EFFECTS OF PHOSPHORUS DEFICIENCY ON SECONDARY METABOLITES AND DISTRIBUTION OF AFRICAN NIGHTSHADE IN SIAYA AND KISII COUNTIES, KENYA

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DECLARATION

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

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DEDICATION

This work is dedicated to my colleague the Late Fabrice Musoni, my mother Margaret Nekesa, my niece Margaret Naluende and my grandmother Mary Mukhwana.

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ABBREVIATIONS AND ACRONYMS

AIDs	Acquired Immune Deficiency Syndrome
AIVs	African Indigenous Vegetables
ALVs	African Leafy Vegetables
AN	African Nightshade
ANs	African Nightshade Species
ANOVA	Analysis of Variance
AOS	Activated Oxygen Species
AST	Agricultural Science and Technology
AVRDC	Asian Vegetable Research Development Center
CBOs	Community Based Organizations
CNBH	Carbon Nutrient Balance Hypothesis
DM	Dry Mass
DPPH	Diphenyl Picryl Hydrazyl
FAO	Food Agricultural Organization
FGDs	Focus Group Discussions
FYM	Farm Yard Manure
GIS	Global Information System
GoK	Government of Kenya
GPS	Global Positioning System
HCDA	Horticultural Crops Development Authority
HIV	Human Immune-deficiency Virus
ITCZ	Inter Tropical Convergent Zone

KARLO	Kenya Agricultural Research and Livestock Organization
LH	Lower Highland
LM	Lower Midland
NEMA	National Environment Management Authority
NGOs	Non Governmental Organizations
NMK	National Museums of Kenya
MoA	Ministry of Agriculture
PCM	Protein Competition Model
PRA	Participatory Rural/Rapid Appraisal
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RUFORUM	Regional University Forum for Capacity Building in Agriculture
SN	*Solanum nigrum*
SS	*Solanum scabrum*
SV	*Solanum villosum*
TAA	Total Antioxidant Activity
TPC	Total Phenolic Content
UM	Upper Midland
UNEP	United Nation Environmental Programme
UNESCO	United Nations Educational Scientific and Cultural Organization
WAT	Weeks After Transplanting
WHO	World Health Organization

ABSTRACT

African Indigenous Vegetables form an integral part of the Kenyan diets, among the most commonly consumed being the African nightshade. These vegetables contain important phenolics that have medicinal values and good health attributes. The abundance of these phenolic substances has strongly been associated with phosphorus use efficiency. In order to investigate the effect of phosphorus stress on African nightshade distribution, a purposive research was done from February 2014 on 70 random farmers growing African nightshade using semi-structured questionnaires in Siaya and Kisii Counties, Kenya. The coordinates, plant and soil samples for the surveyed regions were taken and later on used to map the vegetables according to plant and soil phosphorus, total phenolic content (TPC), total antioxidant activities (TAA) and the two dominant varieties. *Solanum scabrum* had 69% distribution whereas *Solanum villosum* had 21% hence chosen for greenhouse and field experiments. The two varieties were planted in greenhouse and two field cropping seasons (long and short rain seasons) at Kenyatta University farm as split plot arrangements with two varieties being the main plot and phosphorus levels (0, 20, 40 and 60 kg/ha) constituting the subplot in Randomized Complete Block Design. The treatments were replicated four times. Data on plant height, fresh weight, number of secondary buds, leaf and root area was recorded and later the effects resulting from these treatments analysed using ANOVA. In greenhouse and field experiments, plant height, fresh weight, number of secondary buds, leaf and root area, TPC and TAA were significantly ($p \leq 0.05$) affected by the level of phosphorus. TPC and TAA decreased with increase in P, whereas all the growth parameters increased with increase of Phosphorus levels apart from height and weight of plants treated with 60kgP/ha that showed either stagnation or a slight decline. The decline was attributed to zinc and copper deficiency. The highest fresh weight was recorded at 40 kgP/ha, where *Solanum villosum* yielded 6.1t/ha and *Solanum scabrum* yielded 6.35t/ha. *Solanum villosum* had higher TPC and TAA (6.09mg/g and 38.58%) respectively as compared to *Solanum scabrum* that had 5.49mg/g (TPC) and 35.92% (TAA). *Solanum villosum* had more secondary metabolites in the shoots as compared to the roots, the converse was reported for *Solanum scabrum*. Results obtained will be used to educate farmers and extension personnel on ways of improving productivity of African nightshade and will also help develop product labels highlighting their phenolic and antioxidant contents. Farmers are therefore advised to apply phosphorus at the rate 40 kg/ha as it had the highest biomass production. *Solanum villosum* is recommended for soils with limited phosphorus. Further research to be done on other important nutrients for instance anthraquinones and how different levels of macronutrients affect their production.

CHAPTER 1. INTRODUCTION

1.1 Background information

African Indigenous Vegetables form an integral part of the Kenyan diets, among the most commonly consumed being the African nightshade (Mwai and Schipper, 2004). These vegetables contain important phenolics that have medicinal values and good health attributes (Edmonds and Chweya, 1997). Medical evidence increasingly suggests that consumption of a diet rich in phytochemicals has a protective effect against cardiovascular disease and certain forms of cancer (Surch, 2003). They are also an insurance against food insecurity and malnutrition by providing readily available vegetables of high nutritive value which require minimum production input (Mwai and Schipper, 2004). Nutrients contained in African nightshade including proteins, amino acids, vitamins, and fiber; research has shown that secondary metabolites particularly total phenolic content and total antioxidant activities has been reported to be important components in disease prevention (Arts and Hollman, 2005).

Over 60% of the rural populations live below the poverty line, resulting in malnutrition and 1.2 billion of world's population has poor health (GoK, 2012). The poverty situation has been worsened by the high prevalence of HIV/AIDs where 2.5 million Kenyans are infected with about 200,000 new infections per year (GoK, 2012). Kenya is endowed with agricultural biodiversity like African nightshade which could contribute significantly in the management of the HIV/AIDs infected and affected persons (Arts and Hollman, 2005). These vegetables have high micronutrients, medicinal properties and other benefits but have been under utilized for equally along time (Odhiambo and Oluoch, 2010).

Plant phytochemicals can vary widely in their structure and general classification but all share a common feature of containing at least one aromatic ring and one or more hydroxyl groups (Ryans *et al*., 2001). Phytochemical compounds in plants are naturally-occurring antioxidants and their radical scavenging capabilities are thought to play an important function in preventing many chronic illnesses (Rice-Evans *et al*., 1997). Phytochemicals have been shown to inhibit angiogenesis, tumorigenesis, and metastasis (Kohlmeirer *et al*., 1995) and many are known to have antibacterial, antifungal and anti-inflammatory capabilities (Christensen and Brandt, 2006).

Although the nutritional benefits derived from eating phytochemical-rich plant foods are well known, foods and beverages containing the highest phytochemical levels are often lacking or absent in many diets, particularly in Kenya and other developing countries (Beecher, 2003). Therefore, there has been growing interest in developing simple methodologies to increase phenolics and antioxidants concentrations in more commonly consumed plant foods (Schreiner, 2005). This has especially drawn more interesting for the case of the neglected African indigenous leafy vegetables with need to enhancing their overall nutritional value (Kohlmeirer *et al*., 1995).

Phytochemical compounds are produced by plants throughout their development for some certain apparent significance to the plant, among them being: defense against microorganisms, insects, or herbivores (Parr and Bolwell, 2000); nutrient availability (Herms and Mattson, 1992); exposure to ultraviolet radiation (Rozema *et al*., 1997); and because of allelopathic interactions (Mann, 1987). The knowledge on the above mentioned factors can go a long way in enhancing the quantity and quality of the phytochemicals if well researched as this certain contributes to optimal production of these products (too much can also lead to toxicity) without compromising biomass yield (Parr *et al*., 2000).

Availability of key macronutrients during plant growth had significant potential to affect phytochemical accumulation (Parr and Bolwell, 2000). Though N, P, K, and calcium fertilization levels had been shown to affect the production of phytochemical (secondary metabolites) in some plants (Kraus *et al*., 2004), mineral nutrition had little or no effect on phytochemical production in others (Chapin *et al*., 1986). The highest concentration was usually realized when the nutrients are sub-optimal. Other factors such as radiation and water stress too contributed to enhanced production of the secondary metabolites.

This study emphasizes on enhancement of secondary metabolites in light of P status of the soils and thereby finds a trade-off between biomass yield and secondary metabolites, particularly phenolics and related antioxidants. Although P had been previously shown to directly correlate with the growth, yield, and essential oil content of African nightshade (Petersen and Simmonds, 2003), the effect of P availability on the phytochemical composition and antioxidant properties of African nightshade had not yet been determined (Ren *et al*., 2007).

Keeping in view the importance of the valuable indigenous vegetables, the study was undertaken with the aim of evaluating the total phenolic content and total antioxidant activity of leaves and shoot of African nightshade commonly consumed in Kenya through manipulation of P status. The knowledge of the bioactive components of the African nightshade was important in revealing the optimal phenolics and antioxidants contents at different levels of P.

1.2 Problem statement

Food insecurity and malnutrition is an issue of concern in Kenya (GoK, 2009) and other countries in Sub Saharan Africa where rural populations live below the poverty line, resulting in malnutrition and poor health. Agricultural biodiversity like African nightshade in Kenya could contribute significantly in the management of malnutrition and poor health. African nightshades have high micronutrients, medicinal properties and other benefits but it has been under utilized for equally along time.

There has been renewed interest 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). However with sufficient production, there is a growing fear that with the current worldwide rate of fertilizer use, the readily available sources of high grade phosphate rocks maybe depleted within the next 60 to 90 years (Runge-Metzger, 1995). This calls for judicious use of P fertilizers, selection of plants with better P acquisition and use strategies. The current study assessed plant response to P stress effects on the plant distribution based on the secondary metabolites produced. It is apparent that plant secondary metabolites are important antioxidants that are responsible for plant tolerance to these variable environments. These compounds may soon be a major target in vegetable production with the renewed acceptance of nightshade as shown by significance stocking of vegetables in supermarkets (Odhiambo and Oluoch, 2010).

African Leafy Vegetables (ALVs) are African indigenous or traditional vegetables whose leaves, young shoots and flowers are consumed (Maundu *et al*., 1999). These vegetables have been used by communities in Western Kenya for a long time (Grubben and Denton, 2014) though they have been underutilized or neglected for equally long time. There was also a well established and elaborate production to consumption chains of the ANs as compared to any other part in Kenya (Weinberger *et al*., 2011).

In addition, studies indicate that plants produce phenolics and other antioxidants in response to P stress as reported by Wright *et al*. (2010); based on Protein Competition Model (PCM) as well as Carbon: Nutrient Balance Hypothesis (CNBH). These phytochemicals are associated with better and improved health attributes; which is currently priced, especially by the affluent city vegetable consumers (Abukutsa-Onyango, 2007).

In nature, plant species are usually affected by several stress factors at once 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 drought stress. 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 could be good health indices and their quantities need to be documented (Wright *et al*., 2010).

Therefore, the current work assessed African Nightshade Vegetable responses to P on distribution based on the secondary metabolites produced. There are lots of variations of precipitation and P availability hence differentially influencing nightshade distribution. It is apparent that plant secondary metabolites (total phenolic content and total antioxidant activity) are important phytochemicals responsible for plant tolerance to variable environments (Ren *et al*., 2007). The preceding is not equivocal yet these compounds may soon be among our major targets in vegetable production. This work has revealed the significance of P stress and varieties on secondary metabolites accumulation.

Consumers are searching for foods rich in these phenolic compounds and often look for organically produced foods or exotic herbal extracts. But the production practices that directly influence their levels in plants are not well known. A research was therefore necessary to determine the influence of P on the levels of phytochemicals in the African nightshade.

1.3 Justification and Significance of study

This research was aimed at filling existing gaps in scientific knowledge regarding the phytochemical in response to P as a constraint in African nightshades cultivation. It also provides scientific knowledge regarding secondary metabolites for example phenolics in response to phosphorus as biotic stresses as a constraints in African nightshades cultivation. The presence of phenolic compounds in food has garnered much attention in recent years. The primary reason relates to their antioxidant properties and proposed health benefits such as inhibiting certain cancers and promoting cardiovascular health

(Kim *et al*., 1998). This is evident in the increasing use of product labels highlighting their phenolic content (Cohen and Kennedy, 2010). While this marketing strategy may be relatively new, the contribution of phenolics to food quality has been known for some time.

The generated knowledge has given insight into nightshade species plasticity to the above stresses by producing secondary metabolites as a trade-off for yield. Knowledge on biomass per hectare or the phytochemical will be useful in developing guiding principles in vegetable production in segmented Kenyan horticultural market. This research also provides scientific information to small scale vegetable farmers by empowering them with a low cost alternative of P use and efficient technology to earn more income from vegetable nightshade depending on market segments based on phytochemicals and not biomass yield alone hence farmers with small pieces of land would maximize their production by producing African nightshade with high secondary metabolites through the required optimum combination of P.

There is a growing demand for nightshades in supermarkets and there is already a campaign to factor in different communities' tastes (Odhiambo and Oluoch, 2010). These can be achieved through manipulation of external factors such as P; probably depending on species genetic composition. 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. 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 provided insight into factors that influenced phenolic accumulation in plants and what can be done to manipulate the composition of food ingredients.

In western Kenya, soil fertility depletion, particularly of N and P, has been identified as a fundamental root cause of declining crop productivity (Sanchez *et al*., 1997). The need to replenish these nutrients in food production has thus been recognized for a long time (Masinde *et al*., 2009). However, the general belief that traditional vegetables are adapted to low fertility has led to low or no usage of fertilizers on these crops. Furthermore, there is paucity of information on fertilizer recommendations for these vegetables as they have attracted little research attention.

It is only recently that some workers in Kenya have taken keen interest in this research area. Focus has mainly been on N with several workers for instance Masinde *et al*. (2009), Rop *et al*. (2012) and Rao, (1996) demonstrating response to N application in Kenya. However, in many parts of Kenya, P stress is also a major limiting nutrient and response to N and other nutrients is usually inhibited unless P limitations are overcome (Opiyo, 2004). The relative leaf growth rate is one of the most sensitive parameters to P stress and it affects the photosynthetic rate per unit area (Rao, 1996) and hence the yield of vegetables. In this study we are going to take advantage of the P stress; that is among the most common variables constraining agricultural soils and determine at what P levels is the production of phenolics and antioxidants is at its peak so as to enhance labeling.

Further, it is expected that the project empowers farmers by presenting them with an opportunity to identify nightshade cultivars with not only higher biomass yield but also high phytochemical composition in varying phosphorus status. This will contribute to household food and nutrition security. Farmers were engaged in implementation of project activities, including dissemination during the course of research.

1.4 Objectives

1.4.1 General Objective

To enhance secondary metabolites production and distribution of African nightshade in Western Kenya through phosphorus stress manipulation.

1.4.2 Specific Objectives

i. To map out different African nightshade Species in Kisii and Siaya Counties with varying environment in relation to soil phosphorus.

ii. To evaluate the phenolics, antioxidants and yields of African Nightshades from Kisii and Siaya Counties on basis of phosphorus levels.

iii. To determine phenolics and antioxidants partitioning in shoots and roots based on phosphorus stress.

1.5 Research Hypothesis

i. The distribution and richness of African Nightshade varieties is affected by soil phosphorus regimes.

ii. Phosphorus levels differently enhance yield, phenolics and antioxidant production by African Nightshades varieties.

iii. There is variation in retention and release of phenolics and antioxidants between shoot and root as affected by varieties under phosphorus stress.

1.6 Conceptual framework

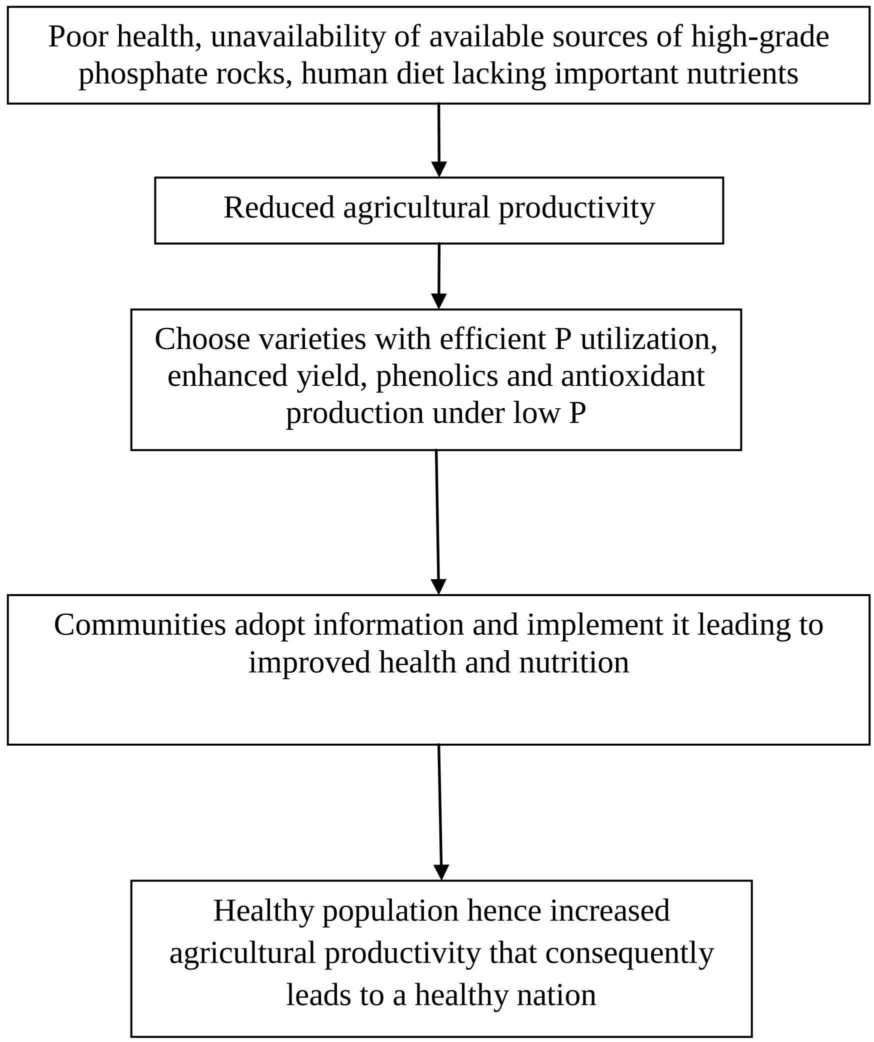

Figure 1.1: Conceptual framework

CHAPTER 2. LITERATURE REVIEW

2.1 African nightshade

Around the world there are over 1000 plant species that are cultivated or harvested from the wild for food (Edmonds and Chweya, 1997). But increasingly, global food security has become dependent on a shrinking basket of selected number of crops (Mwai and Schipper, 2004). Only a few vegetable crops are grown commercially and traded globally and these provide over 50 per cent of all vitamins and calories consumed worldwide (Edmonds and Chweya, 1997). With prices for these vegetable crops having recently doubled or even tripled, it is time to re-focus on the neglected or underutilized vegetable crops that can provide food security and income generation particularly for the poor. This research focuses on the African nightshade that has been overlooked or undervalued but has the potential to provide increased commercial opportunities and increased and/or improved health and nutrition for communities in Kenya and Africa at large (Weinberger *et al*. 2011).

The African nightshades comprise a group of approximately 30 species of genus Solanum including *Solanum villosum fsp* Villosum (Plate 2.2; naming based on National Museum Revised Plant Catalogues), *Solanum villosum fsp* Miniatum and *Solanum scabrum* (Plate 2.1; naming based on National Museum Revised Plant Catalogues) native to North Africa, Europe and West Asia; it is edible and cultivated widely in many regions in Africa (Edmonds and Chweya, 1997). Nightshade is a common name for a diverse group of plants in the family Solanaceae. This family also includes a number of important food crops; including tomato, eggplant and potato (Edmonds and Chweya, 1997).

Plate 2.1*: Solanum scabrum*

Plate 2.2: *Solanum villosum*

African nightshades are some of the most widely consumed traditional leafy vegetables in Africa; where they are important sources of daily nutrition and income for small scale farmers (Edmonds and Chweya, 1997). The leaves contain 87.2 g water, 1.0 mg iron, 4.3 g proteins, 38 K calories, 5.7 g carbohydrates, 1.4 g fibre, 20 mg ascorbic acid, 442 mg calcium, 75 mg P, 3660 μg β-carotene, 0.59 mg riboflavin per 100g fresh weight (K'Opondo *et al*., 2005) and varying concentrations of important phenolics and antioxidants. Consumption, demand and market value of vegetables nightshades have rapidly and steadily risen as consumers become aware of their nutritional, economical and medicinal values (Fontem *et al*., 2004). This study was limited to the broad leaved African nightshade (*Solanum scabrum*) and the narrow leaved (*Solanum villosum*) found in many regions in Africa (Chweya, 1997).

2.2 Origin and diversity of African nightshade

The centre of origin of most nightshade species is South America (Chweya, 1997). The center of diversity for diploid species is South America, and to some extent North America, while the center of origin of most polyploidy species (tetraploids and hexaploids) is either in Europe or Africa (Fontem *et al*., 2004). A large number of distinct taxa in Solanum show their greatest diversity and concentration in the new world tropics, particularly in South America. *Solanum nigrum* L. (Plate 2.3; naming based on National Museum Revised Plant Catalogues), a poisonous nightshade species, is predominantly Eurasian and occurs naturally in South America or Africa (Chweya, 1997).

Plate 2.3: *Solanum nigrum*

Plate 2.4: *Solanum tarderemotum*

Another poisonous species usually mistaken from the edible nightshade is *Atropa belladonna* L. (Deadly nightshade). Edible species such as *S. scabrum* M., *S. villosum* L. and *S. opacum* A. (Plate 2.6; naming based on National Museum Revised Plant Catalogues) are native to Australia. *S. americanum* M. (Plate 2.5; naming based on National Museum Revised Plant Catalogues), *S. tarderemotum* L. (bitter) (Plate 2.4; naming based on National Museum Revised Plant Catalogues) and other species are native to North America and a huge number of taxa occur naturally in South America (Weinberger *et al*., 2011). The most common African representatives of Solanum are also found in Europe and Asia. The most important nightshade species is reported to be *S. scabrum*, it shows considerable diversity in vegetative features and fruits (which are also edible), and it was probably domesticated in Northern Nigeria, where perennial forms still grow wild (Fontem *et al*., 2004).

Plate 2.5: ***Solanum americanum***

Plate 2.6: ***Solanum opacum***

2.3 Importance of African nightshade

In East Africa, nightshades are among the most consumed indigenous plants vegetables (Maundu *et al*., 1999). Despite being rich in vitamins, minerals and trace elements, African nightshade have been increasingly overlooked in preference to cabbages, tomatoes, amongst other more exotic vegetables. And yet, with increasing food prices at local markets, it seems that these indigenous vegetable may yet find their place on plates of rural and urban households (Edmonds and Chweya, 1997).

A diet containing African nightshade rich in secondary metabolites is recommended for malaria patients, people infected with HIV/AIDs and pregnant or nursing mothers (Maundu *et al*., 1999; Mwai and Schippers, 2004). Africans use the leaves of African nightshade to treat duodenal ulcers and stomach upsets boils and swollen glands among other ailments (Edmonds and Chweya, 1997).

A decade ago, these vegetables were mostly growing wild or semi-cultivated. In recent years, the resurgence in popularity has prompted rapid domestication and commercialization of its production, from subsistence to commercialized farming on contract for municipal, urban, super markets and hotel chains (Abukutsa-Onyango, 2007; Mwai and Schippers, 2004; Weinberger *et al*., 2011)

Consumption of African nightshade vegetables in Kenya has experienced rapid shift; in the earlier year times, they were eaten by lower socio-economic groups and hence were grown mainly for subsistence (Onyango *et al.*, 1999) although limited amounts were also sold at the farm level and local markets. However, after it was demonstrated that these vegetables have nutritional and medicinal value (Schippers, 2002), they gained acceptance among the relatively wealthy Kenyans and are now found in the formal markets such as supermarkets and municipal markets where medium and higher socio-economic classes purchase them. This shifting consumer taste for indigenous vegetables therefore offers unique opportunities to diversify farming systems to ensure food security and alleviate poverty, while increasing income and improving human health in Kenya (Schippers, 2002).

Among the African nightshade species, *Solanum scabrum* and *Solanum villosum* have increasingly become important and were the most preferred African nightshade species among the main communities inhabit western Kenya according to a survey conducted (Schippers, 2002). However, the area under its cultivation is still low because it is traditionally produced at subsistence level where it is grown in home gardens around homestead or collected from the wild (Fontem *et al*., 2004). This coupled with the low leaf yields, ranging between 1-3 tons ha^{-1}, have conspired to ensure that consumer demand for the African nightshade is not satisfied (Masinde *et al*., 2009)

The low productivity has been attributed to several factors that include environmental and agronomic constraints and due to these production constraints, plants have evolved adaptive mechanisms to P stress and secondary metabolites production is one such mechanism (Ren *et al*., 2007). There has been reported increase in secondary metabolites concentration under P stress (Ren *et al*., 2007). These products are important components in health fitness and many consumers give emphasis on them; forcing marketers to label the produce (Cohen and Kennedy, 2010).

2.4 Ecological adaption of African nightshade

The worldwide distribution of Solanum could be due to its adaptive ability to flower very early, prolific berry and seed production and the ability to tolerate many diverse habitats (Ojiewo, 2007). Although they perform well in a range of climatic conditions, African nightshades grow better under cool high moisture conditions in medium to high altitudes. Studies by Masinde *et al*. (2009) have shown the broad leaved genotypes are more susceptible to agronomic stress as compared to narrow leaved genotypes. For instance, nightshade responds to high temperature and low moisture by curling of leaves to reduce the leaf surface area thus reducing rate of transpiration, surface for gaseous exchange and photosynthesis resulting in reduction of dry matter accumulation (Masinde *et al*., 2009). Hence *Solanum scabrum* tends to curl most of its leaves as compared to *Solanum villosum* (Ojiewo, 2007).

