my late colleague Fabrice Musoni.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ALV	African leafy vegetables
ANOVA	Analysis of variance
ANS	African nightshades
AOS	Activated Oxygen Species
DPPH	Diphenyl picryl hydrazyl
DNA	Deoxyribonucleic acid
DM	Dry Matter
FAO	Food and Agriculture Organization
GAE	Gallic Acid Equivalent
GOK	Government of Kenya
GPS	Geographical Positioning System
HIV	Human immunodeficiency virus
ROS	Reactive oxygen species
SPSS	Statistical Package for Social Sciences
SAS	Statistical Analysis for Sciences
TPC	Total phenolic content
UN	United Nations

ABSTRACT

Drought is a major abiotic factor limiting crop productivity in many regions of the world. It causes reduction in plant growth, dry matter accumulation and decline in plant water status of the plants and in certain cases interferes with biochemical processes within cultivated crops. The aim of the study was to map out the distribution of different African Nightshade species in Siaya and Kisii Counties of Kenya and to determine water stress effect on total antioxidant capacity and total phenolic content of two selected African nightshade namely, giant nightshade (*Solanum scabrum*) and black nightshade (*Solanum villosum*). Prior to selection of the two varieties, the study involved farmer field visits, mapping of nightshades present and administration of semi-structured questionnaires to farmers to determine the indigenous vegetables being grown, the nightshade species grown and factors affecting their production. Experiments were conducted both in the field and greenhouse conditions. Watering intervals were at 15 cbars, 50 cbars and 85 cbars. Data on number of secondary buds, leaf area, shoot height, shoot and root dry weights were gathered. The total antioxidant capacity was recorded using DPPH radical scavenging method and the total phenolic content using Folin-Ciocalteu method. The data collected were subjected to ANOVA. In both counties production was 100% under small scale, with *Solanum scabrum* being the main variety grown in Siaya County (36%) while in Kisii the main variety was *Solanum villosum* (32%). There were significant differences ($P \leq 0.05$) among treatments in leaf area, plant height, shoot biomass, number of secondary buds, leaf and root total phenolic content and leaf and root antioxidant activity. *Solanum scabrum* had the tallest plants at all stress levels with a maximum height of 45.17cm at 15cbars, while *Solanum villosum* had the shortest plants at all stress levels, with the shortest one being recorded at 16.65 cm at 85 cbars. *Solanum scabrum* also had the highest root dry weight (7.78g), shoot dry weight (50.78g) and highest leaf area of (304.45cm²), however *Solanum villosum* had the highest number of secondary buds at all stress levels with the highest being 24 at 15 cbars. With respect to phytochemicals, *Solanum villosum* had a higher concentration of both the total phenolics and antioxidant activity in the shoots (46.41g GAE/Kg DM total phenolic content and 52.68% total antioxidant activity) while *Solanum scabrum* had higher concentration in the roots (25.06gGAE/Kg DM total phenolic content and 27.18% total antioxidant activity). Water stress led to a decline in all growth parameters but enhanced phytochemical accumulation in nightshade accessions grown. It is therefore recommended that for better yields irrigation should be done at every 15 cbars, however for adequate phytochemical accumulation, irrigation should be carried out at 50 cbars. Further research to explore and quantify other phytochemical components as affected by different watering regimes need to be undertaken.

CHAPTER ONE: INTRODUCTION

1.1 Background information

African Nightshades (*Solanum spp*) are among the African indigenous leafy vegetables reported to be important source of antioxidants such as lycopene, phenolics, and vitamin C in human diet. Antioxidants have been linked to reduced risk of prostate and various other forms of cancer, heart diseases as well boosting the immune system of HIV/AIDS patients (Maundu *et al.*, 1999). Growing demand for dietary antioxidants sources has triggered the search for newer , economical, nutritional and multifunctional sources possessing free radical scavenging potential (Imungi, 2002). With cancer and HIV/AIDS becoming a threat to economic development, a shift has been seen in food consumption trends both in urban centers and rural areas of Kenya, with a majority opting for the traditional vegetables. About 56% of Kenyans live below the poverty line and about 50.6% of the population lack access to adequate food; moreover, even the little they get is of poor nutritional value and quality (Republic of Kenya, 2004). The high poverty levels are manifested in form of malnutrition and poor health particularly for most of the Kenyan rural population. The poverty situation has been worsened by the high prevalence of HIV/AIDs where 2.5 million Kenyans are already infected with an annual infection rate of 200,000 (GoK, 1999). Nevertheless, Kenya is endowed with high agricultural biodiversity like African nightshades (ANS) which could contribute significantly to the management of HIV/AIDS infected and affected persons (Irungu, 2007).

African nightshades have high micronutrient content, medicinal properties (Maundu *et al.*, 1999) and several agronomic advantages and contribute to food and nutrition security and income generation (Schippers, 2002). African nightshades are African

indigenous or traditional vegetables whose leaves, young shoots and flowers are consumed. These vegetables have been used by communities in Western Kenya for a long time (Imungi, 2002). Though they have been underutilized or neglected for a long time, there has been a renewed interest in African nightshades by the policy makers and the international community on the realization that these vegetables have a potential that has yet to be exploited (Olembo *et al.*, 1995).

Phytochemical studies have revealed that the plant contains glycoalkaloids (solanine, solamargine, solanigrine and solasodine), steroidal glycosides (β -solamargine, solasonine and α , β - solansodamine), steroidal saponins (diosgenin), steroidal genin (gitogenin), tannin and polyphenolic compounds (Myung-min oh *et al.*, 2010). In response to drought, plants typically accumulate a wide range of antioxidants, including enzymatic antioxidants (peroxidases, superoxide dismutase, and catalase) and non-enzymatic antioxidants to quench the re- active oxidative species (ROS) induced by water stress (Mittler, 2005). Water stress also induces a number of such phytochemicals, including a-tocopherol, b-carotene, and flavonoids, in a wide range of plant species (Velikova *et al.*, 2000).

Plant is affected by several stress factors (biotic and abiotic) and any particular factor may reinforce or compensate for the effects of others. The current climate change depicted by local changes and annual variations in the precipitation expose many plants to water stress (Gorai *et al.*, 2010). To prevent the accumulation of activated oxygen species (AOS), plants have evolved highly efficient antioxidative defenses, composed of both enzymatic and non-enzymatic antioxidants (Foyer *et al.*, 1994). These products are good health indices whose quantities need to be documented. Therefore, this

research sought to assess ANS responses to effects of water stress on the plant distribution based on the phytochemicals produced. It is apparent that plant phytochemicals for example phenolics are responsible for plant adaptation to variable environments.

1.2 Problem statement

Whereas there is reported research already in relation to contribution of African indigenous vegetables to health in terms of mineral composition, there is very little work reported on phytochemicals, yet they play a crucial role in maintenance of health and protection from coronary heart disease and cancer (Hedges and Lister, 2009). These compounds partially contribute to the bitter taste of certain species of nightshades. With the renewed acceptance of the African Nightshades as shown by significant stocking of these vegetables in supermarkets (Odhiambo and Oluoch, 2008), as well as demand in open air markets, it is imperative that more knowledge and information will be required particularly in terms of the role of plant nutrition, external environment and nightshade species' inherent characteristics in terms of growth and phytochemical contents. The research sought to reveal whether secondary metabolite accumulation depends on environment, species or their interactions.

1.3 Significance of the study

This project aims at filling gaps in scientific knowledge regarding response of phytochemicals (total phenolics content, and total antioxidant activity) content to water status as a constraint in African nightshades cultivation. The generated knowledge will give insight into nightshade species plasticity to the above stress by producing

secondary metabolites as a trade-off for yield. Knowledge on the phytochemicals will be useful in developing guiding principles for vegetable production in the segmented Kenyan horticultural market.

Already there is a growing demand for nightshades in supermarkets and open air markets and there is already a campaign to factor in different communities tastes (Odhiambo and Oluoch, 2008). These can be achieved through manipulation of external factors such as water; probably depending on species genetic composition. The presence of phenolic compounds in food has garnered much attention in recent years (Imungi, 2002). The primary reason relates to their antioxidant properties and proposed health benefits such as inhibiting certain cancers and promoting cardiovascular health (Abukutsa *et al.*, 2005). While this marketing strategy may be relatively new, the contribution of phenolics to food quality has been known for some time. They have obvious sensory impacts ranging from color to bitterness and aroma (Chweya and Eyzaguirre, 1998). These compounds also offer protection to foods in terms of food stability; protecting against oxidation and microbial spoilage. For these reasons the management of phenolics in food is an important aspect of product development. The information presented here should provide insight into factors that influence phenolic accumulation in plants and what can be done to manipulate the phytochemical composition of ANS. Furthermore, the project presents growers especially women with an opportunity to identify nightshade cultivars with relatively higher biomass yield and with high phytochemicals composition in varying abiotic conditions (based on sensory attributes). This will contribute to household food and nutrition security as well as women empowerment.

1.4 General objective

To assess the distribution of different African nightshades species in Siaya and Kisii counties of Kenya and determine effects of water stress on growth parameters and phytochemical accumulation in *Solanum scabrum* and *Solanum villosum*

1.4.1 Specific objectives

- i. To document and map out the distribution of African Nightshade species in Siaya and Kisii counties of Kenya.
- ii. To evaluate biomass yields of African Nightshades species under different watering regimes.
- iii. To determine the concentration of phytochemicals in cultivated species in relation to soil water status and determine trade-off between yield and phytochemicals in plant shoots.

1.4.2 Hypotheses

- i. The distribution and richness of African Nightshade species in Siaya and Kisii Counties of Kenya varies with differences in rainfall distribution in different agro ecologies.
- ii. Water stress lead to differential yield reduction in different plant organs of the African nightshades.
- iii. Water stress enhances phytochemical production in African Nightshades in different nightshade species.

1.5 Conceptual framework

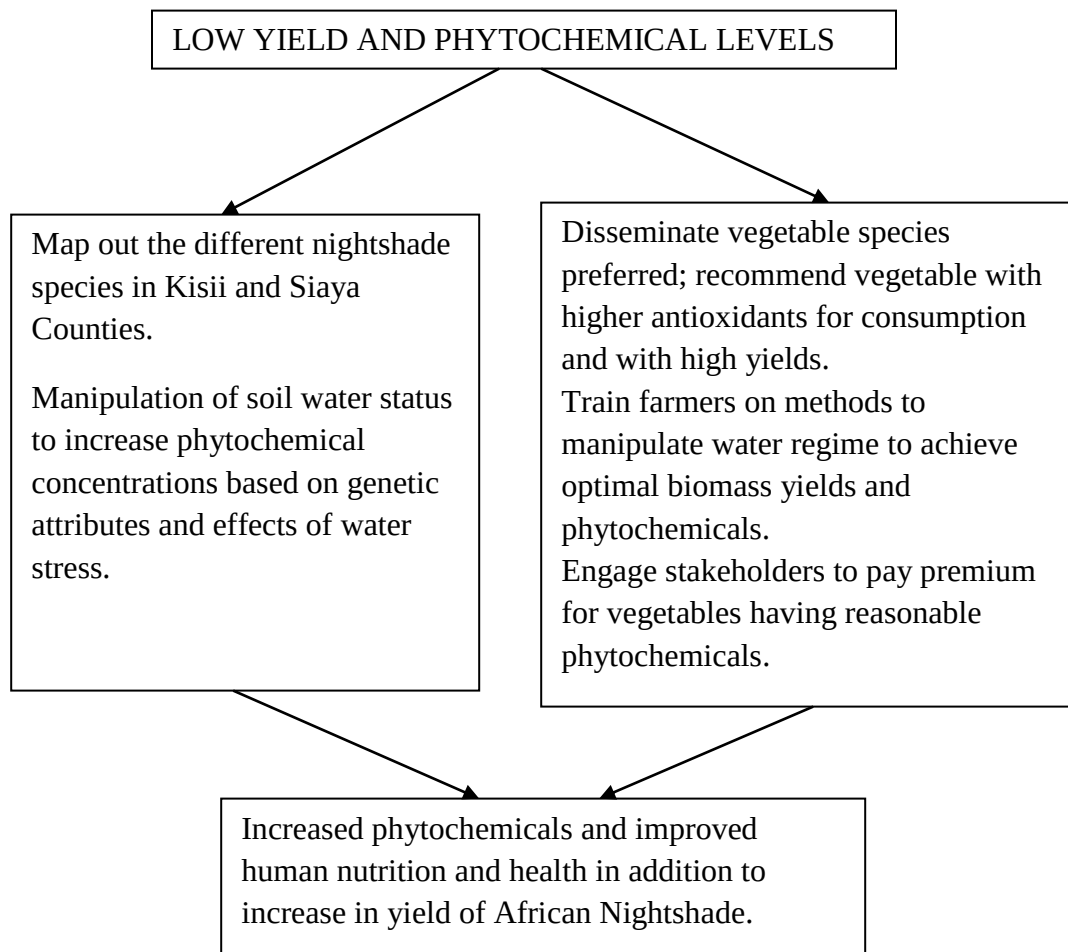


Figure 1.1: Conceptual framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and geographical distribution of Nightshades

The center of origin of most *Solanum* species is South America. The centers of diversity for diploid species are South America, and to some extent North America, while the centers of origin of most polyploidy species (tetraploids and hexaploids) are either in Europe or Africa (Maundu *et al*, 1999). A large number of distinct taxa in section *Solanum* show their greatest diversity and concentration in the new world tropics particularly in South America (Ojiewo *et al.*, 2013). *Solanum scabrum* occurs as a cultivated vegetable from Liberia to Ethiopia, and South to Mozambique and South Africa. It is very common in lowland as well as highland regions in West and East Africa (Chweya and Eyzaguiere, 1999). The wide range of diversity of *Solanum scabrum* found especially in Nigeria and Cameroon suggests that its origin is likely to be in the warm humid forest belt of West and Central Africa. Outside Africa, *Solanum scabrum* can be found in Europe, Asia, New Zealand, North America and Caribbean (<http://www.pfaf.org/database/plants>).

2.2 Health benefits of African Nightshades

Leaves and fresh shoots of *Solanum scabrum* are widely used as a cooked vegetable. As it has a bitter taste, some people prefer not to use salt. Bitterness is reduced by discarding the cooking water and replacing it with fresh water (Irungu *et al*, 2007). In recent years, the potential importance of fruit and vegetables as health-promoting foods has grown in light of the increasing number of epidemiological studies suggesting that the daily intake of fruit and vegetables has significant health benefits in preventing

many chronic and degenerative diseases (Mittler, 2002, Munns, 1988). This is because they are rich in numerous phytochemicals, including antioxidants (Zlatev, 2005).

African nightshades are known to be rich in micronutrients and vitamins. A number of studies have been done on some of these vegetables and compared to exotic leafy vegetables such as cabbage and kales, they have been found to be higher in vitamin content (especially vitamins A, E and C), fiber and minerals (Hedges and Lister, 2009). Some are known to be rich in lysine, an essential amino acid that is lacking in diets based on cereal and fibers, while others are medicinal (Imungi, 2002). The green, leafy African nightshades contain polyphenols which have beneficial physiological effects on humans as antioxidants. They are also known to be anticarcinogenic and anti-arteriosclerotic (Imungi, 2002). A study carried out in Nairobi showed that consumption of African nightshades is associated with the treatment of various diseases including therapy for patients with HIV/AIDS, diabetes, high blood pressure and other common ailments (Kimiye *et al.*, 2006).

Recent literature indicate that natural compounds in ANS are responsible in maintaining redox homeostasis of biological fluids and preventing human diseases such as cardiovascular disease, atherosclerosis and other degenerative pathologies such as cancer, diabetes, alzheimers and Parkinson disease (Irungu *et al*, 2007). It has been reported that the nutrient content of this vegetable can provide 100% of the recommended daily allowance for an adult for calcium, iron, β -carotene, ascorbic acid and 40% of protein if 100g of the fresh vegetable is consumed (Abukutsa, 2003).

2.3 Antioxidants and Phenolics in plants

Antioxidants are nutrients in food that protect cells from damage from free radicals. Free radicals are unstable molecules that can damage cells. The body creates them when food is digested or while breathing in polluted air. This cell damage may increase cancer risk, heart disease, cataracts, diabetes, or infections (Korasaminjad *et al.*, 2011). Free radicals may also affect brain function. Antioxidants are the largest category of phytochemicals and the most widely distributed in the plant kingdom. They exist in the form of polymers and monomers (Ebrahimzadeh *et al.*, 2008). The three most important groups of dietary antioxidants are flavonoids, phenolic acids, and polyphenols. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids.

Antioxidant compounds in food play an important role as a health protecting factor (Hedges and Lister, 2009). Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Hedges and Lister, 2009). Some compounds such as gallates have strong antioxidant activity, while others such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals (Rumit *et al.*, 2010). Synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and

liver (Yordanor *et al.*, 2003). Investigations of antioxidants are therefore focused on naturally occurring substances, especially plant phytochemicals.

Phenolics are a group of over 4000 compounds that occur widely in the plant kingdom, and are usually divided into two classes – the flavonoids and phenolic acids. In the plant they serve a variety of purposes, including protection against fungal disease, insect attack and strong sunlight as well as attracting pollinators and seed dispersers. Often these compounds impart taste (often bitter or astringent) and some also provide aroma and colour (Ebrahimzadeh *et al.*, 2008). The purplish colour of some varieties is attributable to anthocyanins, a flavonoid sub-group. Structurally, phenolics all contain at least one phenol ring and at least one hydroxyl group, which is important as these confer antioxidant activity. They are water-soluble, which affects some of their functional properties (Hedges and Lister, 2009).

Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins (Ayaz *et al.*, 2000). Quantification of food antioxidant levels is just beginning, and preliminary results indicate high variability, even within a given food. As plants grow, phenolics accumulate in cell vacuoles, or polymerize into lignin, which strengthens the secondary cell walls. In addition to this, phenolics possess some physiological functions as they regulate cell elongation (Valentine *et al.*, 2002). Plants differ greatly in their foliar concentrations of these secondary compounds, and the reason for this variation remains poorly understood despite a long history of investigation (Stamp, 2003).

2.4 Water Stress

Water stress is one the major abiotic stress factors that affect all organisms lives including human in terms of health and food. Absence of water from the soil solutions affect the natural evaporative cycle between earth and atmosphere, alternatively affect the amount of rainfall being received. Drought occurs when soil moisture level and relative humidity in air is low while temperature is high. UN reports (2006), estimate that one third of world population has been living in areas where the water sources are poor (Farooq *et al.*, 2008). Water stress resulting from the withholding of water, also changes the physical environment for plant growth as well as crop physiology. Almost every plant process is affected directly or indirectly by water supply (Akinici and Lösel, 2012).

Plants, as one of basic food sources, either in nature or cultivations, in their growing period, require water or at least moisture for germination. Most land plants are exposed to short or long term water stress at some times in their life cycle and have tended to develop some adaptive mechanisms for adapting to changing environmental conditions (Gasper, 2002). Some plants may adapt to changing environment more easily than others giving them an advantage over competitors. Water stress may range from moderate, and of short duration, to extremely severe and prolonged drought that has strongly influenced evolution and plant life. (Apel and Hirt, 2004). Crop yields are restricted by water shortages in many parts of the world. The physiological responses of plants to water stress and their relative importance for crop productivity vary with species, soil type, nutrients and climate. On a global basis, about one-third of potential arable land suffers from inadequate water supply, and the yields of much of the

remainder are periodically reduced by drought (Oktem, 2008). It is estimated that 10 billion people in the world will be hungry and malnourished by the end of this century (FAO, 2011). One of the aims of the researches is to gain an understanding of survival mechanisms which may be used for improving drought tolerant cultivars for areas where proper irrigation sources are scarce or drought conditions are common. In research aimed at improvements of crop productivity, the development of high-yielding genotypes, which can survive unexpected environmental changes, particularly in regions dominated by water deficits, has become an important subject (Chaves *et al.*, 2003).

2.4.1 Water stress and oxidative stress in plants

Oxidative stress, which frequently accompanies many abiotic stresses like high temperature, salinity, or drought stress, causes a serious secondary effect on cells. Oxidative stress is accompanied by the formation of reactive oxygen species (ROSs) such as O^{2-} , O_2 , H_2O , and $OH^\cdot$. ROSs damage membranes and macromolecules affecting cellular metabolism and playing a crucial role in causing cellular damage under drought stress (Ebrahimzadeh *et al.*, 2008).

