

**RESPONSE OF SLENDER LEAF (*CROTALARIA
BREVIDENS* AND *CROTALARIA OCHROLEUCA*) TO
DIFFERENT PHOSPHORUS LEVELS**

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Response of slender leaf (*Crotalaria brevidens* and *Crotalaria ochroleuca*) to different phosphorus levels

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Science in Horticulture in the Jomo Kenyatta University of Agriculture
and Technology**

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my late parents Mr. Kamau Muchekehu and Miriam Wanjiru, my husband Joseph Nduhiu and our three daughters Ruth Wangechi, Grace Wanjiru and Faith Wambui, my brothers and sisters who encouraged me to soldier on even when the work was so involving requiring time that should have been devoted to them .

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LIST OF ABBREVIATIONS/ACRONYMS

AAS	Atomic absorption spectrophotometer
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
ATP	Adenosine triphosphate
°C	Degrees Centigrade
Ca	Calcium
CRD	Completely Randomized Design
EC	Electrical Conductivity
FAO	Food and Agricultural Organization
Fe	Iron
GOK	Government of Kenya
GH	Greenhouse
HCL	Hydrochloric acid
HCDA	Horticultural Crops Development Authority
HNO₃	Nitric acid
HPLC	High Performance Liquid Chromatography

JKUAT	Jomo Kenyatta University of Agriculture and Technology
KDHS	Kenya Demographic and Health Survey
KALRO	Kenya Agricultural and Livestock Research Organization
K	Potassium
KSATN	Kenya Situation Analysis for Transform Nutrition
NPK	Nitrogen, Phosphorus, and Potassium
Mg	Magnesium
mL	Milliliters
P	Phosphorus
N	Normality
NFNSP	National Food Nutrition Security Policy
RELMA	Regional Land Management Unit
UNESCO	United Nations Education, Scientific and Cultural Organization
USDA	United States Department of Agriculture
USAID	United States Agency for International Development
WAP	Weeks after planting

WHO

World Health Organization

Zn

Zinc

ABSTRACT

Crotalaria brevidens and *Crotalaria ochroleuca* are the two most promising slender leaf species that are used as vegetables in Kenya. The young leaves and shoots of the slender leaf have been reported to be a good dietary source of provitamin A, carotenoids, vitamin C, iron, calcium, and proteins. Slender leaf is commonly cultivated and consumed throughout East Africa and to a limited extent in West Africa. Malnutrition is a major challenge in Kenya mostly affecting women and children, and this could be reduced if indigenous vegetables are consumed such as slender leaf. Although slender leaf is commercially produced in Kenya, only 2-4 tons per hectare have been harvested, yet it has the potential of producing 10-12 tons per hectare. This low production has been caused by poor quality seeds, lack of technical production packages and declining soil fertility instigated by continuous cultivation without addition of essential macronutrients such as phosphorus. Although the effect of nitrogen on the growth of slender leaf has been studied in Kenya, limited studies on the effect of phosphorus on the growth of slender leaf have been carried out in Kenya. Thus a study was carried out at Jomo Kenyatta University of Agriculture and Technology farm to evaluate the effect of different phosphorus rates on two *Crotalaria* species in a greenhouse pot study and in the field. The objectives of the study were to evaluate the effect of different phosphorus levels on the growth of two slender leaf species and to determine the effect of phosphorus on the nutritional content of the two slender leaf species at vegetative, reproductive and maturity stages. Eight levels of phosphorus 0, 0.28, 0.56, 0.84, 1.12, 1.40, 1.68 and 1.96 g/pot equivalent to 0, 15, 30, 45, 60, 75, 90, 105 kg P₂O₅ ha⁻¹ were added to the pots filled with red soil and sand mixture in the ratio of 5:1. These were then replicated four times in a completely randomized design. The field study was laid out in 1m x 7 m sq. plots, in split plot design with the species as the main plot factor and fertilizer treatments as sub-plot factor. Watering was done thrice per week to field capacity, while weeding was done to ensure healthy growing plants. Data on the plant height, the number of leaves, the number of branches, the number of pods, was taken

fortnightly. Data on seed weight and seed yield was collected after seeds matured. Leaves for nutritional content analysis were harvested at vegetative, reproductive and maturity stages of growth and kept at 10⁰ Celsius awaiting the determination of nutrient content. The protein and nitrogen content were determined using semi micro Kjeldah method. Beta carotene content was analyzed using column chromatography and UV Spectrophotometry. Determination of minerals was done using dry ashing and atomic absorption spectrophotometry method. Data collected was subjected to the analysis of variance (ANOVA) using SAS 9.1.3 software to establish if the treatments had a significant effect on growth parameters considered and the nutrient content. Results of the study showed that phosphorus had a significant effect on all growth parameters in the greenhouse at $p \leq 0.05$ but not in the field. A significant interaction was found between phosphorus levels and species as far as plant height, number of leaves, number of branches, number of pods and seed weight and seed yield were concerned in the greenhouse study but not in the field. The effect of phosphorus on the nutrient content was only significant ($p \leq 0.05$) for beta carotene but not all other nutrients studied. From the results of this study, it can be concluded that P application had a significant effect on growth in the greenhouse pot study but not in the field conditions. Relatively higher levels of potassium phosphorus, zinc and iron were observed in the vegetative stage of growth. Significantly higher levels of nitrogen, protein magnesium calcium and beta carotene were observed at maturity stage of growth. Significantly higher beta carotene levels were observed in the second field experiment conducted in the months of November 2013-March 2014. *Crotalaria ochloreuca* had significantly higher levels of beta carotene and seed weight than *Crotalaria brevidens*. Application of 60 kg P₂O₅ ha⁻¹ is recommended for enhanced leaf growth in the two slender leaf species

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Slender leaf (*Crotalaria spp*) is commonly known as rattle pod, rattle pea or Ethiopian rattlebox. It is also known as Marejea (Swahili) and Mitoo (Luo). In International trade, it is referred to as a vegetable of small-scale production (Abukutsa- Onyango, 2004). Slender leaf is one of the most promising indigenous vegetables in East and Central Africa that has been grown and consumed for long. The young leaves and shoots are consumed with 100 g fresh weight contributing 4.2-4.9 mg protein, 270 mg calcium, 4 mg iron , 2.9-8.7 mg beta carotene, 115-129 mg and ascorbic acid, (Sikuku et al., 2013). Slender leaf has been reported to have medicinal applications such as treating stomach related ailments, swellings and Malaria (Abukutsa- Onyango, 2004). The plant is also used as green manure (Ontita et al., 2005) and is used in short fallows to reclaim infertile land (Gachene & Kimaru, 2003).

Slender leaf is a legume in the family Fabaceae. The family contains 600 species that grow wild in the Tropical and Sub-tropical areas (Mosjidis and Wang, 2011). The genus *Crotalaria* has 500 species of herbs and shrubs found in Africa. It has been cultivated as a vegetable in Sudan, Kenya, Uganda and Tanzania (Abukutsa Onyango, 2004). *Crotalaria brevidens* and *Crotalaria ochroleuca* are some of the most promising vegetables in East Africa. The two species are almost similar but can be distinguished by their taste and pod size. *C.brevidens* has narrow pods and bitter taste while *C.ochroleuca* has wide pods and mild taste (Sikuku et al., 2013).

1.1.1 Uses of Slender leaf

Slender leaf is grown for its young shoots and leaves that are cooked as vegetables. The young shoots and leaves are consumed when boiled, fried in oil or used as a herb in soups and stews. The leaves may be cooked with other vegetables such as jute mallow (*Corchorus olitorius*). Milk may be added to reduce the bitter taste (Abukutsa Onyango, 2004). The plant has been reported to cure stomach ache, swellings and Malaria. The roots are used to treat sore throat and mouth thrush (Abukutsa-Onyango, 2007; Olembo et al, 1995).

The plant has been used to promote suicidal germination of striga weed (*Striga harmonthica*), a parasitic weed in cereal crops. In the presence of *Crotalaria spp*, the striga seeds germinate then afterwards die due to lack of suitable host (Abukutsa-Onyango, 2007). *Crotalaria* is known to suppress *Meloidogyne* nematode populations (Samba et al, 2002). *Crotalaria* species are used as a source of fibers, silage and green manure (United States Department of Agriculture [USDA], 1999; Abukutsa Onyango, 2004). *Crotalaria* species are also used as cover crops to controls soil erosion and suppress weeds (Wang et al, 2006). It is also used as forage for horses and cattle due to the high amounts of water-soluble gums and proteins in their seeds (Raj et al, 2011).