Nightshade is also known to extend its roots deep when stressed with P and produce root exudates that have a sole purpose of mobilizing the available P for plant uptake (Masinde *et al*., 2009). Small leaved genotypes are less affected in terms of biomass production as compared to the broad leaved (Ojiewo, 2007). Optimum growth temperatures ranges are 15-30 ºC for germination and 20-30 ºC for growth, but most species will grow within the range of 15-35 ºC (Ojiewo, 2007). Low light intensity favours germination and intermittent light enhances germination at less favourable temperatures (Ojiewo, 2007). Nightshades grow on various soil types, but are best adapted to high fertility; they grow well in soils high in N, P and rich in organic matter. Masinde *et al*. (2009) reported that fertilization increased leaf yields 1.5-2.5 folds; but there was no genotypic difference in the level of response, indicating the genetic diversity may not necessarily influence N or P use efficiency (Sanchez *et al*., 1997). Sandy loam to friable clay soils with a pH range of 6.0-6.5 are a particular suitable (Rop *et al*., 2012).

2.5 Phosphorus uptake and concentration

Phosphorus is an essential macronutrient for plant growth, and it is limiting crop production in many regions of the world (Holford, 1997). Global demand for P fertilizer continues to increase while global reserves of P are in decline (Steen, 1998; Cordell *et al*., 2009). The availability of soil P for plants is related to several plant characters, including the release of carboxylates (Ryans *et al*., 2001), rhizosphere pH (Curtin *et al*., 1993; Hinsinger, 2009), morphological traits such as length and surface area of roots (Williamson *et al*., 2001; Li *et al*., 2007), root architecture (Lynch, 1995), root hairs (Gahoonia and Nielsen, 1997), mycorrhizas (Smith *et al*., 2000) and specialized structures such as root clusters (Lambers *et al*., 2003; Shane and Lambers, 2005).

Several investigators have reported that P is a key element that strongly influences the initiation and growth of roots (Shane and Lambers, 2005). In field studies, some grain legumes exhibit higher P-acquisition efficiency than other crops due to root formation and release of carboxylate (Bolland *et al*., 1999). Young roots release large quantities of carboxylates in an exudative burst than mature roots (Watt and Evans, 1999; Shane and

Lambers, 2005). On the other hand, adequate P supply to plants inhibits root formation and so avoids excessive loss of carbon from the root system (Keerthisinghe *et al*., 1998).

Several investigators have reported either initiation or inhibition of root formation to be linked to internal P concentration in the plant (Shane and Lamber, 2005; Li and Liang, 2005; Shen *et al*., 2005). However, the exact location of the signal has not yet been fully elucidated; it might be the shoot P concentration, the P concentration in phloem sap or the P concentration in the root (Shane and Lamber, 2005). Pearse *et al*., (2006) introduced a model that roots in some plant species have a positive effect on P uptake, which enhances P status, but this, in turn, has a negative effect on root formation and exudation.

Phosphorus concentration in plants is balanced by phosphate uptake and plant growth rate (Shen *et al*., 2005). The capacity for P uptake may be affected by several environmentally or genetically controlled factors that differ among plants species (Pearse *et al*., 2006). Further, plant growth rate may be influenced by many ecological or genetic factors. If shoot P concentration is a signal for prevention of root formation, different species with inherent differences in maximum growth rate and P uptake may differ in the foliar P concentration that either initiates or suppresses root formation (Pearse *et al*., 2006). In this way, those plants that grow quickly, even with a higher uptake of phosphate, may accumulate little P in their shoot and stimulate root formation (Shen *et al*., 2005).

By contrast, species that have an inherently low growth rate may accumulate high levels of P in their shoot, even at a relatively low phosphate supply (Ren *et al*., 2007). It is therefore of interest to evaluate changes in P concentration and how this could affect the production of secondary metabolites to deal with P deficiency (Ren *et al*., 2007).

2.6 Phosphorus dynamics in soil

Phosphorus exists in soils of organic and inorganic forms. Organic forms of P are found in humus and other organic material (Masinde *et al*., 2009). Phosphorus in organic material is released by a mineralization process involving soil organisms (Shen *et al*., 2005). Inorganic P which was focused on in study is negatively charged in soil. Almost all phosphorus is taken up by plants as either of two orthophosphate ions (Raghothana and Karthikeyan, 2005). Phosphorus is a key element in many physiological and biochemical processes. Phosphorus is also involved in controlling key enzymes reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). In addition phosphorylation and dephosphorylation of proteins are crucial for signal-transduction pathways in plants (Raghothana and Karthikeyan, 2005).

Phosphorus availability is one of the major constraints to plant growth (Nye, 1981). Phosphate in the soil solution P pool is immediately available but its amount is very small in comparison to the amount needed by plants and to the total P in soils (Shen *et al*., 2005). In addition plants can suffer from P deficiency even though the total P content of the soil appears more than adequate (Masinde *et al*., 2009). The reason for this apparent discrepancy is that the concentration of soluble P is often very low ($< 5 \mu mol/L$) compared to the total amount of P bound to soil minerals and charged sites or fixed into organic forms that are inaccessible to plants (Raghothana and Karthikeyan, 2005).

Phosphorus is affected by chemical properties in soil (Masinde *et al*., 2009). The effect of pH changes on P availability in soils and P uptake has been discussed by some researchers (Nye, 1981; Thomson *et al*., 1990). However, for different soil constituents the relation between pH and P in soil is different; decreasing pH in alkaline soils causes' increased P uptake by plants due to the dissolution of P minerals, whereas in acid soils an increase in pH increases P uptake because of desorbing P from metalloids (Gahoonia and Nielsen, 1997).

2.7 Plants Defense System

Plant damage occurs when the capacity of antioxidant processes and detoxification mechanisms are lower than the amount of ROS production (Rajesh, 2004). Plants have developed complex systems protecting them from ROS, consisting of several enzymes and antioxidants (Rajesh, 2004). Antioxidant protects plant damage because they can scavenge ROS before they cause damage to the various biological molecules, or prevent oxidative damage from spreading (Agrawal *et al*., 2005). These mechanisms can slow down or even stop the oxidation of bio-molecules and block the process of oxidative chain reactions (He *et al*., 2003).

The most important antioxidants are superoxide dismutase (SUD), Catalase (CAT), ascorbate peroxidase (APX) monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (He *et al*., 2003). All of them participate in a highly developed detoxification system named the ascorbate-glutathione cycle (Miller *et al*., 2003). Ascorbate peroxidase gene expression is rapidly induced by various stress conditions including severe nutrient unavailability (He *et al*., 2003 and Miller *et al*., 2003).

2.8 Plant mechanisms to deal with Phosphorus stress

Phosphorus deficiency in soils can be corrected by the use of phosphate fertilizers; a higher dose of which is required for sustaining crop yield because of high P fixation capacity of soils (Marschner, 1995). When plants undergo nutrient deficiency, the amount of exudates released by roots will increase (Marschner, 1995). Enhanced root exudation of organic acids has been reported under phosphorus deficiency (Zhang *et al*., 2008). Root exudates mainly comprising of organic acids, sugars, phenolics and amino acids which affects nutrient dynamics in the rhizosphere and help the plant nutrient status by mobilizing sparingly soluble phosphates into the soil solution (Marschner, 1995). The production of root exudates was assumed to be another mechanism for plants survival under P deficiency (Zhang *et al*., 2008). Rhizosphere acidification has been shown for number of plant species for instance the response of white lupin to P deficiency (Zhang *et al*., 2008). Gardner *et al*., (1983) demonstrated that the proteiod roots of P deficient lupin plants secreted large quantities of citric acid.

During nutrition stress, roots release greater quantities of organic material either passively or actively into the rhizosphere (Curl and True-glove, 1986), although some of the released compounds have been implicated as mechanisms for relieving the external stress (Mori *et al*., 1991). Root architecture, defined as the spatial configuration of root system (Lynch, 1995), may be especially important for P acquisition, since the relative immobility of P makes its acquisition dependent on soil exploration in time and space.

Lateral rooting might be a beneficial root trait conferring P efficiency by increasing soil exploration and P solubility (Zhang *et al*., 2008). An experiment done on white lupin showed concerted regulation of lateral root development in response to low P availability (Zhang *et al*., 2008 and He *et al*., 2003). Plants need to cope with heterogeneous P distribution in soils. Hence, root foraging, root architecture and elongation of lateral roots become important factors in acquisition of P (He *et al*., 2003).

2.8.1 Secondary metabolite production under phosphorus stress

Plant secondary metabolites are products produced by plants that aid in the growth and development of plants but are not required for the plant to survive (Shen *et al*., 2005). Secondary metabolism facilitates the primary metabolism in plants (Ryans *et al*., 2001). The primary metabolism consists of chemical reactions that allow the plants to stay healthy, secondary metabolites plays a pinnacle role in keeping all the plants' systems working properly (Wright *et al.,* 2010). A common role of secondary metabolites in plants is defense mechanisms. They are used to fight off mineral deficiency (Steen, 1998).

In well nourished plants, most of the P is inorganic, stored within the cell in vacuoles (Gahoonia and Nielsen, 1997). Vacuolar P keeps up a constant and rich level in the chloroplast, where biosynthesis begins (Shane and Lamber, 2005). Every molecule produced comes out in a phosphorylated form bonded to a phosphate molecule that gives it the energy it needs for further biosynthesis (Li *et al*., 2007). Phosphorus biosynthesis in some plants has always ensure that plants have defensive mechanisms of repelling pathogens and also enforcing plants structures to prevent nutrient deficiencies, further water loss and pest invasion (Ren *et al*., 2007).

Plants have evolved adaptive mechanisms to P stress and phenolic production is one such mechanism (Ren *et al*., 2007). There has been reported increase in phenolic concentration under P stress (Shen *et al*., 2005). During recent decades, crop production has been on the rise leading to continuous decrease of P in soils (Gahoonia and Nielsen, 1997). These changes and variations in the P concentration in soils expose plants to P stress. Plants have evolved mechanisms to protect themselves and ability to respond is partly genetically inherent (Foyer *et al*., 1994). Several phenolic compounds production such as terpenoids, steroids and production of anthocyanin acid have been evident during such phenomenon (Foyer *et al*., 1994).

In nature, plant species are usually affected by several stress factors at once and any particular factor may reinforce or compensate for the effects of others (Wright *et al.*, 2010). The metabolites such as phenolics and nicotine production in *Nicotiana attenuata* has a fitness cost in terms of seed production in uneaten plants (Steen, 1998) and such products are mainly employed by the plant as defense mechanism against damage by herbivores or defense against radiations and nutrient deficiency. Yet plants differ greatly in their foliar concentrations of these secondary metabolites, and the reason for this variation remains poorly understood despite a long history of investigation (Steen, 1998).

This tenet has explained the distribution of nightshades (either wild or domesticated). Wild species seem to have less demand for P and other nutrients, grow slowly but accumulate more secondary metabolites that deter the grazers and aid in the acquisition of nutrients (Ren *et al*., 2007). These products are just the main products that we may be targeting in horticultural industry in near future (Ren *et al*., 2007). Several hypotheses and models have attempted to explain variation in concentrations of phenolics, two such theories are; Carbon Nutrient Balance Hypothesis (CNBH) and Protein Competition Model (PCM)

The Carbon Nutrient Balance Hypothesis (CNBH) was an early framework for understanding variation in concentrations of phenolic compounds (Cordell *et al*., 2009; Ryans *et al*., 2001). The CNBH predicts that the amount plants invest in secondary compounds depends on the relative supply of carbon and nutrients to the sites of metabolism (Ryans *et al*., 2001). For example, the growth of plants on nutrient-poor soils is postulated to be more limited by nutrients than by the carbon supplied by photosynthesis, and as a result carbohydrates accumulate in the leaves, which are then diverted to form constitutive defenses including phenolic compounds such as condensed tannins (Cordell *et al*., 2009).

Carbon Nutrient Balance Hypothesis predicts that if such plants were provided with more nutrients, then they would accelerate biomass growth and decrease allocation to carbon-based secondary metabolites through lack of carbohydrate substrate materials (Ryans *et al*., 2001). The CNBH has been superseded by the Protein Competition Model (PCM) which is based on a more mechanistic understanding of biochemical pathways

(Fontem *et al*., 2004). Proteins and phenylpropanoids (phenylalanine-derived phenolic compounds, including lignins and condensed tannins) compete for a common precursor, phenylalanine for instance in the shikimate biosynthetic pathway (Ryans *et al*., 2001).

In the work of Wright *et al.* (2010) it is the PCM that explained the role of N in phenolics production while P played very little and in fact the phenolics did not change in P manipulation. According to these authors, a key issue considered in the PCM but not in the CNBH is whether different nutrients have different influences on the production of phenolic compounds. They further extended the PCM prediction to suggest that deficiency of N not P will lead to increased phenolic concentrations, comparing the effects of these nutrients quantitatively (Ryans *et al*., 2001).

2.8.2 Generation of Reactive Oxygen Species under phosphorus stress

When plants are stressed, they are exposed to reactive oxygen species formation (Rajesh, 2004). These incomplete reduced oxygen species are toxic by-products generated at low levels in non-stressed plant cells (Rajesh, 2004). They are generated in chloroplasts and mitochondria and also by cytoplasmic membrane bound or exocellular enzymes involved in redox reactions (especially photosynthetic electron transport processes and respiration). Extra amounts of ROS occur under stressful conditions such as pathogen attacks, wounding, herbivore feeding, UV-light, heavy metals, nutrient unavailability and others (Maqsood *et al*., 2010 and Turner, 2002).

Several metabolic processes may use ROS in a good way (Turner, 2002). Some of the ROS are involved in lignin formation in cell walls (Maqsood *et al*., 2010). They participate in an oxidative burst and act not only as direct protectants against invading pathogens, but also as signals activating further reactions (HR-hypersensitive response or Phytoalexin biosynthesis) (Turner, 2002 and Williamson *et al*., 2001)

2.9 Secondary metabolites on health

Secondary metabolites also known as Phytochemicals are compounds produced by plants that are important for the survival and propagation of the plants that produce them (Kim *et al*., 1998). Secondary metabolites serve as chemical signals enabling the plants to respond to environmental cues or functioning in the defense of the producer against herbivores, pathogens, or competitors (He *et al*., 2003). Others provide protection from radiation or assist in pollen and seed dispersal (Kim *et al*., 1998). They are produced at various sites within the cell and stored primarily within the vacuole. The three major classes of secondary metabolite plant compounds are alkaloids, terpenoids and phenolics that are important in human health.

2.9.1 Total Phenolic Content

All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites is phenolic compounds (Ryans *et al*., 2001). Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups (Ryans *et al*., 2001). They are mainly synthesized from cinnamic acid, which is formed from phenylalanine by the action of L-phenyl-alanine ammonia-lyase PAL, the branch point enzyme between primary (shikimate pathway) and secondary (phenyl-propanoid) metabolism (Ryans *et al*., 2001). The significance of this route can be supported by the fact that, in normal growth conditions, 20% of carbon fixed by plants flows through this pathway (Fig.2.1).

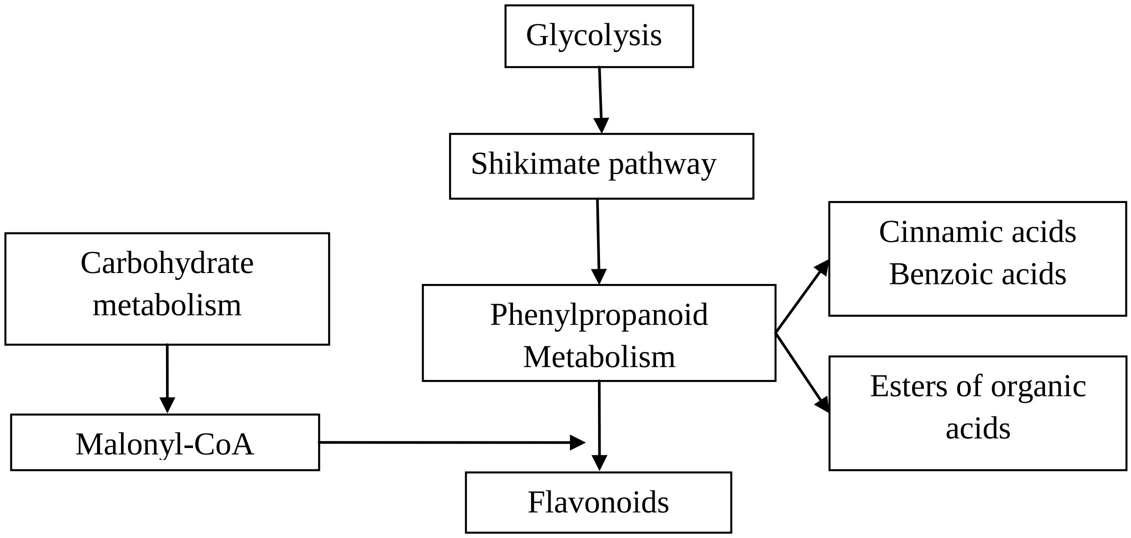

Figure 2.1: Biosynthesis pathways leading to formation of main groups of phenolic compounds (Ryans *et al*., 2001)

Phenolics include simple phenols; phenolic acids (benzoic and cinnamic acid derivatives), flavonoids, chlorogenic acids/ anthraquinones, tannins, steroids, saponins are among the most widely occurring phenolics in African nightshade (Weinberger *et al*., 2011). These act mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective against UV light among others (Ren *et al*., 2007).

The medicinal value is related to the antioxidant properties which are parts of secondary metabolites (Ren *et al*., 2007). Consumers are searching for foods rich in these phenolic compounds and often look to organically produced foods or exotic herbal extracts (Edmonds and Chweya, 1997). But the production practices that directly influence their levels in plants are not well known. The study was therefore conducted to determine the influence of adding more P than usual on the levels of phytochemicals in the African nightshade (Mwai and Schippers, 2004). For centuries, preparations for therapeutics containing phenolic compounds as the principal physiologically active constituents have been used to treat human diseases (Edmonds and Chweya, 1997).

Phenolics are found on various parts of African nightshade. The function of phenolics in flowers is to provide colours to attract pollinators (Harborne and William, 2000). In leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from fungal pathogens and UV radiation (Wright *et al*., 2010). In addition, they are involved in photosensitization, energy transfer, the actions of the plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination (Harborne and William, 2000)

Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of phenolics possessing antifungal, antiviral and antibacterial activity (Edmonds and Chweya, 1997). The vasodilator property of phenolics is highly useful for the treatment of heart diseases (Kim *et al*., 1998). Literature search indicated that biflavones and isoflavones are potential blood circulation enhancers in brain (Bagli *et al*., 2004). Several reviews showed that phenolics possess wide spectrum of biological activities in cardiovascular systems, which include antioxidant, anti-thrombotic, anti-apoptic, anti-ischemic, anti-arrhythmic and anti-hypertensive activities (Cottiglia *et al*. 2001). Flavonoids have also been shown to inhibit the growth of various cancer cell lines invitro and reduce tumor development (Bagli *et al*. 2004).

Phenolics like kaempferol, myricetin and quercetin are strong inhibitors of xanthine oxidase indicated in the treatment of gout, hyperuricemia and reperfusion injury

(Cottiglia *et al*. 2001). Catachines act as an antiulcer agent by inhibiting the H^+/ATPase. Liguritigenin administration in experimental animals showed significant fall in serum cholesterol (Ryans *et al*., 2001). The cardio-toxicity of doxorubicin can be countered by flavonoides like luteolin. Tyrosinase inhibitors like butein (2', 4', 3, 4-tetrahydroxychalcone) and other chalcones have become increasingly important in the cosmetic and medicinal products used in the prevention of hyperpigmentation (Duffy and Vita, 2003).

2.9.2 Total antioxidant activity

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites; so called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants (Agrawal *et al*. 2005). This imbalance leads to damage of important biomolecules and cells, which potentially impact on the whole plant (Harborne and William, 2000). Oxidative stress can damage lipids, proteins, carbohydrates and DNA in cells and tissues resulting in membrane damage, fragmentation and lipid peroxidation (Agrawal *et al*. 2005).

These consequences of oxidative stress construct the molecular basis in the development of cardiovascular diseases, cancer, neurodegenerative disorders, diabetes and autoimmune disorders (Cottiglia *et al*. 2001). ROS are products of a normal cellular metabolism and play vital roles in the stimulation of signaling pathways in cells in response to change in intra and extra cellular environmental conditions. Most ROS are generated in cells by the mitochondrial respiratory chain (Rajesh, 2004). During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion, hydroxyl radical, hydrogen peroxide and organic peroxide as normal products of the biological reduction of molecular oxygen (Agrawal *et al*. 2005).

The electron transfer to molecular oxygen occurs at the level of the respiratory chain, and the electron transfer to molecular oxygen occurs at the level of the respiratory chain, and the electron transport chains are located in the membranes of the mitochondria (Fontem *et al*., 2004) Under hypoxic conditions, the mitochondria respiratory chain also produces nitric oxide, which can generate reactive nitrogen species (RNS) (Pearse *et al*., 2006). ROS/RNS can further generate other reactive species by inducing excessive lipid peroxidation. In order to combat and neutralize the deleterious effects of ROS/RNS, various antioxidant strategies have evolved either the increase of endogenous antioxidant enzyme defenses for example glutathione vitamins through nutrition means (Rajesh, 2004).

Antioxidants can delay, inhibit or prevent the oxidation of oxidisable substrates by scavenging free radicals and diminishing oxidative stress (Ren *et al*., 2007). However, in stress conditions, the defense against ROS is weakened or damaged and the oxidant load increases. In such conditions, external supply of antioxidants is essential to countervail the deleterious consequences of oxidative stress (Pearse *et al*., 2006). It has been proposed that phenolics can act as antioxidants by a number of potential mechanisms (Gahoonia et al., 1992). The free radical scavenging in which the phenolics can break the free radical formation by regulation of enzymes activity or chelating metal ions involved in free radical production are reported to be most important mechanisms of their antioxidant activity (Duffy and Vita, 2003). The interaction between phenolic compounds with other physiological antioxidants is another possible antioxidant pathway for these compounds (Zhang *et al*., 2008).

CHAPTER 3. MATERIALS AND METHODS

3.1 Introduction

This study entailed a survey, greenhouse and field experiments and laboratory analysis of the samples. The survey was aimed at collecting information on the agronomic practices farmers use in the production of African nightshade, preference, reason for planting, source of planting materials, level of education, soil organic amendments and distribution. The survey was conducted in two selected counties namely; Kisii and Siaya whereby 10% of the total number of farmers growing ANs (approximately 300 each County) where choosen at random in each County. The two most common and preferred varieties from the survey were planted in a series of pot experiments conducted in a greenhouse running concurrently with field experiments. These experiments were aimed at determining the response of African nightshade to different P treatments in terms of biomass and secondary metabolites production.

3.2 Study area

The study areas were located in Kisii and Siaya counties, Kenya. Kisii County lies between longitude 34° 46’ 0° E and latitude 0° 41’ 0° S. The county is divided into three ecological zones comprising the upper midland (UM) 75%, Lower Highland (LH) 20%, and Lower Midland (LM) 5% (FAO/UNESCO, 1999).About 78% of the county is arable of which 57% is under crop. Seventy five percent of the county has red volcanic soils (nitosols) which are deep in organic matter. The rest of the county has clay soils which have poor drainage (phaezems), red loams and sandy soils (FAO/UNESCO, 1999).

In the valley bottoms, there exist black cotton soils (verisols) and organic peat soils (phanosols) (FAO/UNESCO, 1999). Kisii County exhibits a highland equatorial climate resulting into a bimodal rainfall pattern with average annual rainfall of 1500m with the long rains between March and June while the short rains are received from September to November. The months of July and January are relatively dry. The maximum temperatures in the county range between 21°C – 30°C while the minimum temperatures range between 15°C – 20°C.

Siaya County lies between longitudes 33° 58’ E and 38° 33’ W and latitude 0° 26’ to 0° 18’ N. The County has three major geo-morphological areas: dissected uplands, moderate lowlands and swamps. The altitude of the district rises from 1140m above sea level in the eastern part to 1400m in the western part (FAO/UNESCO, 1999). The county has two rainy seasons. The geography of the land influences distribution and amount of rainfall. The county is located in the Inter Tropical Convergence Zone (ITCZ) and experiences a modified climate equatorial climate that is influenced by relief and numerous hydrological bodies. It thus has a warm, dry and humid climate. The county is drier in the western part towards Bondo district and is wetter towards the higher altitudes in the eastern part (FAO/UNESCO, 1999).

On the highlands the rainfall ranges between 800-2000mm per annum. The lower areas receive between 800-1600mm. The long rains fall between March and June, with a peak in April and May. Short rains do not last as long and typically fall from late September to November. In the past the pattern was consistent but now it’s unpredictable.

Temperatures also vary with altitude. The mean minimum temperature is 15°C while the mean maximum temperature is 30°C.

The geology of the area is made up of the old Nyanzian rock system made up of exposed volcanic rocks such as basalt, desite and ryolite. These rocks lead to the formation of black cotton loamy and red volcanic soils. The humidity is relatively high with mean evaporation being between 1800mm to 2000mm in a year. The county is located under ecological zones LM1 to LM3, with a small area under UM1. The county is located in the Inter Tropical Convergence Zone (ITCZ) and experiences a modified climate equatorial climate that is influenced by relief and numerous hydrological bodies. It thus has a warm, dry and humid climate (FAO/UNESCO, 1999).

3.3 Survey

Using a sketch map of both Kisii and Siaya Counties respectively (Appendix1 and 2), surveyed areas were identified by first dividing the map areas into three ecological zones and then dividing each ecological zone transversely into five portions to give a total of 6 farmers to be surveyed in each portion. Kisii County was partitioned as follows; Keumbu, Mosocho and Kisii township and Siaya county were; Gem, Dominion and Yala. The purpose of the survey was to document farmers' knowledge, practices and preference of the African nightshade production and taste respectively. To guide the interviews, a semi-structured questionnaire (Appendix 3) was developed. Information gathered included the land preparation techniques, source of planting materials, number of varieties grown, and age group preference on consumption of different varieties. Information on nutrient supply was also gathered by sampling soils and plants from surveyed farmers, and in addition GPS coordinates of the farms where the information was gathered.