Drought creates an imbalance between light capture and its utilization, which inhibits the photosynthesis in leaves. In this process imbalance between the generation and utilization of electrons is created. Dissipation of excess light energy in photosynthetic apparatus results in generation of reactive oxygen species (ROS) (Jaleel *et al.*, 2008). Denaturation of functional and structural macromolecules is the well-known results of ROS production in cells. DNA nicking, amino acids, protein and photosynthetic pigments oxidation, and lipid peroxidation are the reported effects of ROS (Jaleel *et al.*,

2008). As a consequence, cells elicit some responses such as an increase in the expression of genes for antioxidant functions and production of stress proteins, up-regulation of anti-oxidants systems, including antioxidant enzymes and accumulation of compatible solutes. All these responses increase scavenging capacity against ROSs (Shao *et al.*, 2009)

2.4.2 Effects of water stress on photosynthesis

Water stress reduces photosynthesis by decreasing both leaf area and photosynthetic rate per unit leaf area (Yordanov *et al.*, 2008). Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth, or increasing senescence of leaves under drought conditions, may also inhibit photosynthesis in existing leaves (Xu *et al.*, 2002). Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis, and interference with many other basic metabolic processes. Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth, or increasing senescence of leaves under drought conditions, may also inhibit photosynthesis in existing leaves (Kirnak *et al.*, 2003).

In the field, plants are normally not deprived of water rapidly. As water stress increases photosynthesis and transpiration usually decrease at similar rates (Shao *et al.*, 2009). In terms of the relationship between photosynthesis and leaf water status, Akinci and Lösel, (2012), reported that in field conditions, photosynthesis in ambient CO₂ reached a maximum value in the morning and declined later in the day when water potential decreased as leaf to air water vapour pressure deficits increased. In non-watered plants

the decline was larger, and occurred earlier. In most cases stomatal conductance followed a diurnal pattern similar to that of photosynthesis.

2.4.3 Plant Responses to water stress.

The amount of water available to plants is important, since water accounts for 80-90% of the fresh weight of most herbaceous plant structures and over 50% of the fresh weight of woody plants (Akinici and Lösel, 2012). On a global basis, about one-third of potential arable land suffers from inadequate water supply, and the productivity of much of the remainder are periodically reduced by drought. Moreover, water deficits may occur during a plant's life cycle outside of arid and semi-arid regions (Ashraf *et al.*, 2011) even in tropical rainforests. Water is progressively lost from a fully saturated soil, firstly by draining freely, under the influence of gravity, and the rate of loss gradually slows down until no further water drains away, when the soil is said to be at field capacity. Further loss of water by evaporation or by absorption by plant roots reduces the moisture content still further, until no further loss from these causes can occur, a stage known as the wilting point at which plants can no longer obtain the water necessary to meet their needs and they therefore wilt and die from moisture starvation (Kirnak *et al.*, 2003).

Initially, stress conditions occur transiently as cyclic water stress even under adequate soil moisture conditions, and may prevail certain time in the daytime and normalized after reduction of transpiration rate by the night (Apel and Hirt, 2004). Crop yields are restricted by water shortages in many parts of the world and the total losses due to this cannot be estimated with confidence (Kramer and Boyer, 1995). According to Rambal and Debussche (1995), changes in plant conductance under water stress are attributable

to effects on the roots and xylem. As the soil dries, decreased permeability, due to root suberization and or increased loss of fine roots, can reduce the balance between water extraction capacity and transpiring leaf area. Roots of unwatered plants often grow deeper into the soil than roots of plants that are watered regularly (Xu *et al.*, 2002).

Plants exposed to stress due to decreasing supply of water or other resources, or because of climatic changes, show different responses according to species and the nature and severity of the stress (Giannakoul and Ilias, 2013). By altering the chemical and physical composition of tissues, water deficits also modify various aspects of plant quality, such as the taste of fruits and the density of wood (Akinici and Lösel, 2012). Water shortage significantly affects extension growth and the root-shoot ratio at the whole plant level. Although plant growth rates are generally reduced when soil water supply is limited, shoot growth is often more inhibited than root growth and, in some cases; the absolute root biomass of plants in drying soil may increase water use efficiency relative to that of well-watered controls (Sharp, 1996). Almost every plant process is affected directly or indirectly by water supply.

The reduction in water content is accompanied by other changes such as increase in salt concentration and increasing mechanical impedance. The growth of plants is controlled by rates of cell division and enlargement, as well as by the supply of organic and inorganic compounds required for the synthesis of new protoplasm and cell walls. It is well known that water stress not only affects morphological appearance but also changes bio-mass ratio. (Luvaha *et al.*, 2008) reported that water stress drastically decreases root elongation and leaf area expansion but that these two processes are not equally affected. Leaf growth is usually decreased to a greater extent than root growth,

and photosynthate partitioning is altered to increase root-shoot ratio (Tanguilig *et al.*, 1987) reported that the water stress caused major reductions in height, leaf number, leaf area index, fresh and dry weight of cotton plants and some members of the family *Cucurbitaceae*.

2.4.3.1 Osmotic adjustment mechanisms under water stress

Water is essential in the maintenance of the turgor which is essential for cell enlargement and growth and for maintaining the form of herbaceous plants. Turgor is also important in the opening of stomata and the movements of leaves, flower petals, and various specialized plant structures (Kage *et al.*, 2004). Although turgor measurements on segments the non-growing lamina have often appeared to show declining rates of leaf growth with decreasing turgor, turgor measurement in regions of leaves and stems, where cell enlargement usually occurs, often show little or no decrease, even when cell enlargement is largely inhibited due to soil drying (Kamara *et al.*, 2003). This is believed to be due to osmotic adjustment, the process in which solutes accumulate in growing cells as their water potential falls (Kusaka *et al.*, 2005) of osmotic potential arising from the net accumulation of solutes in response to by maintaining turgor in tissues, osmotic adjustment may allow growth to continue at low water potential.

Osmotic adjustment usually depends mainly on photosynthesis to supply compatible solute. As dehydration becomes more severe, photosynthesis is inhibited, resulting in a smaller solute supply for osmotic adjustment. With continued water limitation, osmotic adjustment delays, but cannot completely prevent, dehydration (Lafitte *et al.*, 2007). In

leaves and stems at least, solute accumulation does not fully compensate for the effects of limited water supply on cell enlargement.

The growth of plants is controlled by rates of cell division and enlargement, as well as by the supply of organic and inorganic compounds required for the synthesis of new protoplasm and cell walls (Ayaz *et al.*, 2000). Solutes known to accumulate with water stress and to contribute to osmotic adjustment in non-halophytes, include inorganic cations, organic acids, carbohydrates and free amino acids. In some plants potassium is the primary inorganic cation accumulating during water stress and it is often the most abundant solute in a leaf. Osmotic adjustment is usually not permanent and plants often respond rapidly to increased availability of water.

Loss of osmotic adjustment can occur in less than two days in durum wheat (Oktem, 2008) and both osmotic potentials and concentrations of some individual solutes have been shown to return to prestress levels within ten days after watering (Kameli, 1990). Morgan and Condon (1986) showed that such increase in solute concentration gives tissues a temporary advantage, enabling turgor to be maintained at low water potentials by decreasing their osmotic potentials. The growing tissues throughout the plant may show osmotic adjustment when the soil loses water. In less severe stress, the elongating regions of wheat leaves were found to adjust osmotically by the accumulation of sugars, principally glucose (Munns, 2009). Osmoregulation was very active in races from dry regions (Li *et al.*, 1998).

2.4.3.2 Accumulation of compatible solutes in plant response to water stress

Osmolytes have some important role in plant responses to water stress and resistance. Many studies indicated that the accumulation of compatible solutes in plants causes

resistance to various stresses such as drought, high temperature and high salinity. Osmotic adjustment and turgor regulation are the well-illustrated functions of these compounds in plants and algae since their high solubility in water acts as a substitute for water molecules released from leaves (Shao *et al.*, 2009).

The primary function of compatible solutes is to prevent water loss to maintain cell turgor and to maintain the gradient for water uptake into the cell. These metabolites accumulations in cells lead to increase in the osmotic potential and finally resulted in higher water uptake capacity by roots and water saving in the cells. Natural osmolytes concentrations in plant cells can reach 200 mM or more and such concentrations are osmotically significant. For example, under water stress the proline concentration can reach up to 80% of the total amino acid pool in some plants (Ashraf *et al.*, 2001).

In addition to osmoregulation function, compatible solutes have some other functions in plants such as, protecting of enzymes and membrane structures and integrity, maintain protein conformation at low water potentials, scavenging free oxygen radicals and stabilizing cellular macromolecule's structures such as membrane components (Martinez *et al.*, 2007). A study also indicated the involvement of glycinebetaine in the protection of the transcriptional and translational machinery under stress conditions. Hydroxyl radicals are the most dangerous of all active oxygen species, but no enzyme has been shown to decompose hydroxyl radicals (Nam *et al.*, 2001). Some compatible solutes function as scavengers of hydroxyl radicals such as proline, citrulline and mannitol (Martinez *et al.*, 2007).

Some aspects of these functions of compatible solutes are related to their extremely hydrophilic property, and hence might also replace water molecules around nucleic

acids, proteins and membranes during water shortages. Cell water deficits cause an increase in the concentration of ions that destabilize macromolecules. Compatible solutes might prevent interaction between these ions and cellular components by replacing the water molecules around these components, thereby, protecting against destabilization during drought (Nayyar *et al.*, 2006).

Osmolyte's accumulation in plants is caused, not only by the activation of biosynthesis, but also by the inactivation of degradation. Transformed plants with the higher ability in compatible solutes' production showed a significant increase in plant's tolerance to osmotic stress (Petropoulos *et al.*, 2008). All known compatible solutes was not accumulated in all plant species, for example, accumulation of GlyBet occurs in some, but not in all, higher plant species such as xerophytes and halophytes. Citrulline accumulates in leaves of wild watermelon plants under drought. Organisms other than plants also accumulate compatible solutes; for example, glycerol in yeast and phytoplanktons, ectoine in *Halomonas*, trimethylamine Noxide and urea in shallow-sea animals, and di-*myo*-inositol-1,1'-phos-phate and related compounds in thermophilic and hyperthermophilic bacteria and Archea. Compatible solutes are divided into three major groups - amino acids (e.g. proline), polyamins and quaternary amines (glycinebetaine and dimethylsulfoniopropionate), polyol (mannitol and trehalose) and sugars like sucrose and oligosacharids. Free proline is believed to play a key role in cytoplasmic tolerance in many species and, therefore, in the resistance of the whole plant to severe drought. Sugars can play a role in osmoregulation under a drought condition in many plants such as alfalfa and *Ziziphus mauritiana* (Razmjoo *et al.*, 2008). Many studies indicated that solute accumulation under water stress contributes to

inhibition of shoot growth. It is clear because compatible solute synthesis and accumulation need high energy level (Gorai *et al.*, 2010).

2.4.3.3 Activation of antioxidant systems in plants as response to water stress

Free oxygen radicals, produced as the usual secondary consequence of environmental stresses, are very dangerous for cell components and must be precisely regulated (Sankar *et al.*, 2007). All plants have developed several antioxidant systems, both enzymatic and non-enzymatic, to scavenge these toxic compounds. Among antioxidant enzymes are catalases (CAT), superoxide dismutase (SOD), peroxidases (POD), ascorbate peroxidases (APX), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) are prominent (Ebrahimzadeh *et al.*, 2008). Besides, there are antioxidant molecules such as ascorbic acid (AA), glutathione, tocopherols, flavanones, carotenoids and anthocyanins. Some other compounds like osmolytes (proline), proteins (peroxiredoxin) and amphiphilic molecules (tocopherol) also have ROS scavenging function and may act as the antioxidant (Sankar *et al.*, 2007). Non-enzymatic plant antioxidants are either AA-like scavengers or they are pigments. Some of these compounds are multifunctional; for example, AA can react with H_2O_2 , O_2 , OH^- and lipid hydroperoxidases, acts as the enzyme co-factor and as a donor-acceptor of electron. The degree of activities of antioxidant systems under drought stress is extremely variable (Shao *et al.*, 2008). The defining factors include variation in plant species, in the cultivars of the same species, development and the metabolic state of the plant, and the duration and intensity of the stress (Ayaz *et al.*, 2000).

CHAPTER THREE: MATERIALS AND METHODS

3.1 The study area

The research was carried using two approaches. The initial data collection involved field surveys at Siaya, and Kisii Counties of Kenya. This was carried out in the month of February (long rainy season) and August (short rainy season) 2014. It involved determination of nightshade species grown by farmers in the two counties, the soil moisture content as at the time of mapping, and determination of the phytochemical concentration of the samples collected during the mapping period. In Siaya County, the areas included were; Agoro Oyombe, Ramba, Dominion farms, Yala and Gem Ulamba (Appendix 3). In Kisii the major areas were; Kisii town, Minyinkwa and Keumbu (Appendix 4). Kisii County is located in Western Kenya. It covers an area of approximately 1,317.4 sq km. Kisii lies between 0°41'0"S, 34°46'0"E (www.maplandia.com; google satellite map of Kisii), receives frequent convectional rains and enjoys a great highland climate. It has two rainfall seasons; Short rains (September-November) and Long rains (February-June), and receives rainfall amount of over 1,500mm per annum with temperatures ranging from 16 °C to 27 °C. Siaya County on the other hand covers an area of approximately 2,530.5 Km², has an annual rainfall of between 1,170 mm and 1,450 mm with a mean annual temperature of 21.75 °C and a range of 15 °C and 30 °C, and lies between latitude 0°26' to 0°18'N and longitude 33°58'E and 34°33'W. Finally, Kenyatta university is located between 1°10'52"S, 36°55'38"E and 1.1°S, 36.9°E, receives rainfall amount of about 750mm with temperatures ranging between 25°C to 33°C (wikipedia.org.September, 2014, 12.24).

The second approach involved field experiments which were carried out in Kenyatta University Research and demonstration farm located Nairobi County.

3.2 Field survey

Both qualitative and quantitative data collections were taken. Primary qualitative data were obtained from discussions and detailed interviews with key stakeholders involved in the production and marketing of the African nightshade both in the public and private sectors. The key informants for the study were traders, farmers and agricultural extension officers who were interviewed during the survey.

Quantitative data was collected through the administration of semi-structured questionnaires. This questionnaire targeted randomly selected traders in identified key markets and farmers in different farms. It was carefully pre-tested and adjusted several times before being administered. Direct observation and informal interviews were used to complement the questionnaire and to fill any gaps, during the movement from one study area to the next.

The sampling procedure was purposive and targeted at traders and farmers who were actively participating in African nightshade production and marketing. A total of 20 traders and 60 farmers were randomly selected and interviewed.

The information collected from farmers included; accessions grown, production scale, rainfall or irrigation dependence, drought effect on taste and the rationale for ANS production, while for traders the targeted information included; accessions sold, ANS source and rainfall effect on ANS availability in the market. Plant samples were collected from farmers fields and analyzed for concentration of phytochemicals.

3.3 The greenhouse and field experiment

3.3.1 Materials

The experiment was conducted under both greenhouse and open field conditions at Kenyatta University, main campus. Seeds and fertilizers were obtained from Kiambu agrovets. Other materials included loamy soils and 5 litre plastic pots for the greenhouse experiment.

3.3.2 Tensiometer installation

A portable Irrometer model P tensiometer was used in the spot check of the soil moisture conditions at the onset of the experimental period. The readings were obtained from 24cm root depth at a 3 hourly interval. The treatments included carrying out irrigation at 15 cbars, 50 cbars and 85 cbars. Whenever soil moisture reached the different soil tensions, irrigation was started to 5 cbars which was the field moisture capacity.



Figure 3.1: Portable tensiometer

3.3.3 The greenhouse experiment and design

In the greenhouse, 4.5 kg of loam soils obtained from the field of study and DAP (Diammonium phosphate) fertilizer at a rate of 5g/5 litre plastic pots. Six healthy seedlings were transplanted in each pot. These were then watered daily until establishment before being subjected to the treatments. After one week thinning was done and four plantlets left per pot. Plants were subjected to three watering regimes as follows; Low (15 cbars), moderate (50 cbars), and high (85 cbars). In all the watering regimes irrigation was done to 5 cbars. The treatments were replicated four times and pots arranged in a completely randomized design inside a greenhouse. The pots were randomized after every one week to eliminate errors due to greenhouse conditions. Weeds were controlled by hand pulling. Data collection on various parameters commenced 21 days after the initiation of the experiment.

3.3.4 The field experiment

This was carried out at the Kenyatta university Research and demonstration farm in two distinct seasons. The first season (long rains) was between February and May in 2014 and the second one (short rains) was carried out between the months of August and October same year. The experiment was arranged in a Randomized Complete Block Design (RCBD) replicated four times. This was arranged as split plot with the main plot being two nightshade accessions (*S.villosum* and *S.scabrum*) while the watering regimes at 15, 50 and 85 cbars constituted the sub-plots. In both the seasons, watering regimes commenced two weeks after transplanting.

3.4 Data collection

Data on growth parameters (root length, shoot length, plant fresh and dry weights, the roots dry and fresh weights and leaf area for the different treatments were collected for a period of six weeks in the field.

3.4.1. Number of secondary shoots

The number of secondary shoots emanating from the stem were counted and recorded.

3.4.2 Shoot height

Shoot height was measured using a meter rule from the stem base up to the shoot apex.

This was done before uprooting the plant to counteract errors due to loss of turgidity.

3.4.3 Leaf area

Leaf area was determined using the formula used by Jose et al. (2000), $LA = 0.73 (L_L \times W_L)$, where L_L is the leaf length and W_L is the maximum width measured for each leaf on each plant. Four leaves were randomly measured and leaf area determined from the averages.

3.4.4 Root and Shoot fresh weights

The whole plant was uprooted, rinsed, separated into shoot and root and the weights were measured with an electronic weighing balance. Obtained samples were air dried for two days before being taken to the laboratory for phytochemical analysis.

3.5 Reagents used

Reagents used during the phytochemical analysis are shown in table 3.1

Table 3.1: Reagent used in the study

Chemical reagent	Percentage purity	Company of origin
Gallic acid	99.9	Sigma Aldrich, Germany
Methanol	99.9	Scharlab S.L, Spain
Folin and Ciocalteu phenol reagent	99.7	BDH Limited, Poole- England
Sodium Carbonate	99.5	Central drug house (P) limited, New Delhi-India
DPPH(Diphenyl picryl hydrazyl)	99.9	Sigma Aldrich, Germany

3.6 Preparation of plant materials for phytochemical analysis

Plant shoots and roots were obtained from the experimental plots, weighed and dried in an oven for 48 hours, then reweighed to obtain the dry weight. The samples were crushed separately using electric grinder. Five grams of sample was added to 100ml labeled measuring cylinder. 50ml of methanol was added to each sample as an extract solution, and the cylinders covered with aluminium foil then left for 60 hours. The contents were then filtered using Whatman filter paper No. 1 and the filtrate kept in disposable sampling tubes in a freezer for further analysis.

3.7 Analysis for total phenolic content

The total phenol content of the extracts was determined using the Folin-Ciocalteu method (Risipail *et al.*, 2005). The total phenol content was subsequently calculated using Gallic acid as standard, where 0.5g Gallic acid was dissolved in 10ml of methanol then diluted using distilled water to 1000 ml equivalent to 500 ppm. This was then diluted to 250 ppm, 125 ppm, 62.5 ppm, 31.15 ppm and 15.625 ppm using distilled

water. These provided the calibration solution. Each 1ml of the different Gallic acid solutions was pipetted into separate test tubes and to each 4ml of distilled water was added followed by 0.2 ml Folin reagent and mixed well. After 10 minutes 0.4 ml freshly prepared sodium carbonate (prepared by adding 40 g of Na_2CO_3 in 200ml of distilled water) was added and the solution incubated for 1 hour at 25°C. To determine phenolic content in the obtained plant extracts, 1ml of plant extract was pipetted in separate test tubes, and same procedure for preparation of calibration solution employed. Absorbance of each solution was determined at 765 nm against the blank.

Calculation of total phenolic content (TPC)

$$\text{TPC} = \frac{\text{Gallic acid equivalent (mg/L)} \times \text{total volume of methanol extract} \times \text{sample weight (Kg/g)}}{\text{Dilution factor (L/ml)}}$$

3.8 Antioxidant determination

The free-radical-scavenging ability of the extracts against DPPH (2, 2-diphenyl-1picrylhydrazyl) free radical was evaluated as described by Akter *et al.*, (2010). This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, and 2.0 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was placed in a test tube, and 3ml of methanol added followed by 0.5 ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a UV-vis

spectrophotometer (model Cecil CE: 2041; 2000 series, Shimadzu Corp., Kyoto, Japan).