Slender leaf has the ability to fix nitrogen hence is used as a soil fertility improving agent (Samba et al, 2002). *Crotalaria* species are used as food plants for larvae of *Lepidoptera* species (Raj et al, 2011). The bitter taste due to toxic alkaloids produced by some members of this genus are known to be incorporated by the larva to secure their defense from predators (Bernays et al ., 2003). The alkaloid monocrotalamine, a pyrrolizidine from *Crotalaria* is used to induce pulmonary hypertension in laboratory animals (Raj et al, 2011). The highest quantity of the alkaloid is present in seeds with lesser quantities found in leaves and stems.

1.1.2 Phosphorus Nutrition

Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, the transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to another (Marschner, 1995). Large quantities of P are found in seeds and fruit where it is vital for seed formation and development. Phosphorus is also a constituent of phytin, a major storage form of P in seeds. About 50 percent of the total P in legume seeds and 60 to 70 percent in cereal grains is stored as phytin or closely related compounds. An insufficient supply of P results in reduction of leaf area (Marschner, 1995). It also results in delayed flower initiation, reduced flower and restricted seed formation (Grant et al., 2005).

In the human body, phosphorus is the second most abundant mineral (Holliday, 2007) and is necessary for bone growth, kidney function, cellular health and acid-alkaline balance. It is a component of the major source of energy, adenosine triphosphate (ATP). It is present in every cell membrane as a component of the phospholipid molecule. A maximum 850 g of phosphorus is present in the body of which 85 % is found in the skeleton, 14 % in soft tissues and 1 % in extracellular fluids, intracellular structures and cell membrane (Holliday, 2007). The recommended dietary intake for phosphorus by Food and Nutrition Institute Board of Medicine is 700 mg for adults between ages 19-79 (Holliday, 2007).

Phosphorus is reported to stimulate root and plant growth, initiate nodule formation, as well as influence the efficiency of the rhizobium-legume symbiotic fixation. It is also involved in reactions with energy transfer mainly ATP in nitrogenase activity (Marschner, 1995). Phosphorus application has been found to increase plant biomass according to Patel and Kotecha, (2006). Phosphorus improves the protein content in legumes (Ayub et al., 2012). Deficiency of phosphorus in legumes was found to depress

the activity of nitrogen fixing bacteria (Graham and Vance, 2003; Rahman et al, 2008) and thus the availability of nitrogen in the root zone is also reduced.

1.2 Statement of the problem

Food and nutrition insecurity is closely related to poverty and about half of the Kenyan population lives below poverty living on less than a dollar a day (Abukutsa-Onyango, 2007b). Over 10 million people in Kenya require food assistance at any given time. Nearly 30% of Kenyan's children are classified as undernourished and have micronutrient deficiencies (National Food Security Nutrition Policy [NFSNP], 2011). Malnutrition can be reduced if indigenous vegetables such as slender leaf could be grown and consumed locally. Although slender leaf has been commercially produced in Kenya, only 2-4 tons per hectare have been achieved and yet it has the potential of producing 10-12 tons per hectare (Abukutsa Onyango, 2004). The major constraints in slender leaf production have been poor quality seed, lack of technical production and utilization package, and lack of preservation and processing packages for optimal production (Abukutsa-Onyango, 2010), together with low soil fertility lacking major nutrients mainly phosphorus.

The amount of available phosphorus in the tropics is largely insufficient to meet the demands for legumes resulting in a widespread deficiency in pulse crops such as slender leaf (Turuko & Mohammed, 2014b). Declining land productivity in Kenya is a result of impoverished soils caused by continuous cultivation without the addition of adequate external inputs. The situation is further worsened by soil erosion on steep slopes (Minae & Nyamae, 1988). Continuous cropping of relatively impoverished land with a minimum return of nutrient contributes to declining soil fertility. This is characterized by low amounts of soil organic carbon, nitrogen, and phosphorus, leading to low productivity (Buresh et al, 1997). In many parts of Kenya, phosphorus is a major limiting nutrient affecting food production (Tuwei et al., 2013).

Addition of nitrogen did not have significant effect on yield of leaves and seeds of the slender leaf (Abukutsa-Onyango, 2007a). However, there is limited published information on the effect of phosphorus on the production of slender leaf in Kenya

1.3 Justification

Micronutrient deficiencies are highly prevalent among children below five years old and women (NFNSP, 2011). Malnutrition exists in various forms, such as acute and chronic malnutrition, micronutrient deficiencies, over weight and obesity problems. These problems affect lactating mothers and children under the age of five years, significantly contributing to their morbidity and mortality. Young children suffer from micronutrient deficiencies of iodine, iron, vitamin A and zinc (NFNSP, 2011).

These low micronutrient levels can be improved by the use of readily available and affordable vegetables such as slender leaf an indigenous vegetable that is rich in vitamin A, C, iron and protein. Slender leaf has potential to earn income for the rural poor and possess several advantages and potential that have not been fully exploited (Abukutsa-Onyango et al., 2005)

Declining soil fertility, low soil moisture, soil salinity and sodicity, soil compaction and the formation of hardpans are the major causes of low land productivity (Mureithi et al., 2003a). Many soils in sub-Saharan Africa are characterized by deficient levels of plant available phosphorus. In East Africa high annual nutrient depletion rates exceeding 40 kg N, 6.6 kg ha⁻¹ P and 33.2 kg ha⁻¹ K have been reported (Buresh et al., 1997). Even with diversity in the distribution of parent material and conditions affecting soil formation, soil phosphorus deficiencies result from either inherent low levels of soil phosphorus or depletion of soil phosphorus (Buresh et al., 1997). There is, therefore, need to study the effect of phosphorus on growth and nutritional quality for optimum production in slender leaf species.

1.4 Objectives

1.4.1 General objective of the study

To evaluate the effect of phosphorus application on growth, yield and quality of *Crotalaria brevidens* and *Crotalaria ochroleuca* grown under greenhouse and field conditions.

1.4.2 Specific Objectives

- a) To determine the effect of different phosphorus application rates on growth and yield of two slender leaf species.
- b) To establish the effect of phosphorus application on the nutritional content of two slender leaf species at vegetative, reproductive and maturity stages.

1.4.3 Hypothesis

- a) Different phosphorus application rates have no effect on growth and yield of two slender leaf species.
- b) Different phosphorus application rates have no effect on the nutritional content of the two slender leaf species at vegetative, reproductive and maturity stages of growth.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and distribution

Slender leaf is a legume in the family Fabaceae. The family contains 600 species that grow wild in the Tropical and Sub-tropical areas (Mosjidis & Wang, 2011). The genus *Crotalaria* has 500 species of herbs and shrubs found in Africa. The vegetable originated from Northern Nigeria then spread to Ethiopia and Southern Tanzania. It was later introduced to America. It has been reported to be cultivated as a vegetable in Sudan, Kenya, Uganda and Tanzania (Abukutsa-Onyango, 2004). The center of diversity for *Crotalaria* species is believed to be Africa. Slender leaf occurs in the open and wooded grassland and is occasionally found in seasonal swamps and on termite mounds. Among the 500 slender leaf species growing in Africa, two of the species used as vegetables are *Crotalaria brevidens* and *Crotalaria ochroleuca* (Abukutsa-Onyango, 2007a).

2.2 Botany

Slender leaf is an erect herbaceous annual that branches in the upper portion of the stem. Stems are cylindrical with ridges that end on an inflorescence. Deep yellow flowers develop acropetaly on the inflorescence, which is a terminal open raceme (Mosjidis & Wang, 2011). Plants produce an indeterminate number of flowering stems. The number of flowering stems is much influenced by water availability, temperature and day length. Flowers are visited by a variety of insects and humming birds with two common pollinators being monarch butterfly and bees and these have been found to be species specific (Subramaniam & Pandey, 2013) Fertilization occurs only after the stigmatic surface has been damaged by bees or by mechanical means . The leaves are either simple or compound with 3, 5 or 7 leaflets. Most of the species have numerous yellow

flowers which later on bear tough-skinned seed pods that are inflated. The number of seeds contained in a single pod depends on species but ranges from 5 to 50 seeds. The seeds are kidney-shaped, and their color varies from olive-green to either yellow-red or brown (Mosjidis & Wang, 2011).