A purposive sampling technique was employed targeting farmers producing African nightshade in the selected zones. A total of 5 farmers from the chosen ecological zones in each County were interviewed. The interviews were conducted in a semi-structured way to allow respondents to express themselves openly. All the interviews were conducted at the farmers' plots. This enabled respondents to demonstrate their practices and assisted the recording of measurements where necessary. A total of 70 farmers were interviewed and 70 samples of both soils and plants were collected from the designated Counties. Plant samples picked were dried, packed and labelled in separate sampling bags. The sample soils were also packed and labelled where they were stored in a freezer (-25°C) until their preparation for analysis.

Quantitative information was converted to a standardized form to allow for comparison among farmers and descriptive statistical analysis of the data. Information determining the amount of nutrients individual farmers applied per unit area, the type of fertilizer(s) used and the amount applied per unit area. Quantities of fertilizers were converted to amounts of elemental nutrient using the nutrient composition of the fertilizers (Ahmed, 1989).

3.4 Mapping

An extensive data sheet was developed to acquire detailed information about farming system in each of the visited farms. The following information was listed: name of the farm, Soil P, Global Positioning System (GPS) coordinate, type of African nightshade varieties grown, Plant and soil and tissue P, Plant TPC and Plant TAA. Data were collected from farms in Siaya and Kisii Counties; information of small scale farmers planting African nightshade was developed utilizing Arc-GPS 10.0® software. For the Global Information System (GIS) analysis, the input data were derived from a set of vector data representing location based on the given GPS coordinates. The given information was plotted in maps of the surveyed regions on the points assigned according to the GPS coordinates.

3.5 Greenhouse experiment

A pot experiment was conducted in Kenyatta University greenhouse was located in Kiambu County, Kenya. Two African nightshade variety seeds (*S. scabrum* and *S. villosum*) choosen from the survey based on their relative importance were pre-germinated in a nursery. After 4 weeks, six seedlings were transplanted in each of the 6.25 Kgs plastic pots (34 cms diameter and 30 cms depth) filled with 20 Kgs of sterilized sand. Treatments included four P levels with two varieties of African nightshade replicated four times. This was a split plot design with (*S. scabrum* and *S. villosum*) being the main plot and varying phosphorus levels (0, 20, 40 and 60 kg/ha) constituting the subplot in Completely Randomized Design. The four P application rates in greenhouse experiment; 0, 6, 12 and 18 g/pot were termed as P deficiency (P0), moderate P (P6), high P (P12) and very high P (P18) constituting the subplot. All pots were administered with recommended plant nutrients namely; N, K and other micronutrients through Hoagland solution (Hoagland, 1948). Hoagland solution was made by adding the following quantities of stock solution to 1L of water (Table 3.1)

Table 3.1: Combination of macro and micronutrients during the preparation of Hoagland solution

	Macronutrient stock solution	Quantity
1	0.05M Potassium	10cm^3
2	0.05M Calcium nitrate	10cm^3
3	0.01M Calcium sulfate	200cm^3
4	0.5M Potassium sulfate	5cm^3
5	1M Magnesium sulfate	2cm^3
	Micronutrient stock solution	
1	Boric acid	2.86 gm
2	Zinc sulfate	0.22 gm
3	Manganese chloride	1.81 gm
4	Copper sulfate	0.08 gm
5	85% Molybdic acid	0.02 gm
6	Iron chelate (Fe-EDDHA)	1.5 cm^3

Hoagland solution essential for plant growth was modified by omitting P but including all the other nutrients essential for plant growth and development. Hoagland solution was administered when transplanting at the rate of 15ml of stock solution in 1litre of water per pot. P was also administered when planting. There was no P administration on plants treated with 0 gms/pot, pots treated 6 gms/pot of Triple superphosphate (TSP) was classified as moderate, 12 gms/pot of P was high and finally with 18 gms/pot of Triple superphosphate (TSP) on each pot treated with very high level of P.

The pots were arranged in Completely Randomised Design (CRD) with two varieties of African Nightshade, *Solanum scabrum* and *Solanum villosum* replicated four times. Destructive harvesting was done six weeks after transplanting and was repeated every week until the sixth week from the first harvest. Data on plant height, fresh weight and number of secondary buds, leaf area and root area were recorded. A well calibrated ruler in centimeters, electronic weighing balance in grams and physical counting was implemented.

Plant height was measured from the ground level up to the apex of the youngest leaf. Fresh weight measurement entailed picking all the leaves and tender shoots and weighing them immediately using an electronic weighing balance.

Leaf area was calculated using the formula, leaf area= L x W x K, where L is leaf length, W is leaf width and K is a multiplying factor obtained from the ratio of leaf area as traced on a graph paper (Raghothana and Karthikeyan, 2005).

Root area was calculated (half width of the longest secondary root by length of the tap root) as recommended by Raghothana and Karthikeyan, 2005. The resulting plant was oven dried at 40°C for 72 hours and stored for further analysis. The dry weight was recorded. All data were subjected to ANOVA using SAS version 9 and the means separation using Least Significant Difference (LSD) at $p \leq 0.05$.

3.6 Field experiment

3.6.1 Site description

The field experiment was conducted at Kenyatta university farm, Kiambu County, Kenya. The site lies at an altitude of 1745 meters above sea level and is within latitude 110’ 0.012” S and longitude 3649’59.880” E (FAO/UNESCO, 1999). The average amount of rainfall received is 989 mm per year (FAO/UNESCO, 1999) where 1200 mm rains is recorded during the long rains whereas 780 mm is recorded during the short rains. Temperature ranges between 12.8 degrees Celsius during the cold month and 24.6 degrees Celsius during the hot seasons. The soils are loamy, acidic, well drained and moderately deep.

3.6.2 Initial soil properties and available phosphorus

Selected soil properties at the field experimental site are shown in (Table 3.2). The soil was acidic (pH 5.5) and low in total soil N (<0.2%), organic carbon (<2%) and available P (<10 mg kg^{-1}). All the properties were below the critical levels indicated in the brackets as prescribed by Jaetzold and Schmidt (1982) thus signifying the general infertility of this soil. The mean available P levels were 6.6 and 9.4 mg P kg^{-1} for the treatments with no P application in the first and second seasons respectively. Such low P levels are to be expected in this type of soil which is inherently low in P and also high in P fixation (Sanchez, *et al*., 1997). The increase in available P with increasing P rates in both seasons can be attributed to the fact that P sorption by soil decreases as more P was applied to soil (Sanchez *et al*., 1997) thus maintaining a high P availability.

Table 3.2: Initial soil properties for field experiment site

Parameter	pH	% N	%OlsenP	% org.C	% Clay	% Silt	% Sand	% Zn	% Cu
Value	5.45	0.12	1.49	1.53	33.7	30.3	36	1.11	1.15

3.6.3 Experimental layout and Management

A field experiment was conducted for two cropping seasons, the long rains of May to July 2014 and the short rains season of August to October 2014. A split plot arrangements with two varieties (*S. scabrum* and *S. villosum fsp* villosum) being the main plot and varying phosphorus levels (0, 20, 40 and 60 kg/ha) constituting the subplot in randomized complete block design with four replicates was used with each experimental plot measuring (3x3) m.

Primary tillage was done to a moderate tilth after which 6-week-old seedlings were transplanted. Appropriate rates of Triple Super Phosphate fertilizer were administered into 15 cm deep drilled holes on the respective plots at the time of transplanting.

Calcium Ammonium Nitrate (26% N) at 60 $KgNha^{-1}$ and Muriate of Potash (60 % K_2O) at 30 Kg K ha^{-1} were uniformly administered and incorporated into the soil in both seasons (Shane and Lamber, 2005). The aim was to supply sufficient amounts of N and K to ensure the two nutrients were not limiting factors on plant growth when studying the effects of P.

Twenty four seedlings/plot of the respective African nightshade varieties were transplanted at a spacing of (30x30) cm in 32 plots on 1st May, 2014 (Long rains) in the first season and 1st August, 2014 (Short rains) in the second season. The fields were kept weed free by manually weeding using a hoe. Insect pests and diseases were controlled using abamectin and copper sulfate respectively. Top dressing with N fertilizer was done. Soil samples were collected (0-0.015m depth) (Shane and Lamber, 2005) at 2 WAT, 4 WAT, 6 WAT and 8 WAT from each plot and analyzed for available soil P and pH using the Mehlich 1 method and pH meter respectively as described by (Okalebo *et al*., 2002).

Data on plant height, fresh weight, number of secondary buds, leaf area and root area were recorded. A well calibrated ruler in centimeters, electronic weighing balance in grams and physical counting were used.

Plant height was measured from the ground level up to the apex of the youngest leaf. Fresh weight measurement entailed picking all the leaves and tender shoots and weighing them immediately using an electronic weighing balance. Leaf area was calculated using the formula, leaf area= L x W x K, where L is leaf length, W is leaf width and K is a multiplying factor obtained from the ratio of leaf area as traced on a graph paper (Raghothana and Karthikeyan, 2005).

Root area was calculated (half width of the longest secondary root by length of the tap root) as recommended by Raghothana and Karthikeyan (2009). The resulting plant was oven dried at 40°C for 72 hours (Watson, 2007) and stored for further analysis of total phenolic content and total antioxidant activity. The dry weight was recorded. All data were subjected to analysis of variance (ANOVA) using of SAS version 9 and the means separation using LSD and Turkey at $p \leq 0.05$.

3.7 Analysis of Samples

3.7.1 Phosphorus analysis in soil and plant material

Both regions where the baseline survey was done as well as the field sites were moderately acidic. For this reason, Mehlich 1 method was used to analyse soil P. Mehlich1 extracting solution (0.0125 M H_2SO_4+0.05M HCl) also referred to as dilute double acid. Using a graduated cylinder, 167 ml of concentrated HCl (1.2M) and 28 ml of concentrated H_2SO_4 (1.8M) were added to approximately 35L of de-ionized water in a large polypropylene carboy container. The mixture was made to a final volume of 40L by adding de-ionized water. Bubbling of air through the solution for 3 hours was done to ensure homogenous solution was obtained. 5gms of sieved and air dried soil sample were poured into a 50ml extraction flask. 200mg of charcoal was added to each flask inorder to obtain a colourless filtrate followed by the addition of 20ml of the Mehlich1 extracting solution and shaking for five minutes on a reciprocating shaker set at a minimum of 180 rpm at room temperature. The resulting solution was filtered through a medium porosity using Whatman No.2 and analysed for P by colorimetry using a blank and standards prepared in the Mehlich1 extracting solution. To calculate for extractable P the following formula was used; Mehlich1 extractable P (mg P/kg soil) = Concentration of P in Mehlich1 extract (mg/l) x (0.020L extract ÷ 0.005 kg soil) (Mehlich, 1953).

3.7.2 Extraction of Secondary Metabolites from Plant Material

Methanol extraction was applied for the oven dried plant samples as recommended by Watson, (2007). Five grams of the powdered plant material in a flask was covered with 50ml methanol and allowed to stand for 48-72 hrs. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60°C until methanol free solid powder was obtained. The resulting extracts was then subsequently labeled as methanol extracts and preserved at 5°C in airtight bottles awaiting further total phenolic content and total antioxidant activity analysis (Harborne 1998).

3.7.3 Total phenolic content analysis

To determine TPC, Gallic was used as a standard. 0.5g Gallic acid was weighed and dissolved in 10 ml of methanol and diluted to 100 ml using distilled H_20. 200 g of sodium carbonate was weighed and added in 800 ml of distilled water. The solution was brought to automatic boiling after cooling, few crystals of hydrous sodium carbonate was added and after 24 hours, it was filtered and topped up to 1L using distilled water.

To prepare a calibrated curve 0, 1, 2, 3, 5 and 10 ml of the above gallic acid solution were added into 100 ml volumetric flasks, diluted to volume with water to give the following concentrations; 0, 50, 100, 150, 250 and 500 mg/L gallic acid. From each calibration solution sample or blank, 1 ml was pipetted into separate test tubes and to each, 4 ml of distilled water and 0.2 ml of Folin reagent was added and mixed well. The solutions were left at ambient temperature for 2 hours and the absorbance of each solution was determined at 765 nm against blank. Absorbance verse concentration graph was plotted to determine the equation of regression (R^2).

3.7.4 Determination of antioxidant activity

The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH) radical according to Ayoola *et al*. (2003). This provided 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, 2.0 and 5 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade) to come up with a calibration curve.

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 0.5ml of 1 mM DPPH in methanol. The mixture was then 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-visible spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was calculated using the following formula: % inhibition = {[Ab-Aa]/Ab} x 100. Where: Ab = absorption of the blank sample and Aa = absorption of the extract (Harborne 1998).

3.7.5 Phosphorus, zinc and copper analysis in soil and plant material

An acidified solution of ammonium molybdate containing ascorbic acid and antimony was added to a powdered plant tissue sample/soil solution. The phosphorus/zinc/copper in the plant tissue sample/soil solution reacts with the acidified ammonium molybdate to form an ammonium molydiphosphate/molydi-zinc/molydi-copper complex. A blue coloured solution was generated from the reduction of the ammonium molydiphosphate/molydi-zinc/molydi-copper complex by ascorbic acid. The intensity of the blue colour was proportional to the amount of molybdophosphorus/molydi-zinc/molydi-copper present. Antimony potassium tartrate accelerates the colour development and stabilizes the colour for several hours. The amount of light absorbed by the solution at 660 nm was measured with a visible spectrophotometer (Murphy and Riley, 1962).

To determine the concentration of P, Zn and Cu, 2gms of air dried and ground plant material was weighed and put into 150ml beakers. 2ml of 0.1M HCl was added to the mixture inorder to digest sample using dry ashing method. The samples were quantitatively transferred into 100ml volumetric flasks and 5ml of distilled water added to dilute. Using a dilutor-dispenser, samples were diluted and the 20, 40, 60 and 80 mg P/Zn/Cu/L standards 1:100 with the working solution. Colour was allowed to develop for at least 30 minutes before reading.

To calibrate the spectrophotometer for routine analysis, the working solution was used as the blank and develop 0.80 mg P/Zn/Cu/L standard to establish the slope of the line. Linearity was checked by reading the developed 0.20, 0.40, and 0.60 mg P/Zn/Cu /L standards. When the sample concentration lied above the linear working range, dilution of the samples was done respectively. The concentrations were read at 660 nm with a visible spectrophotometer, the instrument reading were read as percent P/Zn/Cu in the dried plant tissue (Harborne 1998).

3.8 Statistical analysis of data

Data were analysed using SAS version 9 software, where analysis of variance (ANOVA) and correlation among the variable were performed. Where appropriate, means were separated using least significant difference (LSD) test at 5% significant level. Relationship between treatments and variables were established using linear regression, stepwise selection model (Raghothana and Karthikeyan, 2005).

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Field Survey

4.1.1 Sources and reasons for planting African nightshade

According to the survey, farmers in Kisii and Siaya Counties had various reasons for planting African nightshade; these indicated how important African nightshade was in terms of livelihood improvement. In the studied counties, African nightshade was grown for several purposes; 55% of the farmers in Kisii and 50% from Siaya planted African nightshade for commercial purposes, 30% in Kisii and 20% in Siaya for domestic consumption, 10% in Kisii and 25% in Siaya grew naturally (not cultivated) and 5% from both regions had other uses like dye production (Table 4.1).

Table 4.1: Percentage number of farmer's reasons for growing African nightshade

County	Reason for planting African nightshade	%
Kisii	Commercialization purposes	55
	Domestic use	30
	Growing wild	10
	Other purposes	5
Siaya	Commercialization purposes	50
	Domestic use	20
	Growing wild	25
	Other purposes	5

Popularity of African nightshade has been increasing since early 2000s exceeding other common AIVs like Amaranths, Spider plant and Jute mellow (Masinde *et al.*, 2007). According to the survey, African nightshade had 45% production in Kisii and 40% in Siaya, Amaranths had 35% in Kisii and 25% in Siaya, spider plant had 10% in Kisii and 15% in Siaya and Jute mellow had 10% in Kisii and 20% in Siaya (Table 4.2).

Table 4.2: Percentage acreage of land under indigenous vegetables

County	Percentage acreage of land under indigenous vegetables	%
Kisii	Spider plant (*Cleome gynandra*)	10
	African nightshade (*Solanum sp.*)	45
	Amaranths (*Amaranthus sp.*)	35
	Jute mallow (*Corchorus olitorius*)	10
Siaya	Spider plant (*Cleome gynandra*)	15
	African nightshade (*Solanum sp.*)	40
	Amaranths (*Amaranthus sp.*)	25
	Jute mallow (*Corchorus olitorius*)	20

Recent campaigns by World Bank and World Health Organization (GoK, 2012) in the study regions on the importance of African nightshade explain reasons for planting and assigned acreage of African nightshade over other AIVs as shown in table 4.1 and 4.2. African nightshade and other indigenous vegetables traditionally used to be collected from the wild in surrounding bushes or weed forms in cultivated fields in rural areas (Masinde *et al*., 2009). Town dwellers would receive small portions normally as souvenirs by neighbours or relatives travelling from rural areas (Irungu *et al*., 2007). This trend slowly changed due to; awareness creation and promotion activities by NGOs and research organizations, increased general health awareness in the population, promotion of peri-urban production, provision of free and subsidized African nightshade planting materials, increased domestic and commercial demands and increased capacity for self organization with producer groups (Maundu *et al*. 1999).

Adaptability of African nightshade to varied environment and climatic condition had made it possible for the plant to be cultivated in nearly all types of soils and this has encouraged a lot of research in breeding of improved varieties (Weinberger *et al*. 2011). The ability of African nightshade to do better under low nutrients and limited crop husbandry had led to increased acreage as compared to other AIVs (Edmond and Chweya, 1997). Its acceptability by the community in terms of taste, propagules availability, readily available customers (consumers) was a few of the many reasons for increased acreage. Work by Abukutsa-Onyango (2003) in Western Kenya showed African nightshade was the most grown AIV. Similar results were also obtained by Asian Vegetable Research Development Center (AVRDC, 2004) reported ANs had higher cultivation in Western Kenya as compared to other AIVs.

4.1.2 Education, extension services and macronutrients application to manage African nightshade

The survey showed that farmers attained various levels of education. The level of education attained highly determined farm management practices by the farmer. African nightshade farmers who completed tertiary education were 20% in Kisii and 15% from Siaya where as 15% in Kisii and 25% in Siaya completed secondary education. Fifty five percent in Kisii and 40% in Siaya completed primary education. Ten percent and 25% of farmers from both Kisii and Siaya respectively never attended school (Table 4.3).

Extension services frequency in the two study counties highly influenced the spread, management and cultivation of African nightshade. According to the survey, farmers received varied frequencies of extension services. Forty percent of farmers in Kisii and 5% in Siaya were visited more than two times a year, 30% in Kisii and 20% in Siaya were visited twice a year, 10% in Kisii and 40% in Siaya were visited once a year. Twenty percent and 30% from Kisii and Siaya respectively were never visited (Table 4.3).

Types of macronutrients used based on fertilizers used affected production of African nightshade. According to the survey, Nitrogen used by farmer was 50% in Kisii and 40% in Siaya, those using Phosphorus used were 25% in Kisii and 10% in Siaya, Potassium based plant nutrition were 5% in both Kisii and Siaya and 20% in Kisii and 45% in Siaya never used any kind of fertilizer (Table 4.3).

Table 4.3: Percentage number of farmer's education, extension services and macronutrient use in African nightshade production

County	Level of Education	%	Extension service	%	Macronutients used in kg/ha	%
Kisii	Completed tertiary	20	> 2 times@year	40	Nitrogen	50
	Completed secondary	15	2 times@yaer	30	Phosphorus	25
	Completed Primary	55	1 time@ year	10	Potassium	5
	Never	10	Never	20	None	20
Siaya	Completed tertiary	15	> 2 times@year	5	Nitrogen	40
	Completed secondary	25	2 times@yaer	20	Phosphorus	10
	Completed Primary	40	1 time@ year	40	Potassium	5
	Never	25	Never	35	None	45

Farmers' education level and availability of extension services highly affected production and management of African nightshade (Chweya and Ezyaguirre, 1999). Farmers with higher level of education (above secondary education) had shown increased productivity of African nightshade in the study counties since they have knowledge on ways of management and improve ANs production. The results were in agreement with the survey by Ojiewo *et al*. (2010) who showed farmers with above secondary education in Western Kenya had better management and production skills of AIVs contributing to their value addition in market chain.

Farmers in Kisii received more extension services than in Siaya because Siaya farmers had organization of African nightshade based groups hence any information given to them by extension officer was disseminated in groups as compared to Kisii where extension officers visited farmers as individuals in their farms. Higher extension service in Kisii translates to increased productivity and productive sustainability of ANs in Kisii where African nightshade present in the market was not dependent on seasonality as compared to Siaya where availability of ANs was dependent on seasonality of the crop. Similar results were obtained by Ojiewo, (2007) who showed an increase of 3 fold in production of ANs after extension service had been offered to farmers after a period of three months.

Nitrogen, phosphorus and potassium are important plant nutrients that were required in fairly large amounts (Mwai and Schippers, 2004). In many cultivated soils, these nutrients were inadequately available, necessitating their addition in the form of fertilizers and organic manure. The amounts that needed to be added to the soil varied, depending on the type of crop and the nutrient content of the soil. Consequently, the study revealed that nitrogen and phosphorus were the highest macronutrients administered by farmers in the study counties. This was due to the existence of both organic and inorganic forms of Nitrogen and phosphorus which were easily available, for instance, organic waste from kitchen and animal shed offered substantial amount of Nitrogen in soil and also availability of rock Phosphate in some of the study counties made contributions to Phosphorus (Runge-Metzger, 1995). Phosphorus was the least available macronutrient in soils of the studied regions since sources of phosphorus were utilized without replenishment. The results were in agreement with Van Averbeka and Khosa, (2004) who profiled tropical soils and major N P K sources as organic home waste.

4.1.3 Dominant and preferred African nightshade varieties

Survey showed *Solanum nigrum* had the highest percentages dominance of 62% and 50% in Kisii and Siaya County respectively. *Solanum scabrum* had 15% in Kisii and 35% dominance in Siaya; *Solanum villosum* had 18% in Kisii and 5% dominance in Siaya. Other varieties had less than 5% dominance in all study regions (Table 4.4).

The highly preferred variety of African nightshade by the majority of the population (15 to 45 years) was *S. scabrum* with 78% and 65% preference in Kisii and Siaya respectively. *S. villosum* had 16% preference in Kisii and 25% in Siaya, the least preferred by the same group is the *S. nigrum* with 6% and 10% preference in both Kisii and Siaya respectively. The older population (over 46) preferred *S. villosum* that had 40% in Kisii and 45% in Siaya, *S. nigrum* had 22% in Kisii and 35% in Siaya. *Solanum scabrum* had 38% and 20% preference in Kisii and Siaya respectively (Table 4.4).

Table 4.4: Percentage number of farmers growing solanum and varietal preferences among age groups

County	Variety	Dominance (%)	Age	Variety	Preference (%)
Kisii	*S. nigrum*	62	15-45	*S. scabrum*	78
	S. villosum	18		*S. villosum*	16
	S. scabrum	15		*S. nigrum*	6
	S. americanum	3	Over 46	*S. scabrum*	38
	S. opacum (W)	1		*S. villosum*	40
	S. tarderemotum (W)	1		*S. nigrum*	22
Siaya	*S. nigrum*	50	15-45	*S. scabrum*	65
	S. villosum	35		*S. villosum*	25
	S. scabrum	5		*S. nigrum*	10
	S. americanum	5	Over 46	*S. scabrum*	20
	S. opacum (W)	3		*S. villosum*	45
	S. tarderemotum (W)	2		*S. nigrum*	35

W-wild

Percentage dominance was determined by number of variety growing without agronomic management (unattended) over the total of all the varieties growing without agronomic management (Chweya, 2007). The unattended varieties of the African nightshade were mostly observed on the unploughed fields or initially cultivated but abandoned lands especially in Siaya County.

Unattended varieties had distinctive features that were differentiated from domestic varieties for instance trichome on their leaf lamina, decreased leaf surface i.e. slender leaves, decreased biomass in terms of reduced leaf size and number (Mwai and Schipper, 2004). *Solanum nigrum* was among the first varieties of African nightshade with bitter taste but due to cross pollination with other varieties, *Solanum nigrum* had lost its bitter taste and some of its distinctive morphological characterists were fading with time (Maundu *et al*., 1999).

Population over 46 years preferred the narrow leaved varieties (*S. villosum* and *S. nigrum*) due to their bitter taste. They attributed bitter taste to medicinal value hence improved health and nutrition were the reason for their preference over the broad leaved variety (*S. scabrum*). The narrow leaved varieties were well adapted to soils with limited nutrients and minimal agronomic management hence for them to survive in such condition, they produce bitter compounds known as phenolics to enable them acquire nutrients, prevent infestation by pest and prevent infections from pathogens (Ren *et al*., 2007). These were self defence mechanisms that led to bitter taste. The results were in agreement with Edmonds and Chweya, (1999) who showed absolute preference of the bitter ANs by older population who regarded the bitterness as anti-ulcer.

Population of 15 to 45 years preferred the broad leave varieties (*S. scabrum*) due to the lack of bitter taste. They attribute the lack of bitter taste to palatability and ease in cooking which was energy saving since intensive boiling was not required to reduce bitter taste. These were the reasons for their preference over the narrow leaved varieties (*S. villosum* and *S. nigrum*) (Mwai and Schipper, 2004). Broad leaved varieties (SS) were not adapted to soils with limited nutrients and minimal agronomic management hence for them to thrive in such condition, farmers need to ensure good soil nutrient and perform routine agronomic managements. Masinde *et al*. (2007) also gave the same observation that the newly improved SS required farmer participation for their continuous yield improvement

4.1.4 Distribution and Occurrence of African nightshade

The baseline survey showed African nightshade varieties were available in the two Counties; Siaya and Kisii, determined in terms of distribution and occurrence. Percentage distribution was calculated as the distance covered from the first farmer to be interviewed to the next farmer over the total distance covered for the whole survey in the subject region. Percentage occurrence was calculated as the total number of varieties found on one piece of land over the number of varieties prevalent to the regions. In Siaya County, the highest distribution was observed in Yimbo east, followed by Central Gem and West Gem (Fig.4.1).