All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

$$\% \text{ total antioxidant activity} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

3.9 Data analysis

Analysis of variance (ANOVA) was carried out using SAS statistical computer package version 9.00 TS Level 00M0 XP-PRO platform, while SPSS software version 21 was used for the analyses of data gathered from survey. Means were separated using least significance difference (LSD) test at the 95% level of probability.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Survey Findings

4.1.1 Indigenous vegetables and nightshade accessions grown and traded in Siaya and Kisii counties

The indigenous vegetables grown in the two counties included; *Vigna unguiculata*, *Crotalaria brevidens*, *Gynandropsis gynandra*, *Brassica carinata*, *Solanum sp*, *Corchorus olitorius*, *Amaranthus graciczans* and *Cucurbita sp*. The nightshade species grown included; *S. nigrum*, *S. villosum*, *S. americanum*, *S. florulentum*, *S. scabrum*, *S.grossidentatum*, *S. tanderemotum* and *S. nigrum* (Table 4.1). The major indigenous vegetable grown in the two counties was nightshade at 32% and 29% for Siaya and Kisii counties respectively.

Table 4.1: Indigenous vegetables and nightshade species in Siaya and Kisii counties

Indigenous vegetables grown	Siaya (%)	Kisii (%)	Indigenous vegetables traded in market	Siaya (%)	Kisii (%)	Nightshade accessions grown	Siaya (%)	Kisii (%)
<i>Vigna unguiculata</i>	20	28	<i>Vigna unguiculata</i>	20	18	<i>S. nigrum</i>	9	15
<i>Crotalaria brevidens</i>	5	10	<i>Crotalaria brevidens</i>	3	4	<i>S. villosum</i>	21	32
<i>Gynandropsis gynandra</i>	2	6	<i>Gynandropsis gynandra</i>	12	7	<i>S. americanum</i>	5	4
<i>Brassica sp</i>	5	3	<i>Brassica sp</i>	7	3	<i>S. florulentum</i>	12	4
<i>Solanum sp</i>	32	29	<i>Solanum sp</i>	19	43	<i>S. scabrum</i>	36	25
<i>Corchorus olitorius</i>	8	9	<i>Corchorus olitorius</i>	12	8	<i>S.grossidentatum</i>	4	4
<i>Amaranthus graciczans</i>	18	13	<i>Amaranthus graciczans</i>	17	12	<i>S. tanderemotum</i>	4	0
<i>Cucurbita sp</i>	10	2	<i>Cucurbita sp</i>	10	5	<i>S. nigrum</i>	9	15

Cowpea was the second most dominant vegetable in the two counties at 20% and 28% for Siaya and Kisii counties respectively; this was followed by Amaranthus at 18% and 13% respectively for Siaya and Kisii counties of Kenya respectively. Similar results were obtained by Maundu *et al.*, (1999). There was variation in the preferences of other

African indigenous vegetables across the two counties (Table 4.1). In terms of market share in Siaya County, the most traded in African indigenous vegetable was cowpeas at 20%, followed very closely by nightshade at 19% and Amaranthus at 17%. In Kisii County however, nightshade took the most shares of the indigenous vegetables being traded at 43% followed by cowpea at 18% and Amaranthus at 12%. There was variability in the trade of pumpkin, jute mallow, slender leaf, spider plant and African kale in the two counties. The variation in production and trade levels is attributed to consumer preference. However, certain consumers preferred cooking the different African vegetables together as a way to reduce the bitterness of others and add flavor to taste.

Siaya County had eight African nightshade species being grown compared to Kisii which had seven. *S. scabrum* was the major species in Siaya County at 36.15% and *S. villosum* was the dominant accession in Kisii County at 32.31%. Dominion farm bordering Yala swamp had the wild relatives of black nightshades growing by themselves within the bushes. Results show that there was variability in terms of production and trade of African indigenous vegetables. Some farmers produced the AIVs for their own consumptions in their kitchen gardens especially in Kisii County. The dominance of *S. scabrum* in Siaya County is attributed to farmers and consumer preference. *S. scabrum* is less bitter and has larger leaves as compared to *S. villosum*, this lead to a general decline in *S villosum* production and consumption in Siaya County. In Kisii County, *S. villosum* dominates as its seeds are readily available as production is from the landraces; less knowledge is available concerning the availability of improved seeds in agrovets.

During the field survey, it was observed that Siaya County was richer in diversity in African nightshade accessions as compared to Kisii County (Figures 4.1 and 4.2). The results were in agreement with the findings of Schippers, (2002) and Abukutsa, (2003).

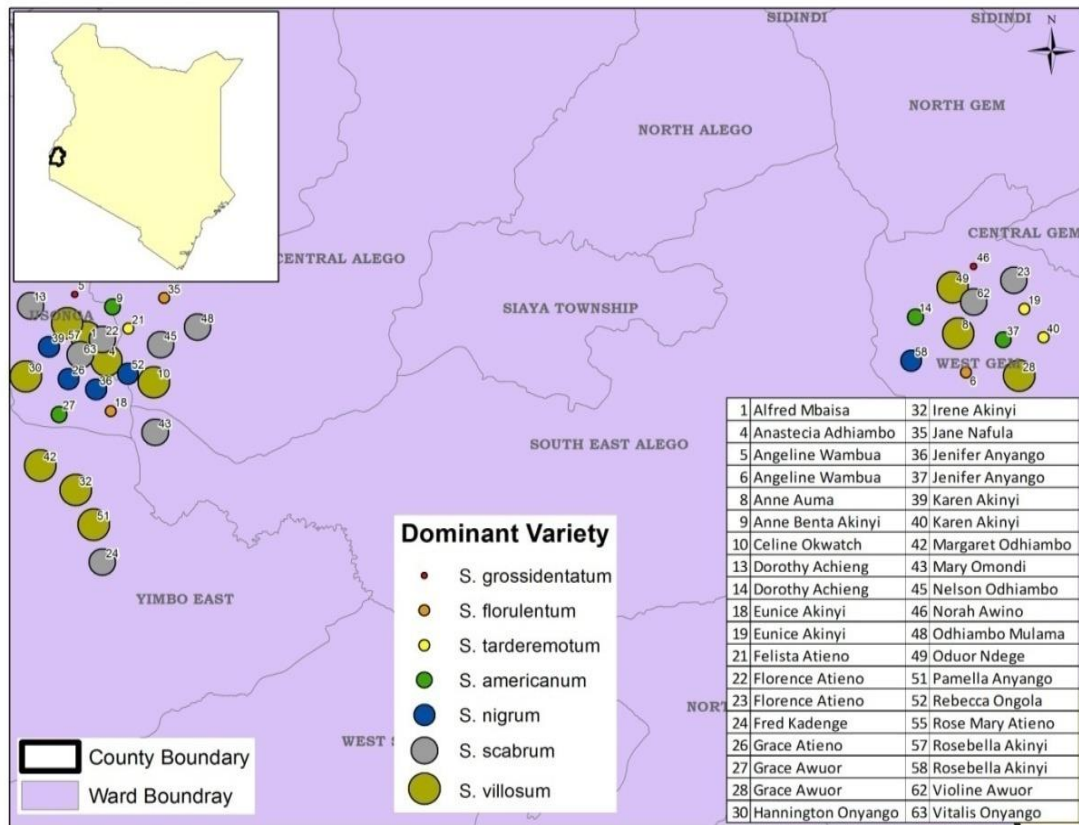


Figure 4.1: Nightshade accessions as distributed in Siaya County

Some of the species were not preferred by certain farmers as they described them as being very bitter. Bitterness is probably associated with a higher degree of phytochemical accumulation hence they were the most preferred by HIV/AIDS patients within Siaya County (Irungu *et al.*, 2007). Giant nightshade (*Solanum scabrum*) however dominates most farms in Siaya County after it was introduced and recommended by the World bank in 2012. Though common, its leaves prolificacy tend

to reduce at a faster rate compared to other grown accessions especially after the first harvest.

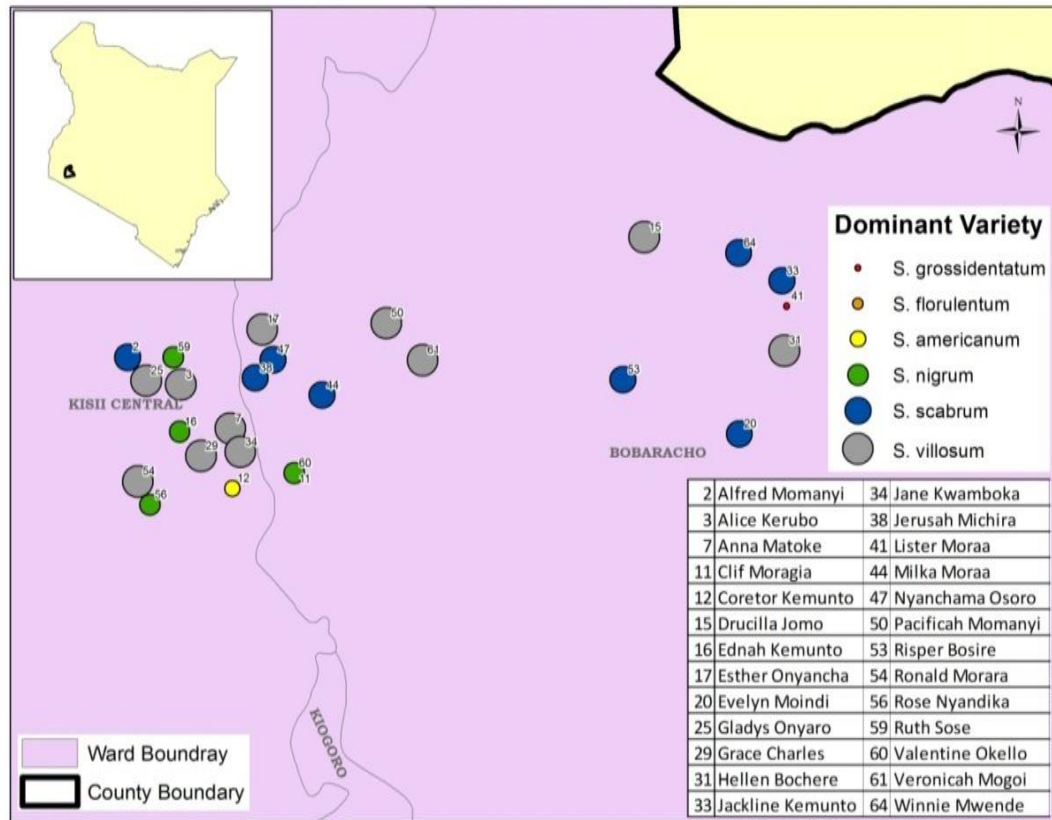


Figure 4.2: Nightshade accessions as distributed in Kisii County

Wild relatives of black nightshade were found along the hedges of live fences in Kisii County, however they were considered to be source of herbal medicine rather than sources of vegetables. The black nightshade (*Solanum villosum*) dominated most farms in Kisii region as they were preferred by consumers. Other accessions found in the two counties were mapped. All the Siaya markets had both the *Solanum scabrum* and *Solanum villosum* being sold, however all Kisii markets had only the *Solanum villosum* variety. Seventy two point seven percent of traders in Siaya County sourced the ANS from their own farms while 27.3% had them supplied from other areas such as

Kadenge, Anduro, Awello and Umalla, 40% of traders in Kisii County were producers while 60% sourced the vegetables from Kilgoris, Transmara, Magena and Kiamabundo. Production in Kisii county does not meet consumers demand due to fragmentation of farms to more income generating crops like sugarcane. This leaves little land for ANS production, hence to satisfy consumer demand the traders import ANS from the neighbouring counties.

4.1.2 Gender and production system in Siaya and Kisii counties

Both men and women were involved in the production of ANS (Table 4.2). In Siaya county 55% and 45% of males and females respectively were involved in the production of African nightshades. Kisii County on the other hand had 15% males and 85% females involved in the farming of African nightshades.

In the two counties, the production of ANS by farmers in terms of acreage was below one acre. Thirty three point three percent of farmers in Siaya County had irrigation systems in place while no farmer had an irrigation system developed in Kisii County. In terms of rainfall dependence, 100% of farmers in Kisii county were entirely rainfall dependant while in Siaya county 57.7% of farmers did not dependent on the onset of rainfall in ANS production.

Table 4.2: Gender and production systems in Siaya and Kisii counties

Gender	Siaya (%)	Kisii (%)	Production scale	Siaya (%)	Kisii (%)	Irrigation system	Siaya (%)	Kisii (%)	Rainfall Dependence	Siaya (%)	Kisii (%)
Male	55	15	Below 1 acre	100	100	present	33.3	0	Dependant	43.3	100
Female	45	85	Above 1 acre	0	0	absent	66.7	100	Not dependant	57.7	0

In terms of gender composition in ANS production in Siaya County, the findings tend to differ with those of Abukutsa *et al*, (2005) who reported that 25% males and 75%

females were African Indigenous Vegetable (AIV) farmers. The shift in composition in terms of gender could be attributed to the high returns from AIVs sales, high poverty and unemployment levels in Siaya County (GoK, 1999), making majority of males to turn to farming especially production of ANS. Increase in demand is due to WHO effort in creating awareness of the health benefit of ANS to HIV/AIDS infected patients in Siaya County which has an HIV/AIDS prevalence rate of 17.8% (GoK, 1999). All the farmers interviewed agreed that ANS fetched higher returns compared to exotic vegetables such as kales and cabbages with prices escalating to \$100 per bag in certain markets such as Awelo.

African nightshade production in Siaya County takes place along rivers or on swampy ground. The rivers provides water for irrigation as practiced in Agoro Oyombe, Gem Kanyibuop and Gem Kathomo, however in the vast Yala swamp region, especially farms surrounding Dominion region, production usually takes place on ever moist soil and this does not require irrigation.

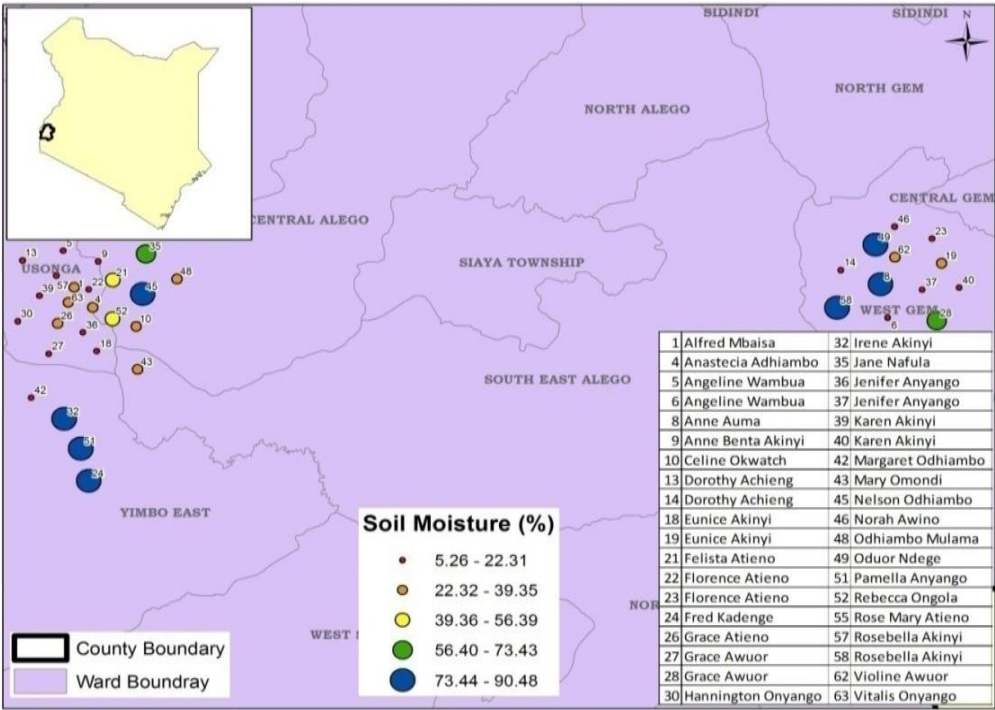


Figure 4.3: Soil moisture levels during the survey in Siaya County.

Kisii County on the other hand lies within a high altitude region and receives well distributed rainfall throughout the year and this promotes ANS production in the entire year.

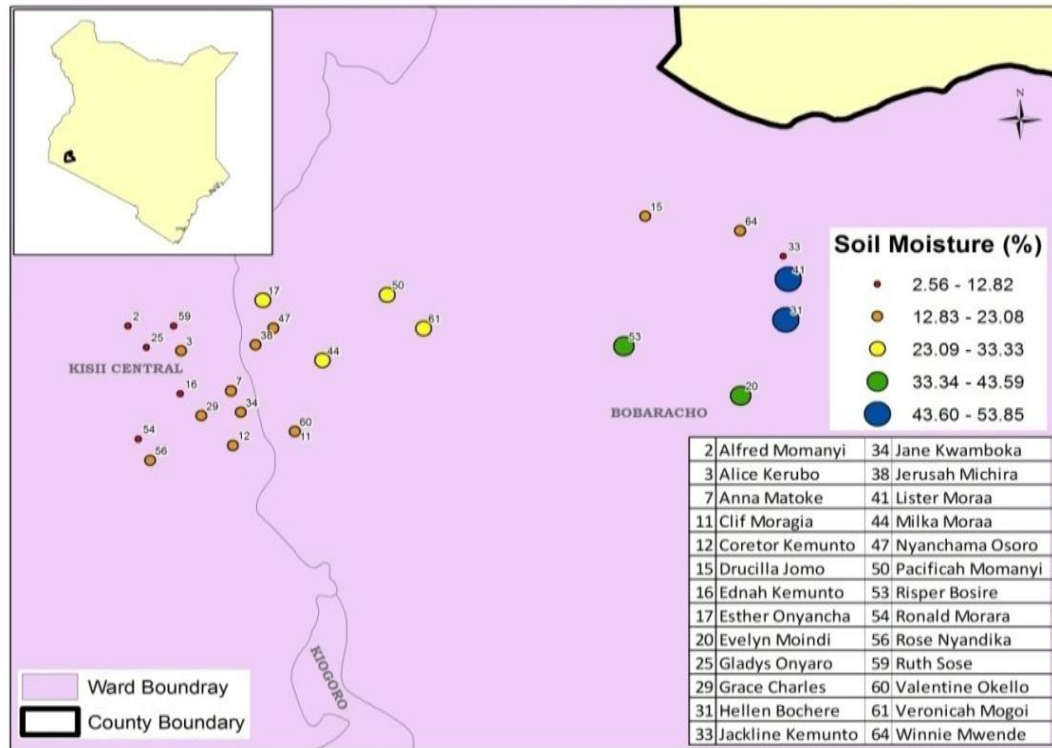


Figure 4.4: Soil moisture levels during the survey in Kisii County.

Soil moisture content plays a direct influence on the phytochemical concentration among plant parts (Akinci and Lösel, 2012), however as admitted by farmers in the growing regions the roles of specific genes on phytochemical accumulation cannot be ruled out. Areas with the highest soil moisture content were either lying within Yala Swamp (Figure 4.3) Yimbo East, or were under daily irrigation as in some parts in West Gem in Siaya county. Kisii County had no irrigation practices being carried out; hence the soil moisture content may be directly influenced by differences in the altitudinal ranges and the prevailing climatic conditions. However Kisii County recorded a lesser variation in soil moisture content as compared to Siaya County (Figure 4.4).

4.1.3 Age, education levels and optimal African nightshade production

There was variability in terms of age, level of education, reason behind production of ANS and the constraints involved in ANS production among interviewed farmers as shown in Table 4.3. Majority of interviewed farmers were between the ages of 20 to 30 years old with a percentage composition of 40% and 35% in both Siaya and Kisii counties respectively. The second dominant group was between the ages of 30 and 40 years. Their percentage composition was 24% and 33% in Siaya and Kisii counties respectively. Among interviewed farmers, 65% in Siaya County and 51% in Kisii County had basic primary education. Twenty percent and 10% of the interviewee in Siaya County had secondary and college education respectively. On the other hand 29% and 12% of the interviewed farmers in Kisii County had secondary and college education respectively. However 5% in Siaya County and 8% Kisii County admitted to having had no education at all. This agrees with Irungu, (2007) and Mwaura *et al.*, (2013), that ANS farmers are more educated than the other categories of traders, implying that the production of ANS is a field for those endowed with human capital.