2.3 Cultivation

Slender leaf does well, at altitudes of 500-2700 meters above sea level. The plant grows best in well drained soils with a pH 5-7.5 (USDA, 1999). *Crotalaria* seeds are drilled in rows 30 cm apart. Germination takes 3-4 days. Thinning is done to maintain a spacing of 15-20 cm between the plants. Slender leaf responds well to manure and an application of 20 ton per hectare is recommended. The initial growth of slender leaf is slow but the plant is ready for harvesting in eight weeks. Harvesting may continue for up to four months. The plant matures in eight weeks and has the ability to produce seed under tropical conditions. It performs well in low nitrogen soils and drought conditions (Abukutsa -Onyango, 2004).

2.4 Diseases and Pests

Under wet conditions, the crop may be destroyed by blight just before flowering. Rotar and Joy, (1983) observed cases of powdery mildew (*Oidium erysiphoides*) that affected the plants during the cold wet months. Aphids (*Aphis gossypii*) and thrips (*Frankliniella occidentalis*) are occasionally observed but are not a serious threat. During pod development, pod borers may enter and interfere with seed development (Osman & Elgersma, 2002; Abukutsa Onyango, 2004)

2.5 Harvesting

Slender leaf is uprooted just before flowering when the stems are 40 cm tall and 8 weeks old. Farmers use thinning as the first harvest after 6 weeks and use a ratoon system

afterwards. In the ratoon system, the first shoot is plucked at 8 weeks-stage followed by subsequent harvesting of new side shoots. The main shoot is cut at 15 cm above the ground leaving three leaves. The new shoots are harvested after two weeks. When the rainfall is adequate, harvesting may be done fifteen times. Yields of up to 10 tons have been achieved with an area of 1 m squared producing a kilo of leaves (Abukutsa Onyango, 2004).

2.5.1 Post harvest handling

Fresh leaves and young shoots are highly perishable and do not keep well. At 20-30°celsius the leaves last for one day (Abukutsa-Onyango, 2004). For longer storage the fresh leaves should be kept at temperatures below 20° C. To reduce deterioration, the shoots are tied in bundles that are regularly sprinkled with clean water. To prolong the use of slender leaf after harvest, they should be spread on a clean surface dried in the sun for 3-4 days during sunny days while during the rainy days they should be dried for 6-7 days. These are then packed in containers and sold in the market during the dry season (Abukutsa Onyango, 2004).

2.6 Economic importance of the slender leaf

Slender leaf is mainly grown for domestic market. The total area under production in 2012 was 286 ha increasing to 370 ha in 2013, and this production increased to a total of 533 ha in 2014 (Figure 1). The volume and total revenue continue to increase indicating an increase in demand and consumption of slender leaf in Kenya.

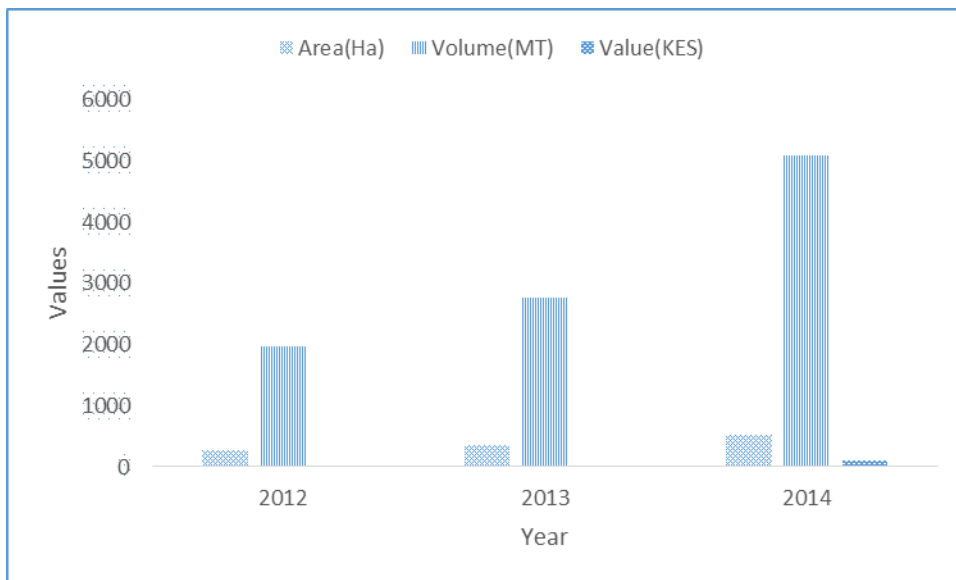


Figure 1: Production of slender leaf in Kenya 2012-2014. Horticulture Validated Report, HCDA, (2014)

2.7 The role of phosphorus in plants

Phosphorus is an important plant macronutrient, making up about 0.2 % of a plant's dry weight. It is a component of key molecules such as nucleic acids, phospholipids and adenosine triphosphate (ATP). It is involved in several plant functions, including controlling enzyme reactions, regulation of metabolic pathways, energy transfer, photosynthesis and transformation of carbohydrates as well as the formation of oils (Marschner, 1995). The optimum rate of P application is important in improving yields of most crops (Rashid & Rehim, 2007). Phosphorus deficiencies can adversely affect the symbiotic rhizobia establishment and nodule functions in legumes (Osman et al., 2002).

Van Averbek et al.(2007) reported that black night shade required 40 kg ha⁻¹ for optimum production while crops in the brassica family required 100-260 kg N ha⁻¹, 100-205 kg P·ha⁻¹ and 160-205 kg K·ha⁻¹ to achieve optimum yield. In cowpea production,

phosphorus was found to be the most limiting fertility nutrient in soils of the semi-arid Tropics of West Africa (Bationo et al., 2003).

In a study on the response of phosphorus to legumes species used as green manure in two KARI stations in Kenya, Kakamega and Kabete, phosphorus had a significant effect on the biomass production. Higher insignificant nodulation was recorded with the application of phosphorus than without P at Kakamega, while at Kabete, application of 20 kg P ha¹ increased biomass yield of slender leaf from 0.2-0.7 tons per hectare (Ojiem et al., 1997).

2.8 Effect of phosphorus on nutritional quality

Several factors directly or indirectly affect the nutritional quality of crops such as slender leaf. Among these are soil factors, that include soil pH, available nutrients, texture, organic matter content and soil-water relationships. Climatic factors that include temperature, rainfall and light intensity also affect the nutritional quality. Other factors include the crop and cultivar, postharvest handling and storage, fertilizer applications and cultural practices (Hornick, 1992).

In a study on the effects of phosphorus on the yield, quality and nutrient status of pigeon peas, phosphorus levels significantly affected the number of pods per plant, the number of grains per pods as well as crude protein percent (Deshbhratar et al., 2010). Black nightshade was found to require phosphorus at the rate of 40 kg ha⁻¹ for optimum production (Van Averbeke et al., 2007). Fertilizer affects the productivity and nutrient quality of crops. Inadequate levels of primary nutrients that include nitrogen, phosphorus and potassium results in weak vegetative growth, poor fruit setting, undesirable fruit quality and low nutritional quality as noted by Oloyede, (2012). There is limited published information on the effect of phosphorus on the nutritional content of the slender leaf.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Greenhouse pot and field experiments were carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) demonstration farm which is in Juja. The farm is situated 36 km North-East of Nairobi along the Thika-Nairobi highway. Juja is in the Upper midland zone 4, which is semi-humid to semi-arid. It is elevated at 1530 meters above sea level. The area is mainly covered by cambisols, vertisols and plinthisols. The area receives an annual rainfall of 1074 mm with annual mean temperature of 20.5°C (Batjes, 2006 ; Sombroek et al., 1982)