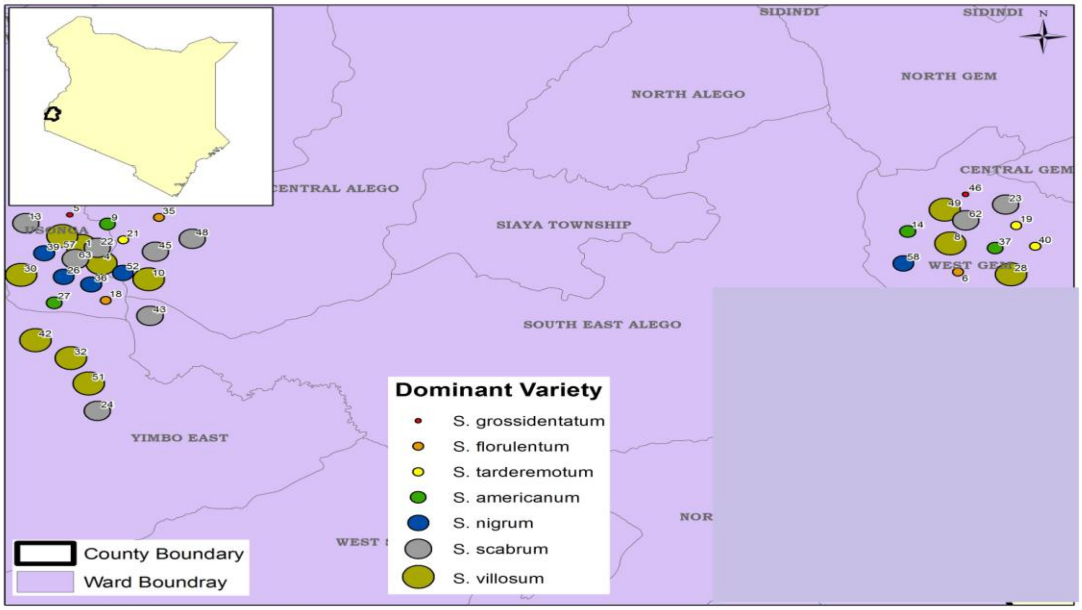

Figure 4.1: Map of Siaya County showing the distribution of African nightshade varieties (The size of the circle represents percentage distribution)

Kisii County recorded an even occurrence of African nightshade of 35%, 35% and 30% in Keumbu, Kisii and Mosocho respectively (Fig. 4.2).

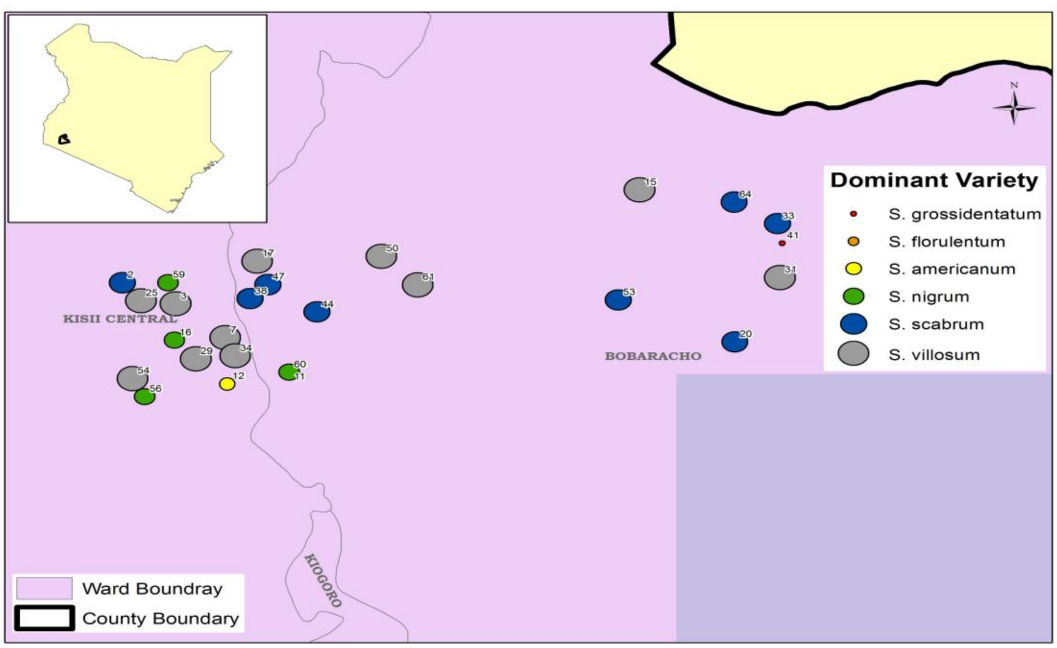

Figure 4.2: Map of Kisii County showing the distribution of African nightshade varieties (The size of the circle represents percentage distribution)

There were differences in African nightshade distribution and occurrence between the two counties surveyed (Fig.4.1 and 4.2). This was attributed to the plants adaptive ability to flower early, prolific seed production and the ability to tolerate many diverse habitats (Ojiewo *et al*., 2013). Although they perform well in a range of climatic conditions, African nightshade grow best under optimum P as evidenced in the survey where highest occurrence and distribution was observed in Siaya county (Yimbo East).

Table 4.5: African nightshade variety distribution and occurrence in different districts of Siaya and Kisii Counties, Kenya

County	District	Mean Distribution (%)	Mean Occurrence (%)
Siaya	Dominion	78	95
	Yala	13	4
	Gem	9	1
Kisii	Keumbu	45	35
	Kisii township	11	35
	Mosocho	44	30

Dominion region (Yimbo East) recorded the highest % soil phosphorus as compared to other surveyed regions (Figure 4.1 and Table 4.5). High occurrence and distribution of African nightshade in Dominion region was attributed to the recently reclaimed swamp lands of Kanyaboli. The region's soils are rich in organic matter, nutrients and moisture content making its lands a perfect seedbed for both wild and domesticated African nightshade varieties found in the region were; *Solanum scabrum*, *Solanum villosum*, *Solanum nigrum*, *Solanum florulentum*, *Solanum americanum* and *Solanum grossidentatum* (Abukutsa-Onyango, 2007).

Yala region (Central Gem) followed closely after Dominion. The region was characterized by many pit blogs with rich silt-loamy soils eroded from River Yala.

There was also plenty of irrigation water from River Yala hence this elevates the levels of extractable soil phosphorus (Masinde *et al*., 2009).

Gem region (West Gem) was characterized by vast lands with limited cultivation. Population was sparse hence shift cultivation was still practised. Gem region recorded the least % soil phosphorus levels (Fig. 4.3) and this was attributed to the poor soils, lack of irrigation water, lack of fertilizer use and poor land and agricultural management practices. The declining soil P in Gem region was due to lack of fertilizer use and continued nutrient utilization without replenishment. It is estimated that African nightshades remove 9.5 kgP/ha which is higher than additions in terms of P fertilization by the resources-poor farmers (Kaihura *et al*., 2001). The African nightshade varieties thriving in Gem are those adapted to limited soil phosphorus as also observed by (Rop *et al*., 2012).

The situation was however different in Kisii. There was existence of three common varieties that were distributed evenly, attributed to the land tenure system in Kisii where land has been fragmented and it is always cultivated throughout the cropping seasons (Edmond and Chweya, 1997). This has led to reduced diversity of exotic species that grow wild. The high existence and distribution of *Solanum scabrum*, *Solanum villosum* and *Solanum nigrum* was as a result of farmers planting them for subsistence and commercialization purposes. The results were in agreement with Abukutsa-Onyango, (2007) and Rao, (1996) who both ranked the three ANs as the most common in Western Kenya.

The averagely distributed % soil P in Kisii (Fig. 4.4) was as a result of erosion of rock phosphate from the hill top and leaching of phosphorus based fertilizers from tea farms that are major cash crops grown in Kisii (NEMA, 2013). When extractable P is absorbed from the soil, there is a gradual replenishment from the above named sources hence this explains the high mean % soil phosphorus in Kisii County as also reported by Masinde et al. (2009).

Studies by Maundu *et al*. (1999) showed that the broad leaved genotypes (*S. scabrum)* are more susceptible to P stress as compared to the narrow leaved genotypes (*S. nigrum* and *S. villosum*) as evidenced by the existence of both *S. villosum* and *S. nigrum* in both Kisii and Siaya counties. Both moisture and temperature affect the uptake of P from soil; since phosphorus uptake requires optimum temperature and moisture Maundu *et al*. (1999).

4.1.5 Mapping based on Soil and plant tissue phosphorus in Siaya and Kisii Counties

Soil analysis done on the surveyed regions in Kisii and Siaya showed varied amounts of extractable P. Survey showed that soils in Siaya had low concentrations of extractable soil P ranging from 0.27-1.42 %. (Fig. 4.3) (smallest circle 0.27%, biggest circle 1.42%).

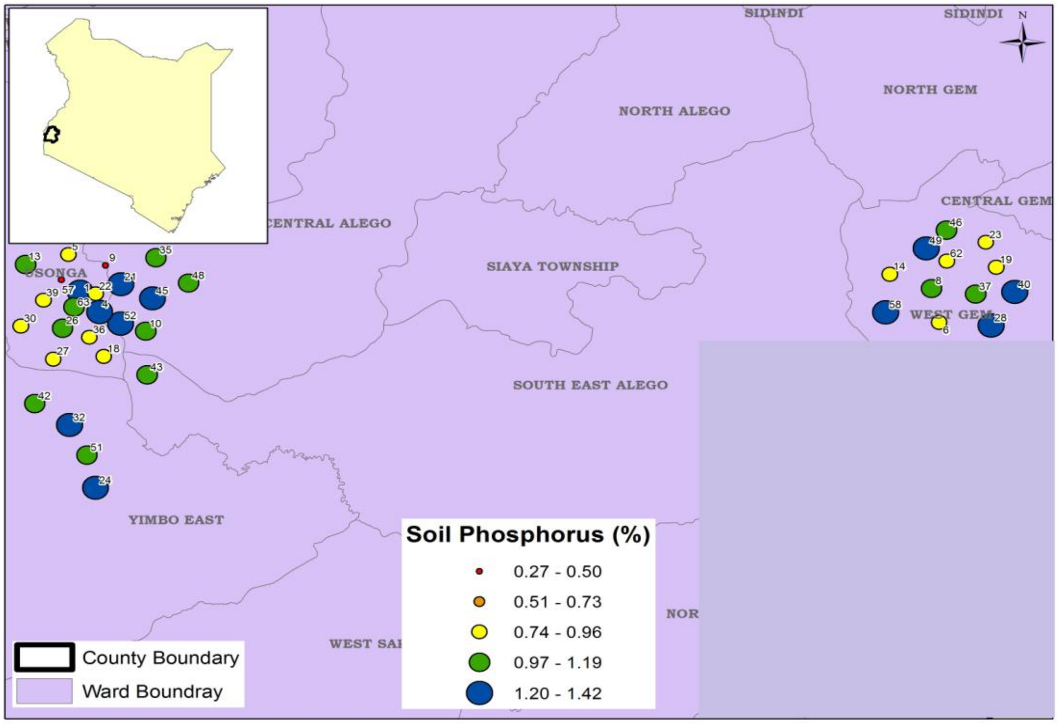

Figure 4.3: Map of Siaya County showing the distribution of soil phosphorus (The size of the circle represents the amount of P in soil)

Low soil P was as a result of low use of fertilizer that now stands at an average rate of 1.5 kgP/ha. This was attributed to high cost of fertilizers and transport, untimely availability, unpredictable rainfall, inadequate supply, and lack of credit which was also reported by Chweya, (1997). Despite low soil P in Siaya County, a few regions reported high soil P. High soil P in these regions were attributed to cool climate, leached phosphate fertilizer from rice fields, availability of water and fertile soils that resulted from recently reclaimed swamp lands .

Generally, soil moisture and temperature affect P availability (Masinde *et al*., 2009). In cool and wet soils, P availability and movement was enhanced (Jones *et al*., 2003). As a result, crops were more responsive to phosphate fertilizer in cool, wet spring conditions than in warmer, drier spring conditions (Chapin *et al*., 1986). Optimal soil moisture and temperature can help accelerate microbe activity, thereby releasing more P from organic matter. Adequate soil moisture enhanced availability of extractable P in soil, promoted plant growth; hence soil P and other nutrient requirements were generally higher for crops grown under irrigation or in higher rainfall areas (Masinde *et al*., 2009). High soil extractable P translates to high P concentration in Plant tissue (Raghothana and Karthikeyan, 2005).

Kisii County reported a higher and even distribution of extractable soil P from 1.3-1.7% (Fig. 4.4) (smallest circle 0.13% and biggest circle 1.31%) as compared to Siaya.

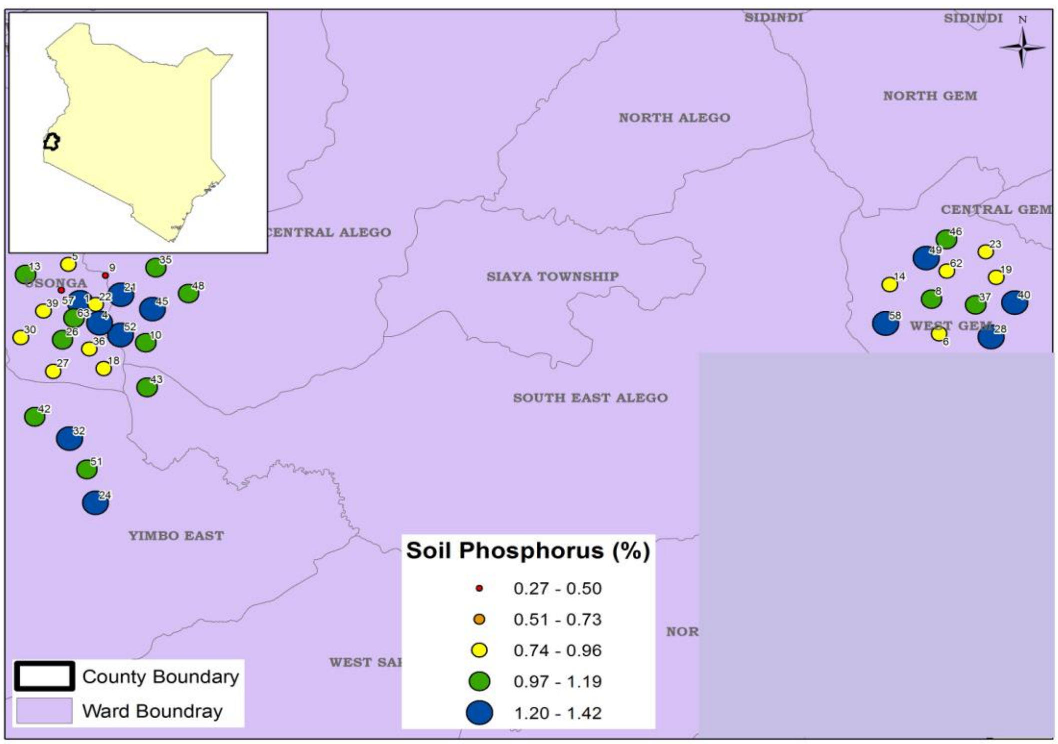

Figure 4.4: Map of Kisii County showing the distribution of soil phosphorus (The size of the circle represents the amount of P in soil)

This was probably because most of the farmers in Kisii County grow tea. According to the KTDA advice, phosphate fertilizer should be administered every three months. The fertilizer was probably leached through the soil strata and found its way to low lying African nightshade farms (NEMA, 2013). Erosion of rock phosphate from the hilltops may also be another contributing factor (Chweya, 2007). These led to high and even distribution of extractable soil P. When extractable P was absorbed from the soil, there was a gradual replenishment from the above named sources hence this partly explained the high soil P in Kisii County. High extractable soil P translates to high plant P (Raghothana and Karthikeyan, 2005).

4.1.6 Mapping of Total Phenolic Content and Total Antioxidant Activity in Siaya and Kisii County

According to the survey, there was variation in plant analysis of TPC and TAA from the regions sampled in both Siaya and Kisii. In Siaya, TPC varied between 6.56-31.71 mg/g whereas in Kisii it varied from 15.43-38.23 mg/g (Fig.4.5 and 4.6)

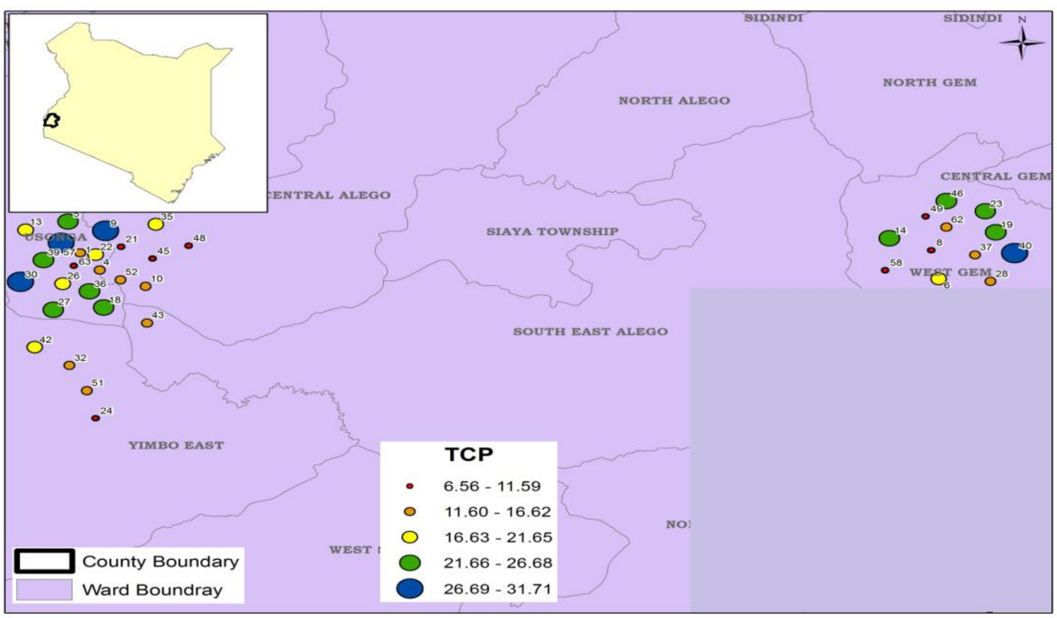

Figure 4.5: Map of Siaya County showing the distribution of Plant TPC (The size of the circle represents the amount of TPC in plant)

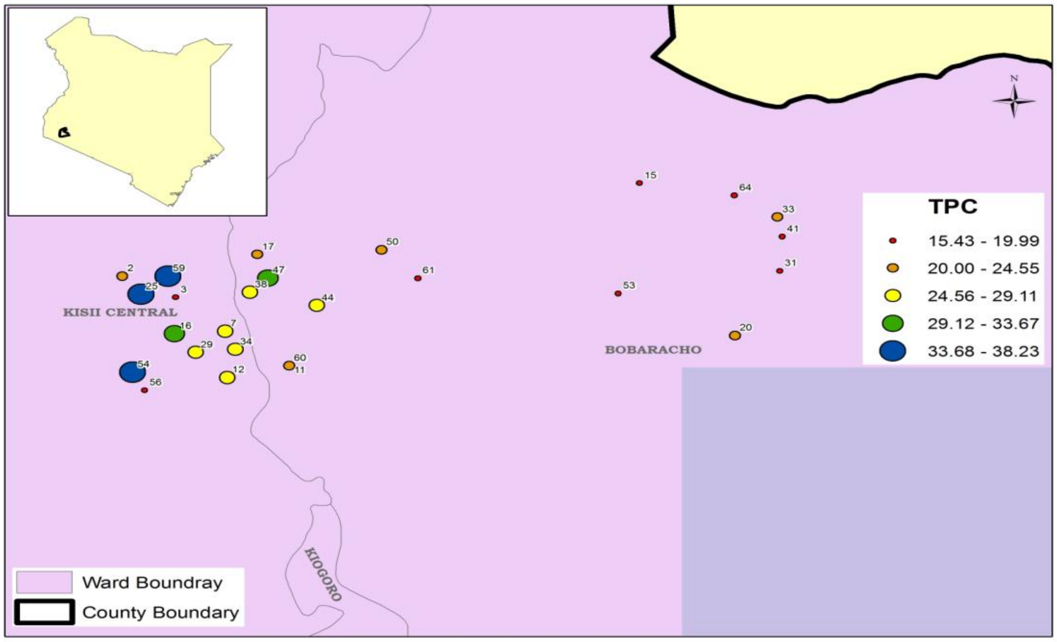

Figure 4.6: Map of Kisii County showing the distribution of Plant TPC (The size of the circle represents the amount of TPC in plant)

TAA in Siaya and Kisii varied between 15.60 to 56.00% and 24.00 to 61.00% respectively (Fig.4.7 and 4.8).

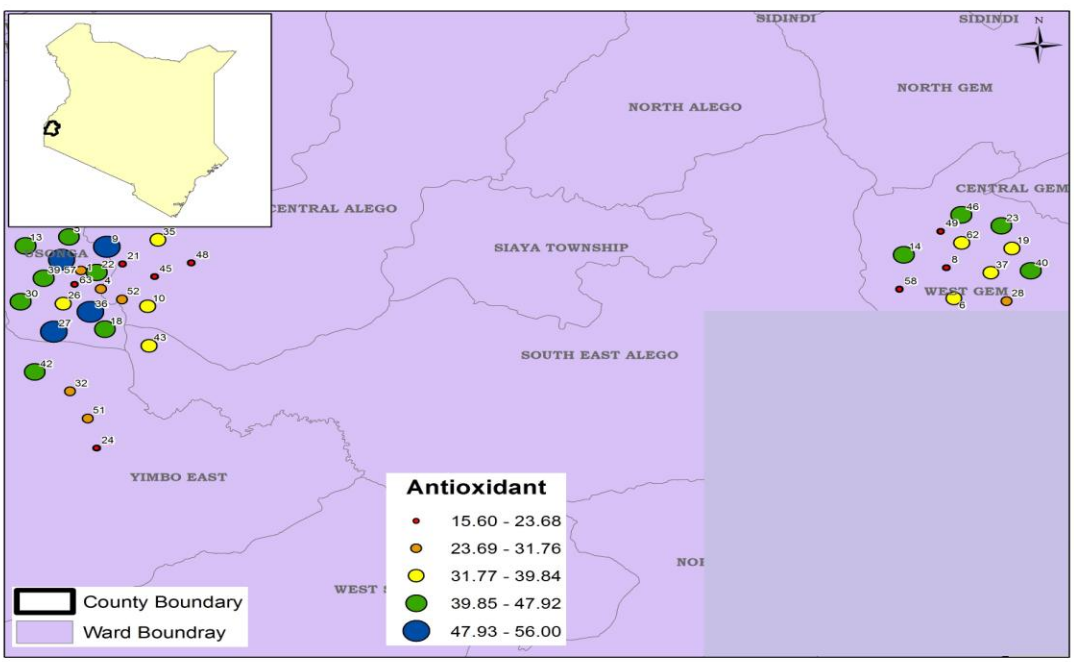

Figure 4.7: Map of Siaya County showing the distribution of Plant TAA (The size of the circle represents the amount of TAA in plant)

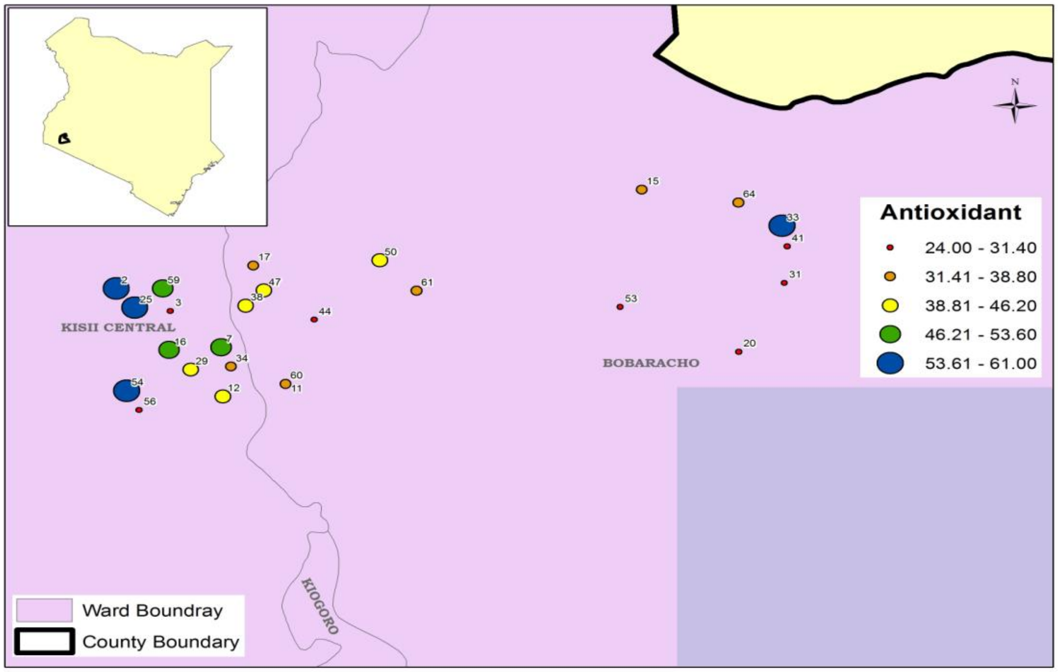

Figure 4.8: Map of Kisii County showing the distribution of Plant TAA (The size of the circle represents the amount of TAA in plant)

Kisii had higher TPC and TAA than Siaya County, though it had averagely higher extractable soil P. According to Masinde *et al*. (2007), plants produce less secondary metabolites when grown in soils with sufficient nutrients but this depends on other factors such as temperature and moisture. Different climatic conditions experienced by plants grown in soils with sufficient nutrients have been known to have increased production of secondary metabolites (Ren *et al*., 2007). In Kisii County, increased production of TPC and TAA was probably attributed to moisture deficiency and high temperatures despite the higher extractable soil P during the sampling period hence the sample plants responded to water stress and elevated temperatures through production of phenolics and antioxidants.