Table 4.3: Age, education level and optimal African nightshade production

Age	Siaya (%)	Kisii (%)	Education level	Siaya (%)	Kisii (%)	Reason for production	Siaya (%)	Kisii (%)	Production challenges	Siaya (%)	Kisii (%)
20-30	40	35	Primary	65	51	Health benefit	30	20	Poor quality seeds	60	80
30-40	24	33	Secondary	20	29	Consumer demand	35	25	Technical production	15	8
40-50	18	21	College	10	12	High returns	35	55	Marketing system	5	2
50-60	12	11	None	5	8	—			Inadequate research	20	10
60-Above	6	—	—			—			—	—	—

Farmers in Siaya County began producing ANS commercially due to high consumer demand and high returns both at 35%. However only 30% of interviewed farmers in Siaya County produced ANS due to health benefit associated with them. In Kisii however the main reason behind ANS production was the high returns constituting 55% of the interviewed farmers. Only 20% of interviewed farmers in Kisii County knew about the health benefits of the African nightshades. Sixty percent and 80% of interviewed farmers in Siaya and Kisii counties respectively admitted that the major constraint to ANS production was poor quality seeds. This is in agreement with Abukutsa *et al.*, (2005). Most farmers complained that seeds obtained from the agrovets were low yielding due to high rate of fruiting after second or third harvest.

Fifteen percent in Siaya County and 8% in Kisii County had technical production problems. Twenty percent of interviewed farmers in Siaya County and 10% of interviewed farmers in Kisii County felt that inadequate research was being done to promote the production of ANS, though only 5% of interviewed farmers in Siaya County and 2% of interviewed farmers complained of poor market systems. Kenya has unemployment rate of above 40% (GoK, 1999), hence more youths are opting for self

employment, and one of the ways is through indulging in Agriculture. This explains to the high percentages of the youth involved in ANS production. The high returns due to increasing ANS demand among consumers might be a reason behind increase in the number of youthful farmers in the two counties. Finally the low acquisition of tertiary education among the farmers does not render them competitive enough in the ever shrinking job market; therefore they opt to farming (Schippers, 2002, Otieno *et al.*, 2009).

The green, leafy African nightshades contain polyphenols which have beneficial physiological effects on humans as antioxidants. They are also known to be anticarcinogenic and anti-arteriosclerotic (Imungi, 2002). A study carried out in Nairobi showed that consumption of African nightshades is associated with the treatment of various diseases including therapy for patients with HIV/AIDS, diabetes, high blood pressure and other common ailments (Kimiye *et al.*, 2006). Majority of farmers in Siaya County are aware of this due to WHO campaigns on ANS consumption. The awareness has created an increase in demand which has alternatively led to high ANS prices in the markets in Siaya County. However in Kisii, there has been a tradition in the consumption of ANS hence the demand has always been there leading to ever increasing returns from ANS production.

Lack of quality seed has been a major hindrance to sustainable production and utilization of indigenous vegetables (Abukutsa, 2003) as also shown in table 4.3. Some of the vegetables perpetuate themselves untended, they were harvested whenever they occurred and this system of seed procurement heavily depended on the soil borne seed pool and the ability of these species to reproduce. Seed production has for a long time

virtually remained in the hands of farmers, although seed sale in markets was common. For a very long time these vegetables were harvested from the wild, but as the pressure on land increased, they were domesticated and the need for quality seed set in. Normally ANS are grown as a subsistence crop and most farmers save their own seed from season to season, and sell surplus to other growers. The quality of such seeds is poor in terms of purity, viability and seed dormancy issues. There is need for production and supply of quality seed to increase yields and quantities produced to meet the unsatisfied market demands of priority indigenous vegetables especially in urban centres (Irungu *et al.*, 2007).

African nightshades have often been grown as intercrops with other vegetables or staples; however there has been hardly any technical information on optimal production and appropriate cropping systems. There has been lack of agronomic and preparation packages and access to technical information has been very limited, therefore extension workers have limited knowledge to advise African nightshade growers. This necessitates research on development of optimal production packages for African nightshades and recipe development.

Marketing of African nightshade vegetables has been poorly organized leading to great losses of the produce in transit or in markets. The major constraint of marketing was abundance of ANS vegetables during the rainy season leading to low prices and scarcity during the dry season. This calls for identification and creation of markets for ANS vegetables and possibly linking farmers to appropriate markets. The results were in concurrence with those of Abukutsa, (2003).

African nightshades have short harvesting period but are more prolific in seed production. This is discouraging to producers, however less or no research has been carried out to come up with varieties that are high yielding in terms of leaves, and are low seed producers.

4.1.4 Phytochemical concentration

Phytochemical analysis of plant samples collected from different farms revealed that different nightshade accessions have different phytochemical concentration (Figures 4.5 to 4.8).

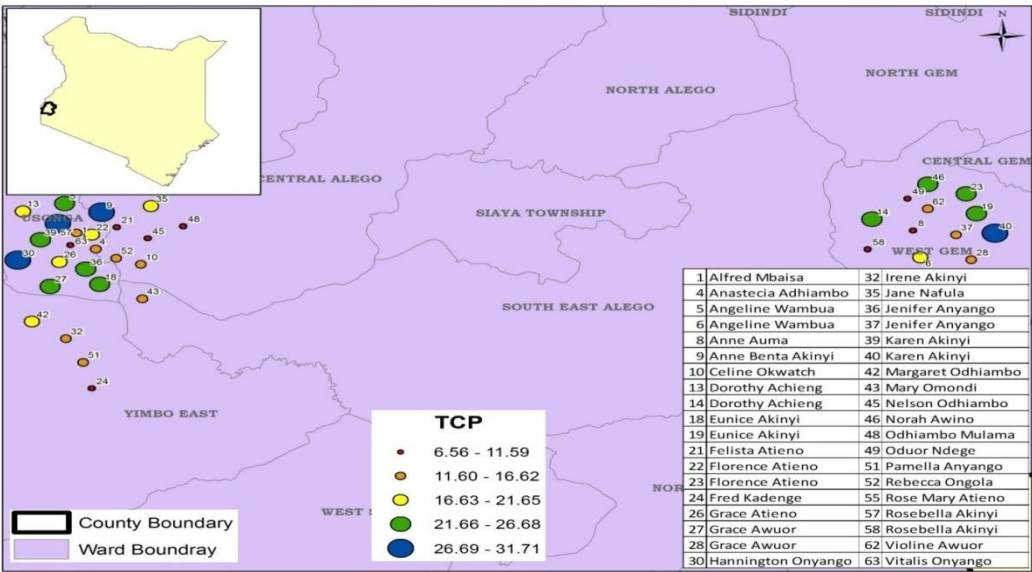


Figure 4.5: Total phenolic content of different nightshade species as distributed in Siaya County

Solanum villosum had the highest phytochemical concentration in both Kisii and Siaya counties at 61% and 55% total antioxidant activity respectively and total phenolic content of 38.8gGAE/Kg DM and 31g GAE/Kg DM respectively. The lowest total antioxidant activity in Kisii county was recorded in *S. grossidentatum* at 24% with a

total phenolic content of 3.57gGAE/Kg DM while the lowest total antioxidant activity in Siaya county was recorded in *S. tarderemotum* at 16% with total phenolic content of 7.46gGAE/Kg DM however, there was great variability in the phytochemical concentration among night accession samples collected from the field (Appendix 4).

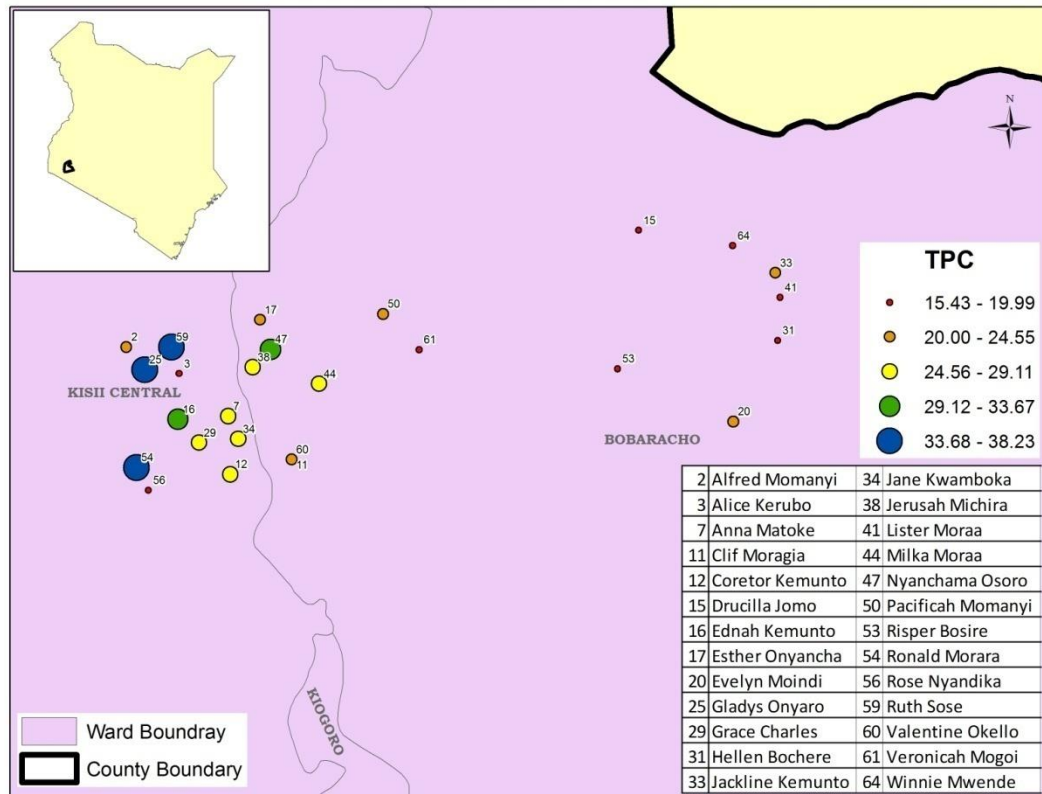


Figure 4.6: Total phenolic content of different nightshade species as distributed in Kisii County

This shows that the production of various phytochemicals (total phenolic content and total antioxidant activity) may be genetically regulated hence cells activate some responses such as an increase in the expression of genes for antioxidant functions and production of stress proteins, up-regulation of anti-oxidants systems, including

antioxidant enzymes and accumulation of compatible solutes differently in different plants.

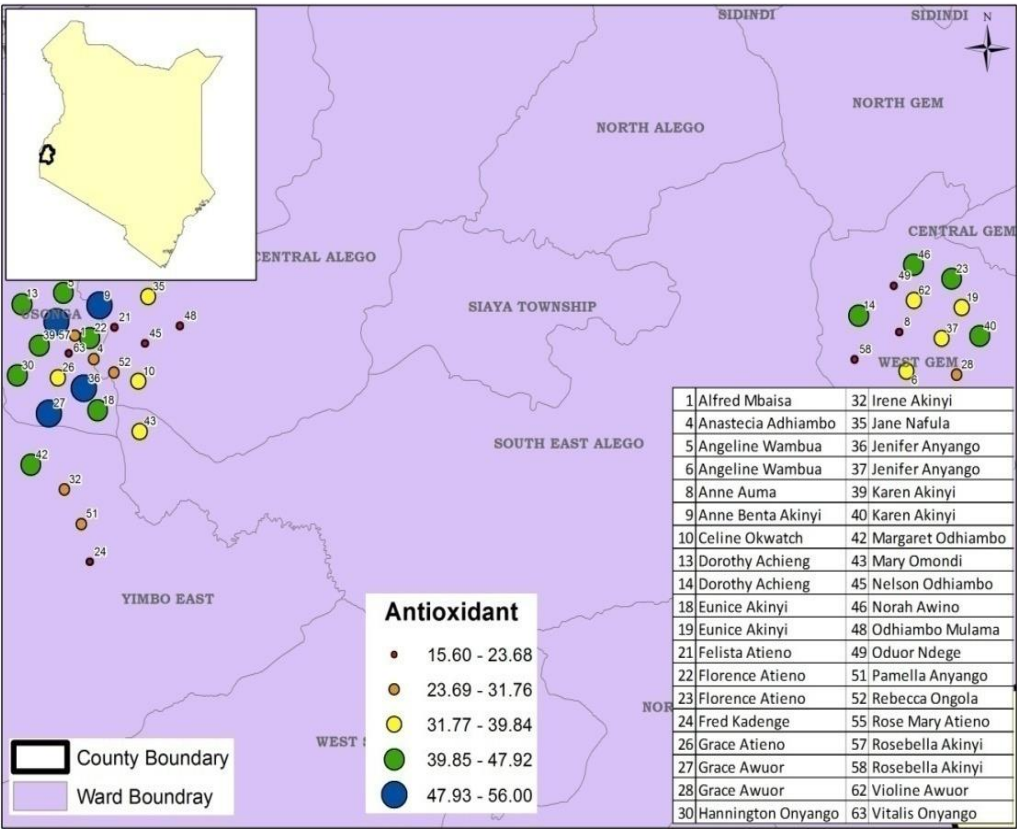


Figure 4.7: Total antioxidant activity of different nightshade species as distributed in Siaya County

Among antioxidant enzymes are catalases (CAT), superoxide dismutase (SOD), peroxidases (POD), ascorbate peroxidases (APX), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) are prominent (Sairam *et al.*, 2008). Besides, there are antioxidant molecules such as ascorbic acid (AA), glutathione, tocopherols, flavanones, carotenoids and anthocyanins. Some other compounds like osmolytes (proline), proteins (peroxiredoxin) and amphiphilic molecules (tocopherol) also have ROS scavenging function and may act as the antioxidant (Mustapha *et al.*,

2014). Non-enzymatic plant antioxidants are either AA-like scavengers or they are pigments. Some of these compounds are multifunctional; for example, AA can react with H_2O_2 , O_2 , OH^- and lipid hydroperoxidases, acts as the enzyme co-factor and as a donor-acceptor of electron (Apel and Hirt, 2004).

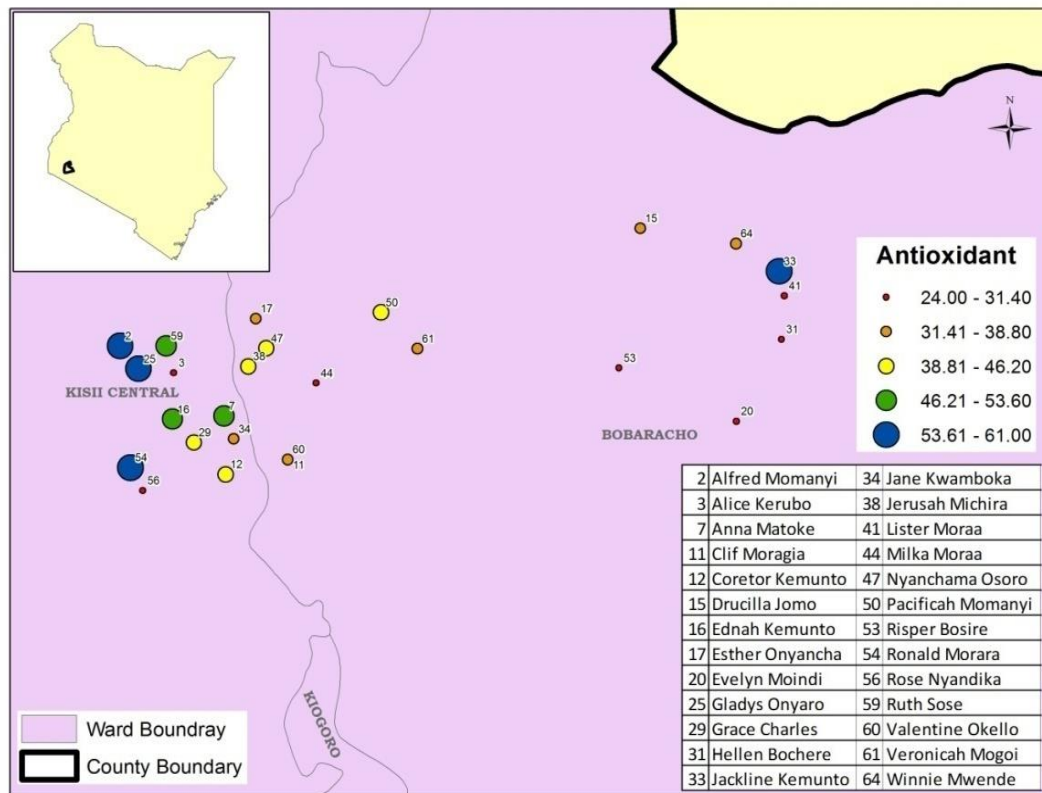


Figure 4.8: Total antioxidant activity of different nightshade species as distributed in Kisii County.

The degree of activities of antioxidant systems under drought stress is extremely variable as the defining factors may include variation in plant species, in the cultivars of the same species, development and the metabolic state of the plant, and the duration and intensity of the stress.

4.2 Effect of water stress on growth parameters of nightshades

4.2.1 Number of secondary buds

There was significant effect of water stress on the number of secondary buds at $P \leq 0.05$ in greenhouse, during long rains and short rains (Figure 4.9). Numbers of secondary buds were significantly affected by the different irrigation intervals.

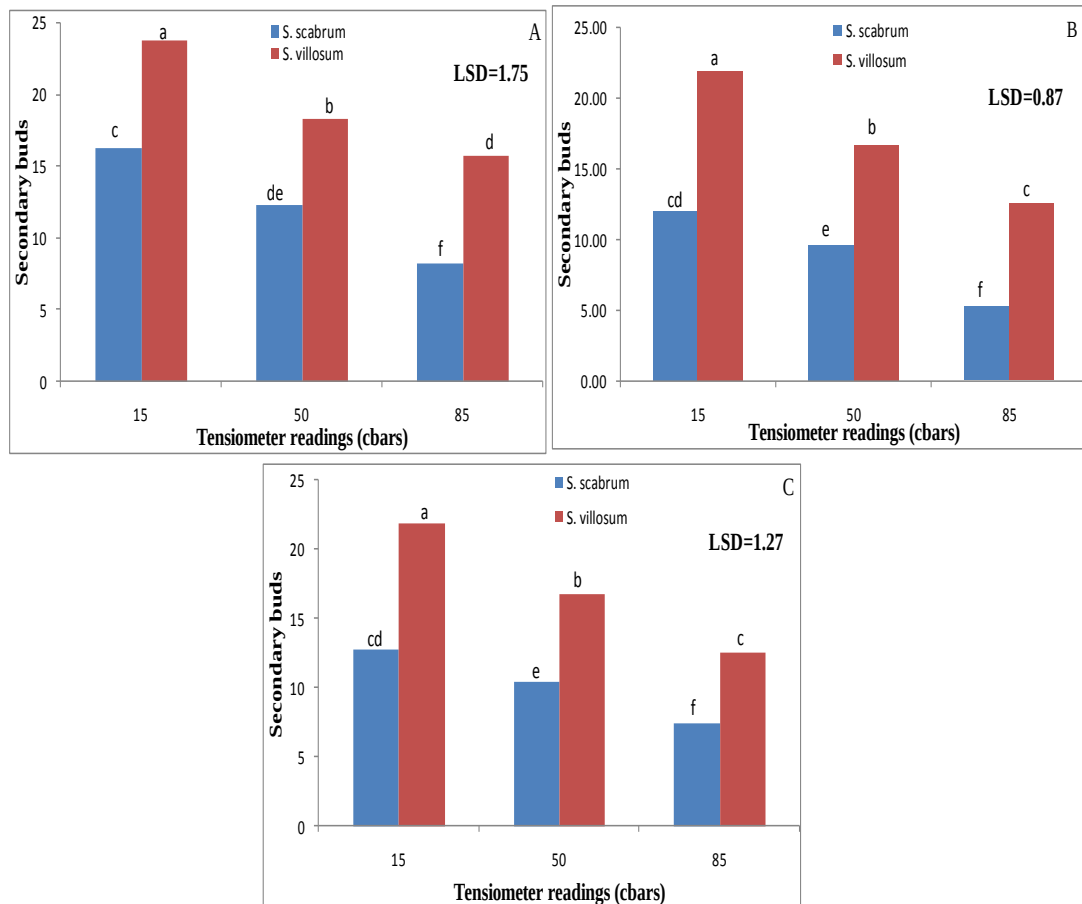


Figure 4.9: Effects of water stress on the number of secondary buds in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$).