3.1.1 Greenhouse pot experiment

Red soil (Rhodic Nitisols), (FAO-UNESCO, 1988) was collected from Ni-kanini farm, situated half a kilometer from JKUAT. The soil was thoroughly mixed with river sand in the ratio of 5:1. Three composite samples of the mixture were taken to JKUAT Horticultural Laboratory for measurement of EC and pH. Analysis of nitrogen, phosphorus and potassium (NPK) was done according to the method described by Okalebo, (2002). A two factor experiment consisting of two slender leaf species and eight phosphorus levels replicated 4 times was set up in the greenhouse. The growing media was filled in 2 kg plastic pots and these were arranged in a completely randomized design in the greenhouse. Eight fertilizer rates consisting of 0, 0.28, 0.56, 0.84, 1.12, 1.40, 1.68 and 1.96 g of Triple super phosphate (TSP) equivalent to 0, 15, 30, 45, 60, 75, 90 and 105 kg P₂O₅ ha⁻¹ were applied into the pots. *Crotalaria brevidens* and *Crotalaria ochloreuca* seeds obtained from African Indigenous Vegetables Project at JKUAT were sown in germinating trays in row 10 cm apart. Water application was done daily to field capacity until the seeds germinated. Each seedling was pricked into the pot

that contained the growing media. After pricking the seedlings, watering was done to field capacity three times per week. The plants were maintained weed free by hand pulling of any emerging weeds. Data collection commenced three weeks after germinating and continued weekly until the 15th week when the seeds matured and were harvested. Data collected included, plant height, the number of leaves, the number of branches, the number of pods, seed yield and seed weight (g/1000). The first greenhouse experiment was conducted in the month of January 2013 to May 2013 and the second experiment was carried out in the month of June to November 2013.



Plate 1: *Crotalaria brevidens*



Plate 2: *Crotalaria ochloreuca*



Plate 3: *Crotalaria ochloreuca* growing in field. **Plate 4:** *Crotalaria brevidens*



(Mature pods before harvest).

3.1.2 Field experiment

3.1.2.1 Experimental design

Field layout was carried out in a field which had been ploughed and harrowed at JKUAT University Tuition farm at the African indigenous vegetable section. Split plot experimental design was used where species was the main plot factor while eight phosphorus levels were the subplots. Plots of 1m x 7m were measured out for the two *Crotalaria* species, and this were replicated four times resulting in a factorial experiment with two slender leaf species and eight fertilizer levels. Four rows 30 cm apart were made in each plot. Different fertilizer rates of 0, 15, 30, 45, 60, 75, 90 and 105 kg P₂O₅ ha⁻¹ were applied per plot respectively. Seeds were drilled in the rows and covered thinly. Watering was done to field capacity, thrice per week using overhead irrigation method. Thinning was done to maintain a spacing of 30 cm between plants. Weeding was carried out to maintain weed free fields. Data collection commenced three weeks after germination just like in the Greenhouse study earlier explained in section 3.1.1. The first field study was carried out in July 2013 to October 2013, and this was repeated from November 2013 to March 2014.



Plate 5: Weeding slender leaf in Field 1



Plate 6: Growing slender leaf in Field 2

3.1.3 Soil sample collection and analysis

Soil samples taken from 0 to 15 cm depth, were collected from representative spots of the entire experimental field by using diagonal sampling method and the composite samples were obtained. The samples were taken to JKUAT Horticultural University Laboratory for analysis. Analysis for nitrogen was done using micro Kjeldahl method (AOAC, 1984). Analysis for potassium was done using flame photometer, while phosphorus was extracted using Na HCO₃ Olsen method according to (Okalebo, 2002). Calcium, iron, magnesium and zinc were determined using atomic absorption spectrophotometry. The soil pH and electrical conductivity were measured according to the method by Okalebo, (2002).

Results of soil analysis in Table 1 and 2 showed that the level of phosphorus was below the critical P level that is adequate for plant growth. Plants require phosphorus above 10 parts per million in order to support plant growth. Addition of phosphorus was required to support the growth of the two species of slender leaf and enhance production. The soil pH range was slightly below 6-7.5 values that are ideal for P-availability. At acidic pH values, P ions react with aluminium (Al) and iron (Fe) to form less soluble compounds while at alkaline pH values phosphate ions react with calcium (Ca) and magnesium (Mg) to form less soluble phosphates unavailable to the plants (Marschner, 1995).

Table 1: Chemical soil analysis for Greenhouse pot study

Chemical	Amount	
pH	5.69	
EC(dS/m)	0.09	
N%	0.05	
P (ppm)	0.09	

Table 2: Chemical soil analysis for the field studies

Parameter	Field 1 (July-October 2013)			Field 2 (Nov.2013-March 2014)			Optimal ranges for nutrients in soil		
pH	6.81			6.38			5.5-6.8		
EC	0.13			0.01			<0.8		
N %	0.32			0.28			>0.25		
K cmole ⁺ /Kg	2.36			1.95			0.2-2		
P(ppm)	0.05			0.11			>0.2		
Mg cmole ⁺ /Kg	1.85			1.67			1.0-4.0		
Ca cmole ⁺ /Kg	4.22			3.27			4.0-11.0		
Fe mg kg ⁻¹	0.09			0.06			2.0-2000		
Zn mg kg ⁻¹	0.11			0.07			1.0-20.0		

Table 3: Thika Sub-county weather data for 2013 (KALRO).

Year		2013											
Parameter	Unit	Jan.	Feb.	March	April	May	June	July	Aug	Sep.	Oct.	Nov.	Dec.
Temperature	°C	20.6	21.05	22.25	21.85	20.55	18.95	18.1	18.1	20.45	21.65	21.05	19.8
Relative humidity	%	66.5	58.7	65	71.5	68.5	70.5	68	71	59	52.5	68	71.5
Rainfall	mm	717	0	1944	4294	206	91	20	69	95	14	1134	1102
Evaporation	mm	135	159.5	145.9	123.8	95.1	69.1	81.5	72.6	32.9	153.6	118.9	111.7
Mean Radiation	m/jm ²	26.6	25.23	21.68	19.03	17.53	12.21	16.6	14.59	19.58	23.12	18.19	17.96

Table 4: Thika Sub-county weather data for 2014 (KALRO).

Year				2014			
Parameter	Unit	Jan.	Feb.	March	April	May	June
Temperature	°C	20.55	21.65	22	21.15	21.2	19.5
Relative humidity	%	57.5	65	64.5	70	68.5	70.5
Rainfall	mm	0	963	1284	972	399	359
Evaporation	mm	155	135.3	152	189.2	94	77.4
Mean Radiation	m/jm ²	28.19	23.51	23.75	18.93	17.76	12.54

3.2 Measurement of plant growth parameters

Plant height was taken by selecting three plants per plot, and these were tagged and numbered. Random numbering of the pre-tagged plants from the two inner rows in each plot was done. Measurement of plant height was done from the ground level to the apical bud using a ruler in centimeters. The number of branches, leaves, and pods were counted and recorded.

3.2.1 Measurement of seed yield in (g/plant) for greenhouse experiments.

In the green house experiments, seeds from individual plants were harvested before shattering, these were shelled, winnowed and all unhealthy and broken seeds were removed. These were then air dried in the shade packed and weighed using an electric weighing balance and the weight recorded. Seed yield and seed weight were taken from each plant.

From the two field experiments, seeds from plants in each plot (an average 84 plants), were harvested when mature before shattering. These were then air- dried threshed and winnowed. The seeds were then oven dried at 70°C for 24 hours to 8 % moisture content. The seeds were then weighed using an electric weighing balance model Chyo JL-180. Seed yield per plot was recorded, and the seed yield transformed to seed yield kilograms per hectare (kg ha^{-1}), using the following formula:

$$\text{Seed yield - kg ha}^{-1} = \text{Seed yield per plot (kg)} * 10000 \text{ m}^2 / \text{area of plot m}^2$$

3.2.2 Determination of seed weight (g/1000 seeds)

A thousand seed weight was taken by counting 1000-seeds thrice from each sample, and these were weighed using an analytical balance model Chyo JL-180. The average weight of the samples was calculated and recorded.

3.3 Nutrient content analysis

3.3.1 Harvesting of leaves in Field 1 and 2.

Leaves were harvested at the vegetative (week four), reproductive (week seven) and maturity stages (twelve week) of growth. The leaves were harvested by hand plucking. They were then put in polythene bags labelled and stored in a cold room at 10°celsius prior to analysis.

3.3.2 Determination of minerals

Determination of minerals was done by dry ashing and atomic absorption spectrophotometry (AAS), according to (AOAC, 1984) . The minerals determined were potassium, calcium, magnesium, zinc, iron and phosphorus.