These results were in agreement with Edmonds and Chweya (1997) who showed that leaves harvested from stressed plants were bitter (less TPC and TAA) as compared to those harvested from plants grown in full sun. African nightshade tolerates shade but grows better when exposed to full sun as long as adequate water and soil nutrients were available (Edmonds and Chweya, 1997). Broad-leafed types (*S. scabrum*) were generally intolerant to water stress and poor soil nutrients while those with narrow leaves (*S. villosum* and *S. nigrum*) tolerated water stress and poor soil nutrients better (Masinde *et al*., 2006)

Annual rainfall of 500-1200 mm was adequate for growth of African nightshade (Chweya and Ezyaguirre, 1999). It also grows on various soil types, but were best adapted to high fertility as they grow well in soils with high phosphorus and rich in organic matter. Masinde *et al*. (2010) reported that phosphorus increased leaf yields upto 3 fold, but there were no genotypic differences in the levels of response, indicating that genetic diversity may not necessarily influence phosphorus use efficiency (Fontem *et al*., 2004). Increased production of TPC and TAA were reported when plants were stressed (Ren *et al*., 2007). Stress in plants was experienced when there was nutrient or moisture deficiency also observed by Edmonds and Chweya, (1997).

4.2 Effect of phosphorus rate and variety on growth parameters of the ANs.

4.2.1 Leaf fresh weight

There was significance (P≤ 0.05) increase in leaf fresh weight in plants grown in the greenhouse, long and short rains in response to P. The fresh leaf weight increased with increase in P in the greenhouse, long and short rains (Fig. 4.9) probably confirming that P was limiting in the soils of field experiment.

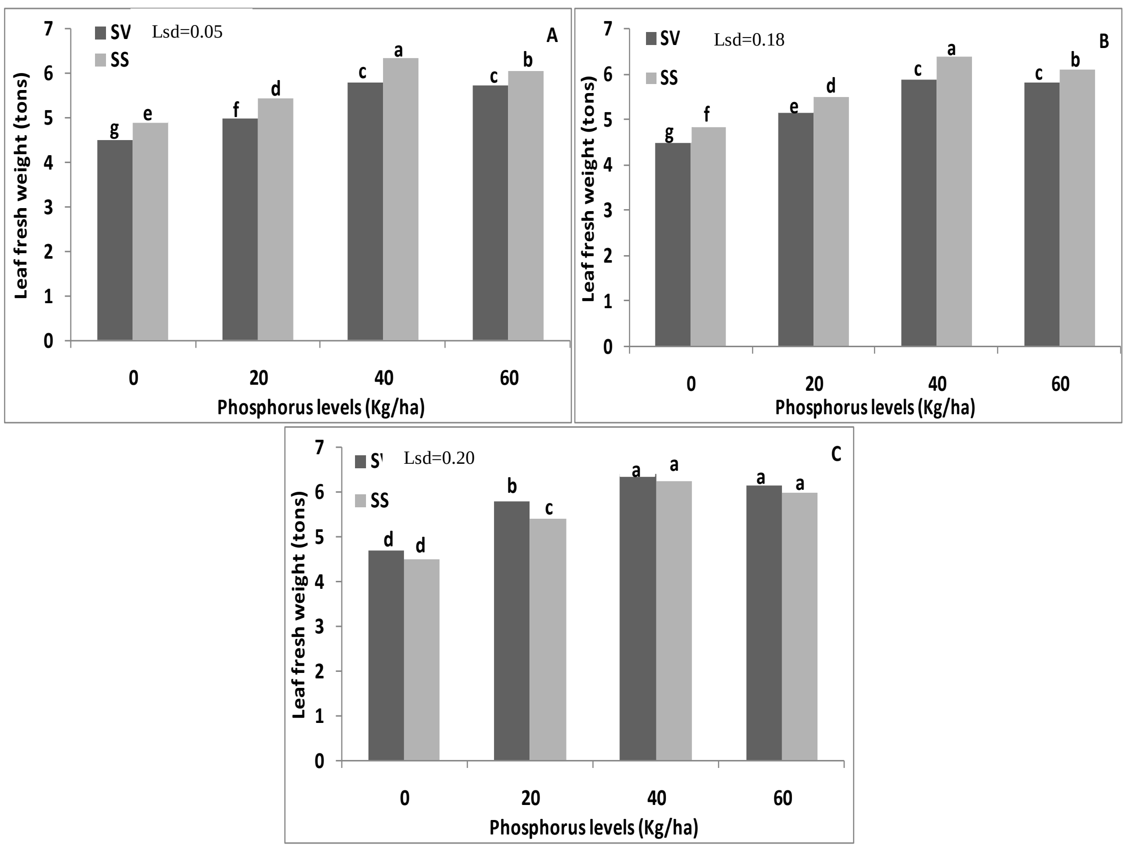

Figure 4.9: Effects of Phosphorus levels on leaf fresh weight of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Fresh weight was significantly affected by different levels of Phosphorous. Results showed that maximum fresh weight (5.8t/ha for *S. villosum* and 6.35t/ha for *S. scabrum*) was produced by the treatments of 40 kg/ha, followed by 60 kg/ha (5.75t/ha for *S. villosum* and 6.05t/ha for *S. scabrum*) then by 20 kg/ha (5.0t/ha for *S. villosum* and

5.45t/ha for *S. scabrum*).There was no significant differences between plants treated with 40 kg/ha and 60 kgP/ha in all experiments. As expected, the control treatment (0 kgP/ha) resulted in the lowest leaf fresh weight (Fig. 4.9). *Solanum villosum* had higher fresh weight during the short rains as compared to *S. scabrum* that had higher fresh weight in the long rains.

Phosphorus application of 40kg/ha resulted in the greatest leaf fresh weight as compared to other P levels. This indicated that P at 40 kg/ha was the optimum rate that led to desirable increase in production per unit area. However at 60 kgP/ha, there was a decrease in fresh weight rendering it uneconomical and a waste of resources. Optimum supply of P was associated with increased root growth, resulting in plants exploration in more soil nutrients and moisture (Khan *et al.*, 1999). Lack of P affects root growth of plants. This negatively affects other physiological functions leading to reduced fresh weight as also reported by Sharma and Sharma, (1996). Increase in fresh weight as a result of P application has also been reported by other previous worker (Maqsood *et al.*, 2001). Jones *et al*. (2003) and Uarrota, (2010) also reported that fresh yield of *Solanum nigrum* increased with increase of P application.

Decline in leaf fresh weight at 60 kg/ha was thought to be attributed to deficiencies of other micronutrients. Crop response to Phosphorus was affected by unavailability of micronutrients (Stuckenholtz *et al*., 1966). An interaction of the excess phosphorus with labile available micronutrients, particularly Zinc and Copper, made them unavailable for plant use. Soil analysis test done before and after harvesting showed decrease in Zinc and Copper percentage quantity (Fig. 4.10, 4.11 and 4.12). Results are similar to those of Thorne (1957) and Stuckenholtz *et al*. (1966) experimenting on Amaranths where high levels of available phosphorus induced zinc and copper deficiency.

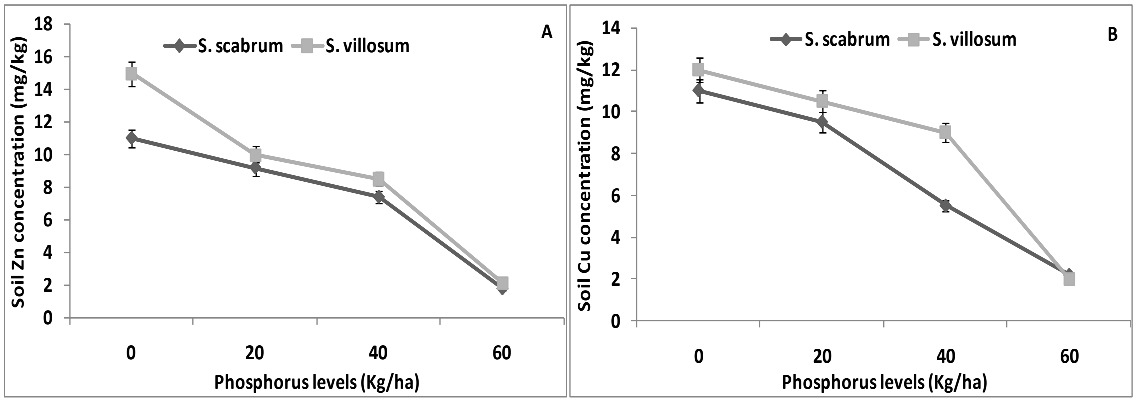

Figure 4.10: Effect of phosphorus levels on soil zinc (A) and copper (B) concentration in greenhouse, 40 days after transplanting

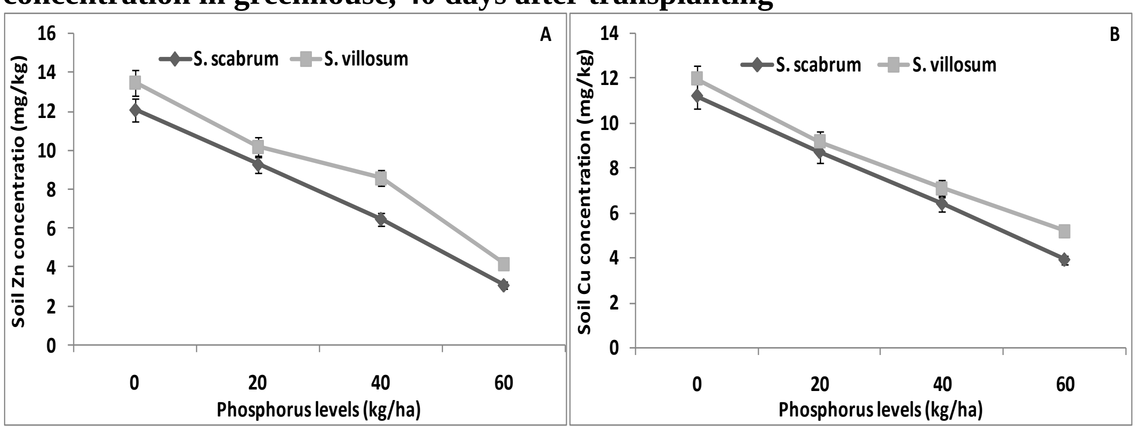

Figure 4.11: Effect of phosphorus levels on soil zinc and copper concentration in long rains, 40 days after transplanting

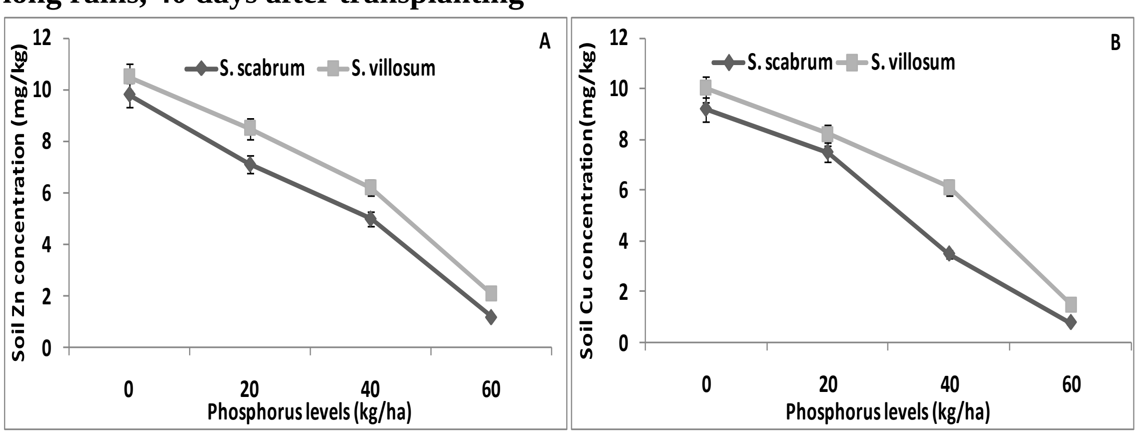

Figure 4.12: Effect of phosphorus levels on soil zinc and copper concentration in short rains, 40 days after transplanting

The results revealed an interaction between varieties and P on leaf fresh weights for greenhouse, long and short rains are presented in Table 4.6. There was significant interaction at (P≤ 0.05) between variety and phosphorus rate on fresh leaf weights indicating that varieties responded similarly to P application interms of leaf fresh weight. Given that varieties responded similarly to P application, the differences in their fresh weight at similar P rates was attributed to their inherent morphological characteristics; *S. scabrum* has inherently bigger leaves and thick stem as compared to *S. villosum* (Edmonds and Chweya, 1997).

Table 4.6: Effect of phosphorus rate and variety on fresh leaf weight (tons) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Fresh weight	Fresh weight	Fresh weight
S.villosum	0	4.5^{g}	4.52^{g}	4.7^{d}
	20	5^{e}	5.17^{e}	5.8^{b}
	40	5.8^{c}	5.9^{c}	6.42^{a}
	60	5.75^{c}	5.82^{c}	6.15^{a}
S.scabrum	0	4.92^{f}	4.85^{f}	4.5^{d}
	20	5.45^{d}	5.52^{d}	5.4^{c}
	40	6.35^{a}	6.41^{a}	6.27^{a}
	60	6.05^{b}	6.12^{b}	6^{a}
LSD		0.05	0.18	0.2
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

4.2.2 Leaf area and number of secondary buds

Leaf area was significantly affected at (P≤0.05) by the different levels of phosphorus in greenhouse, long and short rains. There was no statistical difference between 40 and 60 kgP/ha in leaf area from greenhouse and short rains. Mean values of the data indicated that maximum leaf area (150.35 cm^2/plant for *S. villosum* and 166.52 cm^2/plant for *S. scabrum*) was recorded on plants with P applied at 60 kg/ha followed by P at 40 kg/ha (145.94 cm^2/plant for *S. villosum* and 161.44 cm^2/plant for *S. scabrum*) then by P at 20 kg/ha (108.26 cm^2/plant for *S. villosum* and 122.52 cm^2/plant for *S. scabrum*) and finally the control (Fig. 4.13).

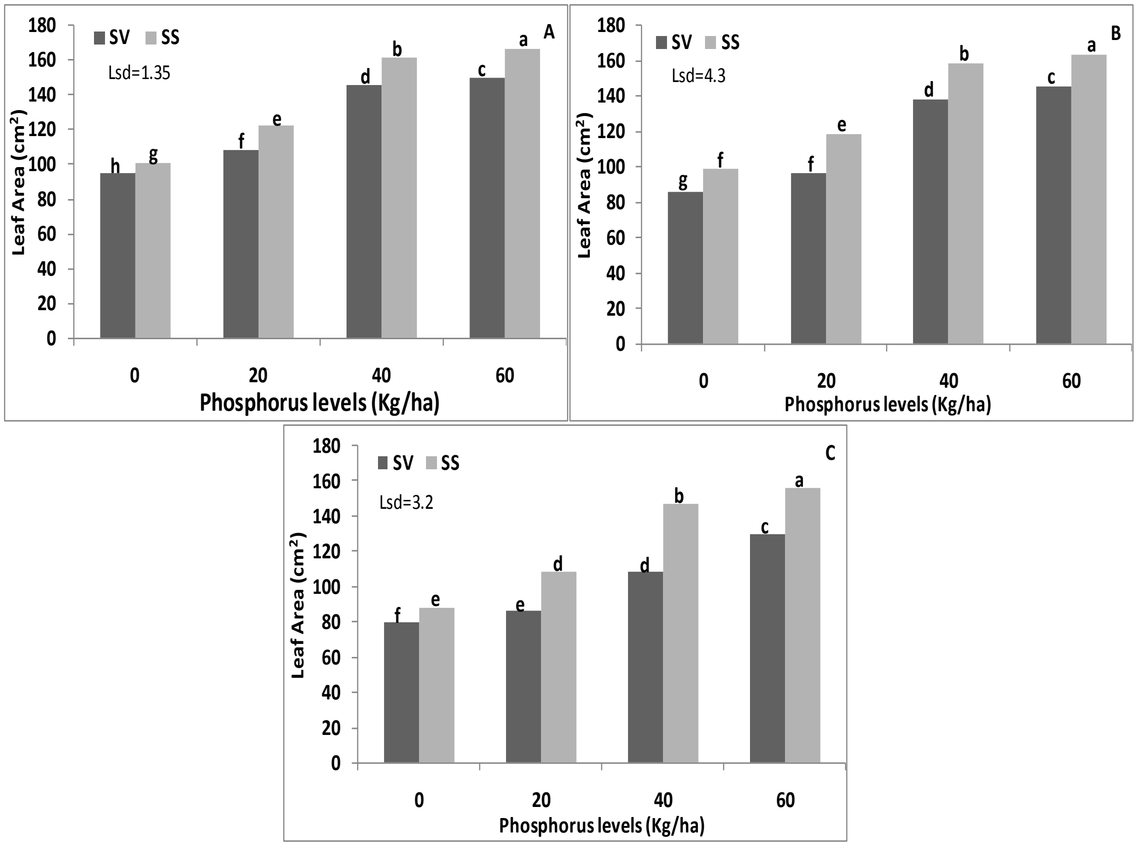

Figure 4.13: Effects of Phosphorus levels on leaf area of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Phosphorus at 40 kg/ha was the optimum rate for best results in maximum leaf area. This was because increasing P level from 40 to 60 kg/ha had a directly proportional effect on the maximum leaf area but with no significant difference.

Plants with P deficiency (0 kgP/ha) partition large fraction of resources to the root system and as a result, leaf growth and expansion become restricted leading to less carbon assimilation resulting in low shoot biomass (Poorter and Nagel, 2000). Similar results were also reported by Hussain *et al.* (2006) who reported that fresh yield increased with phosphorus application and plots receiving 50kg/ha gave maximum fresh yield as compared to the lower P treatments.

Leaf area for greenhouse, long and short rains presented in Table 4.7 showed significant interaction at ($P \leq 0.05$) between variety and phosphorus rate on leaf area in greenhouse, long and short rains indicating that varieties responded similarly to P application in terms of fresh leaf weight.

Table 4.7: Effect of phosphorus rate and variety on leaf area (cm^2) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Leaf area	Leaf area	Leaf area
S.villosum	0	95.8^{h}	85.6^{g}	79.6^{f}
	20	108.3^{f}	96.4^{f}	86.2^{e}
	40	146^{d}	138.2^{d}	108.3^{d}
	60	150.4^{c}	145.9^{c}	129.9^{c}
S.scabrum	0	100.6^{g}	98.8^{f}	88.2^{e}
	20	122.5^{e}	118.7^{e}	108.5^{d}
	40	161.4^{b}	158.41^{b}	147.2^{b}
	60	166.5^{a}	163.4^{a}	156.1^{a}
LSD		1.35	4.3	3.2
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). * Significant F values at $P \leq 0.05$ and NS-Not significant.

Given that varieties responded similarly to P application, the differences in their leaf area at similar P rates were attributed to their inherent morphological characteristics; *S. scabrum* has inherently bigger leaves than *S. villosum*.

Data revealed that different levels of phosphorus had significant effect ($P \leq 0.05$) on number of secondary buds of African nightshade (Fig. 4.10). The application of phosphorus at 60 kg/ha resulted in the highest number of secondary buds (20 for *S. villosum* and 17 for *S. scabrum*), which was consistent with all the other treatments of P application except the control (10 for *S. villosum* and 7 for *S. scabrum*). This indicates that P at 60 kg/ha was the optimum rate for improvement in number of secondary buds which ultimately had a direct effect on the number of leafs.

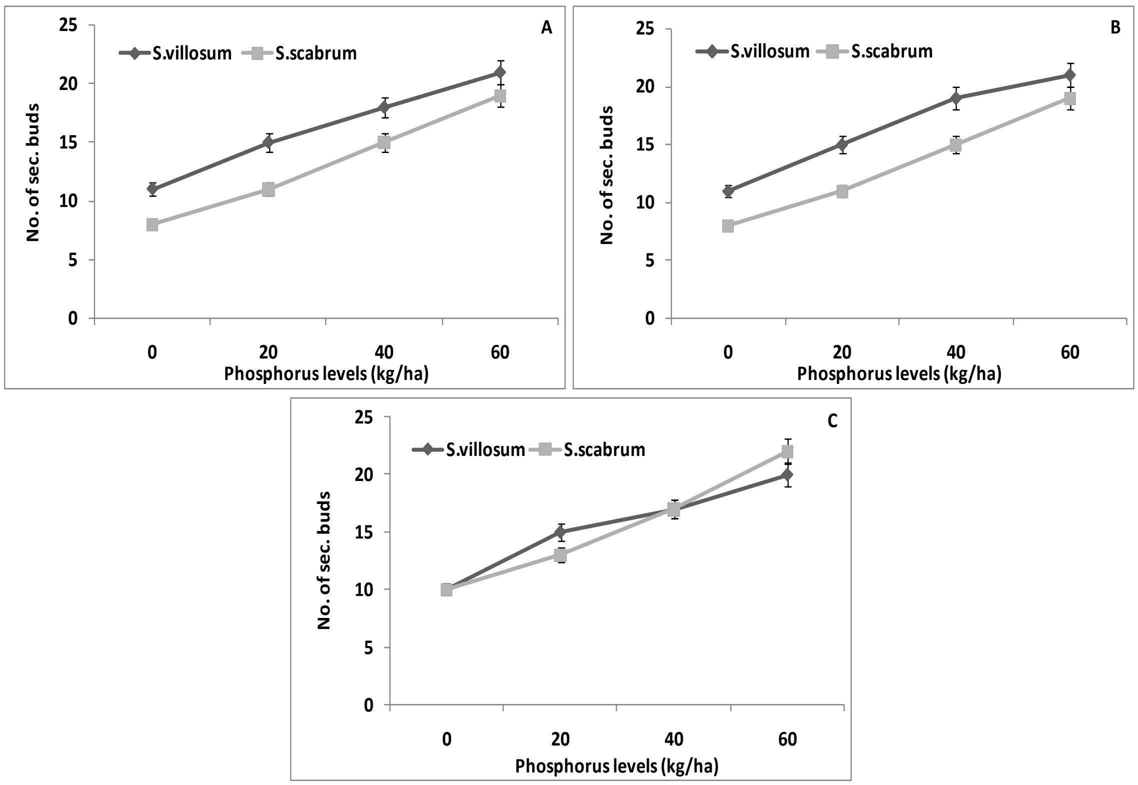

Figure 4.14: Effects of Phosphorus levels on number of secondary buds of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

The direct proportionality of phosphorus and the number of secondary buds was explained by the functions of phosphorus in plant. Phosphorus seem to responsible for good root growth which directly affects the overall plant performance; the regimes of phosphorus at the rate of 0 kg/ha resulted in the least number of secondary buds (Fig. 4.14).

These results were in accordance with Gurmani *et al*. (2006) who reported that phosphorous fertilizer applications significantly affected the number of tillers produced by vetch fodder. Muthomi and Musyimi, (2009) also reported that numbers of secondary buds were influenced significantly with phosphorus application; the higher the application the greater the number of secondary buds in African nightshade.

The numbers of secondary buds planted in the greenhouse, long and short rains presented in Table 4.8 showed significant interaction at ($P \leq 0.05$) between variety and phosphorus rate on numbers of secondary buds indicating that varieties responded similarly to P application in terms of number of buds.

Table 4.8: Effect of phosphorus rate and variety on number of secondary buds in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	No. of sec. buds	No. of sec. buds	No. of sec. buds
S.villosum	0	11^{c}	11^{c}	10^{c}
	20	15^{b}	15^{b}	15^{b}
	40	18^{a}	18^{a}	17^{b}
	60	21^{a}	21^{a}	20^{a}
S.scabrum	0	8^{d}	8^{d}	10^{c}
	20	11^{c}	11^{c}	13^{b}
	40	15^{b}	15^{b}	17^{b}
	60	19^{a}	19^{a}	22^{a}
LSD		2	2	2
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). * Significant F values at $P \leq 0.05$ and NS-Not significant.

Given that the varieties responded similarly to P application, the differences in the numbers of secondary buds at similar P rates was attributed to their inherent morphological characteristics; *S. villosum* has inherently more number of secondary buds than *S. scabrum*.

4.2.3 Plant height

Data regarding plant height indicated that different levels of phosphorus had significant difference at ($p \leq 0.05$) on plant height in greenhouse, during long and short rains.

Phosphorus application at 40 kg/ha resulted in long stature plants (53.5 cm for *S. villosum* and 61 cm for *S. scabrum*) followed by P at 60 kg/ha (45.2 cm for *S. villosum* and 51 cm for *S. scabrum*), P at 20 kg/ha (48.2 cm for *S. villosum* and 55 cm for *S. scabrum*) and finally P applied at 0 kg/ha (38 cm for *S. villosum* and 44.87 cm for *S. scabrum*) (Fig. 4.15).