Solanum villosum had the highest number of secondary buds when irrigation was done at tensiometer reading of 15 cbars. However, *Solanum scabrum* developed the least number of secondary buds in the greenhouse and in both the seasons at tensiometer

reading of 85 cbars. The highest mean number of secondary buds of 24 was attained by *Solanum villosum* under irrigation at water tension of 15 cbars, while the lowest mean number of secondary buds of 5 was attained by *Solanum scabrum* under irrigation at water tension of 85 cbars. *Solanum villosum* had the highest number of secondary buds at all stress levels. There was a significant interaction between water stress and number of secondary buds (Table 4.4).

Table 4.4: Interactions between irrigation interval and variety on the number of secondary buds

Variety	Tensiometer readings (cbars)	Green house	Long rainy season	Short rainy season
		Secondary buds	Secondary buds	Secondary buds
<i>Solanum scabrum</i>	15	16.25 ^c	12 ^d	13 ^d
	50	12.25 ^e	9.6 ^e	10.47 ^e
	85	8.25 ^f	5.25 ^f	7.75 ^f
<i>Solanum villosum</i>	15	23.75 ^a	21.9 ^a	22.47 ^a
	50	18.25 ^b	16.75 ^b	17.25 ^b
	85	15.75 ^{cd}	12.55 ^c	13.75 ^c
LSD		1.75	0.87	1.74
T X V		*	*	*

*Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

From the obtained data, moisture stress at vegetative stage significantly reduced branch production in this study. This is in line with the work of Mustapha *et al.*, (2014) who found that water stress reduced branching in selected soya bean genotypes; and Summerfield *et al.*, (1976) who reported that water stressed cowpea plants were reduced in branching. Reduction in the number of secondary buds can be a phenomenon by the plants to reduce surface area over which transpiration takes place.

4.2.2 Leaf Area

There was significant effect at $P \leq 0.05$ between watering regimes and leaf area in greenhouse, season one (long rains) and season two (short rains) (Figure 4.10).

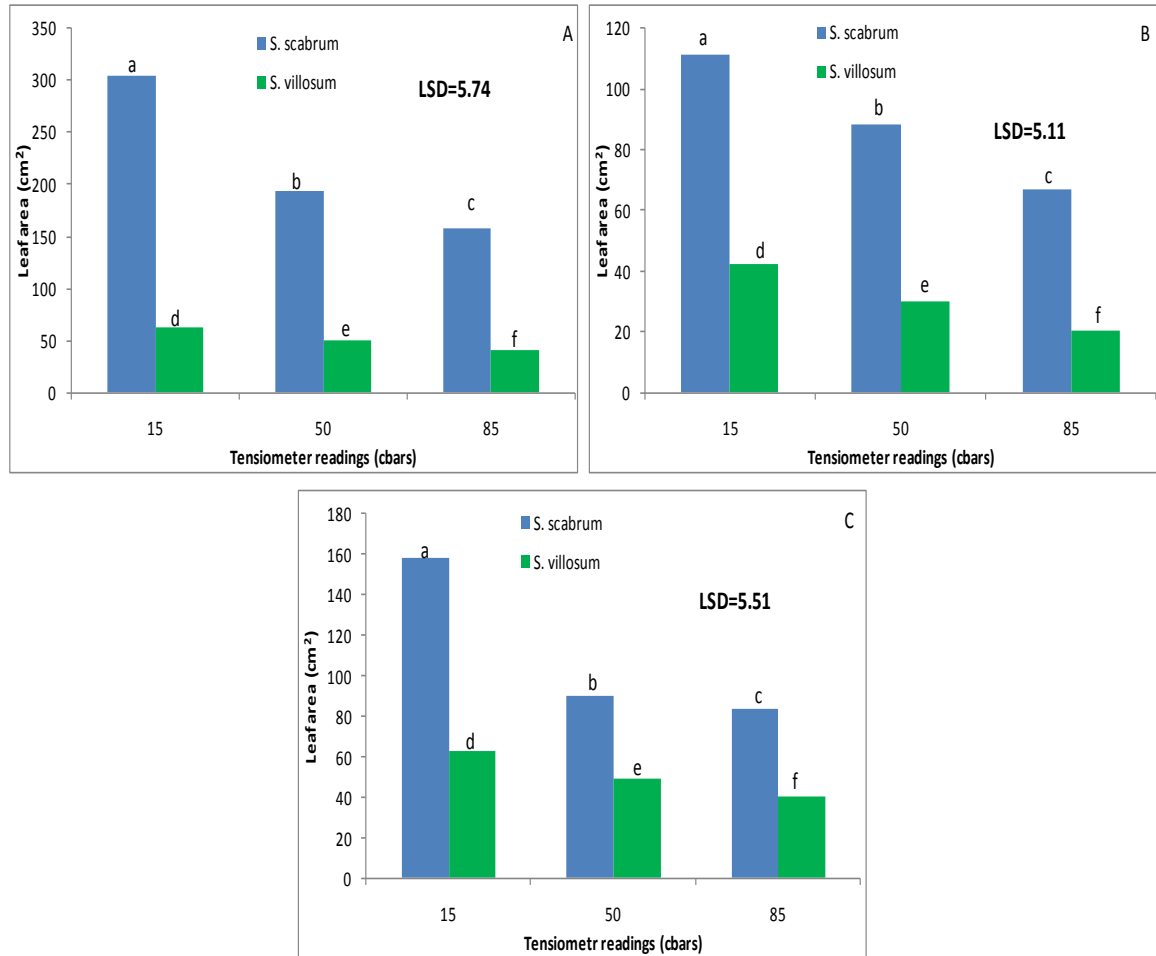


Figure 4.10: Effects of water stress on leaf area in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$).

Solanum scabrum had higher total leaf area of 304.45cm² than *Solanum villosum* of 62.45cm² under irrigation at water tension of 15 cbars. Lowest leaf area of 20.62cm² was attained in *Solanum villosum* under irrigation at water tension of 85 cbars. In moderate water deficit, leaf area among varieties had a decline order of *Solanum*

scabrum > *Solanum villosum*. In all the water stress levels, *S. scabrum* leaves had the highest leaf area. As soil water tension increased, the leaf area reduced. The reduction in leaf area was more pronounced in *S. scabrum* when subjected to water stress than in *S. villosum*. There was a significance interaction between irrigation interval and variety on leaf area (Table 4.5).

Table 4.5: Interactions between irrigation interval and variety on leaf area

Variety	Tensiometer readings (cbars)	Green house	Long rainy season	Short rainy season
		Leaf area	Leaf area	Leaf area
<i>Solanum scabrum</i>	15	304.45 ^a	111.27 ^a	158.64 ^a
	50	193.61 ^b	88.45 ^b	90.37 ^b
	85	158.45 ^c	66.92 ^c	83.41 ^c
<i>Solanum villosum</i>	15	62.45 ^d	42.50 ^d	56.88 ^d
	50	49.55 ^e	30.16 ^e	47.26 ^e
	85	40.81 ^f	20.62 ^f	35.51 ^f
LSD		5.74	5.11	5.51
T X V		*	*	*

*Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

Leaf area development is directly related to the yield of African nightshades since the edible part is the leaf. Water stress reduces leaf growth by reducing rates of cell division and expansion due to turgor loss (Akinci and Lösel, 2012). It is well known that as soil water availability is limited, plant growth is usually decreased. This was previously considered to be due to turgor loss in the expanded cells. More recent studies, however, show that stem and leaf growth may be inhibited at low water potential despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment (Tanguilig *et al.*, 1987). This suggests that the growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development

of transpiring leaf area in water stressed plants. The impact of water stress on leaf growth can be explained as a method of adaptation to the conditions of water shortage to limit the rate of transpiration, in order to maintain the water supply in the soil around plant roots to increase the chance of survival of the plant. The mechanism, by which plant leaf area is reduced under water stress, is through the reduction of cell elongation, which leads to the reduction of cell size and therefore the reduction of leaf area (Markhart, 1985).

Emam *et al.*, (2010) reported a reduction in leaf area of dry beans when the plants were exposed to drought stress during vegetative growth stage. Furthermore, Nielsen and Nelson (1998), observed significant leaf area reductions in black bean (*P. vulgaris* L) under drought stress in the vegetative phase. Markhart, (1985) also found significant reductions in the leaf area under drought conditions at 23 days after planting for two bean species (*P. vulgaris* and *P. acutifolius*). Muthomi and Musyimi, 2009, found leaf area reduction on *Solanum scabrum* when they subjected the seedlings to water stress. Indeed, loss of leaf area, which could have resulted from reduced size of younger leaves and inhibition of the expansion of developing foliage, is also considered as an adaptation mechanism to moisture deficit (Emam *et al.*, 2010)

4.2.3 Shoot height

Water stress significantly affected shoot height at $P \leq 0.05$ in greenhouse, long rains and short rains as in Figure 4.11. *Solanum scabrum* produced the tallest plants at all water stress levels.

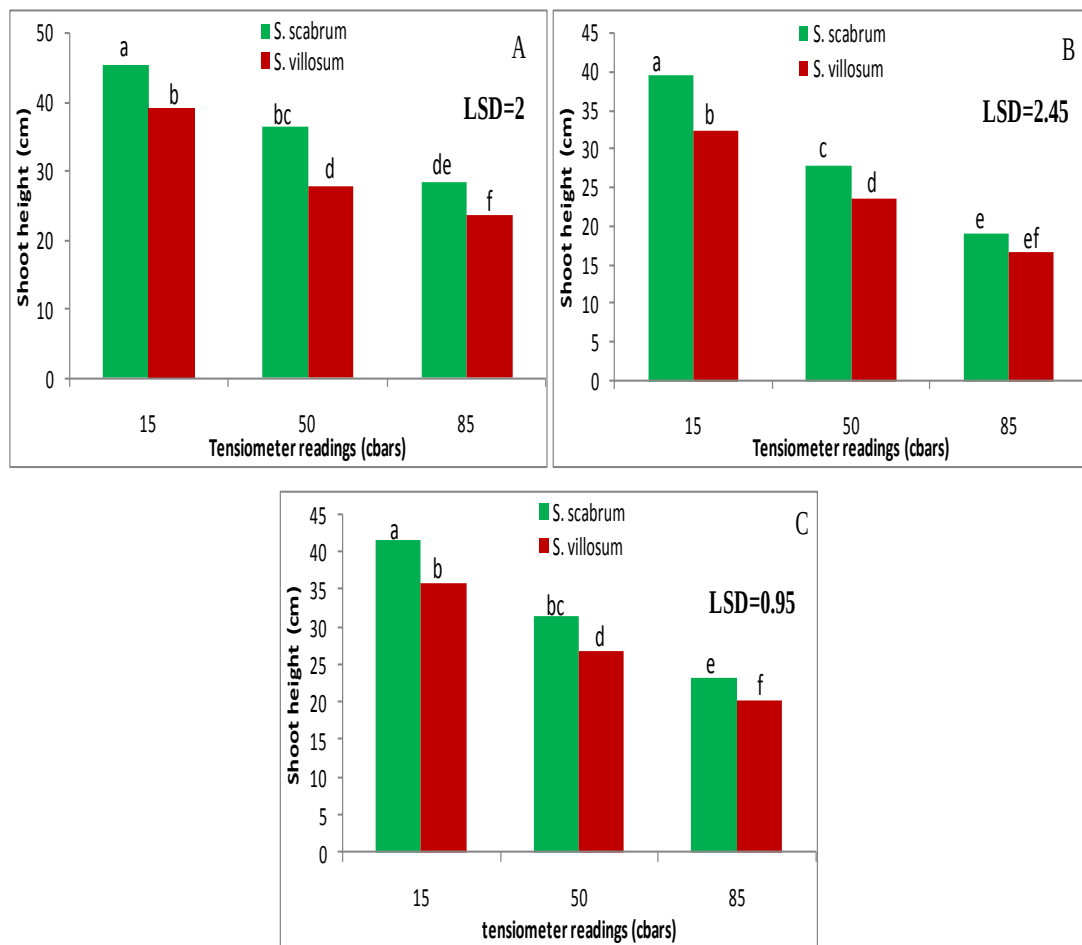


Figure 4.11: Effects of water stress on shoot height in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$).

This was related to the growth habit of this species, however, water stress depressed plant height of both accessions and the shortest plants were produced at higher water stress levels. With regard to plant height, the two varieties differed in height with *Solanum scabrum* being the tallest (40.17cm) under irrigation at 15 cbars while *Solanum villosum* were the shortest (20.24cm) under irrigation at 85 cbars. The growth reduction in shoot length in irrigation interval at 85 cbars treatment compared to 15 cbars could be associated with decrease in the cytokinin transport from roots to shoot or the increase in the amount of phytohormone abscisic acid (ABA) (Kannan and

Kulandaivelu, 2011). The hormone imbalance leads to changes in the cell wall extensibility, and decline in the concentration of photosynthetic enzymes, which results in reduced growth. (Kannan and Kulandaivelu, 2011). In Table 4.6, interaction between water stress and varieties on shoot height were statistically significant ($P \leq 0.05$).

Table 4.6: Interaction between irrigation interval and species on shoot height

Variety	Tensiometer readings (cbars)	Green house	Long rainy season	Short rainy season
		Shoot height	Shoot height	Shoot height
<i>Solanum scabrum</i>	15	45.17 ^a	39.49 ^a	41.42 ^a
	50	36.24 ^{bc}	27.85 ^c	31.24 ^{bc}
	85	28.29 ^e	19.09 ^e	23.29 ^e
<i>Solanum villosum</i>	15	39.03 ^b	32.22 ^b	35.64 ^b
	50	27.79 ^d	23.58 ^d	26.79 ^d
	85	23.45 ^f	16.65 ^{ef}	20.24 ^f
LSD		2	2.45	0.95
T X V		*	*	*

*Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

The effect of water stress on plant height was highly significant. Plant height was decreased at vegetative stage. This is in concurrence with the findings of Desclaux *et al.*, (2000) who found that water stress reduced the number of nodes and main stem height of soybeans. The finding is also in agreement with that of Hiler *et al.*, (1972) who found that plant height in cowpea was substantially reduced by water stress. Similarly, reduction in plant height in response to soil water deficit has been reported for wheat (Day and Intalap, 1978; Abayomi, 1992), barley (Day *et al.*, 1978), grain sorghum (Blum *et al.*, 1989 and cowpea (Aderolu, 2000). This result is in agreement with results of Emam, (2010), Kannan and Kulandaivelu, (2011) Nielsen and Nelson, (1998) and Shenkut and Brick, (2003) who reported on depression of plant height as a

result of severe stress treatment. Influence from environmental factors such as water stress. Drought stress affects both elongation and expansion growth (Jaleel *et al.*, 2008). Water deficient significantly suppresses cell growth and expansion due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions (Shao *et al.*, 2009). The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence (Bhatt and Rao, 2005). Stress inhibits the efficiency of the translocation and assimilation of photosynthetic products (Xiong and Zhu, 2002) and might have caused reduction in shoot growth.

4.2.4 Fresh and dry shoot weights

There was significant (at $P \leq 0.05$) effect of water stress and the fresh and dry shoot weights (Figure 4.12). In well-irrigated soils (at 15 cbars), *S. scabrum* variety had the highest fresh and dry weights of 152.27 and 50.78 respectively.

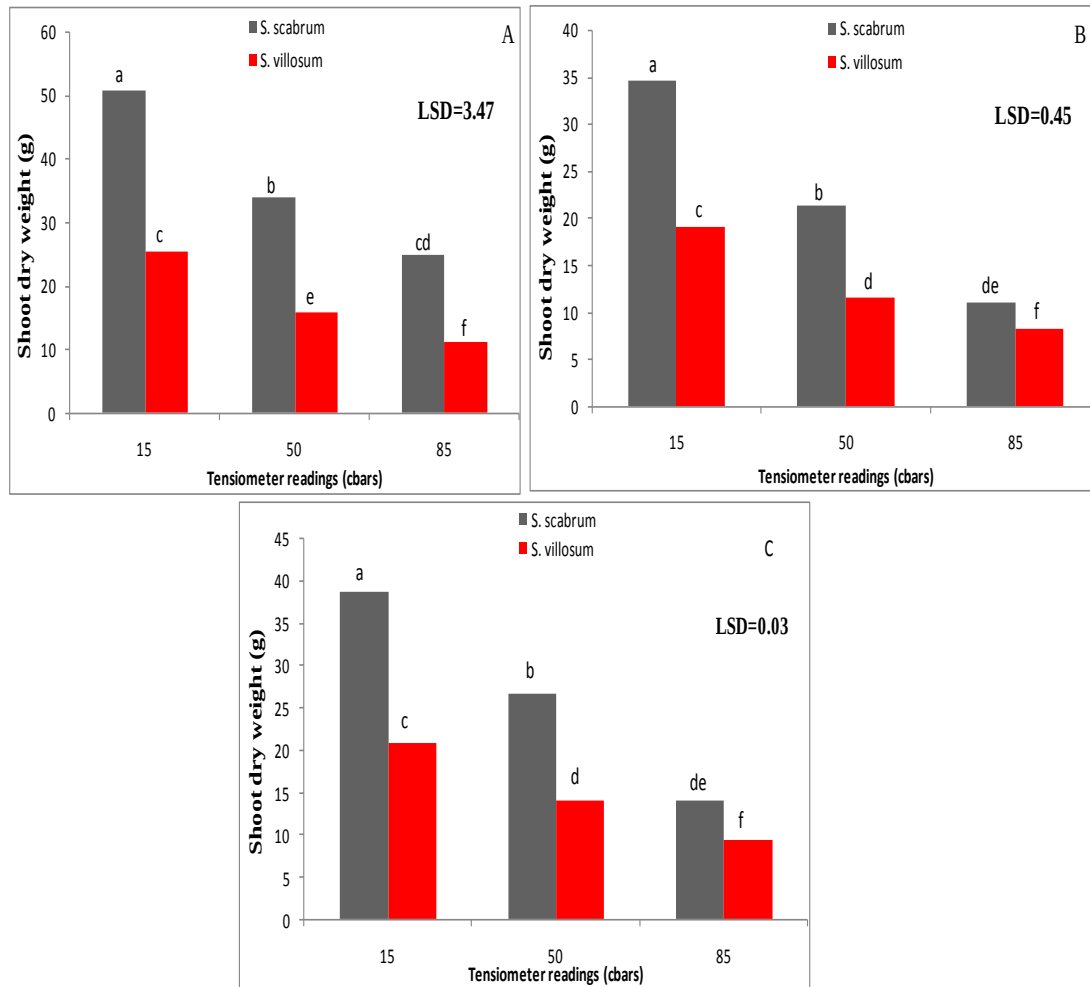


Figure 4.12: Effects of water stress on shoot dry weight in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$).

The difference in the weights was significant ($P \leq 0.05$) among *S. scabrum* and *S. villosum* varieties. In moderate water deficit, the fresh and dry shoot weights among the two accessions had a decline order of *S. scabrum* > *S. villosum*. There was great reduction in weight between fresh and dry shoot weights of *S. scabrum* than *S. villosum*. This is an indication that *S. scabrum* harbors more water in the tissues and is more susceptible to water stress as seen in the rapid reduction in fresh weight at different irrigation intervals.

Moisture stress reduced shoot dry weight (Table 4.7). Plants under irrigation at 15 cbars had large leaf area hence absorbed sufficient sunlight and so likely produced large quantities of dry matter through photosynthesis resulting to high fresh and dry weights compared to those under irrigation at 85 cbars. The reduction in the number of secondary buds, leaf area and shoot height also contributed significantly to the reduction in shoot fresh and dry weights.