Clean, dry crucibles were weighed and 1g plant sample was weighed into it. The crucibles were placed in a hot plate under a fume hood where the temperature increased slowly until smoking ceased and the samples were charred. These were then transferred into a muffle furnace, and the temperature increased gradually to 250°C heated for an hour then increased to 500°C and incinerated to complete ashing for seven hours. The temperature was decreased to 300°C, and the crucibles were removed and cooled at room temperature. The ash was transferred quantitatively to 100 ml beaker using 1N HCL. It was then heated at 80-90°C on a hot plate for 5 minutes. The contents were transferred to 100 ml volumetric flask and filled to mark using 1N HCL. The contents were filtered using Whatman No.42. The filtrate was put in polyethylene bottles. The absorbance was read by Atomic Absorption Spectrophotometer (AAS). The various mineral standards were prepared to make calibration curves. The digest was used to determine potassium, magnesium, calcium, iron and zinc according to AOAC, (1984).

3.3.2 Determination of nitrogen and protein content

Protein was determined using the semi-micro Kjeldhal method as described by Rodriguez-Amaya and Kimura, (2004). Approximately 2 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g potassium sulphate and 0.5 g of copper sulphate and 15 mL of sulphuric acid. The mixture was heated in a fume hood till the digest color turned blue. This signified the end of the digestion process.

The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalyst was also made. Exactly 10 mL of the diluted digest was transferred into the distilling flask and washed with distilled water. Fifteen mL of 40 % NaOH was added and this also washed with distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N HCL to an orange color of the mixed indicator, which signified the end point. The nitrogen content in the sample was calculated as:

$$\% \text{ Nitrogen} = (V_1 - V_2) \times N \times F \div (V \times 100) \text{ Sample weight, Where:}$$

V₁ is the titre for the sample in ml, V₂ is titre for blank in ml; N= normality of standard HCL (0.02); F= factor of standard HCL solution; V= volume of diluted digest taken for distillation (10 ml); S= weight of sample taken for distillation (2 g). The protein content was then calculated as % *Protein* = *Nitrogen* × *Protein factor* (6.25).

3.3.3 Determination of Beta carotene

Beta carotene content was analyzed using column chromatography and UV Spectrophotometer. Extraction was done by acetone and petroleum ether as described by Rodriguez-Amaya and Kimura, (2004).

Approximately 2 grams of fresh sample was weighed, finely chopped and placed in a mortar with about 10 mL of acetone. This was thoroughly ground and the acetone extract transferred into 100 mL volumetric flask. The residue was again extracted with 10 mL acetone, and the extract was added to the contents of the volumetric flask. The extraction with acetone was continued until the residue was colorless. The combined extract was made to a volume of 100 mL with acetone.

Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator. The residue was dissolved in 10 mL petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether, and the absorbance was read at 450 nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Beta carotene standard was also prepared to make a calibration curve.

Total carotenoid was calculated using the formula: *Total carotenoids content* (μg) = $A \times \text{Volume (mL)} \times 104 A_1 \% 1 \text{ cm} \times \text{Sample wt (g)}$

Where A= absorbance; volume = total volume of extract (25 mL); $A_1 \% 1 \text{ cm}$ = absorption coefficient of β -carotene in Petroleum Ether.

3.4 Data analysis

Collected data was subjected to analysis of variance, (ANOVA) to establish if the treatments had a significant effect on the growth and nutrient content of the two slender leaf species at 5 per cent level of significance. Means were separated using Student Newman Keuls test using SAS 9.1.3 software.

CHAPTER FOUR

RESULTS

4.1 Effect of phosphorus on the growth of two slender leaf species in the greenhouse experiments 1 and 2.

4.1.1 Plant height (cm)

There was significant ($p \leq 0.05$) interactions between species and phosphorus, with respect to plant height (Table 5). The effect of phosphorus application was significant ($p \leq 0.05$) on the plant height. Increasing phosphorus levels increased plant height for *C.brevidens* up to 60 kg P₂O₅ ha⁻¹ when maximum plant height of 37.46 cm was achieved. However, this height was not significantly different from the height at 90 and 105 kg P₂O₅ ha⁻¹ but was significantly different from the height at all other P levels (Table 5). The lowest plant height 28.4 cm was observed at the control. During the second greenhouse experiment, maximum plant height for *Crotalaria brevidens* 114.02 cm was recorded at the control though this was not significantly different from the height at 30 kg P₂O₅ ha⁻¹ but was different from all other levels. Minimum plant height for the species 78.83 cm was recorded at 90 kg P₂O₅ ha⁻¹ and this plant height was not significantly different from the height at 45-105 kg P₂O₅ ha⁻¹ (Table 5).

In the first greenhouse experiment, maximum plant height of 70.77 cm was observed at 15 kg P₂O₅ ha⁻¹ for *Crotalaria ochroleuca*, even when more phosphorus was applied there was no more increase in plant height. The minimum plant height of 56.10 cm was recorded at 60 kg P₂O₅ ha⁻¹. During the second experiment, maximum plant height of 97.33 cm was recorded at 105 kg P₂O₅ ha⁻¹ for *C.ochloreuca*. This plant height was not significantly different from the height at 60 and at 75 kg P₂O₅ ha⁻¹. However, the lowest plant height 70.92 cm for the species was recorded at 15 kg P₂O₅ ha⁻¹.

Table 5: Effect of phosphorus on plant height (cm) in two slender leaf species

Greenhouse 1 experiment (January -May 2013)				Greenhouse 2 experiment (June -November 2013)		
Plant height (cm)				Plant height (cm)		
P ₂ O ₅ kg ha ⁻¹	<i>C. brevidens</i>	<i>C. ochloreuca</i>	P means	<i>C. brevidens</i>	<i>C. ochloreuca</i>	P means
0	28.40 ± 3.6 ^c	63.60±9.16 ^{abc}	46.00± 5.20 ^c	114.02±13.52 ^a	95.37± 8.69 ^a	104.69±8.05 ^a
15	33.95±4.32 ^{ab}	70.77 ± 9.21 ^a	52.36± 5.37 ^a	87.69 ± 9.09 ^b	70.92 ± 7.09 ^d	79.30 ± 5.80 ^c
30	31.02±3.72 ^{bc}	59.73± 7.88 ^{bc}	45.38± 4.56 ^c	112.96±15.41 ^a	85.41 ± 7.88 ^b	99.18±8.72 ^{ab}
45	31.32±3.75 ^{bc}	59.54± 7.81 ^{bc}	45.43± 4.53 ^c	93.88 ± 9.69 ^b	76.83 ± 6.93 ^c	85.35±5.99 ^{cde}
60	37.46 ± 4.23 ^a	56.10 ± 6.73 ^c	46.78±4.06 ^{bc}	93.54 ± 9.40 ^b	94.78 ± 8.21 ^a	94.16± 6.21 ^{bc}
75	31.21±3.65 ^{bc}	56.59± 6.66 ^{bc}	43.90 ± 3.98 ^c	82.42 ± 9.29 ^b	97.10 ± 8.59 ^a	89.76±6.34 ^{bcd}
90	35.46 ± 3.91 ^a	66.38± 7.79 ^{ab}	50.92±4.60 ^{ab}	78.83 ± 8.40 ^b	86.23 ± 7.85 ^b	82.53± 5.73 ^{de}
105	35.09 ± 3.96 ^a	58.37± 6.79 ^{bc}	46.73±4.08 ^{bc}	86.48 ± 9.92 ^b	97.33 ± 8.68 ^a	91.91±6.58 ^{bcd}
Species means	32.99 ± 1.37 ^b	61.38 ± 2.75 ^a		93.73 ± 3.85 ^a	87.99 ± 2.85 ^b	

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.1.2 Number of leaves.

There was significant ($p \leq 0.05$) interaction between phosphorus and species in respect to the number of leaves. The effect of phosphorus on the number of leaves was significantly different ($p \leq 0.05$). *Crotalaria brevidens* recorded 115.87 mean number of leaves that was significantly higher than 49.69 observed in *Crotalaria ochloreuca*. The number of leaves increased with increase in phosphorus levels (Table 6). Maximum number of leaves for *Crotalaria brevidens* 141.85 was observed at 105 kg P₂O₅ ha⁻¹ and this number of leaves was not significantly different from the leaf number at 45-90 kg P₂O₅ ha⁻¹ but was significantly different from the leaf numbers observed at 0-30 P₂O₅ kg ha⁻¹. The lowest number of leaves for the species was 71.27 observed at the control.