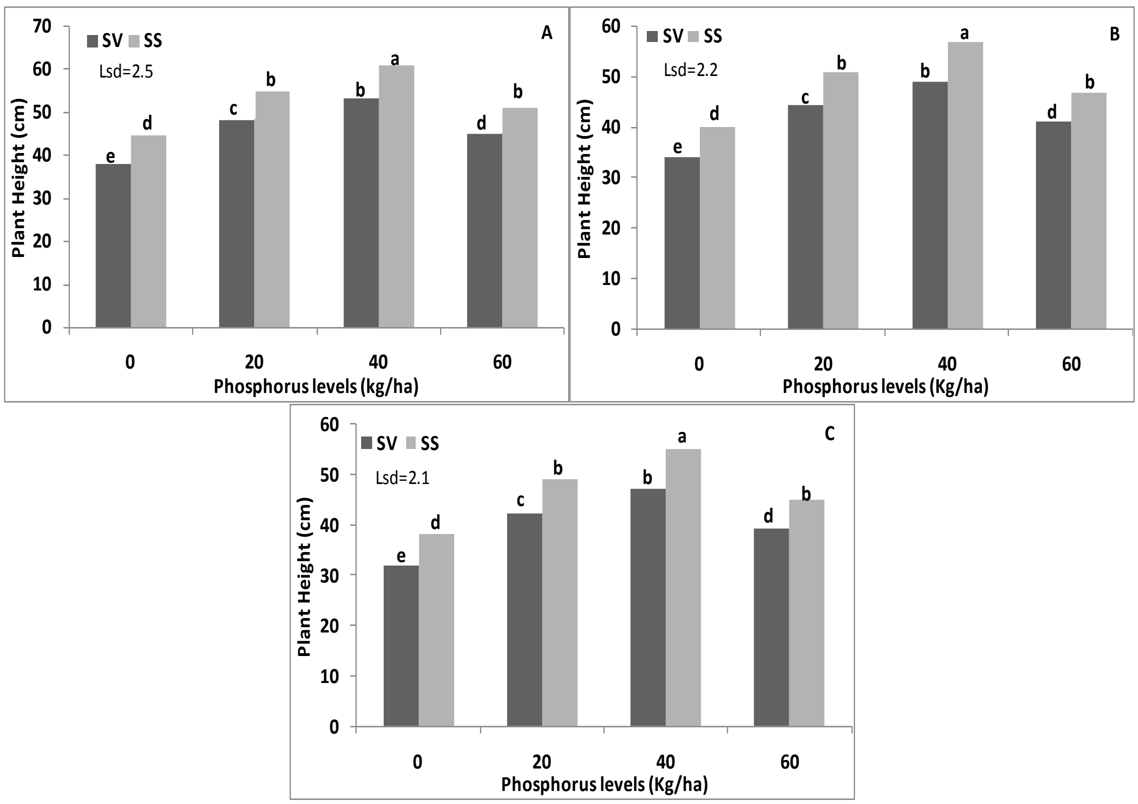

Figure 4.15: Effects of Phosphorus levels on plant height of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Increase in plant height with increase in phosphorus level was attributed to phosphorus ability to improve root growth which had a great effect on the overall plant growth performance; therefore the regimes of P at the rate of 0 kg/ha resulted in the shortest stature plants. Promotion effect of high P level on plant height was due to better development of root system and nutrient absorption (Turner *et al*., 2002). Indira, (2005) and Wolf, (1999) reported plant height of cowpea increased with increase in P application.

The heights of varieties administered with the same P rate, in the first season followed the order *S. scabrum* > *S. villosum* converse to the second season. The trend changed during short rains. *S. villosum* proved to be drought tolerant as compared to *S. scabrum* as it had taller plants in the absence of sufficient water. Decline in plant height at 40 kgP/ha (63.5 cm) to (61.95 cm) at 60 kgP/ha was attributed to deficiencies of other micronutrients same as in fresh weight.

Results of plant heights for greenhouse, during long and short rains are presented in Table 4.9 show no significant interaction at (P≤ 0.05) between variety and phosphorus rate on plant height, indicating that varieties responded similarly to P application in terms of plant height.

Table 4.9: Effect of phosphorus rate and variety on plant height (cm) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Plant height	Plant height	Plant height
S.villosum	0	38[e]	34[e]	32[e]
	20	48.2[c]	44.5[c]	42.3[c]
	40	53.5[b]	49.1[b]	47.1[b]
	60	45.2[d]	41.2[d]	39.3[d]
S.scabrum	0	44.87[d]	40.2[d]	38.2[d]
	20	55[b]	51[b]	49[b]
	40	61[a]	57[a]	55[a]
	60	51[b]	47[b]	45[b]
LSD		2.5	2.2	2.1
PxV		NS	NS	NS

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

4.2.4 Root area

Root area was significantly affected (P≤0.05) by different levels of Phosphorous in the greenhouse, during long and short rains. Mean values of the data showed maximum root area was recorded at 60 kgP/ha (59.27 cm^2 for *S. villosum* and 44.52 cm^2 for *S. scabrum*) followed by P at 40 kg ha^{-1} (55.2 cm^2 for *S. villosum* and 40.31 cm^2 for *S. scabrum*) then P at 20 kg ha^{-1} (45.8 cm^2 for *S. villosum* and 31.35 cm^2 for *S. scabrum*) (Fig. 4.16).

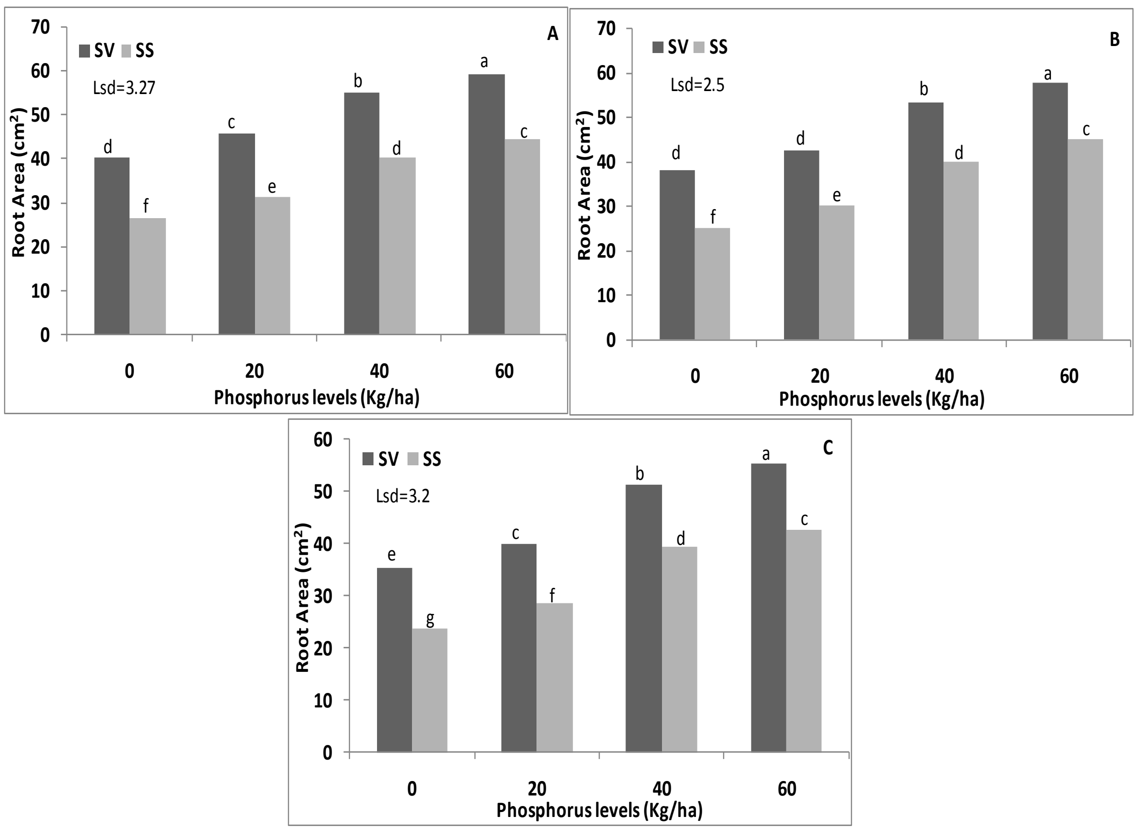

Figure 4.16: Effects of Phosphorus levels on root area of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Phosphorus at 0 kg/ha (40.5 cm^2/plant for *S. villosum* and 26.7 cm^2/plant for *S. scabrum*) resulted in minimum root area. Phosphorus application at 40 and 60 kg/ha resulted in long statured plants, big leaved plants, and more secondary buds, which resulted in greater fresh weight yield as compared to other P levels. This revealed P at 60 kg/ha was the optimum rate to cause a desirable increase in root area.

Increase in root proliferation due to P application was also documented (Onyango *et al.* 1999 and Opiyo, 2004). Merinyo, (1996) reported that root area in black nightshade increased with increase in P application that is black nightshade treated with 60 kg/ha exhibited maximum root area as compared to plants receiving lower P treatments.

Optimum supply of P was associated with increased root growth that is related to plants exploration of more soil nutrients and moisture. The root areas were lowest in plants treated with 0 kgP/ha. Lack of P reduced the root growth of plants which negatively affected the other physiological functions.

Root areas for greenhouse, during long and short rains presented are in Table 4.10 showed significant interaction ($P \leq 0.05$) between variety and phosphorus rate on root indicating that varieties responded differently to P application in terms of root area.

Table 4.10: Effect of phosphorus rate and variety on root area (cm^2) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Root area	Root area	Root area
S.villosum	0	40.5^{d}	38.3^{d}	35.5^{e}
	20	45.8^{c}	42.7^{d}	40.1^{c}
	40	55.2^{b}	53.5^{b}	51.3^{b}
	60	59.27^{a}	57.8^{a}	55.3^{a}
S.scabrum	0	26.7^{f}	25.2^{f}	23.7^{g}
	20	31.35^{e}	30.38^{e}	28.6^{f}
	40	40.31^{d}	40.2^{d}	39.4^{d}
	60	44.52^{c}	45.3^{c}	42.6^{c}
LSD		3.27	2.5	3.2
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). * Significant F values at $P \leq 0.05$ and NS-Not significant.

Given that varieties responded similarly to P application, the differences in their root areas at similar P rates were attributed to their inherent morphological characteristics; *S. villosum* has inherently bigger root areas than *S. scabrum* (Opiyo, 2004). *S. villosum* are known to do well in soils with nutrients deficiencies and limited water due to their extensive roots that help in acquisition of water and nutrients sources from deep soil strata (Merinyo, 1996).

4.2.5 Concentration of Phosphorus in shoot

Shoot phosphorus was significantly affected ($P \leq 0.05$) by different levels of phosphorous in the greenhouse, long and short rains. Mean values of the data showed that maximum shoot P was recorded on plants with P applied at 60 kg/ha (24.3 mg/kg for *S. villosum* and 21.3 mg/kg for *S. scabrum*).There after by P at 40 kg/ha (19.8 mg/kg for *S. villosum* and 17.2 mg/kg for *S. scabrum*) then P at 20 kg/ha (18.7 mg/kg for *S. villosum* and 16.1 mg/kg for *S. scabrum*) (Fig. 4.17). The control (16.3 mg/kg for *S. villosum* and 15 mg/kg for *S. scabrum*) resulted in minimum shoot P.

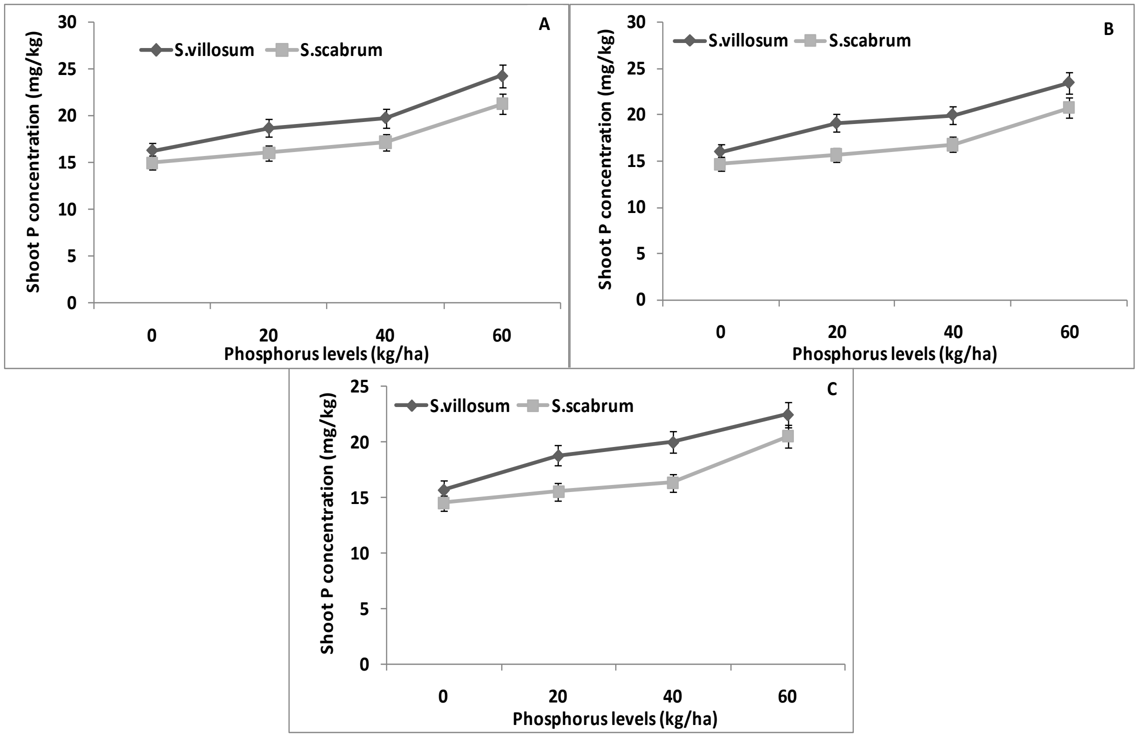

Figure 4.17: Percentage shoot phosphorus concentration in (A) greenhouse, (B) long rains and (C) short rains respectively 40 days after transplanting

Shoot P concentration increased with increase in soil P availability, therefore, shoot P concentration was more pronounced at higher P treatment (60 kg/ha) that recorded 23.7 mg/kg for *S. villosum* and 21.5 mg/kg for *S. scabrum*. Plant P uptake depends not only on P availability but also on physiological traits of different plant genotypes for instance, *S. villosum* has a higher shoot P concentration of 23.7 mg/kg as compared to 21.5 mg/kg for *S. scabrum*.

There was significant interaction (P≤ 0.05) between variety and phosphorus rate for plants in greenhouse, long and short rains indicating that varieties responded differently to P application in terms of shoot P (Table 4.10). Given that the varieties responded similarly to P application, the differences in their shoot P at similar P rates was attributed to their inherent morphological characteristics; *S. villosum* has plant adaptation and properties such as root architecture, possession of adventitious roots and exudation of anions in the rhizosphere that enables it to acquire more P from the soil than *S. scabrum.* Exudation of organic compounds by different varieties of Lupin as a way of acquiring P from soils was also reported by Hinsinger (2009).

Table 4.11: Effect of phosphorus rate and variety on shoot phosphorus (mg/kg) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	% Shoot P	% Shoot P	% Shoot P
S.villosum	0	16.3^{e}	16^{f}	15.7^{f}
	20	18.7^{d}	19.1^{d}	18.8^{d}
	40	19.8^{c}	20^{c}	20^{c}
	60	24.3^{a}	23.5^{a}	22.5^{a}
S.scabrum	0	15^{f}	14.7^{g}	14.5^{g}
	20	16.1^{e}	15.7^{f}	15.5^{f}
	40	17.2^{e}	16.8^{d}	16.3^{e}
	60	21.3^{b}	20.8^{b}	20.5^{b}
LSD		1	0.7	0.3
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

The difference between the genotypic shoot P concentrations indicated that *S. villosum* had plant adaptation and properties such as root architecture, possession of adventitious roots and exudation of organic compounds in the rhizosphere that enables it to acquire more P from the soil. Studies by Miller *et al*. (2003) and Hinsinger (2009) also confirmed different plants absorbed Phosphorus from the soils and gave different mechanisms including release of organic compounds exhibited by root. The difference in P uptake among the genotypes shows the diversity in efficiency with which plants were able to absorb P from soils with varying P availability (Hinsinger, 2009). Thus, P uptake was a good indicator for identification and selection for plant breeding.

4.3 Effect of phosphorus rate and variety on secondary metabolites

4.3.1 Total Phenolic Content in shoot and root.

Shoot and root TPC were significantly affected ($P \leq 0.05$) by different levels of Phosphorous in greenhouse, long and short rains. Mean values of the data showed maximum shoot TPC recorded on plants with P applied at 0 kg/ha (6.09 mg/g for *S. villosum* and 5.49 mg/g for *S. scabrum*) whereas root TPC was 2.18 mg/g for *S. villosum* and 5.09 mg/g for *S. scabrum*. Shoot TPC was 4.93mg/g for *S. villosum* and 3.88mg/g for *S. scabrum* at 20 kgP/ha whereas root TPC had 1.77mg/g for *S. villosum* and 4.78mg/g for *S. scabrum*.

Phosphorus applied at 40 kg/ha resulted in shoot TPC of 3.31mg/g for *S. villosum* and 2.54mg/g for *S. scabrum* whereas root TPC resulted in 1.11mg/g for *S. villosum* and 2.22mg/g for *S. scabrum* for (Fig. 4.14 and 4.15). The lowest shoot and root TPC (1.62 mg/g for *S. villosum* and 1.20 mg/g for *S. scabrum*) and (0.41 mg/g for *S. villosum* and 1.42 mg/g for *S. scabrum*) respectively were produced by plants treated with 60 kgP/ha (Fig. 4.18 and 4.19).

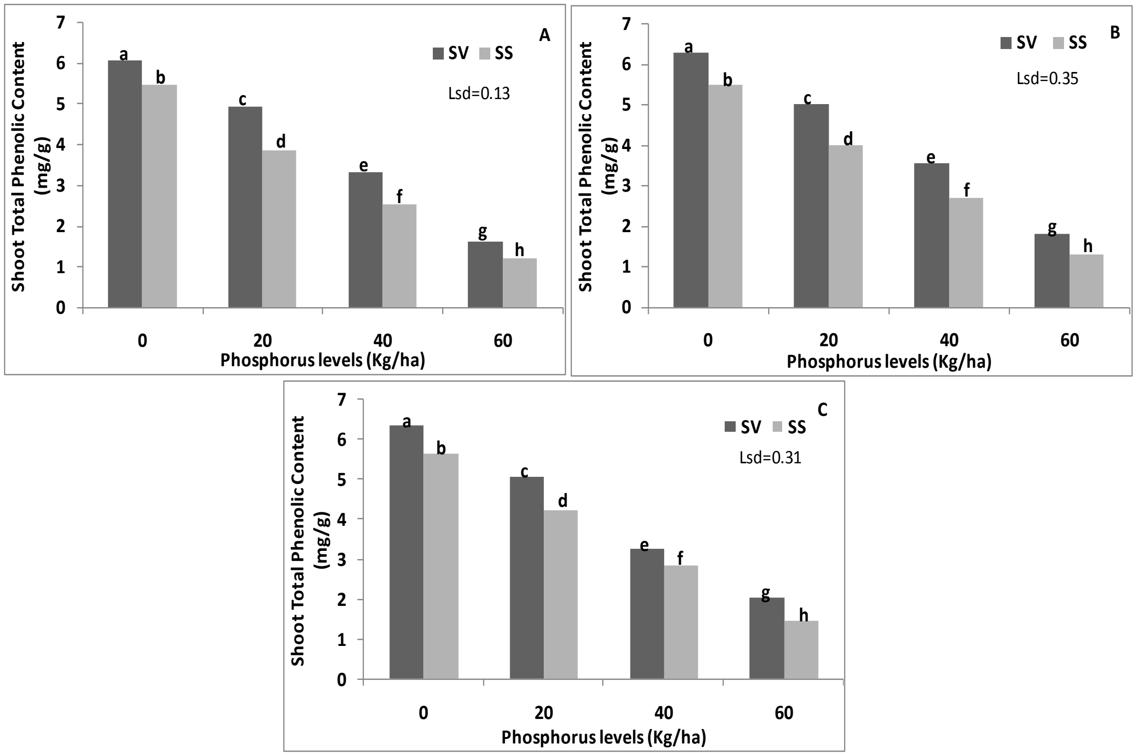

Figure 4.18: Effects of Phosphorus levels on shoot total phenolic content of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

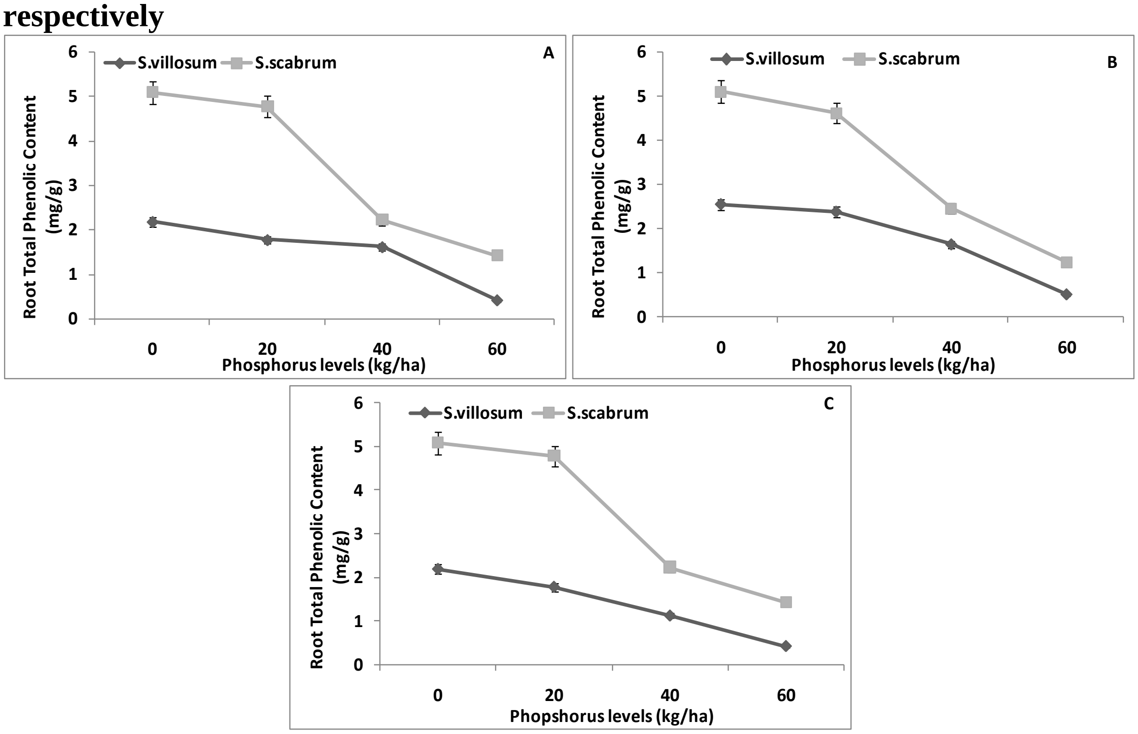

Figure 4.19: Effects of Phosphorus levels on root total phenolic content of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Plant species have different ways of mobilizing phosphorus from the soil (Holford, 1997). During phosphorus deficiency, plants secrete organic compounds such as mucilage, organic acids, phosphatases, and some specific signaling substances, which are key drivers of various rhizosphere activities (Hinsinger, 2009). The chemical and biological processes in the rhizosphere not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient use efficiency of crops, thus profoundly influencing crop productivity (Hinsinger, 2009; Richardson *et al*., 2009; Wissuwa *et al*., 2009; Zhang *et al*., 2008).

Phosphorus at 0 kg/ha recorded the highest TPC. High amounts of phosphorus required by the plants triggers the release of plant phenolics in accordance with the plant phosphorus requirement thus the more severe the deficiency, the higher the release of phenolics. Different plant species have different reservoirs for phenolics (Ryans *et al*., 2001). For instance, in the case of this study, *S. scabrum* reserves more phenolics in the roots as compared to *S. villosum* that stores more in shoots hence when P deficiency was experienced in *S. scabrum*, it probably releases phenolics from the roots that aid in mobilization of available P from the rhizosphere making it available to the plant.

The release of phenolics in form of organic acids in soil led to the lowering of root rhizosphere pH (Fig. 4.20). Similar results were obtained by Kajjidoni *et al.* (2002) who indicated that genotypes exhibited greater variation for root morphological traits and phenolic exudation under varied P sources. He further reported that genotypes varied widely in P uptake under P deficiency.

During P deficiency, P was mobilized from the older leaves to the younger leaves; the resulting leaves were abscissed on the surface of the soil above the rhizosphere (Cordell *et al*., 2009). The decomposition process of the leaves releases phenolics that were stored in the leaf vacuole to the rhizosphere that would consequently leach to the soil to facilitate mobilization of available P (Ryans *et al*., 2001). The release of organic acids in form of phenolic content causes acidification of the rhizosphere. Therefore soils were analysed for pH before transplanting and 40 days after transplanting and the results indicated lowered pH levels Fig. 4.20.