Table 4.7: Interactions between irrigation interval and species on shoot fresh and dry weights

Variety	Tensiometer readings (cbars)	GREEN HOUSE		LONG RAINS		SHORT RAINS	
		Shoot fresh weight.	Shoot dry weight.	Shoot fresh weight.	Shoot dry weight.	Shoot fresh weight	Shoot dry weight.
<i>Solanum scabrum</i>	15	152.27 ^a	50.78 ^a	84.21 ^a	34.53 ^a	87.44 ^a	38.54 ^a
	50	96.72 ^b	33.87 ^b	54.91 ^c	21.39 ^b	60.28 ^{bc}	26.55 ^b
	85	66.35 ^c	24.73 ^{cd}	37.86 ^{ef}	11.02 ^{de}	45.41 ^c	14.01 ^d
<i>Solanum villosum</i>	15	73.69 ^{cd}	25.23 ^c	58.48 ^d	18.98 ^c	61.26 ^b	20.7 ^c
	50	47.68 ^e	15.82 ^e	38.25 ^e	11.45 ^d	44.01 ^d	13.99 ^{de}
	85	28.63 ^f	11.07 ^f	26.73 ^f	8.16 ^f	30.27 ^e	9.3 ^f
LSD		7.34	3.47	2.42	0.45	0.98	0.03
T X V		*	*	*	*	*	*

*Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

These results are in agreement with the findings of Bradford and Hsiao, (1982) and Chartzoulakis *et al.*, (1993). Chartzoulakis *et al.*, (1993) reported similar effects of water stress on dry matter in Kiwi fruit. Other researchers who have reported similar effects of water stress on shoot biomass reduction for a range of other agricultural and horticultural crops including sorghum (Chaudhuri and Kanemasu, 1982), tomato (Rumit *et al.*, 2010; peach (Tan and Buttery, 1997) and strawberry (Kirnak *et al.*, 2003).

Reduction in fresh and dry weights of plants subjected to water stress can be attributed to reduction in the plant shoot height, the decrease in plant leaf areas and reduction in the number of secondary buds.

4.2.5 Fresh and dry root weights

Water stress significantly affected fresh and dry root weights of the two accessions. The effect on the dry and fresh weights was statistically significant at $P \leq 0.05$.

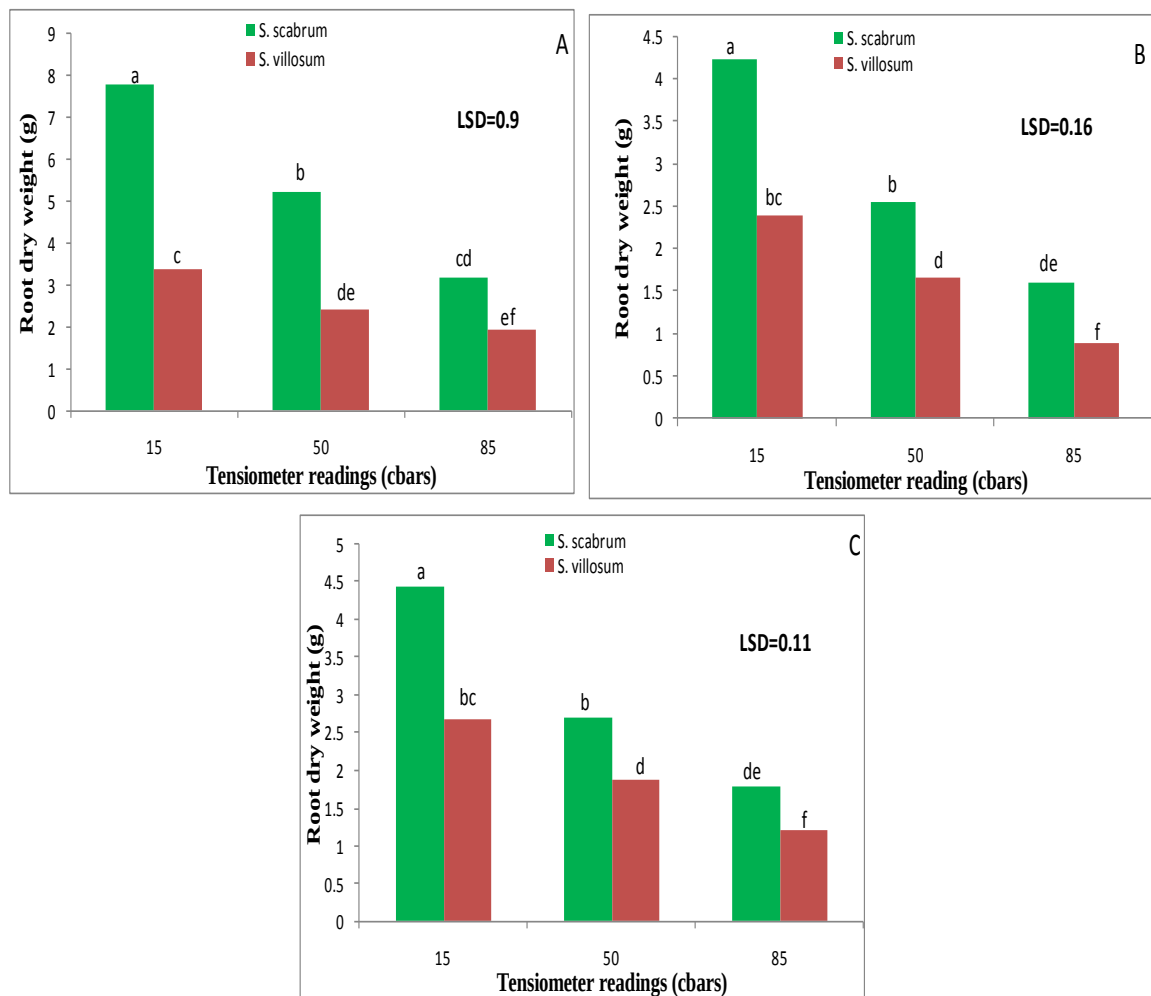


Figure 4.13: Effects of water stress on root dry weight in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different at $P \leq 0.05$

S. scabrum had the highest fresh and dry root weights (18.51 and 7.78 respectively) at irrigation interval of 15 cbars. The lowest fresh and dry root weights (1.92 and 0.89

respectively) were recorded in *S. villosum* at irrigation interval of 85 cbars. However in terms of root weight, the reduction due to water stress was minimal compared to the same effect on shoot weight. Table 4.8 below shows the interaction between the different irrigation intervals and fresh and dry root weight which was found to be significant ($P \leq 0.05$).

Table 4.8: Interactions between irrigation interval and variety on root fresh and dry weights

Variety	Tensiometer readings (cbars)	GREEN HOUSE		LONG RAINS		SHORT RAINS	
		Root fresh weight.	Root dry weight.	Root fresh weight.	Root dry weight.	Root fresh weight.	Root dry weight.
<i>Solanum scabrum</i>	15	18.51 ^a	7.78 ^a	7.78 ^a	4.23 ^a	12.41 ^a	4.43 ^a
	50	8.49 ^b	5.23 ^b	5.23 ^b	2.55 ^b	7.50 ^{bc}	2.69 ^b
	85	5.17 ^{bd}	3.17 ^{cd}	3.17 ^c	1.59 ^{de}	5.03 ^e	1.78 ^{de}
<i>Solanum villosum</i>	15	8.25 ^{bc}	3.36 ^c	3.36 ^{cd}	2.39 ^{bc}	8.26 ^b	2.67 ^{bc}
	50	4.47 ^{ce}	2.41 ^{de}	2.41 ^e	1.66 ^d	6.23 ^d	1.86 ^d
	85	3.49 ^{cf}	1.92 ^{ef}	2.23 ^f	0.89 ^f	2.80 ^f	1.19 ^f
LSD		3.32	0.9	0.76	0.16	0.81	0.11
T X V		*	*	*	*	*	*

*Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

Root growth was less inhibited than shoot growth under water stress. This is in agreement with studies conducted by Kirnak *et al.*, (2003) who reported that some roots continue to elongate at low water potentials that completely inhibited shoot growth. In plants growing in drying soil, the development of the root system is usually less inhibited than shoot growth and may even be promoted. In addition, roots growing at low water potential become thinner and this change in morphology is believed to be adaptive such that roots can further concentrate their use of resources (Sharp, 1996).

4.3 Effects of water stress on phytochemical accumulation in nightshades

4.3.1 Total shoot and root phenolic content

There was significant effect of water stress effect ($P \leq 0.05$) on the total leaf phenolic content (Figure 4.14).

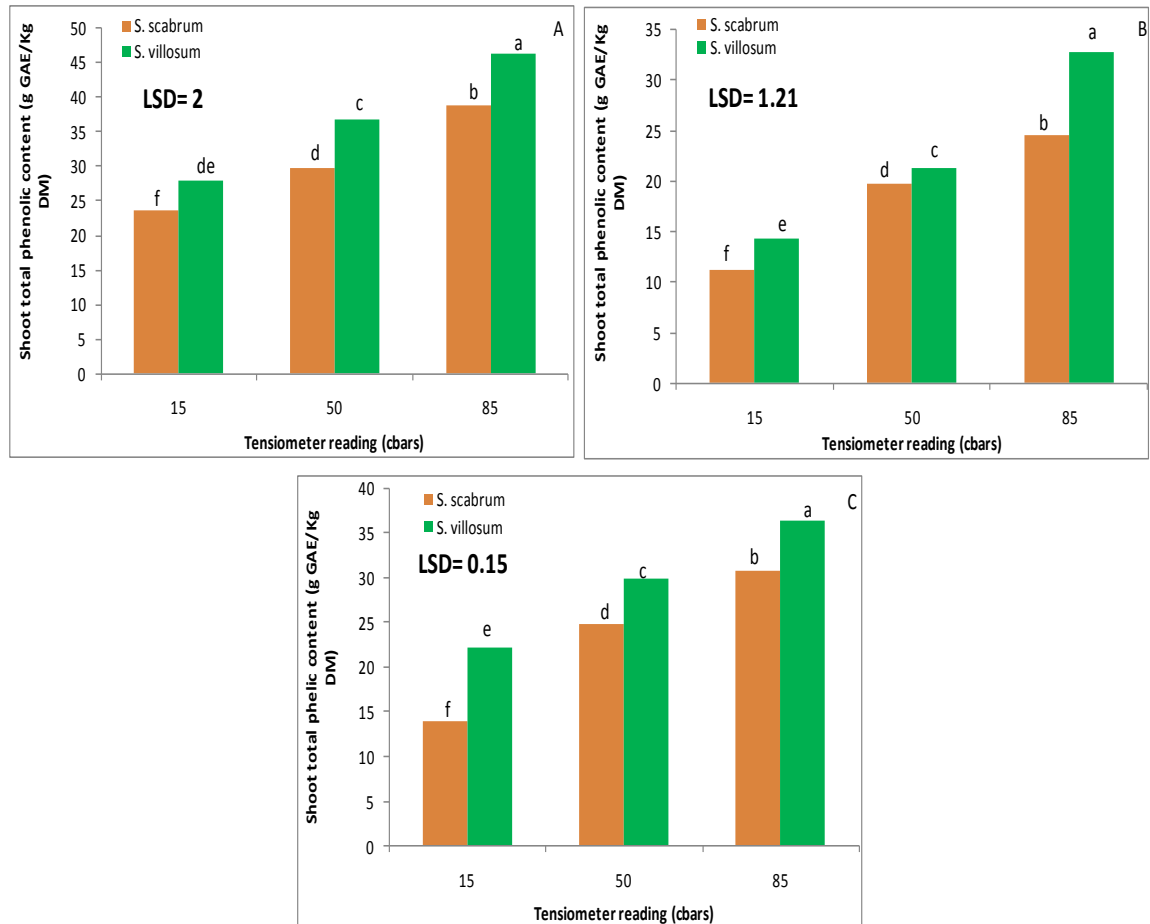


Figure 4.14: Effects of water stress on shoot total phenolic content in (A) greenhouse, (B) long rains (B) and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$)

Shoot and root total phenolic content were significantly affected by the different irrigation intervals ($P \leq 0.05$). Data showed that the highest concentration of total phenolic compounds was in the shoots of *S. villosum* at 46.41gGAE/Kg DM and roots of *S. scabrum* at 25.06gGAE/Kg DM. The phenolic contents of both the roots and

leaves increased with increasing water stress levels; hence the highest results were obtained from plants irrigated at 85 cbars. Despite variation in the phenolic contents in different seasons, the trend however remained the same (Figure 4.15).

Table 4.9: Interactions between irrigation interval and variety on shoot and root total phenolic content

Variety	Tensiometer readings (cbars)	Green house		Long rainy season		Short rainy season	
		STPC	RTPC	STPC	RTPC	STPC	RTPC
<i>Solanum scabrum</i>	15	23.55 ^f	9.49 ^e	11.26 ^f	8.56 ^e	13.78 ^f	6.92 ^e
	50	29.86 ^d	16.45 ^c	19.71 ^d	13.96 ^c	24.65 ^d	13.87 ^b
	85	38.91 ^b	21.81 ^a	24.53 ^b	20.17 ^a	30.66 ^b	16.67 ^a
<i>Solanum villosum</i>	15	27.86 ^{de}	7.59 ^f	14.24 ^e	7.83 ^f	22.15 ^e	5.57 ^f
	50	36.75 ^c	15.28 ^{cd}	21.37 ^c	10.65 ^d	29.87 ^c	10.40 ^d
	85	46.41 ^a	18.39 ^b	32.81 ^a	16.62 ^b	36.26 ^a	13.29 ^{bc}
LSD		2	1.17	1.21	0.51	0.15	1.23
T X V		*	*	*	*	*	*

*Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$. (STPC- shoot total phenolic content, RTPC- root total phenolic content).

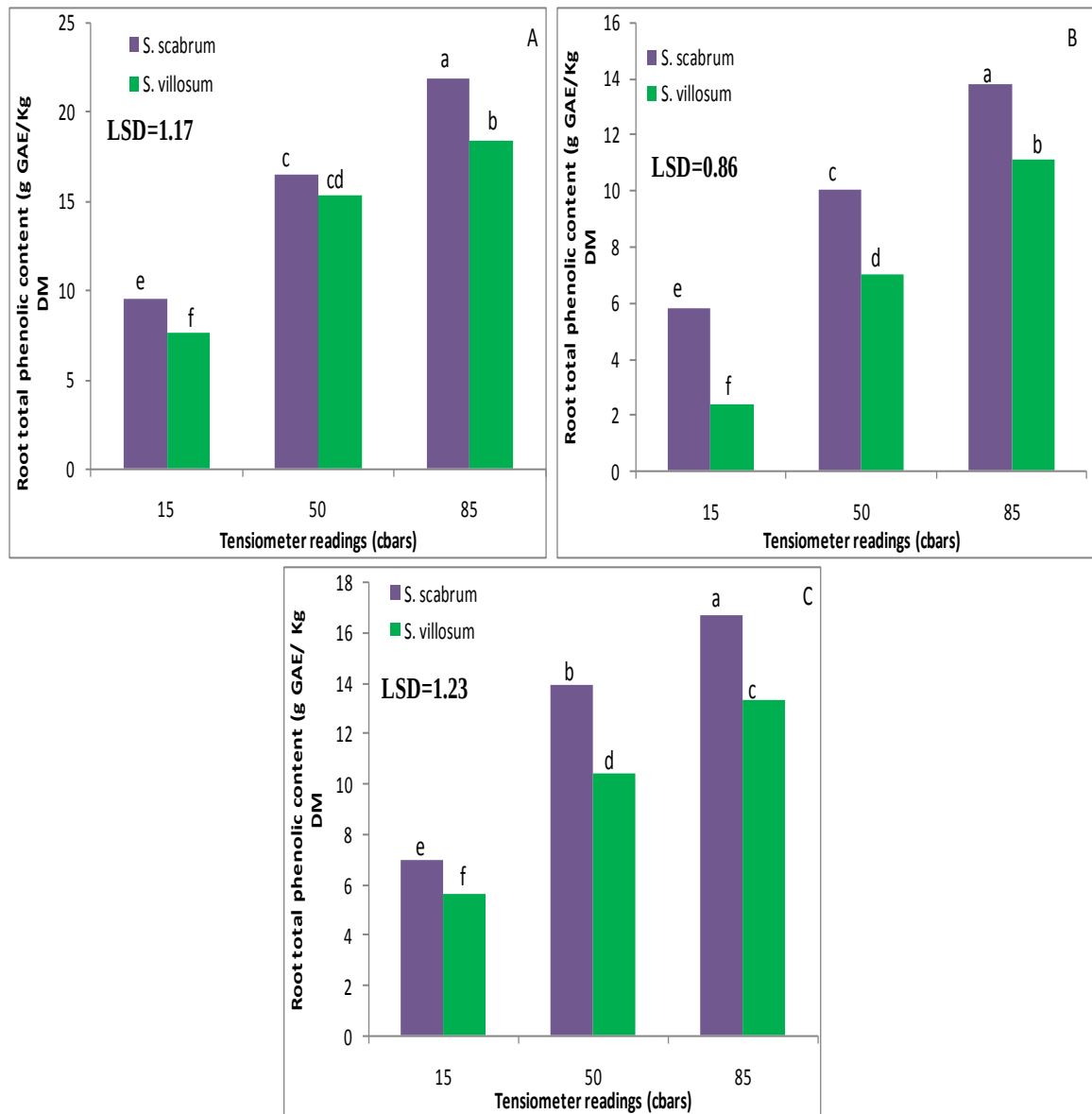


Figure 4.15: Effects of water stress on root total phenolic content in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$)

Water stress, plants activate the synthesis of phenolic compounds (anthocyanin, proline and flavonoids), carotenoids and ascorbic acid (Mandal *et al.*, 2009). Thus, phenolic compounds provide important physiological and ecological functions, being mainly involved in protection against different types of stress (Ayaz *et al.*, 2000). Besides

numerous enzymes (superoxide dismutase, peroxidase etc.), phenolic compounds are strong antioxidants that help plants to survive stress conditions (Mittler, 2002). Antioxidant compounds such as phenolic compounds are able to prevent oxidative burst of plant cells and thus protect plants from damage of proteins and lipids, DNA and RNA molecules (Apel and Hirt, 2004). In the study, the nightshade species grown revealed higher contents of total phenols under water stress conditions. The promotion of the synthesis of phenolic compounds due to drought has been documented in numerous studies (Ayaz *et al.*, 2000; Alexieva *et al.*, 2001; Sánchez-Rodríguez and Rubio-Wilhelmi, 2010).

However *Solanum scabrum* and *Solanum villosum* responded differently to the water stress conditions. *Solanum scabrum* had higher phenolic content in the roots while *Solanum villosum* had higher phenolic content in the shoots. This can be explained as differential partitioning of phenolics in different plant species when under stress. *Solanum scabrum* portioned more phenolics in the roots to act as osmolytes to maintain root cell turgor and an increase in osmotic potential that helps maintain the gradient for water uptake. The higher phenolic content in the shoots of *Solanum villosum* would likely help in protecting enzymes, membrane structure and integrity, scavenging free radicals and stabilizing cellular macromolecule structures.

4.3.2 Shoot and root total antioxidant activity

There was significant influence of watering regimes and the total leaf and root antioxidant activity in greenhouse, during long rains and short rains (Figure 4.16) and (Table 4.10).