The maximum number of leaves 64.25 for *C.ochlorea* was observed at 105 P₂O₅ kg ha⁻¹. However, the number of leaves at this level was significantly different from the leave numbers at 0-45 kg P₂O₅ ha⁻¹ but was not significantly different from the leave numbers at all other phosphorus levels. The lowest number of leaves 27.85 was observed at the control (Table 6).

In the second greenhouse experiment, the effect of phosphorus was not significant (p>0.05) on the number of leaves for the two slender leaf species. Higher leave numbers were observed at lower phosphorus levels and the leave numbers continued to reduce as the P levels increased in the two slender leaf species.

Table 6: Effect of phosphorus on the number of leaves in two slender leaf species in the greenhouse.

P ₂ O ₅ kg/ha-1	Greenhouse experiment 1(January-May 2013)			Greenhouse experiment 2 (June-November 2013)		
	<i>Crotalaria brevidens</i>	<i>Crotalaria ochlorea</i>	P means	<i>Crotalaria brevidens</i>	<i>Crotalaria ochlorea</i>	P means
0	71.27 ± 12.34 ^c	27.85 ± 5.09 ^d	49.56 ± 7.00 ^d	96.00 ± 15.45 ^a	123.25 ± 22.25 ^a	101.24 ± 8.33 ^a
15	84.50 ± 13.29 ^c	40.06 ± 7.67 ^c	62.28 ± 7.97 ^c	93.75 ± 20.20 ^a	76.50 ± 15.29 ^a	74.85 ± 5.82 ^c
30	106.50 ± 17.16 ^b	49.04 ± 9.05 ^b	77.77 ± 10.09 ^b	89.00 ± 19.52 ^a	91.75 ± 7.85 ^a	91.98 ± 8.61 ^{ab}
45	122.31 ± 19.67 ^{ab}	45.40 ± 7.56 ^{bc}	83.85 ± 11.20 ^b	104.00 ± 16.56 ^a	110.25 ± 4.70 ^a	87.66 ± 6.46 ^{bc}
60	135.15 ± 20.57 ^a	60.06 ± 10.46 ^a	97.60 ± 12.11 ^a	105.00 ± 17.24 ^a	82.75 ± 4.77 ^a	75.26 ± 5.47 ^c
75	127.92 ± 21.42 ^{ab}	52.04 ± 9.28 ^b	89.979 ± 12.25 ^{ab}	82.75 ± 23.64 ^a	86.50 ± 14.38 ^a	81.45 ± 7.10 ^{bc}
90	137.46 ± 23.45 ^a	58.77 ± 9.81 ^a	98.12 ± 13.27 ^a	80.00 ± 9.34 ^a	89.00 ± 18.40 ^a	76.33 ± 5.65 ^c
105	141.85 ± 25.06 ^a	64.250 ± 11.78 ^a	103.05 ± 14.33 ^a	95.75 ± 18.34 ^a	63.75 ± 6.98 ^a	76.47 ± 6.53 ^c
Species mean:	115.87 ± 6.98 ^a	49.69 ± 3.22 ^b		93.25 ± 3.94 ^a	73.06 ± 2.75 ^b	

Means with the same letter along the column are not significantly different at p≤0.05 by Student Newman Keuls

4.1.3 Number of branches

Significant ($p \leq 0.05$) interaction between phosphorus and species with respect to the number of branches was observed in the first greenhouse experiment (Table 7). *Crotalaria ochloreuca* had significantly ($p \leq 0.05$) higher mean number of branches than *Crotalaria brevidens* in the two greenhouse experiments. During the first experiment, the number of branches increased as more phosphorus was added for the two species. Maximum number of branches 11.34 per plant for *Crotalaria brevidens* were observed at 90 kg P_2O_5 ha⁻¹. The number of branches at this level was not significantly different from the number of branches at 105 kg P_2O_5 ha⁻¹. The minimum mean number of branches was 8.0 was recorded at the control (Table 7). However, for *Crotalaria ochloreuca*, the maximum number of branches 12.27 was recorded at 105 kg P_2O_5 ha⁻¹ but the number of branches was not significantly different from the number of branches at 60-90 kg P_2O_5 ha⁻¹. The lowest mean number of branches 7.0 was recorded at zero.

Different results were observed in the second experiment where for the two slender leaf species, application of different phosphorus levels had no significant ($p > 0.05$) effect on the number of branches recorded (Table 7). However, maximum number of branches 10.79 for *C. brevidens* was observed 105 kg P_2O_5 ha⁻¹ although the level was not significantly different from the number of branches from all other P levels. For *C. ochloreuca*, maximum number of branches 11.2 was observed at the control but the number of branches at this level was not significantly different from all other phosphorus levels. The species means were significantly different in both greenhouse experiments with the highest number of branches observed in *C. ochloreuca*.

Table 7: Effect of phosphorus on the number of branches/plant for slender leaf species GH experiments.

Greenhouse experiment 1 (January-May 2013)				Greenhouse experiment 2 (June -November 2013)		
P ₂ O ₅ kg ha ⁻¹	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	P means	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	P means
	No. of branches	No. of branches		No. of branches	No. of branches	
0	8.00± 0.99 ^e	7.07± 0.96 ^c	7.536 ± 0.69 ^d	9.96±0.55 ^a	12.48±1.10 ^a	11.219 ± 0.63 ^a
15	8.93± 1.04 ^d	9.77± 1.23 ^b	9.348 ± 0.80 ^c	10.29±0.59 ^a	10.88±1.06 ^a	10.583 ± 0.61 ^{ab}
30	9.82± 1.09 ^{bcd}	9.73± 1.10 ^b	9.777 ± 0.77 ^c	10.69±0.85 ^a	10.73 ± 0.97 ^a	10.708 ± 0.64 ^{ab}
45	9.61± 1.03 ^{cd}	9.70± 1.08 ^b	9.652 ± 0.74 ^c	10.29±0.6 ^a	12.15 ± 0.91 ^a	11.219 ± 0.55 ^a
60	10.30± 1.09 ^{bc}	11.96 ± 1.17 ^a	11.125 ± 0.8 ^{ab}	10.23±0.59 ^a	10.71 ± 0.96 ^a	10.469 ± 0.56 ^{ab}
75	9.43± 1.01 ^{cd}	11.84± 1.26 ^a	10.634 ± 0.81 ^b	10.19±0.67 ^a	10.67± 0.93 ^a	10.427 ± 0.57 ^{ab}
90	11.34± 1.24 ^a	11.68± 1.19 ^a	11.509 ± 0.86 ^a	9.19±0.46 ^a	10.69 ± 0.83 ^a	9.938 ± 0.48 ^b
105	10.75± 1.18 ^{ab}	12.27± 1.10 ^a	11.509 ± 0.81 ^a	10.79±0.64 ^a	10.40±0.94 ^a	10.594 ± 0.56 ^{ab}
Sp means	9.772±0.38 ^b	10.50±0.41 ^a		10.203±0.22 ^b	11.086±0.34 ^a	

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.1.4 Number of pods.

There was significant ($p \leq 0.05$) interaction between phosphorus levels and slender leaf species with respect to the number of pods. The effect of phosphorus on the number of pods was significant ($p \leq 0.05$). The slender leaf species too were significantly different with respect to the number of pods. In the first greenhouse experiment, maximum number of pods per plant for *Crotalaria brevidens* 18.38 was observed at 45 kg P₂O₅ ha⁻¹. Application of P beyond 45 kg ha⁻¹ did not increase the number of pods. However, the number of pods at the level were only significantly different from number of pods at 60 kg P₂O₅ ha⁻¹. The minimum number of pods 8.13 was observed at 60 kg P₂O₅ ha⁻¹. Different response was observed during the second experiment where the maximum

number of pods 14.3 for the *C.brevidens* was observed at 15 kg P₂O₅ ha⁻¹ while the minimum 4.25 was recorded at 75 kg P₂O₅ ha⁻¹(Table 8).