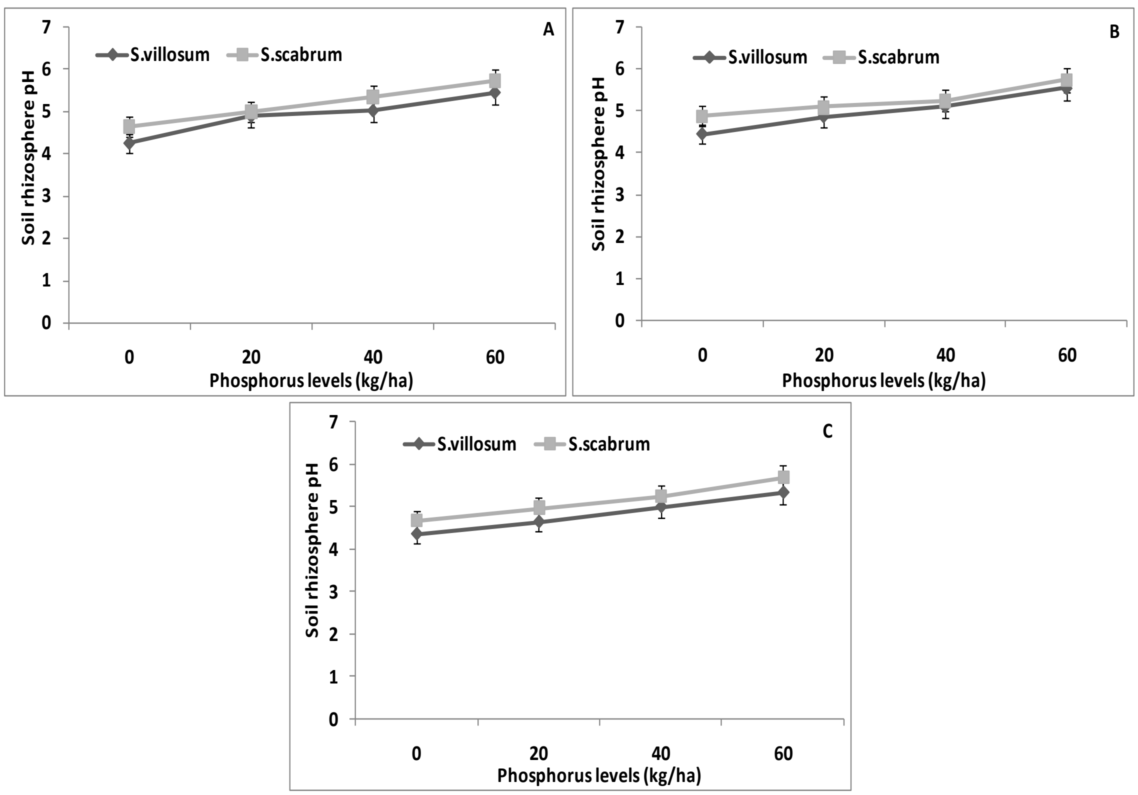

Figure 4.20: Effect of phosphorus levels on soil rhizosphere pH of (A) greenhouse, (B) long rains and (C) short rains respectively 40 days after transplanting

Leaf total phenolic content for greenhouse, long and short rains are presented in Table 4.11 showed significant interaction (P≤ 0.05) between variety and phosphorus rate on leaf TPC indicating that varieties responded differently to P application in terms of Leaf TPC.

Table 4.12: Effect of phosphorus rate and variety on shoot total phenolic content in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Shoot TPC	Shoot TPC	Shoot TPC
S.villosum	0	6.09[a]	6.29[a]	6.35[a]
	20	4.93[c]	5.02[c]	5.07[c]
	40	3.31[e]	3.57[e]	3.27[e]
	60	1.62[g]	1.8[g]	2.02[g]
S.scabrum	0	5.49[b]	5.52[b]	5.63[b]
	20	3.88[d]	4.01[d]	4.21[d]
	40	2.54[f]	2.71[f]	2.85[f]
	60	1.2[h]	1.32[h]	1.45[h]
LSD		0.13	0.35	0.31
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). * Significant F values at $P \leq 0.05$ and NS-Not significant.

Given that varieties responded similarly to P application, the differences in their shoot TPC at similar P rates was attributed to their inherent secondary metabolite composition; *S. villosum* had higher secondary metabolite in leaves than in roots that enabled it repel herbivores during stressful situations and also to acquire more P from the soil than *S. scabrum* (Wissuwa *et al*., 2009). The converse is true for *S. scabrum.*

4.3.2 Total Antioxidant Activity in root and shoot

Shoot and root TAA were significantly ($P \leq 0.05$) affected by different levels of Phosphorous in the greenhouse, long and short rains. Mean values of the data showed that maximum shoot TAA was recorded on plants with P applied at 0 kg/ha (38.58% for *S. villosum* and 30.92% for *S. scabrum*) whereas root TAA was 37.25% for *S. villosum* and 40.11% for *S. scabrum*. Phosphorus applied at 20 kg/ha produced shoots TAA (34.49% for *S. villosum* and 25.38% for *S. scabrum* whereas root TAA was 33.36% for *S. villosum* and 50.11% for *S. scabrum*). Phosphorus applied at 40 kg/ha resulted in shoot TAA of 30.75% for *S. villosum* and 17.5% for *S. scabrum* while root TAA was

30.44% for *S. villosum* and 39.33 for *S. scabrum* (Fig. 4.17 and 4.18). The lowest Shoot TAA (23.71% for *S. villosum* and 10.38% for *S. scabrum*) and root TAA (20.92% for *S. villosum* and 22.7% for *S. scabrum*) were produced by plants treated with 60 kgP/ha (Fig. 4.21 and 4.22).

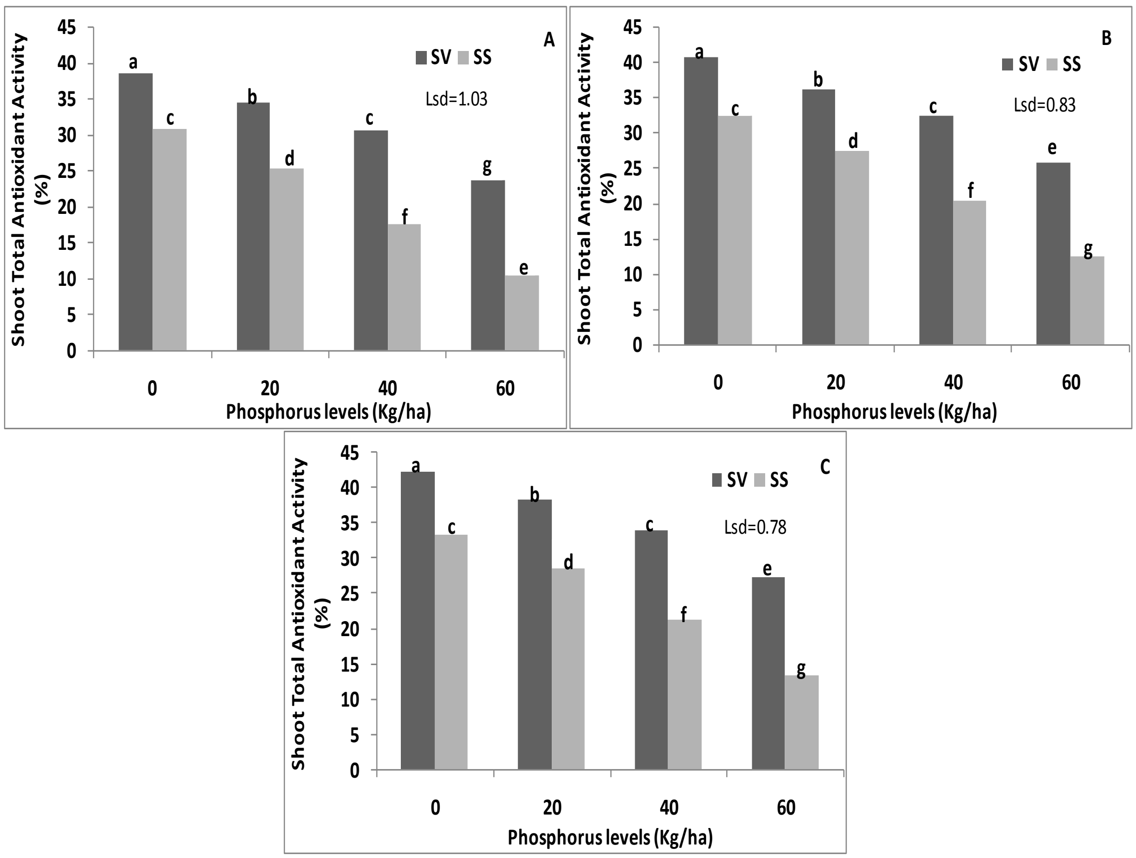

Figure 4.21: Effects of Phosphorus levels on shoot total antioxidant activities of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

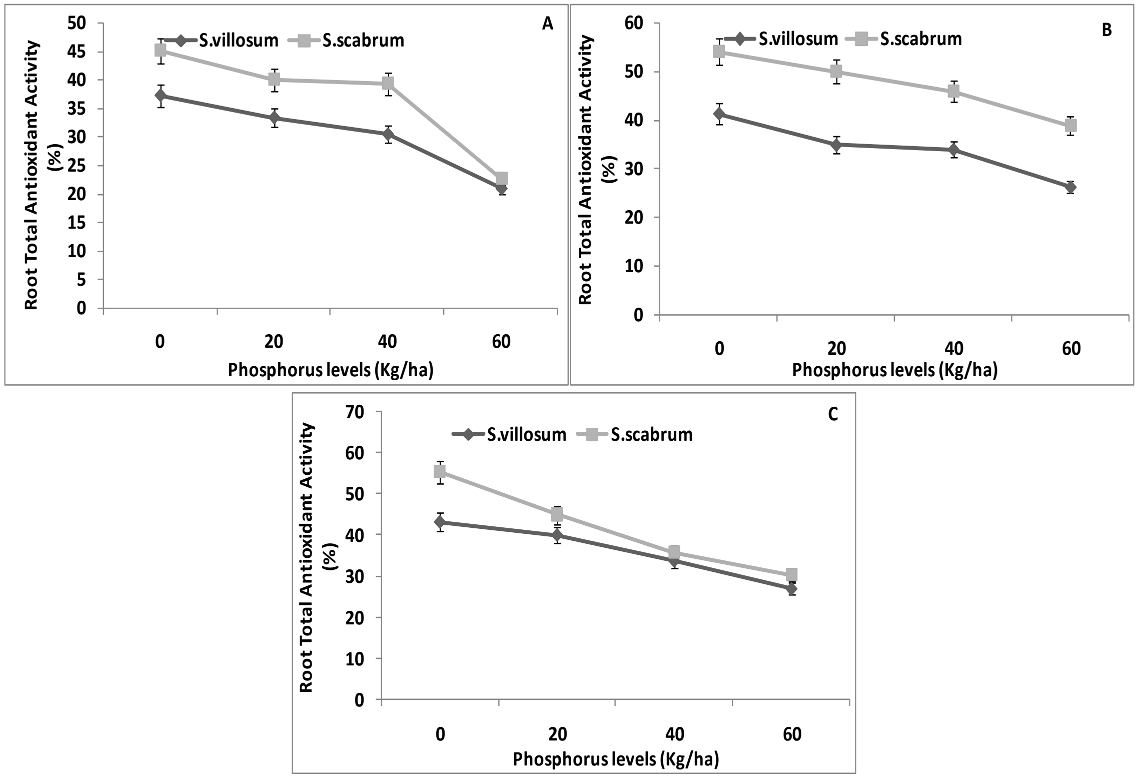

Figure 4.22: Effects of Phosphorus levels on root total antioxidant activities of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

Phosphorus plays an important role in nutrients stress condition for plant survival (Holford, 1997). In shortage of phosphorus, plants sensitivity increases (Cakmak, 2003). Plants treated with 0 kgP/ha had the highest release of reactive oxygen species (ROS) (Ren *et al*., 2007). ROS production in plants involves the release of cognate enzymes such as Super Oxide Dismutase (SOD), Ascorbate Peroxidase and Glutation Reductase (Cakmak and Marschner, 2005) that catalyzed the reaction responsible for mobilizing the available phosphorus stored in plant. The release and reactions of the enzymes cause digestion of other tissues within the plant hence this calls for their regulation (Cakmak, 2003).

To prevent excessive destruction of tissue by the ROS enzymes, there was the release of antioxidants that counter-reacts the activities of ROS thus preventing further tissue damage (Mishra *et al*., 1995). Therefore, plants treated with 0 kgP/ha had the highest antioxidant activity (38.58% for *S. villosum* and 35.92% for *S. scabrum*) followed by 20kgP/ha then 40 kgP/ha and finally 60 kgP/ha. The more severe the deficiency the higher the production of antioxidant thus this explains the reduction of antioxidant with the decrease in phosphorus deficiency severity (Figure 4.21 and 4.22).

The difference in antioxidant activity between shoots and roots was as a result of the location of the plant phosphorus reservoir. *S. villosum* reserves most of its secondary metabolites in the leaves whereas *S. scabrum* reserves in the roots. Similar results were obtained by Habibi *et al*. (2004) in the study of Sunflower varieties under phosphorus deficiency which showed the increase in antioxidant activity.

Shoot total antioxidant activities for greenhouse, long and short rains are presented in Table 12. There was significant interaction at ($P\leq 0.05$) between variety and phosphorus rate on shoot TAA indicating that varieties responded differently to P application in terms of shoot TAA.

Table 4.13: Effect of phosphorus rate and variety on shoot total antioxidant activity (%) in greenhouse, long and short rains

		GREENHOUSE	LONG RAINS	SHORT RAINS
Varieties	P levels	Shoot Antioxidant	Shoot Antioxidant	Shoot Antioxidant
S.villosum	0	38.58[a]	40.76[a]	42.3[a]
	20	34.49[b]	36.3[b]	38.4[b]
	40	30.75[c]	32.4[c]	33.9[c]
	60	23.71[e]	25.9[e]	27.3[e]
S.scabrum	0	30.92[c]	32.6[c]	33.4[c]
	20	25.38[d]	27.6[d]	28.5[d]
	40	17.5[f]	20.4[f]	21.2[f]
	60	10.38[g]	12.5[g]	13.3[g]
LSD		1.03	0.83	0.78
PxV		*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

Given that varieties responded similarly to P application, the differences in their leaf TAA at similar P rates was attributed to their inherent secondary metabolite composition; *S. villosum* has higher secondary metabolite that enables it repel herbivores during stressful situations and also acquire more P from the soil than *S. scabrum.* Similar results were obtained by Wissuwa *et al*. (2009) who worked with production of phytochemicals on medicinal plants.

4.4 Relationship between phosphorus level and plant variables

Both Total Antioxidant Activity and Total phenolic concentration were negatively correlated with phosphorus levels (R^2=0.996 and 0.987 respectively) (Fig. 4.23) while yield and shoot P had a positive correlation with the phosphorus levels (R^2=0.809 and 0.997 respectively) Fig. 4.24. A correlation between % shoot P with TAA and TPC was done resulting in negative correlation (R^2= 0.964 and 0.963 respectively) (Fig. 4.25)

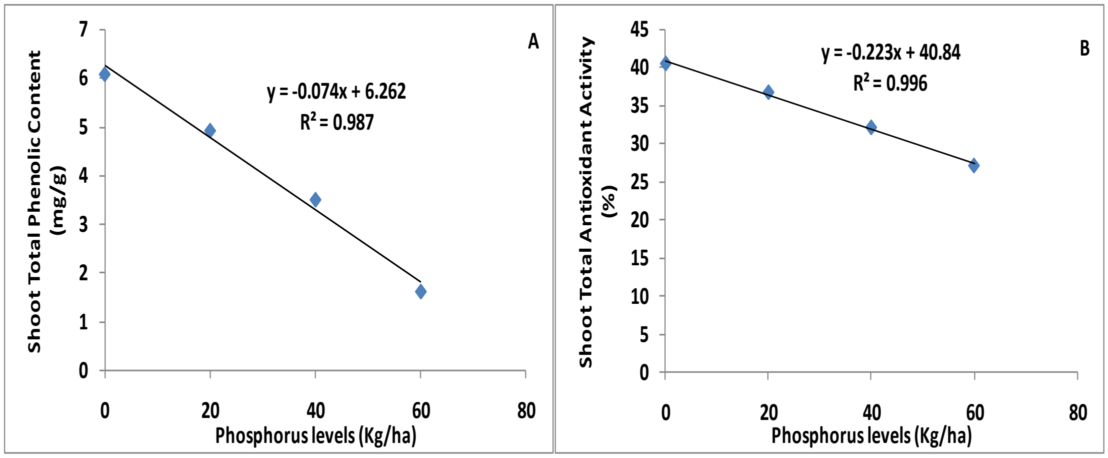

Figure 4.23: Relationship of phosphorus treatments with TPC (A) and TAA (B). Data analyzed using linear regression, stepwise selection model

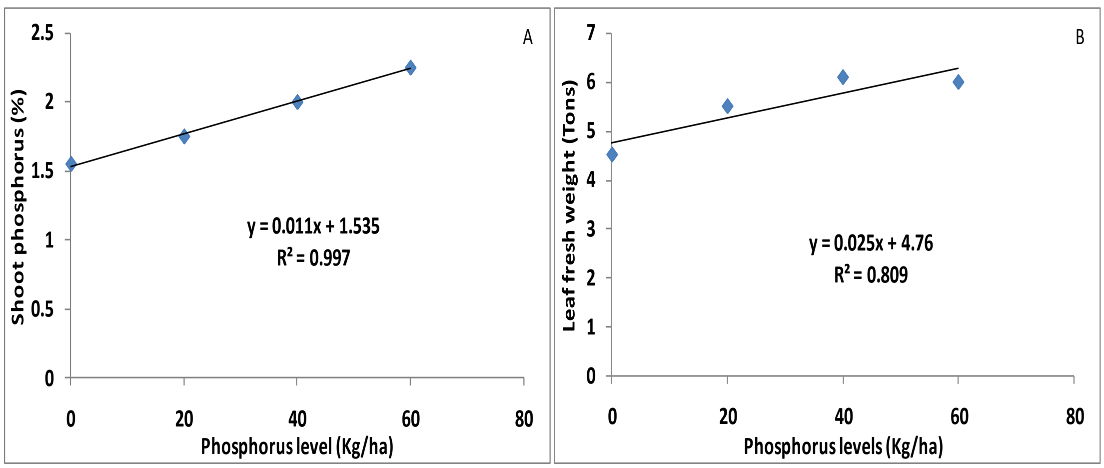

Figure 4.24: Relationship of phosphorus treatments with % shoots P (A) and Yield (B). Data analyzed using linear regression, stepwise selection model

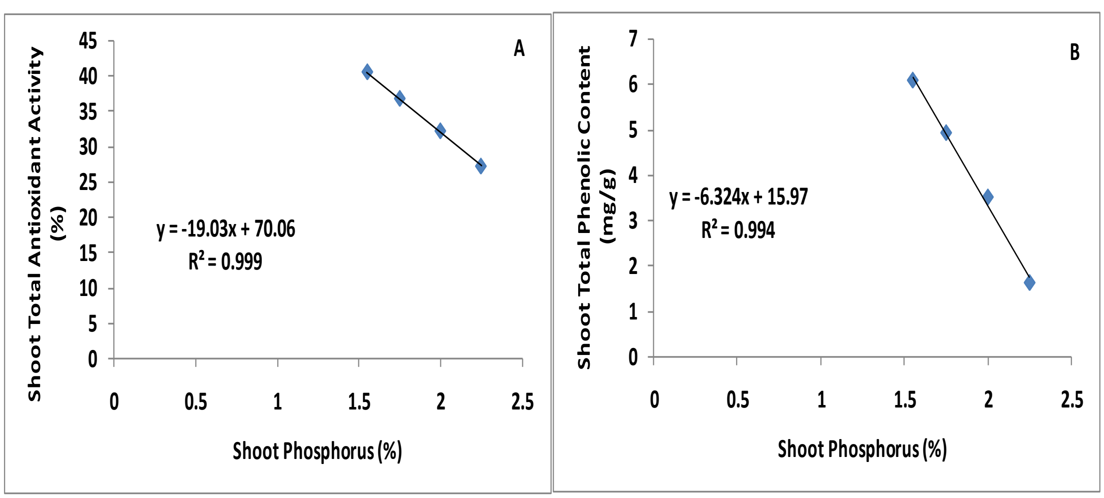

Figure 4.25: Relationship of % shoots P with TPC (A) and TAA (B). Data analyzed using linear regression, stepwise selection model

Plants have survival mechanisms to deal with nutrient deficiency (Ren *et al*, 2007). For instance, when plants experience P deficiency, they release secondary metabolites that help in the mobilization of available P from the rhizosphere (Hinsinger, 2009). An example of secondary metabolite released by plants was phenolics. At 0 kgP/ha plants produced maximum TPC as compared to the other treatments. TPC was only produced when shoot P was at its lowest level in the plant hence the negative linear relationship (R^2=0.963) trend of P treatment with TPC produced by plants (Fig. 4.23A). Similar results were obtained by Richardson and Mullaney (2009) who reported an increase in anthraquinones (phenolics) in black gram plants grown under P deficiency.

When plants undergo nutrient deficiency, there was the production of ROS that facilitates mobilization of available P within the plant. The release of excessive enzymes triggered the release of antioxidants that deactivates the enzymes to prevent further damage resulting from enzyme digestion during the process of P acquisition from within the plant reserves (Ren *et al*., 2007).

Severity of deficiency led to increased enzyme secretion and antioxidants production (Ren *et al*, 2007). However with the presence of absorbable P in soil, there was limited production of antioxidants hence the negative linear relationship (R^2=0.996) trend of P treatment with TAA produced by plants (Fig. 4.23B). Similar results were obtained by (Habibi *et al*., 2004) in the study of Sunflower variety in P deficiency which showed increase of vitamin C.

Uptake of P by plants depends on many factors but the most important factor was the availability of absorbable P in the rhizosphere (Curtin *et al*., 1993). The availability of extractable soil P in the rhizosphere facilitates maximum absorption of P by plants thus when plants were treated with high levels of P (60 kgP/ha), shoot P was expected to be high. Increase in absorbable P consequently leads to increase in shoot P hence the positive linear relationship (R^2=0.997) trend of P treatment with % shoot P produced by plants (Figure 4.24A). An experiment conducted by Miller *et al*. (2003) showed that maximum tissue P was obtained on Lentils treated with 90 kgP/ha as compared to those treated with 50 kgP/ha.

Plant macronutrients nutrients have been known to have different functions when it comes to plants metabolism and the more the plant macronutrients the higher the productivity (Ren *et al*., 2007). At high nutrient availability, plants partition large fractions of their resource to the shoot system and as a result, leaf growth and expansion become maximized such that there was an increase in the above ground biomass and eventually increases yield (Indira, 2005).

This suggests that P (60 kg/ha) efficiency encourages leaf expansion and consequently, more carbon assimilation resulting in high shoot biomass under high P (Maqsood et al., 2001). Decrease in P leads to decrease in shoot biomass leading to decreased yield hence the positive linear relationship (R^2=0.809) trend of P treatment with productivity (Figure 4.24B). Similar results were obtained Poorter and Nagel (2000) who showed a positive correlation in maize yield and Phosphorus level.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Soil phosphorus affected the distribution of varieties in the study Counties such that where there was a general increased abundance of ANs with increase in soil available P. Phosphorus deficiency also significantly affected the distribution of the plant phenolics and antioxidants in both Counties.

The results also showed a corresponding increase in harvestable yield with soil P content. This yield was optimal between 40-60kg/ha. Beyond 60kg/ha, the increase in yield was not realized and to some extent there was yield reduction. *Solanum scabrum* had higher yield response than *Solanum villosum*, though P stress had a significant effect on *Solanum scabrum*.

The two Solanum varieties showed difference in leaf total phenolics and antioxidant, with higher concentration being recorded in *Solanum villosum* than *Solanum scabrum*. However *Solanum* scabrum was superior on root total phenolics and antioxidants. This could have led to mobilization of P and hence partly explain higher vegetative yield. The P supply of about 40kg/ha was ideal as trade-off between yield, phenolics and antioxidants.

5.2 Recommendations

There is need for further mapping, include more parts of the country other than Kisii & Siaya and also include more indigenous vegetables across the country. In addition, further experiments can be done interms of other elements including Aluminum.

Solanum scabrum had higher yield as compared to *Solanum villosum,* but yield reduction was more predominant in *Solanum scabrum* under P stress meaning that

Solanum scabrum was higher yielding but not tolerant and hence the breeding of the two can be done to transfer P tolerance from *Solanum villosum* to higher yielding *Solanum scabrum*.

Where biomass harvest is of importance, P at 40kg/ha is recommended. However, it may be advisable to grow African nightshade with 40 kg/ha P if the grower is targeting modest leaf yield and modest phenolics and antioxidant. There is need to profile a range of specific phenolics, there partitioning in different organs and check whether there is excretion of these phenolics in different nightshades and indigenous vegetables in general.