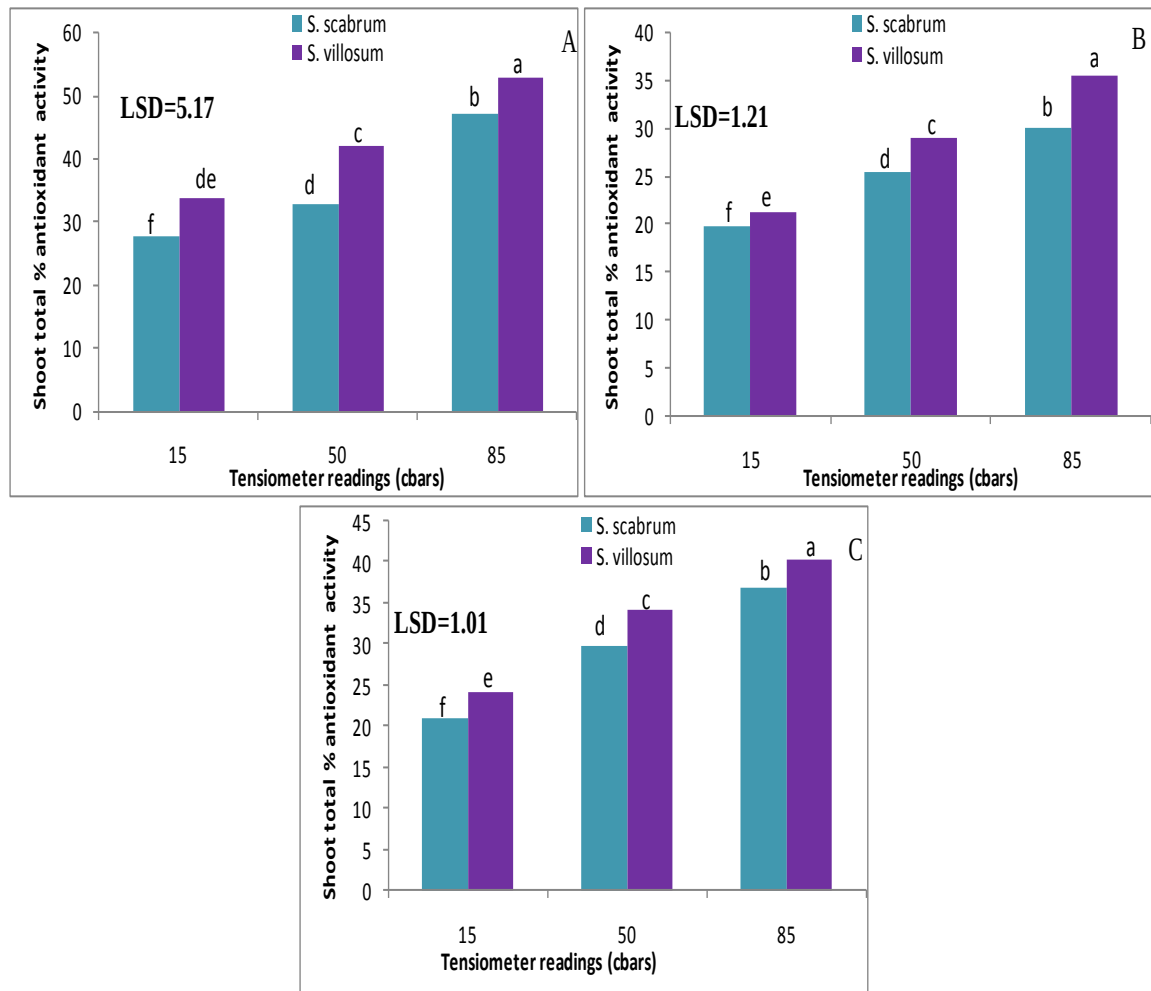


Figure 4.16: Effects of water stress on the shoot total antioxidant activity in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$)

S. villosum had the highest total antioxidant activity in the leaves at 52.68%, while *S. scabrum* had the highest concentration of the same in the roots at 27.18% (Figure 4.17).

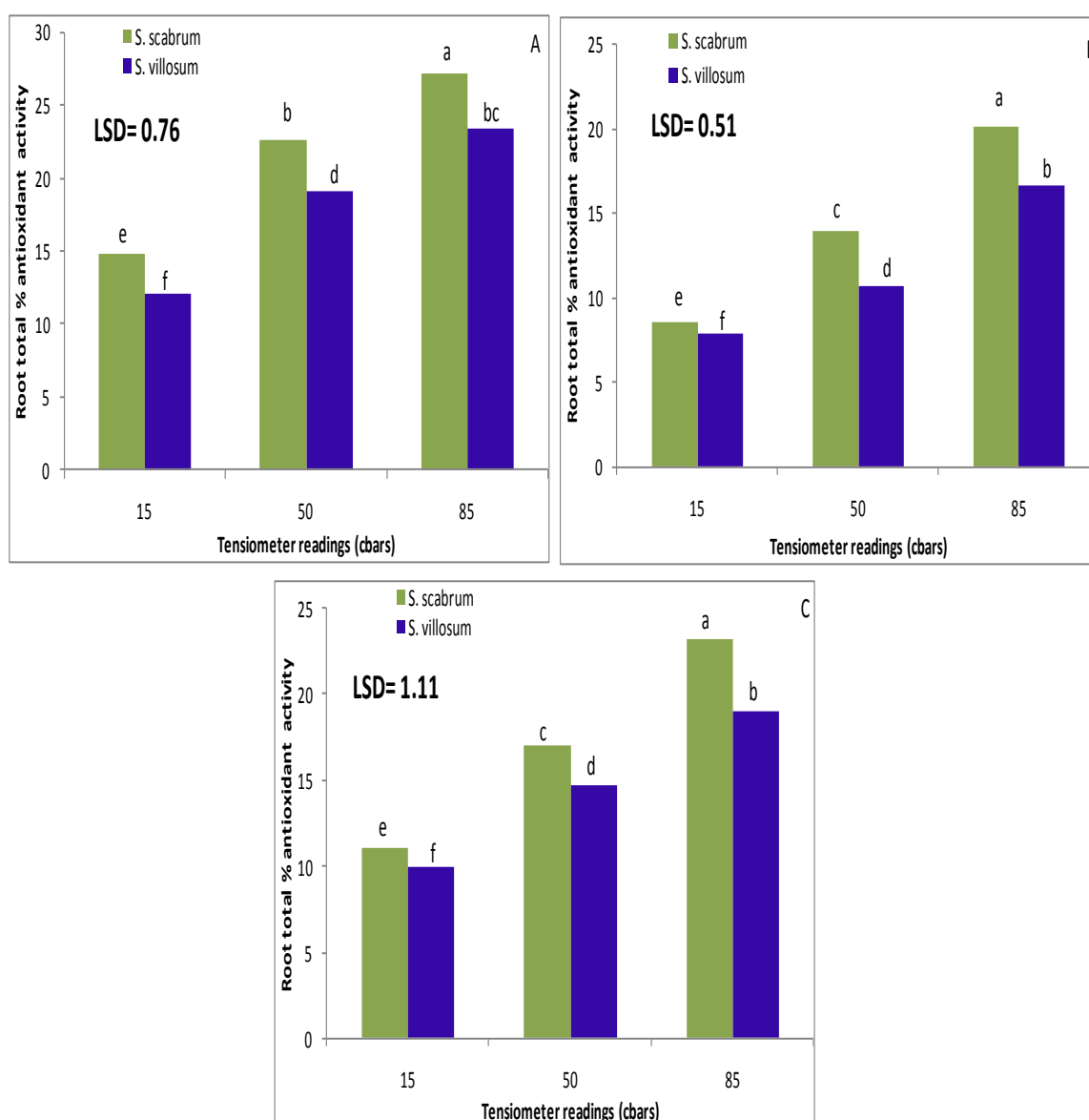


Figure 4.17: Effect of water stress on the root total antioxidant activity in (A) greenhouse, (B) long rains and (C) short rains. Bars with different letters were significantly different ($P \leq 0.05$)

Changes in the antioxidant capacity of water stressed plants paralleled those in the total phenolic compounds. The changes in antioxidant capacity of nightshade plants were roughly reflective of the changes in the total phenolic content. At 50 cbars, the total antioxidant activity in the roots among the two species had an increasing order of *Solanum scabrum* greater than *Solanum villosum*, while in the leaves the order was

Solanum villosum > *Solanum scabrum*. The lowest levels of total antioxidant activity were recorded in the plants irrigated at 15 cbars. This shows that increase in water stress led to increase in phenolic content in the species grown. The differential in concentration of total antioxidant activity depended on nightshade species grown, which determines their differential in partitioning as in total phenolic content.

Table 4.10: Interactions between irrigation interval and variety on the total shoot and root antioxidant activity

Variety	Tensiometer readings (cbars)	Green house		Long rainy season		Short rainy season	
		STAA	RTAA	STAA	RTAA	STAA	RTAA
<i>Solanum scabrum</i>	15	27.7 ^f	14.84 ^f	19.83 ^f	8.56 ^e	21.1 ^f	11.05 ^e
	50	32.66 ^{de}	22.59 ^{bc}	25.41 ^d	13.96 ^c	29.89 ^d	17.01 ^c
	85	47.04 ^b	27.18 ^a	30.14 ^b	20.17 ^a	36.87 ^b	23.22 ^a
<i>Solanum villosum</i>	15	33.64 ^d	12.06 ^e	21.34 ^e	7.83 ^f	24.19 ^e	9.95 ^f
	50	41.87 ^{bc}	19.11 ^d	28.95 ^c	10.65 ^d	34.22 ^c	14.74 ^d
	85	52.68 ^a	23.35 ^b	35.6 ^a	16.62 ^b	40.35 ^a	19.04 ^b
LSD		5.17	0.76	1.21	0.51	1.01	1.11
T X V		*	*	*	*	*	*

*Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$. (STAA- shoot total antioxidant activity, RTAA- root total antioxidant activity)

Drought affects not only water relations, but also induces stomatal closure and decreases the photosynthetic rate and growth. Closure of stomata decreases CO_2 concentration in leaf mesophyll tissue and results in an accumulation of NADPH. Under such conditions, where NADP is a limiting factor, oxygen acts as an alternate acceptor of electrons from the thylakoid electron transport chain, resulting in the formation of superoxide radical (O_2^-) (Cadenas, 1989). Superoxide radical and its reduction product H_2O_2 are potentially toxic compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic hydroxyl radical (OH^-) (Sairam *et al.*, 1998).

Under optimal conditions leaves are rich in antioxidant enzymes and metabolites and can cope with reactive oxygen species (ROS), thus minimizing oxidative damage. A large number of reports deal with the deleterious effects of ROS, whose production is stimulated under water stress conditions (Malenčić *et al.*, 2000, Blokhina *et al.*, 2003, Foyer and Noctor, 2005). ROS cause lipid peroxidation and consequently membrane injuries, protein degradation, enzyme inactivation (Sairam *et al.*, 2005), thus induce oxidative stress. Tolerant genotypes, therefore, should not only be able to retain sufficient water under drought, but should also have a highly active system to protect against oxidative injury, and *Solanum villosum* exhibits more of this than *Solanum scabrum*. Plants possess several tissue antioxidant enzymes for protection against ROS, as superoxide dismutase (SOD), ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX), reductase (GR) and catalase (CAT). These enzymes either quench toxic compounds or regenerate antioxidants with the help of reducing power provided by photosynthesis (Zacchini *et al.*, 2003).

During drought conditions high activities of antioxidant enzymes are associated with lower levels of lipid peroxidation, being related to drought tolerance (Bowler *et al.*, 1992). An increased metabolic capacity of these enzymes may be part of a general antioxidative system in plants involving regulation of protein synthesis or gene expression (Foyer *et al.*, 1994, Scandalios *et al.*, 1997). Low-molecular weight antioxidants are presented by carotenoids, tocopherols, glutathione and ascorbic acid. Apart from their obvious role as enzyme substrates, they can react chemically with almost all forms of ROS. Among substances able to protect plant cell from oxidative attack, a specific role of polyamines in preventing photooxidative damages is reported

(Tadolini, 1988; Løvaas, 1997). Genotypes of the same species respond differentially to environmental stresses and oxidative injury, as a result of genetic based differences in their antioxidant systems as in *Solanum villosum* and *Solanum scabrum*. That provides an important tool to have an insight into the physiological mechanisms operative in stress tolerant genotypes (Sairam *et al.*, 1998).

According to Foyer *et al.*, (1997) much of the injuries caused by exposure to biotic and abiotic stresses are associated with oxidative damage at a cellular level, the chloroplasts being an important site of H₂O₂ generation. Zlatev *et al.*, (2005) established that, at the end of drought period, an increased H₂O₂, and OH⁻ production was observed in young bean plants, therefore revealing a state of oxidative stress in cells. H₂O₂ is a strong oxidant produced mainly as a result of scavenging of superoxide radical, and its higher concentration is injurious to cells, resulting in a localized oxidative damage, lipid peroxidation, and disruption of metabolic function and losses of cellular integrity at sites where it accumulates (Menconi *et al.*, 1995; Velikova *et al.*, 2000).

It is well known that H₂O₂, similar to glutathione, has multi-functional interactive roles in the early stages of plant stress response. H₂O₂ can diffuse to relatively long distances, causing changes in the redox status to surrounding cells and tissues where, at relatively low concentrations, may trigger an antioxidative response (Foyer *et al.*, 1997). Rather than just the scavenging capacity, a fine-tuning of H₂O₂ levels is essential for an efficient control. The rationale of this assumption is that H₂O₂, whilst deleterious to some cellular components, is essential to plants in various biosynthetic reactions and, as suggested by some authors, possibly also in signal transduction pathways, which could contribute to plant defense (Schreck and Baeuerle, 1991). In fact, the drought induced

production of H_2O_2 in the mesophyll cells may be associated with changes in the cell wall structure (Scandalios *et al.*, 1997). Furthermore, H_2O_2 is necessary for the peroxidase-mediated oxidative polymerization of cinnamil alcohols to form lignin, and several enzymatic systems have been proposed as responsible for hydrogen peroxide production, on the surface of plant cells (Lütje *et al.*, 2000).

It may be therefore suggested that the increased level of H_2O_2 observed by many authors in the drought treated plants is due to oxidative damages, but eventually may also have a signal function. H_2O_2 , OH^- and other ROS can be expected to be responsible for the lipid peroxidation (Zacchini *et al.*, 2003). As reported by Sgherri and Navari-Izzo, (1995), the increase in the activity of scavenging enzymes could be due either to an adaptive change in catalytic properties or to the transcription of the corresponding silent genes. This could be related to enhanced levels of free radicals or other ROS in plant cells and correlate with a temporal coordination of the production of H_2O_2 via SOD and destruction of this peroxide by APOX and CAT. Such coordinated responses are believed to promote plant tolerance to oxidative stress (Foyer *et al.*, 1994). It is also possible that increased SOD activity could alter the expression of other metabolic processes associated with water stress. Gupta *et al.*, (1993) have demonstrated that enhanced activity of Cu, Zn SOD in transgenic plants was associated with increased activity of APOX. Some other authors also reported an increase in SOD activity in plants under oxidative stress (Gupta *et al.*, 1993; Zlatev *et al.*, 2005).

It appears that relative tolerance of plant genotypes, as reflected by its lower lipid peroxidation and higher membrane stability, is related with the levels of its antioxidant enzymes activity. APOX, Cu, Zn-SOD and CAT are involved in overcoming of

oxidative stress. The increased activities of antioxidant enzymes act as a damage control system and, thus, provide protection from oxidative stress, resulting in lower LPO and higher membrane stability in tolerant genotypes. The literature analyzed in this review complexity of tolerance of plants to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its acclimation to changes in environmental conditions is a first essential step in stress avoidance (Yordanov *et al.*, 2000). The wider the range of adaptation capacity of plants, the better they are protected against various stresses. The changes in program of plant development are always associated with changes in their physiological and biochemical program and activity.

In spite of intensive investigation of the problem of water deficit tolerance, many of its aspect remain to be explored. Water deficit induces expression of particular genes and this is associated in most cases with adaptive responses of stressed plants. The functions of many of them are still not established. Similar results were obtained by Myung min oh, (2010) on lettuce seedlings subjected to different water stress levels.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

African nightshades are widely distributed in Kisii County, however in Siaya County, the production is mainly along streams and rivers as in Yala swamp. The plant total phenolic content and total antioxidant activity distribution is species specific. There is increasing demand of African nightshades in both the counties.

Water stress significantly affected the growth parameters of African nightshades differentially, the effect was more pronounced in *S. scabrum* than *S. villosum*, where water stress reduced the number of secondary buds, leaf area; shoot height, which consequently reduced biomass production. Irrigation at 15 cbars gave the best results with the yields harvest is of importance; irrigation should be carried out at 15 cbars.

Shoot and root total phenolic content and total antioxidant activity increase with increasing soil water tension in both *S. scabrum* and *S. villosum*. But there was more accumulation of both phenolics and antioxidants in the shoots of *S. villosum* than *S. scabrum* while these compounds were significantly higher in roots of *S. scabrum* than *S. villosum*.

5.3 Recommendations

- The study dwelt majorly on two counties- a study where mapping of many counties with very contrasting agroecologies should be considered to give a more conclusive report. However, African nightshades grown as source of the vital antioxidants should be watered at 50 cbars but for more accumulations irrigation at 85 cbars interval is recommended. It should however be noted that a

tradeoff between yield and phytochemicals (phenolic and antioxidant activity) would be achieved when *S. scabrum* and *S villosum* is given at between 15 and 50 cbars intervals. Consequently, irrigation with water regimes between 15cbars and 50cbars should be recommended to farmers grown African Nightshade so as to benefit from the shoot total phenolics and antioxidants for the purposes nutraceutical attributes without compromising on leafy yield.

- A further experiment may be considered to include profiling all types of phenolics and antioxidants instead of shoot total phenolics and antioxidants.

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APPENDICES

Appendix 1 Nightshade accessions, production and packaging

*S.florulentum**S.villosum**S.scabrum*

Production



Seed source



Transplants

Packaging and transport



Sorting and packaging



Packed nightshade ready for sale

Appendix 2; Field experiment and laboratory work



Effects of water stress at T2- 50 cbars and T1- 15 cbars(R-replication and V2- *S. villosum*).

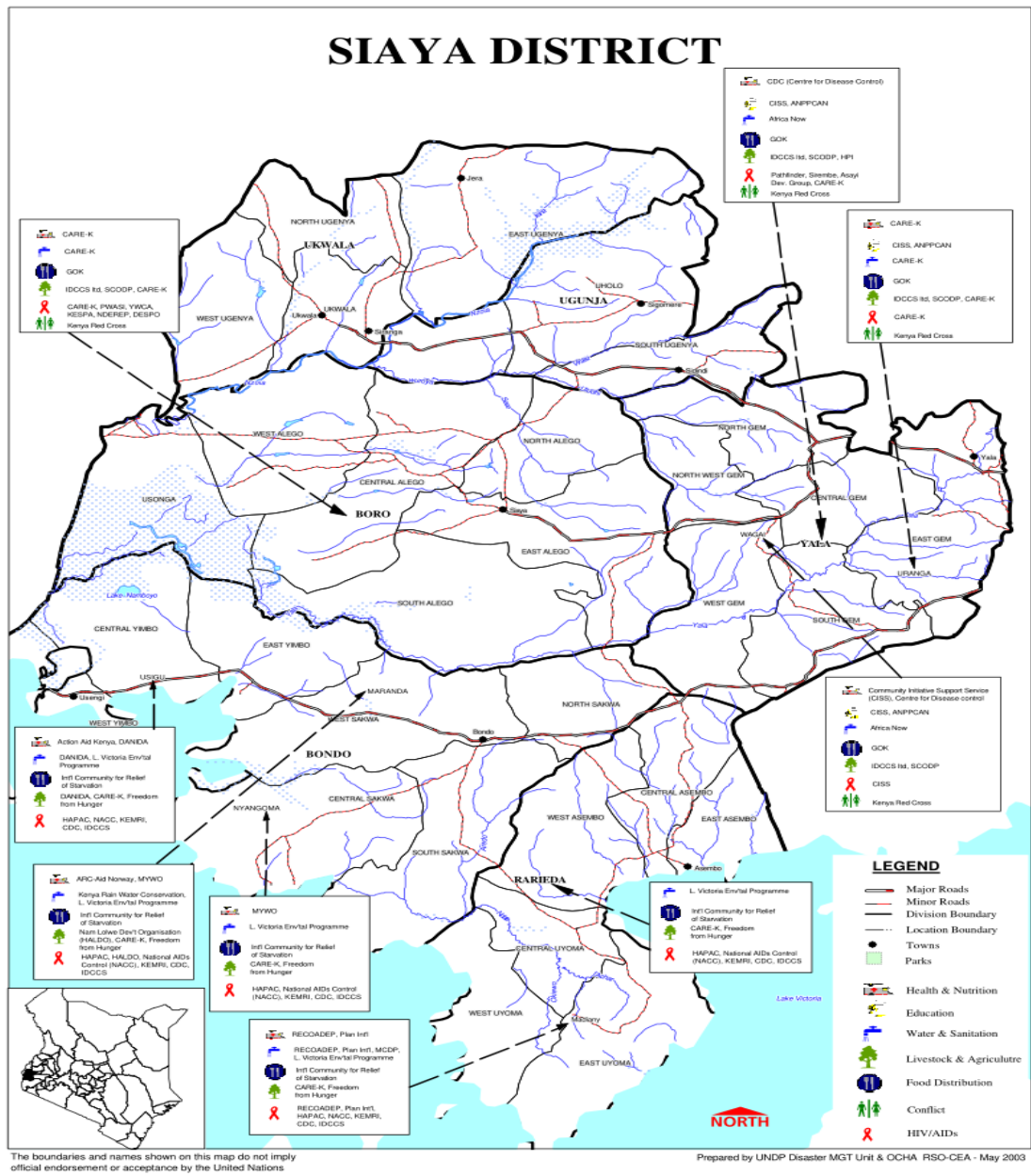


The greenhouse experiment



Laboratory analysis (shoots and root methanol extracts)