In the first greenhouse experiment, maximum number of pods 18.8 for *Crotalaria ochroleuca* was observed at 105 kg P₂O₅ ha⁻¹ and this level was only significantly different from the control. Minimum number of pods 1.94 was observed at the control. When the experiment was repeated, maximum number of pods at 20.35 was observed at 60 kg P₂O₅ ha⁻¹for the species. .When the P level increased to 75 kg the number of pods decreased rapidly. The lowest mean number of pods 8.2 was recorded at the control (Table 8).

Table 8: Effect of phosphorus on the number of pods in two slender leaf species.

P ₂ O ₅ kg ha ⁻¹	Greenhouse experiment 1 (January-May 2013)			Greenhouse experiment 2 (June - November 2013)		
	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	P means	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	Pmeans
0	13.19 ± 5.97 ^{ab}	1.94 ± 1.37 ^b	7.56 ± 3.18 ^b	13.00 ± 2.45 ^{ab}	8.20 ± 1.69 ^d	10.600 ± 1.517 ^{ab}
15	11.56 ± 5.16 ^{ab}	5.94 ± 2.71 ^{ab}	8.75 ± 2.91 ^{ab}	14.30 ± 1.58 ^a	13.40 ± 3.67 ^{ba}	13.85 ± 1.97 ^{ab}
30	10.88 ± 5.29 ^{ab}	6.69 ± 2.40 ^{ab}	8.78 ± 2.88 ^{ab}	9.30 ± 2.22 ^{abcd}	16.55 ± 2.66 ^{ba}	12.93 ± 1.81 ^{ab}
45	18.38 ± 5.64 ^a	11.00 ± 4.06 ^{ab}	14.69 ± 3.48 ^{ab}	12.80 ± 2.24 ^{ab}	14.95 ± 3.05 ^{ba}	13.88 ± 1.88 ^{ab}
60	8.13 ± 4.57 ^b	6.63 ± 2.78 ^{ab}	7.38 ± 2.63 ^b	11.05 ± 1.75 ^{abc}	20.35 ± 4.34 ^c	15.70 ± 2.43 ^a
75	16.19 ± 6.53 ^{ab}	8.56 ± 3.48 ^{ab}	12.38 ± 3.71 ^{ab}	4.25 ± 1.24 ^d	12.15 ± 2.89 ^{ba}	8.20 ± 1.68 ^b
90	14.63 ± 6.94 ^{ab}	18.25 ± 5.65 ^a	16.44 ± 4.41 ^a	7.90 ± 2.02 ^{bcd}	15.85 ± 2.89 ^{ba}	11.88 ± 1.86 ^{ab}
105	12.06 ± 5.15 ^{ab}	18.88 ± 7.63 ^a	15.47 ± 4.57 ^a	6.15 ± 1.29 ^{cd}	15.70 ± 6.01 ^{ab}	10.93 ± 3.13 ^{ab}
Species means	13.13 ± 1.98 ^a	9.73 ± 1.53 ^b		9.84 ± 0.71 ^b	14.64 ± 1.28 ^a	

Means with the same letter along the column are not significantly different at p≤0.05 by Student Newman Keul.

4.1.5 Seed yield (g/plant)

There was significant ($p \leq 0.05$) interaction between phosphorus and the species with respect to the seed yield. For all phosphorus levels, *C.ochlorea* had higher seed yield than *Crotalaria brevidens* in the two greenhouse experiments. The effect of phosphorus on seed yield was significant ($p \leq 0.05$). In the first greenhouse experiment, as the phosphorus levels increased the seed yield increased significantly until the maximum mean seed yield of 18.55 g was attained at 75 kg P₂O₅ ha⁻¹ for *Crotalaria brevidens*. The seed yield at 75 kg was not significantly different from the yield at 45 kg P₂O₅ ha⁻¹ (Table 9). The minimum seed yield 9.17 g was observed at the control. Maximum mean seed yield for *Crotalaria ochlorea* 22.34 g was recorded at 90 kg P₂O₅ ha⁻¹ and the lowest 10.94 was recorded at 15 kg P₂O₅ ha⁻¹ although this was not significantly different from the yield at the control.

During the second experiment, despite the different rates of phosphorus applied, significantly low seed yield was recorded for the two slender leaf species. The effect of phosphorus on seed yield for both species in the second experiment was not significantly different ($p > 0.05$). However, the maximum seed yield for *C. brevidens* 4.71 was observed at 45 kg P₂O₅ ha⁻¹ while for *C.ochlorea* the maximum seed yield 3.87 was observed at 75 kg P₂O₅ ha⁻¹ (Table 9).

Table 9: Effect of phosphorus on the seed yield (g/plant) in greenhouse experiments

P ₂ O ₅ kg ha ⁻¹	Greenhouse 1 (January-May 2013)		Greenhouse 2 (June-November 2013)		
	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	
0	9.17±1.66 ^a	12.01±1.61 ^a	3.19±1.52 ^a	4.33±1.55 ^a	
15	9.56±1.66 ^a	10.94±1.61 ^a	2.27±0.64 ^a	3.96±0.83 ^a	
30	13.17±1.66 ^a	14.85±1.61 ^a	3.42±0.35 ^a	2.79±0.40 ^a	
45	15.11±1.66 ^a	16.17±1.61 ^a	2.59±0.49 ^a	4.71±0.80 ^a	
60	14.25±1.66 ^a	18.5±1.61 ^a	3.36±1.24 ^a	4.35±1.02 ^a	
75	18.55±1.66 ^a	19.2±1.61 ^a	3.87±0.77 ^a	3.89±0.76 ^a	
90	14.57±1.66 ^a	22.34±1.61 ^a	3.07±0.69 ^a	2.77±0.46 ^a	
105	15.84±1.66 ^a	18.7±1.61 ^a	1.53±0.72 ^a	3.70±0.92 ^a	

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.1.6 Seed weight (g/1000 seeds)

Significant ($p \leq 0.05$) interaction between phosphorus and species in respect to the seed weight was observed during the first greenhouse experiment. The effect of phosphorus on the seed weight was also significantly different ($p \leq 0.05$) during the first experiment for the two slender leaf species but not in the second experiment (Table 10). The maximum seed weight for *Crotalaria brevidens* 6.34 g was recorded at 90 kg P₂O₅ ha⁻¹, and this weight was significantly different from the weight 4.29 g recorded at the control. In *Crotalaria ochloreuca* maximum seed weight 6.89 g was observed at 30 kg P₂O₅ ha⁻¹ although this weight was not significantly different from the seed weight at 90 kg P₂O₅ ha⁻¹. The minimum weight for *C.ochloreuca* 5.05 g was observed at 60 kg P₂O₅ ha⁻¹. During the second greenhouse experiment the effect of phosphorus was not significantly different for the two species ($p > 0.05$). For all phosphorus levels the seed weight was not significantly different for the two slender leaf species (Table 10).

Table 10: Effect of phosphorus on seed weight (g/1000 seeds) of two slender leaf species.

Greenhouse experiment 1 (January-May 2013)				Greenhouse experiment 2 (June-November 2013)		
Seed weight (g/1000 seeds)				Seed weight (g/1000 seeds)		
P ₂ O ₅ kg ha ⁻¹	<i>Crotalaria brevidens</i>	<i>Crotalaria ochroleuca</i>	P mean	<i>Crotalaria brevidens</i>	<i>Crotalaria ochroleuca</i>	P means
0	4.29 ± 0.12 ^b	5.92 ± 0.18 ^{ab}	5.11±0.13	5.55 ± 0.71 ^a	7.29 ± 0.56 ^a	6.42 ± 0.53
15	5.01 ± 0.43 ^{ab}	5.94 ± 0.14 ^{ab}	5.47±0.24	5.20 ± 1.0 ^a	6.45 ± 1.03 ^a	5.82 ± 0.70
30	4.51 ± 0.20 ^{ab}	6.89 ± 0.25 ^a	5.70±0.225	3.18 ± 0.48 ^a	7.26 ± 0.96 ^a	5.22 ± 0.92
45	5.12 ± 0.33 ^{ab}	6.20 ± 0.28 ^{ab}	5.66±0.305	5.55 ± 0.09 ^a	5.40 ± 1.77 ^a	5.47 ± 0.82
60	5.42 ± 0.28 ^{ab}	5.76 ± 0.51 ^{ab}	5.59±0.395	4.55± 1.15 ^a	5.40 ± 1.81 ^a	4.98 ± 1.00
75	5.69 ± 0.56 ^{ab}	6.63 ± 0.17 ^{ab}	6.16±0.365	2.80 ± 1.23 ^a	7.03 ± 0.45 ^a	4.92 ± 1.00
90	6.34 ± 0.59 ^a	5.05 ± 0.75 ^b	5.70±0.67	4.29 ± 0.87 ^a	6.38 ± 0.50 ^a	5.33 ± 0.61
105	5.48 ± 0.36 ^{ab}	5.95 ± 0.19 ^{ab}	5.7±0.28	5.69 ± 0.38 ^a	4.69 ± 1.76 ^a	5.19 ± 0.86
Species means	5.24 ± 0.16 ^b	6.04 ± 0.15 ^a		4.60 ± 0.32 ^b	6.24 ± 0.42 ^a	