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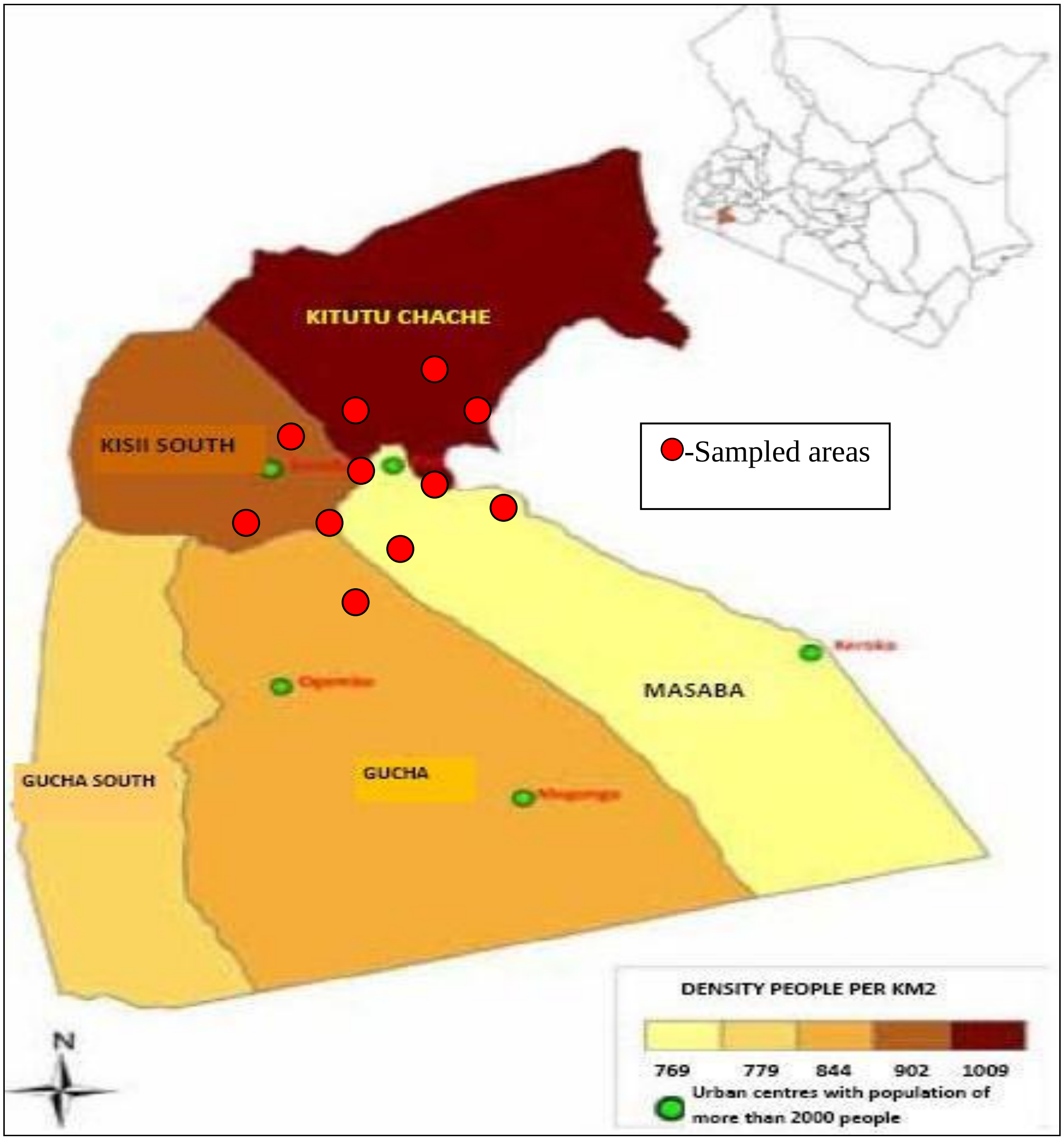
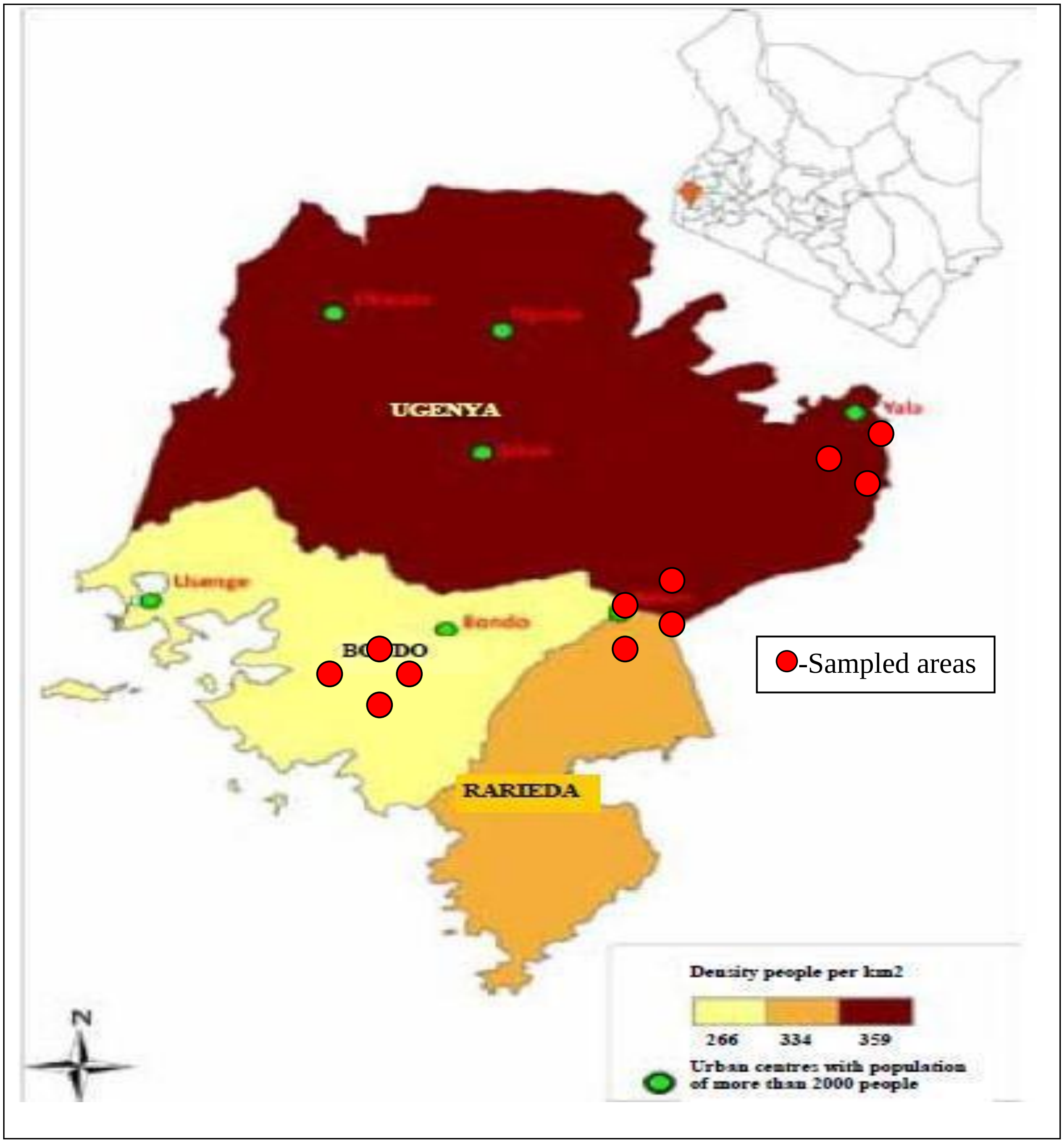
APPENDICES

APPENDIX 1: Map of Kisii County showing sites where samples were collected.



(Adopted from Kenya Mpya County maps, 2012)

APPENDIX 2: Map of Siaya County showing sites where samples were collected.



(Adopted from Kenya Mpya County maps, 2012)

APPENDIX 3: Study Questionnaire

Objectives of the questionnaire

1. To characterize African nightshade on the basis of consumer preference.
2. To gather information on fertilizer use during planting and growing of ANs.
3. To map out the locality of African nightshade with the aid of Global Positioning System technology.

Personal Information

Name of respondent: ………………………………………………Gender………..

Education level: …………………………………Occupation………………………

Location Details

County: ……………….District: ……………….Location: ………………….

GPS Coordinates: …………………………….

Date: ……………………………….

Nearest market/town: …………………………………………………..

Introduction

Do you know when African nightshades were introduced in this market?

1 = Yes, 2 = No

If yes, which year? ....

Which African nightshade were the first to be traded in this market?

………………………

When did you yourself start trading in African nightshade? ....

What made you start? ....

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

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

1= Mobile trader (State markets)

2= Permanent in this market

3=Occasional trader (specify)…………………………………………….

B8. Where did you get most of your supplies of African nightshade?

Species of African nightshade	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 centres in the farming area (farmer groups)

4= from same market (wholesalers)

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

If source is from own farm, ask the following and/or otherwise skip to the next section

Do you grow other African nightshade that you do not sell? 1 = Yes, 2 = No

What is the total acreage of your farm? ……………… Acres

Acreage under crops …………………………………

Acreage under African nightshade ………………………………….

When did you start growing African nightshade? ………………. (Year)

Changes in other crops production including African nightshade. (*Include all from own farm)*

Species Grown	Type within the species	2006 Area in acres	2001 Area in acres	1996 Area in acres

Area in acres

Do you have visits from extension officers or advisory persons?

1=Yes…….2=No………

If yes, where do they come from? ……………………..

If yes, how many times in a year? ……………………..

Has extension services provided you with any information concerning improved species of African nightshade?

1=Yes……2= No…….

If yes, which species? ....

Agronomic practices

Have you ever conducted soil analysis? 1=Yes…….2=No………

If yes, at what interval? ....

What were you analysing? ....

Do you use fertilizer when the production of African nightshade?

1=Yes…….2=No………

If yes, what quantity per acre? ....

How frequent? ....

Do you grow other indigenous vegetable? 1=Yes…….2=No………

If yes, rank them (Rank them, from the most highly preferred to the least)……………………………………………………………………………………….

Which ANs are preferred by the following age group?

10 to 20…………………..Reason……………………………………………………………

21 to 30…………………..

Reason…………………………………………………………………

31 to 40…………………..

Reason…………………………………………………………………

41 to 50…………………..

Reason…………………………………………………………………

51 and over………………

Reason…………………………………………………………………

Which of your African nightshade types sell fast? (Rank them, from the fastest to the slowest)

…………………………………………………………………………………………

…………

Do you consumer African nightshade?

……………………………………………………………………………..

If yes,

why………………………………………………………………………………………

…..

Do you practice any agronomic management to ensure improve quality of African nightshade? [ ] 1= Yes; 2= No.

If yes, which ones?

……………………………………………………………………………………

Income share

Do you market other farm produce apart from the African nightshade? [ ] 1= Yes; 2= No

(If yes, CONTINUE, if no skip to 14)

Which ones?

LIST………………………………………………………………………………..

Which of your produce (African nightshade, exotic vegetables and other farm produce) sell fast? (Rank them, from the fastest to the

slowest)…………………………………………………………………

What is the daily NET profit from your marketing activities? .... (PROMPT for average)

What proportion of your net profit do the African nightshades contribute? .... (PROMPT 10%, 15 %....)

Help or Influence

Have you been helped or influenced by any institution, organization, or individual in the marketing of African nightshade? 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)……………………………………………………………………………………

………………………………………………………………………………………………

…………………..

G2. Which form of support do you consider most beneficial to you?

………………………………………………………………………………………………

………..

G3. Do you experience any constraints in marketing African nightshade? [ ] 1= Yes, 2= No

G4. If yes, which ones? (Rank them with the most important first)

1………………………………………………………………………………………

2………………………………………………………………………………………

3………………………………………………………………………………………

APPENDIX 4: Summary of tables showing means of plant variables as affected by Phosphorus, variety and interaction of both.

1. Effect of varieties on growth parameter of two varieties of African nightshade grown in the green house, long and short rains.

		Green house			
Variety	Leaf area	Plant height	Freshweight	No. of sec. buds	Root area
S. villosum	121.77b	53.41a	45.77b	14a	47.25a
S.scabrum	126.66a	54.77a	48.68a	11b	43.43b
LSD	1.61	2.18	0.99	1.09	1.13
		Season 1			
S. villosum	122.84b	50.64a	42.57b	15a	42.84a
S.scabrum	124.11a	51.52a	45.43a	11b	44.11b
LSD	1.26	2.12	0.81	1.5	1.26
		Season 2			
S. villosum	112.91b	52.61a	48.57a	14a	45.59a
S.scabrum	119.61a	49.09a	46.21b	11b	40.23b
LSD	2.56	3.63	1.27	0.81	1.23

Means followed by the same letter within the same column are not significantly different ($p \leq 0.05$)

2. Effect of varieties on secondary metabolites of two varieties of African nightshade grown in the green house, long and short rains.

		Green house		
Variety	Leaf TPC	Root TPC	Leaf Antioxidant	Root Antioxidant
S.villosum	4.21a	1.57a	36.6a	44.89b
S.scabrum	2.72b	3.58b	33.31b	57.86a
LSD	0.65	0.54	2.608	9.5591
		Season 1		
S.villosum	4.71a	2.1b	40.76a	33.02b
S.scabrum	3.88b	3.66a	34.74b	46.04a
LSD	0.66	0.45	2.64	4.07
		Season 2		
S.villosum	5.08a	1.57b	42.57a	48.43b
S.scabrum	4.09b	3.58a	36.38b	61.54a
LSD	0.62	0.54	2.53	9.8

Means followed by the same letter within the same column are not significantly different ($p \leq 0.05$)

3. Effect of varieties on shoot P and plant rhizosphere of two varieties of African nightshade grown in the green house, long and short rains.

		Green house		
Variety	% Shoot P	% Zn Soil	% Cu Soil	Rhizosphere pH
S. villosum	1.98a	0.75a	0.86a	4.73a
S. scabrum	1.74b	0.73a	0.69a	4.93a
LSD	0.21	0.05	0.19	0.37
		Season 1		
S. villosum	2.01a	0.87a	1.01a	4.85a
S. scabrum	1.82b	0.85a	0.97a	4.91a
LSD	0.15	0.03	0.05	0.07
		Season 2		
S. villosum	2a	0.93a	1.02a	4.75a
S. scabrum	1.89b	0.9a	1.03a	4.81a
LSD	0.09	0.05	0.02	0.07

Means followed by the same letter within the same column are not significantly different ($p \leq 0.05$).

4. Effect of phosphorus levels on growth parameters of two varieties of African nightshade grown in the greenhouse

		GREENHOUSE				
Vaieties	P levels	Leaf area	Plant height	Fresh weight	No. of sec. buds	Root area
S.villosum	0	95.8h	38e	4.5g	10d	40.5d
	20	108.3f	48.2c	5e	14c	45.8c
	40	146d	53.5b	5.8c	17b	55.2b
	60	150.4c	45.2d	5.75c	20a	59.27a
S.scabrum	0	100.6g	44.87d	4.92f	7e	26.7f
	20	122.5e	55b	5.45d	10d	31.35e
	40	161.4b	61a	6.35a	14c	40.31d
	60	166.5a	51b	6.05b	18b	44.52c
LSD		1.35	2.5	0.05	1.5	3.27
T x V		*	NS	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

5. Effect of phosphorus levels on growth parameters of two varieties of African nightshade grown in long rains

		SEASON1				
Varieties	**P levels**	**Leaf area**	**Plant height**	**Fresh weight**	**No. of sec. buds**	**Root area**
S.villosum	**0**	**85.6g**	**34e**	**4.52g**	**11c**	**38.3d**
	20	**96.4f**	**44.5c**	**5.17e**	**15b**	**42.7d**
	40	**138.2d**	**49.1b**	**5.9c**	**18a**	**53.5b**
	60	**145.9c**	**41.2d**	**5.82c**	**21a**	**57.8a**
S.scabrum	**0**	**98.8f**	**40.2d**	**4.85f**	**8d**	**25.2f**
	20	**118.7e**	**51b**	**5.52d**	**11c**	**30.38e**
	40	**158.41b**	**57a**	**6.41a**	**15b**	**40.2d**
	60	**163.4a**	**47b**	**6.12b**	**19a**	**45.3c**
LSD		**4.3**	**2.2**	**0.18**	**2**	**2.5**
T X V		*	**NS**	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

6. Effect of phosphorus levels on growth parameters of two varieties of African nightshade grown in short rains

		SEASON2				
Varieties	P levels	Leaf area	Plant height	Fresh weight	No. of sec. buds	Root area
S.villosum	0	79.6f	32e	4.7d	10c	35.5e
	20	86.2e	42.3c	5.8b	15b	40.1c
	40	108.3d	47.1b	6.42a	17b	51.3b
	60	129.9c	39.3d	6.15a	20a	55.3a
S.scabrum	0	88.2e	38.2d	4.5d	10c	23.7g
	20	108.5d	49b	5.4c	13b	28.6f
	40	147.2b	55a	6.27a	17b	39.4d
	60	156.1a	45b	6a	22a	42.6c
LSD		3.2	2.1	0.2	2	3.2
T X V		*	NS	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

7. Effect of Phosphorus level on secondary metabolites of two varieties of African nightshade grown in green house

GREENHOUSE					
Varieties	**P levels**	**Leaf TPC**	**Root TPC**	**Leaf Antioxidant**	**Root Antioxidant**
S.villosun	**0**	6.09a	2.18b	38.58a	37.25c
	20	4.93c	1.77c	34.49b	33.36d
	40	3.31e	1.61b	30.75c	30.44e
	60	1.62g	0.41c	23.71e	20.92g
S.scabrun	**0**	5.49b	5.09a	30.92c	45.14a
	20	3.88d	4.78a	25.38d	40.11b
	40	2.54f	2.22b	17.5f	39.33b
	60	1.2h	1.42c	10.38g	22.7f
LSD		0.13	0.35	1.03	1
T x V		*	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

8. Effect of Phosphorus level on secondary metabolites of two varieties of African nightshade grown in long rains

SEASON ONE					
Varieties	**P levels**	**Leaf TPC**	**Root TPC**	**Leaf Antioxidant**	**Root Antioxidant**
S.villosum	**0**	6.29a	2.38e	40.76a	41.38d
	20	5.02c	1.64f	36.3b	34.94e
	40	3.57e	2.55c	32.4c	34.02e
	60	1.8g	0.51h	25.9e	26.3f
S. scabrun	**0**	5.52b	5.11a	32.6c	54.13a
	20	4.01d	4.62b	27.6d	50.05b
	40	2.71f	2.47d	20.4f	46.02c
	60	1.32h	1.25g	12.5g	38.94d
LSD		0.35	0.03	0.83	3.01
T x V		*	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

9. Effect of Phosphorus level on secondary metabolites of two varieties of African nightshade grown in short rains

SEASON TWO					
Varieties	**P levels**	**Leaf TPC**	**Root TPC**	**Leaf Antioxidant**	**Root Antioxidant**
S.villosum	**0**	6.35a	2.18b	42.3a	43.24b
	20	5.07c	1.77b	38.4b	40.07b
	40	3.27e	1.11b	33.9c	33.81b
	60	2.02g	0.41d	27.3e	27.02b
S.scabrum	**0**	5.63b	5.08a	33.4c	55.29a
	20	4.21d	4.78a	28.5d	44.93b
	40	2.85f	2.22b	21.2f	35.78b
	60	1.45h	1.42b	13.3g	30.42b
LSD		0.31	0.41	0.78	4.38
T x V		*	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

10. Effect of Phosphorus level on % shoot P, % Zn and Cu soil and root rhizosphere pH of two varieties of African nightshade grown in green house, long and short rains.

		GREENHOUSE			
Varieties	**P levels**	**% Shoot P**	**% Zn Soil**	**% Cu Soil**	**Rhizosphere pH**
S.villosum	**0**	1.63e	1.12a	1.18a	4.25g
	20	1.87d	0.95b	1.15a	4.76e
	40	1.98c	0.78c	0.95b	4.95c
	60	2.43a	0.15d	0.17d	5.15a
S.scabrum	**0**	1.5f	1.17a	1.16a	4.71f
	20	1.61e	0.95b	0.96b	4.82d
	40	1.72e	0.78c	0.47c	4.94c
	60	2.13b	0.15d	0.16d	5.05b
LSD		0.1	0.06	0.16	0.02
T x V		*	NS	NS	NS

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

11. Effect of Phosphorus level on % shoot P, % Zn and Cu soil and root rhizosphere pH of two varieties of African nightshade grown in green house, long and short rains.

		SEASON.ONE			
Varieties	**P levels**	**% Shoot P**	**% Zn Soil**	**% Cu Soil**	**Rhizosphere pH**
S.villosum	**0**	1.6f	1.15a	1.22a	4.63a
	20	1.91d	0.98a	1.17a	4.75a
	40	2c	0.87a	0.92a	4.91a
	60	2.35a	0.21c	0.37c	5.01a
S.scabrum	**0**	1.47g	1.19a	1.21a	4.75a
	20	1.57f	1.03a	1.07a	4.82a
	40	1.68d	0.71b	0.63b	4.99a
	60	2.08b	0.25c	0.24c	5.07a
LSD		0.07	0.15	0.15	0.12
T x V		*	NS	NS	NS

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

12. Effect of Phosphorus level on % shoot P, % Zn and Cu soil and root rhizosphere pH of two varieties of African nightshade grown in green house, long and short rains.

		SEASON.TWO			
Varieties	**P levels**	**% Shoot P**	**% Zn Soil**	**% Cu Soil**	**Rhizosphere pH**
S.villosum	**0**	1.57f	1.16a	1.27a	4.78b
	20	1.88d	1.01a	1.12a	4.91b
	40	2c	0.91a	0.81b	5.08a
	60	2.25a	0.35b	0.41c	5.15a
S.scabrum	**0**	1.45g	1.21a	1.24a	4.82b
	20	1.55f	1.08a	1.11a	4.95b
	40	1.63e	0.83a	0.78b	5.11a
	60	2.05b	0.31b	0.33c	5.25a
LSD		0.03	0.14	0.13	0.11
T x V		*	NS	NS	NS

Means followed by the same letter within the same column are not significantly different (P≤0.05). * Significant F values at P≤0.05 and NS-Not significant.

APPENDIX 5: Pearson Correlation Coefficients Tables

1. Pearson Correlation Coefficients greenhouse, N=32

	Trtmnt	L.Area	Plant hght.	F.weight	sec. buds	R.area	L.TPC	R.TPC	L.A.A	R.A.A	% Shoot P	% Zn Soil	% Cu Soil	Rhiz.pH
Trtmnt	1	0.418	0.344	0.457	0.445	0.253	-0.1738	-0.724	-0.529	-0.238	0.249	-0.2157	-0.123	0.259
L.Area	_	1	0.423	0.521	0.629	0.794	-0.343	-0.127	-0.311	-0.321	-0.427	-0.172	-0.231	0.322
Plant hght	_	_	1	0.673	0.2173	0.3524	-0.492	-0.139	-0.139	-0.421	0.5123	0.436	0.524	0.412
F.weight	_	_	_	1	0.273	0.433	-0.1304	-0.1171	-0.341	-0.526	0.891	0.397	0.491	0.217
sec.buds	_	_	_	_	1	0.1271	-0.3362	-0.239	-0.521	-0.142	0.1274	0.657	0.433	0.507
R.area	_	_	_	_	_	1	-0.1031	-0.1241	0.2146	-0.257	0.817	0.264	0.521	0.443
L.TPC	_	_	_	_	_	_	1	0.279	0.359	0.2678	-0.142	-0.321	-0.271	-0.172
R.TPC	_	_	_	_	_	_	_	1	0.493	0.3174	-0.151	-0.456	-0.311	-0.071
L.A.A	_	_	_	_	_	_	_	_	1	0.567	-0.279	-0.539	-0.432	0.184
R.A.A	_	_	_	_	_	_	_	_	_	1	-0.198	-0.173	-0.179	0.096
% Shoot P	_	_	_	_	_	_	_	_	_	_	1	0.313	0.272	0.017
% Zn Soil	_	_	_	_	_	_	_	_	_	_	_	1	0.339	0.123
% Cu Soil	_	_	_	_	_	_	_	_	_	_	_	_	1	0.227
Rhiz.pH	_	_	_	_	_	_	_	_	_	_	_	_	_	1

2. Pearson Correlation Coefficients long rains, N=32

	Trtmnt	L.Area	Plant hght.	F.weight	sec. buds	R.area	L.TPC	R.TPC	L.A.A	R.A.A	% Shoot P	% Zn Soil	% Cu Soil	Rhiz.pH
Trtmnt	1	0.452	0.424	0.521	0.313	0.247	-0.1924	-0.721	-0.481	-0.241	0.351	-0.2487	-0.224	0.284
L.Area	_	1	0.325	0.531	0.731	0.801	-0.349	-0.133	-0.321	-0.421	-0.594	-0.203	-0.324	0.416
Plant hght	_	_	1	0.741	0.2249	0.4109	-0.51	-0.24	-0.281	-0.43	0.4332	0.537	0.664	0.407
F.weight	_	_	_	1	0.284	0.437	-0.1404	-0.1107	-0.37	-0.62	0.421	0.279	0.487	0.213
sec.buds	_	_	_	_	1	0.2104	-0.1071	-0.24	-0.63	-0.15	0.1333	0.547	0.434	0.493
R.area	_	_	_	_	_	1	-0.197	-0.143	0.274	-0.36	0.727	0.273	0.532	0.454
L.TPC	_	_	_	_	_	_	1	0.314	0.32	0.2771	-0.145	-0.249	-0.284	-0.202
R.TPC	_	_	_	_	_	_	_	1	0.527	0.3175	-0.167	-0.507	-0.321	-0.083
L.A.A	_	_	_	_	_	_	_	_	1	0.582	-0.276	-0.624	-0.501	0.174
R.A.A	_	_	_	_	_	_	_	_	_	1	-0.178	-0.631	-0.241	0.104
% Shoot P	_	_	_	_	_	_	_	_	_	_	1	0.232	0.321	0.023
% Zn Soil	_	_	_	_	_	_	_	_	_	_	_	1	0.351	0.213
% Cu Soil	_	_	_	_	_	_	_	_	_	_	_	_	1	0.313
Rhiz.pH	_	_	_	_	_	_	_	_	_	_	_	_	_	1

3. Pearson Correlation Coefficients short rains, N=32

	Trtmnt	L.Area	Plant hght.	F.weight	sec. buds	R.area	L.TPC	R.TPC	L.A.A	R.A.A	% Shoot P	% Zn Soil	% Cu Soil	Rhiz.pH
Trtmnt	1	0.431	0.423	0.531	0.321	0.251	-0.2107	-0.801	-0.672	-0.314	0.314	-0.2241	-0.141	0.341
L.Area	_	1	0.476	0.516	0.679	0.813	-0.353	-0.1039	-0.315	-0.388	-0.507	-0.207	-0.343	0.357
Plant hght	_	_	1	0.683	0.223	0.413	-0.645	-0.1025	0.287	-0.435	0.5568	0.523	0.535	0.451
F.weight	_	_	_	1	0.228	0.512	-0.2013	-0.1207	-0.409	-0.0531	0.887	0.372	0.474	0.219
sec.buds	_	_	_	_	1	0.1341	-0.2117	-0.312	-0.604	-0.145	0.1379	0.682	0.521	0.511
R.area	_	_	_	_	_	1	-0.183	-0.171	0.207	-0.261	0.821	0.267	0.301	0.451
L.TPC	_	_	_	_	_	_	1	0.327	0.461	0.279	-0.156	-0.394	-0.424	-0.0213
R.TPC	_	_	_	_	_	_	_	1	0.487	0.324	-0.503	-0.468	-0.527	-0.1031
L.A.A	_	_	_	_	_	_	_	_	1	0.601	-0.314	-0.589	-0.623	0.207
R.A.A	_	_	_	_	_	_	_	_	_	1	-0.207	-0.209	-0.182	0.103
% Shoot P	_	_	_	_	_	_	_	_	_	_	1	0.421	0.407	0.1011
% Zn Soil	_	_	_	_	_	_	_	_	_	_	_	1	0.423	0.124
% Cu Soil	_	_	_	_	_	_	_	_	_	_	_	_	1	0.331
Rhiz.pH	_	_	_	_	_	_	_	_	_	_	_	_	_	1