Appendix 3; Siaya map



Siaya County, formerly Siaya District (source: google/maplandia/Siaya county map)

Appendix 4: Kisii Map



Kisii County, formerly Kisii District (source: [google/maplandia/Kisii county map](https://www.google.com/maps/@0.0,0.0,15z/data=!3m1!1e3!3m2!1sKisii+County+Kenya))

Appendix 5: Mapping

COUNTY	Latitude	Longitude	Antioxidant	TPC	Dominant variety
Kisii	-0.69898	34.770521	50.00	37.72	<i>S. nigrum</i>
	-0.70229	34.770809	49.00	32.02	<i>S. nigrum</i>
	-0.70311	34.772456	46.00	26.78	<i>S. villosum</i>
	-0.70314	34.772578	46.33	26.32	<i>S. villosum</i>
	-0.70321	34.772764	40.00	25.85	<i>S. americanum</i>
	-0.70319	34.773551	38.00	25.52	<i>S. villosum</i>
	-0.70411	34.775968	35.00	22.09	<i>S. nigrum</i>
	-0.70411	34.775968	32.00	20.12	<i>S. florulentum</i>
	-0.6998	34.774997	30.00	24.78	<i>S. scabrum</i>
	-0.69979	34.774991	46.00	26.09	<i>S. scabrum</i>
	-0.69908	34.775016	45.67	29.45	<i>S. scabrum</i>
	-0.69843	34.775319	37.00	21.32	<i>S. villosum</i>
	-0.69747	34.780134	39.00	23.64	<i>S. villosum</i>
	-0.6991	34.781768	32.00	19.67	<i>S. villosum</i>
	-0.69997	34.790793	30.00	19.25	<i>S. scabrum</i>
	-0.70239	34.796039	31.00	20.51	<i>S. scabrum</i>
	-0.69868	34.798067	25.00	16.87	<i>S. villosum</i>
	-0.69671	34.798183	24.00	3.57	<i>S. grossidentatum</i>
	-0.6956	34.797958	54.00	23.89	<i>S. scabrum</i>
	-0.69435	34.796022	37.00	15.43	<i>S. scabrum</i>
	-0.69365	34.791745	38.00	19.67	<i>S. villosum</i>
	-0.6996	34.769943	30.00	17.81	<i>S. villosum</i>
	-0.70553	34.769472	30.00	16.82	<i>S. nigrum</i>
	-0.70449	34.768924	60.00	38.02	<i>S. villosum</i>
	-0.70002	34.769298	61.00	38.23	<i>S. villosum</i>
	-0.69985	34.769351	55.00	23.71	<i>S. scabrum</i>
Siaya	0.044851	34.141597	50.00	26.58	<i>S. nigrum</i>
	0.045412	34.140683	50.33	24.67	<i>S. americanum</i>
	0.045412	34.140684	42.00	26.34	<i>S. florulentum</i>
	0.045435	34.140704	45.00	23.57	<i>S. nigrum</i>
	0.045459	34.140819	43.67	20.43	<i>S. scabrum</i>
	0.045713	34.140801	56.00	27.84	<i>S. americanum</i>
	0.045702	34.140403	55.00	31.71	<i>S. villosum</i>
	0.045495	34.140143	46.00	22.78	<i>S. grossidentatum</i>
	0.045456	34.139745	40.00	19.34	<i>S. scabrum</i>
	0.042156	34.142002	35.00	15.89	<i>S. scabrum</i>
	0.043102	34.142256	33.00	16.45	<i>S. villosum</i>
	0.043829	34.141999	45.00	29.65	<i>S. villosum</i>
	0.043963	34.142319	33.97	17.32	<i>S. nigrum</i>

	0.044133	34.142423	28.93	14.35	<i>S. scabrum</i>
	0.044397	34.142282	31.33	15.03	<i>S. villosum</i>
	0.044389	34.142282	21.12	8.23	<i>S. scabrum</i>
	0.044338	34.142667	16.00	7.46	<i>S. tarderemotum</i>
	0.044081	34.142978	27.00	15.52	<i>S. nigrum</i>
	0.044497	34.144385	34.76	19.24	<i>S. florulentum</i>
	0.044904	34.147324	23.33	9.87	<i>S. scabrum</i>
	0.045052	34.148454	23.00	9.33	<i>S. scabrum</i>
	0.00058	34.137433	17.00	6.56	<i>S. scabrum</i>
	0.0027	34.135352	24.00	13.89	<i>S. scabrum</i>
	0.004051	34.13441	28.67	15.03	<i>S. villosum</i>
	0.004051	34.134409	42.00	21.07	<i>S. villosum</i>
	0.062286	34.404454	33.20	14.46	<i>S. scabrum</i>
	0.063495	34.4041	15.60	8.87	<i>S. villosum</i>
	0.065423	34.404598	45.20	25.64	<i>S. grossidentatum</i>
	0.058982	34.408627	43.00	22.73	<i>S. scabrum</i>
	0.051661	34.420602	45.00	28.03	<i>S. tarderemotum</i>
	0.051837	34.42054	36.00	24.56	<i>S. tarderemotum</i>
	0.049077	34.418801	27.00	14.55	<i>S. villosum</i>
	0.050389	34.400216	32.00	15.54	<i>S. americanum</i>
	0.050606	34.400049	21.45	10.01	<i>S. scabrum</i>
	0.044173	34.393858	17.50	9.87	<i>S. nigrum</i>
	0.049336	34.40264	34.20	19.67	<i>S. florulentum</i>
	0.051087	34.399941	43.25	21.95	<i>S. americanum</i>

Appendix 6: Weather data

Kisii County

2013						2014				
Month	R.A	temp		R.H		R.A	temp		R.H	
		Max	min	at06z	at12z		max	min	at06z	at12z
January	69.3	26.1	15.9	49	31	6.4	27.9	16.1	78	52
February	60.9	27.8	15.9	52	33	70.4	28.7	16.2	69	40
March	272.0	27.1	16.2	59	37	143.8	28.3	16.1	65	70
April	399.6	25.1	15.4	70	66	327.6	24.9	15.8	76	63
May	205.9	25.4	15.1	74	70	238	24.8	15.8	72	58
June	105.1	25.4	14.9	74	62	254.8	24.4	15.2	63	51
July	105.0	25.6	14.7	70	56	89	24.4	15.4	66	59
August	143.6	24.9	14.4	62	52	177.8	24.7	15.3	62	55
September	262.8	25.9	15.2	57	47	225.2	25.6	15.4	73	61
October	151.0	26.4	15.0	63	62	207.1	25.9	15.8	79	66
November	224.2	25.2	14.9	69	70					
December	125.7	25.4	15.2	74	34					

Siaya County

2013						2014				
Month	R.A	temp		R.H		R.A	temp		R.H	
		max	min	at06z	at12z		max	min	at06z	at12z
January	54.2	29.8	17.3	69	5	0	31.8	16.3	50	35
February	31.3	31.8	17.3	61	4	47.5	31.9	17.7	51	31
March	238.4	30.9	18.7	73	47	94.7	31.4	18.4	59	42
April	203.0	28.8	18.6	79	57	308.9	27.5	18.7	77	57
May	99.6	28.7	17.9	81	44	131.2	27	18	75	57
June	63.7	28.9	17.8	56	32	98.3	27.8	17.7	71	56
July	35.9	29.9	16.8	76	56	37	27.5	17.4	72	5
August	93.0	28.8	17.3	72	17	98.2	28.8	17.3	64	5
September	151.0	30.2	17.5	67	5	258.3	26	12.4	77	5
October	77.9	30.3	18.2	69	5	101.2	30.7	18	77	5
November	102.1	29.8	18.0	65	7					
December	135.0	29.1	18.0	69	50					

Data source: Kenya metrological department, Nairobi.

Appendices 7: Pearson Correlation tables

Table1; Pearson Correlation Coefficients for greenhouse. N=24

GREENHOUSE												
	Trt.	S.F. Wt.	S. D.Wt.	R.F.Wt.	R.D.Wt.	S. height	Sec. buds	LArea	LTPC	RTPC	LTAA	RTAA
Trt.	1	-0.5341	-0.4791	-0.5772	-0.5943	-0.6693	-0.506	-0.3068	0.6483	0.5162	0.5694	0.541
S.F. Wt.	—	1	0.9601	0.9607	0.9224	0.9079	0.4484	0.8667	-0.1105	0.3266	-0.8806	0.1761
S. D.Wt.	—	—	1	0.9379	0.8762	0.831	0.2638	0.9476	-0.1757	0.3048	-0.1568	0.1312
R.F.Wt.	—	—	—	1	0.9611	0.8777	0.4758	0.8325	-0.1367	0.2046	-0.1326	0.0763
R.D.Wt.	—	—	—	—	1	0.8708	0.5026	0.7721	-0.1469	0.1891	-0.1126	0.0789
S. height	—	—	—	—	—	1	0.7327	0.6775	-0.024	0.2661	0.0452	0.2098
Sec. buds	—	—	—	—	—	—	1	0.0656	0.2921	0.1669	0.3559	0.2678
LArea	—	—	—	—	—	—	—	1	-0.1433	0.3616	-0.1411	0.1789
LTPC	—	—	—	—	—	—	—	—	1	0.7742	0.9619	0.8731
RTPC	—	—	—	—	—	—	—	—	—	1	0.7833	0.9539
LTAA	—	—	—	—	—	—	—	—	—	—	1	0.8904
RTAA	—	—	—	—	—	—	—	—	—	—	—	1

(Trt- treatment, S.F.wt- shoot fresh weight, S.D. Wt- shoot dry weight, S.height- shoot height, Sec. buds- secondary buds, L.Area- Leaf area, LTPC- leaf total phenolic content, RTPC- root total phenolic content LTAA- leaf total antioxidant activity, and RTAA- root total antioxidant activity)

Table2; Pearson Correlation Coefficients for season one. N=24

SEASON 1												
	Trt.	S.F. Wt.	S. D.Wt.	R.F.Wt.	R.D.Wt.	S. height	Sec. buds	LArea	LTPC	RTPC	LTAA	RTAA
Trt.	1	-0.6062	-0.07252	-0.6701	-0.6285	-0.6872	-0.556	-0.4355	0.5244	0.6137	0.4961	0.209
S.F. Wt.	—	1	0.9694	0.9247	0.9012	0.8992	0.5598	0.7946	-0.4622	-0.4015	-0.5121	0.4678
S. D.Wt.	—	—	1	0.9457	0.9366	0.9366	0.6449	0.734	-0.4945	-0.4412	-0.5347	0.3584
R.F.Wt.	—	—	—	1	0.8833	0.9792	0.6887	0.7093	-0.4938	-0.3919	-0.5641	0.5143
R.D.Wt.	—	—	—	—	1	0.9743	0.6887	0.7093	-0.4938	-0.3919	-0.5641	0.5143
S. height	—	—	—	—	—	1	0.7026	0.7331	-0.4697	-0.4010	-0.5322	0.4371
Sec. buds	—	—	—	—	—	—	1	0.1074	-0.1956	-0.3909	-0.2446	0.2548
LArea	—	—	—	—	—	—	—	1	-0.4789	-0.2774	-0.5133	0.4279
LTPC	—	—	—	—	—	—	—	—	1	0.8718	-0.9772	-0.3909
RTPC	—	—	—	—	—	—	—	—	—	1	0.8485	-0.2534
LTAA	—	—	—	—	—	—	—	—	—	—	1	-0.4939
RTAA	—	—	—	—	—	—	—	—	—	—	—	1

(Trt- treatment, S.F.wt- shoot fresh weight, S.D. Wt- shoot dry weight, S.height- shoot height, Sec. buds- secondary buds, L.Area- Leaf area, LTPC- leaf total phenolic content, RTPC- root total phenolic content LTAA- leaf total antioxidant activity, and RTAA- root total antioxidant activity).

Table3; Pearson Correlation Coefficients for season two. N=24

SEASON 2												
	Trt.	S.F. Wt.	S. D.Wt.	R.F.Wt.	R.D.Wt.	S. height	Sec. buds	LArea	LTPC	R.TPC	LTAA	R.TAA
Trt.	1	-0.489	-0.6091	-0.5055	-0.5594	-0.566	-0.3771	-0.3031	0.5077	0.4803	0.4368	0.0089
S.F. Wt.	—	1	0.9275	0.9173	0.9003	0.77909	-0.1384	0.8336	-0.4844	-0.3164	-0.4999	0.6022
S. D.Wt.	—	—	1	0.9383	0.9302	0.9344	0.1864	0.763	-0.474	-0.3154	-0.5008	0.5802
R.F.Wt.	—	—	—	1	0.9249	0.8745	0.1174	0.8052	-0.4467	-0.2577	-0.4734	0.7094
R.D.Wt.	—	—	—	—	1	0.9364	0.2472	0.7215	-0.5009	-0.4052	-0.5337	0.7547
S. height	—	—	—	—	—	1	0.4584	0.6201	-0.4546	-0.3445	-0.4975	0.6825
Sec. buds	—	—	—	—	—	—	1	-0.178	-0.1025	-0.1796	-0.1238	0.173
LArea	—	—	—	—	—	—	—	1	-0.4225	-0.2666	-0.4466	0.5872
LTPC	—	—	—	—	—	—	—	—	1	0.8772	0.9713	-0.1642
R.TPC	—	—	—	—	—	—	—	—	—	1	0.8846	-0.0852
LTAA	—	—	—	—	—	—	—	—	—	—	1	-0.241
R.TAA	—	—	—	—	—	—	—	—	—	—	—	1

(Trt- treatment, S.F.wt- shoot fresh weight, S.D. Wt- shoot dry weight, S.height- shoot height, Sec. buds- secondary buds, L.Area- Leaf area, LTPC- leaf total phenolic content, RTPC- root total phenolic content LTAA- leaf total antioxidant activity, and RTAA- root total antioxidant activity)

Appendix 8: Questionnaires

Study Questionnaire for farmers

Objectives of the questionnaire

1. To find out the irrigation systems employed in the production of African nightshades in the three different counties.
2. To find out the different nightshade accessions being grown by farmers

Interview data (to be filled – in by the interviewer)

Name of interviewer	
Duration of the interview (min)	
Interview date	
Interviewee gender	Male ☐ Female ☐

Personal details (farmer)

Farmers name (optional):

Age: 20-30 (1) 30-40 (2) 40-50 (3) 50-60 (4) 60-100 (5)

County: _____

GPS coordinates: _____ Education level: primary-1, secondary-2, college-3, none-4

INFORMATION FROM FARMERS

A1. Which different African nightshades (ANS) accessions do you produce in your farm?

Improved-1, traditional-2

A2.What is your scale of production of ANS? Subsistence-1, large scale-2

A3.Is there an irrigation system developed for the production of ANS? Yes-1, No-2

A3.1.If YES how regularly do you irrigate and why? Everyday-1, twice a week-2, everyweek-3, none-4

A3.2. If NO, why?

A4.Do you depend on rainfall for ANS production? Yes-1, No-2

A5.Does rainfall seasonality affects availability of ANS? Yes-1, No-2

A5.1. If NO, why?

A6.Does rainfall availability affects the taste of ANS? Yes-1, No-2

A6.1. If YES, in what way?

A7.How long does the different accessions take to mature? 0-1(1), 1-2(2), 2-3(3)

A8.Which accessions are most grown and

why_____

—

A9.How many acres have you set aside for ANS production?

A10.For how long have you been involved in ANS production? 2005-2010 (1), 2010-2015 (2)

A11.Do you produce other vegetables apart from ANS? Yes-1, No-2

A11.1.If YES which ones; _____

A11.2.If YES which vegetables gives you higher returns; _____

A12. What prompted you to start nightshade production in your farm

Health benefits-1, consumer demand-2, high returns-3, drought resistance-4

Study Questionnaire for traders

Objectives of the questionnaire

To find out the different nightshade accessions sold in the selected agro-ecologies

To determine consumer preference of the marketed nightshade accessions.

Sampling is to be done on the spot. The enumerator is to do a physical count with an informer. Should include those who are always there but may be absent at the moment)

Total traders: _____ Sample size: _____

Interview data (to be filled – in by the interviewer)

Name of interviewer	
Duration of the interview (min)	
Interview date	
Interviewee gender	Male ☐ Female ☐

Personal details (Trader)

Traders name (optional):

Age: 20-30 (1) 30-40 (2) 40-50 (3) 50-60 (4) 60-100 (5)

County: _____

Market name: _____ GPS coordinates: _____

Education level: Primary-1, Secondary-2, College-3, None-4

INFORMATION FROM TRADERS

B1. Do you know when African nightshades (ANS) were introduced in this market?

1 = Yes, 2 = No

B1.1. If yes, which year? _____

B2. Which ANS accessions were the first to be traded in this market? Traditional-1, improved-2

B3. When did you yourself start trading in ANS? ----- (Year)

B4. What made you start? High income-1, consumer demand-2

B5. What specific role do you play in the market? []

1= Producer wholesaler 2= Producer retailer

3= Wholesaler only [1st Level] 4= Wholesaler only [2nd Level]

5= Retailer only

B6. How would you describe your mode of operation? _____

1= Mobile trader (State markets) _____

2= Permanent in this market

3=Occasional trader (**specify**) _____

B7. Where did you get most of your supplies of ANS?

Species of ANS	Type (within the species)	Source (code and name)	Distance to the Market

Code for source

1= from own farm 2= from other farms (including harvesting

3= from collection centers in the farming area (farmer groups)

4= from same market (wholesalers)

5= from different market(s) (specify _____)

If B7 for source is from own farm, ask the following, otherwise skip to B10

Producer traders

B8. Do you grow ANS that you do not sell? 1 = Yes, 2 = No

B8.1. If YES, What is the total acreage of your farm? _____ Acres

B8.1.1. Acreage under crops _____

B8.1.2. Acreage under ANS _____

B9. When did you start growing ANS? _____ (Year)

Area in acres

Accession Grown	Type within the Species	2014	2013	2012
		Area in acres	Area in acres	Area in acres

B10. do you source for ANS from elsewhere apart from your own production? Yes-1,
No-2

B10.1. If YES, from where?

B11. Do you have visits from extension officers or advisory persons?
1=Yes___2=No_____

B11.1. If yes, where do they come from? _____

B11.2. If yes, how many times in a year?. _____

B12 Has extension services provided you with any information concerning improved
species of ANS? 1=Yes___2= No_____

B13. On average PER DAY, which species of ANS have you been trading in (state the
quantities and selling prices in Kshs over time?

B14. If the species traded are more than one, why do you deal with the combination?

B15. Which of your ANS types sell fast? (**Rank them, from the fastest to the slowest**)
Improved-1, Wild-2, All-3

B16. How did you know that there was a market for the ANS? _____

B17. What did you do initially in order to arrive at the initial buying and/or selling
prices?

When buying _____

When selling _____

B18. What have you done or do you do when you want to get market information for
better prices of ANS or increase your sales? _____

Income share

B19. Do you market other farm produce apart from the ANS? [] 1= Yes; 2= No

(If yes, *CONTINUE*, if no skip to F14)

F19.1. Which ones? LIST

F19.2. What are the reasons for combining different produce the way you do? _____

20. Which of your produce (ANS, exotic vegetables and other farm produce) sell fast?
(**Rank them, from the fastest to the slowest**)

B21. What is the daily NET profit from your marketing activities? 1-200(1), 200-400(2), 400-600(3), 600-1000(4)

B22. What proportion of your net profit do the ANS contribute? _____

Help or Influence

B23. Have you been helped or influenced by any institution, organization, or individual in the marketing of ANS? 1= Yes 2= No

If yes: answer the following, if NO skip to G3

Source of Influence	When (Year)	Form of influence (check codes)

Codes for form of influence

1= BDS (Business Development Services) 2= Credit

3= Linkup with traders' organization 4= Locating source of supply

5= Transportation 6= Market information

7=Other(Specify)

B24. Which form of support do you consider most beneficial to you?

Financial-1, Extension service-2, Research-3

B25. Do you experience any constraints in marketing ANS? [] 1= Yes, 2= No

B26. If yes, which ones? (Rank them with the most important first)

1_____

2_____

3_____

B27. Does rainfall seasonality affect ANS availability in this market? Yes-1, No-2

27.1. If NO, why? _____