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.2 Effect of phosphorus on growth of two *Crotalaria* species in the field study

4.2.1 Plant height

No interaction was observed in the field experiments between phosphorus and species with respect to plant height. The effect of phosphorus on plant height was not significant ($p > 0.05$). In the first field experiment, as phosphorus increased the plant height for *Crotalaria brevidens* increased to 63.13 cm at 60 kg P₂O₅ ha⁻¹. When the P level increased beyond 60 kg there was a decline in plant height, while the minimum plant height 46.17 cm was observed at the control. When the experiment was repeated in Field

2, maximum plant height of 60.42 cm was observed at 75 kg P₂O₅ ha⁻¹ for *C.brevidens* while the lowest 40.0 cm was recorded at the control (Table 11). In the first field experiment, maximum plant height for *C.ochloreuca* was 82.76 cm observed at 30 kg P₂O₅ ha⁻¹ while the lowest 62.68 cm was observed at 90 kg P₂O₅ ha⁻¹. When the experiment was repeated in Field 2, maximum plant height of 92.04 cm was recorded for the species at 75 kg P₂O₅ ha⁻¹ while the lowest plant height 79 cm for the species was observed at 60 kg P₂O₅ ha⁻¹. In both field experiments, *Crotalaria ochloreuca* recorded higher plant height than *Crotalaria brevidens* at all phosphorus levels (Table 11) Maximum plant height during the second experiment for the two species was observed at 75 kg P₂O₅ ha⁻¹.

Table 11: Effect of phosphorus on plant height of two slender leaf species Field experiments 1 and 2.

Field 1 experiment(JULY-OCTOBER 2013)		Field 2 experiment (NOV 2013-MARCH 2014)		
<i>Crotalaria brevidens</i>		<i>Crotalaria ochroleuca</i>	<i>Crotalaria brevidens</i>	<i>Crotalaria ochroleuca</i>
P ₂ O ₅ kg/ha	Plant height(cm)	Plant height(cm)	Plant height(cm)	Plant height(cm)
0	46.17±3.38 ^a	78.22 ± 6.14 ^a	40.44 ± 3.47 ^a	80.39±7.42 ^a
15	59.51±4.35 ^a	70.35 ± 6.21 ^a	46.39 ± 3.86 ^a	84.44±8.56 ^a
30	57.78±3.99 ^a	82.76 ± 7.08 ^a	48.36 ± 3.63 ^a	80.39±7.85 ^a
45	56.99±3.62 ^a	69.83 ± 5.52 ^a	49.58 ± 3.65 ^a	87.42±8.25 ^a
60	63.13±4.67 ^a	79.35 ± 6.59 ^a	55.78 ± 5.55 ^a	79.00±7.80 ^a
75	59.47±4.76 ^a	69.93 ± 5.63 ^a	60.42 ± 6.24 ^a	92.04±8.5 ^a
90	46.17±3.38 ^a	62.68 ± 5.09 ^a	53.25 ± 0.60 ^a	83.81±7.39 ^a
105	58.39±3.95 ^a	67.15 ± 5.54 ^a	45.19±4.35 ^a	82.25±8.98 ^a

Means with the same letter along the column are not significantly different at p≤0.05 by Student Newman Keuls

4.2.2 Number of leaves

No significant ($p>0.05$) interaction between phosphorus and species in the first field experiment in respect to the number of leaves was observed. The effect of phosphorus on the number of leaves in the two slender leaf species was not significant ($p>0.05$). In the first field experiment, the maximum number of leaves for *Crotalaria brevidens* was 84.89 observed at 30 kg P₂O₅ ha⁻¹, while the minimum number of leaves 58.86 was observed at 15 kg P₂O₅ ha⁻¹. The leaf number increased as phosphorus levels increased up to 30 kg P₂O₅ ha⁻¹ after which even when the level of P levels increased that level the leaf numbers did not increase (Table 12). During the second experiment, the maximum number of leaves for *C. brevidens* was 139.17 observed at 60 kg P₂O₅ ha⁻¹, when more P was added the leaf number decreased. The lowest number of leaves was recorded at the control (Table 12).

During the first field experiment, the effect of phosphorus application was not significant ($p>0.05$) on the number of leaves for *C. ochlorea*. However, the maximum number of leaves for *C. ochlorea* 63.13 was observed at 60 kg P₂O₅ ha⁻¹ while the minimum number of leaves 46.17 was observed at the control. When the experiment was repeated in the second experiment conducted in the months of November 2013-March 2014, the maximum number of leaves 121.06 was observed at 30 kg P₂O₅ ha⁻¹ while the minimum 94.13 number of leaves was observed at the 90 kg P₂O₅ ha⁻¹ (Table 12).

Table 12: Effect of P on the number of leaves in two slender leaf species

Field 1 Experiment (JULY-OCTOBER 2013)			Field 2 Experiment (NOV 2013-MARCH 2014)	
	<i>C. brevidens</i>	<i>C. ochroleuca</i>	<i>C. brevidens</i>	<i>C. ochroleuca</i>
P_2O_5 kg/ha ¹	Number of leaves	Number of leaves	Number of leaves	Number of leaves
0	71.94 ± 8.93 ^a	46.17±3.38 ^a	98.67±17.80 ^a	106.81±14.50 ^a
15	58.86 ± 6.89 ^a	59.51±4.35 ^a	116.39±19.9 ^a	106.64±16.68 ^a
30	84.89 ± 9.54 ^a	57.78±3.99 ^a	134.19±21.1 ^a	121.06±19.1 ^a
45	69.14 ± 6.98 ^a	56.99±3.62 ^a	126.53±21.0 ^a	106.83±16.5 ^a
60	78.56 ± 8.81 ^a	63.13±4.67 ^a	139.17±23.5 ^a	112.97±15.9 ^a
75	65.11 ± 8.13 ^a	59.47±4.76 ^a	101.92±15.6 ^a	103.61±14.34 ^a
90	64.92 ± 7.46 ^a	46.17±3.38 ^a	114.28±16.3 ^a	94.13±10.55 ^a
105	60.81 ± 7.82 ^a	58.39±3.95 ^a	108.92±19.2 ^a	112.861±15.9 ^a

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.2.3 Number of branches

No significant ($p > 0.05$) interaction between phosphorus and species in respect to the number of branches was observed. The effect of phosphorus on the number of branches in the two experiments in the field was not significant ($p > 0.05$). The maximum number of branches for *Crotalaria brevidens* 13.4 were observed at 15 kg P_2O_5 ha⁻¹ during the first experiment. The number of branches reduced as more phosphorus was applied. The lowest number of branches 7.67 was recorded at 105 kg P_2O_5 ha⁻¹ (Table 13). When the experiment was repeated, the maximum number of branches for *Crotalaria brevidens* 10.56 was observed at 90 kg P_2O_5 ha⁻¹, while the lowest number of branches 8.64 was recorded at 105 kg P_2O_5 .

In the first field experiment, the maximum number of branches for *Crotalaria ochroleuca* 12.80 was observed at 15 kg P₂O₅ ha⁻¹. The number of branches reduced as more P was applied beyond 15 kg P₂O₅ ha⁻¹. The minimum number of branches 8.64 was recorded at 90 kg P₂O₅ kg ha⁻¹. When the experiment was repeated, the maximum number of branches for *Crotalaria ochroleuca* 16.39 was observed at 75 kg P₂O₅ ha⁻¹ while the least number of branches 11.33 was observed at 60 kg P₂O₅ ha⁻¹(Table 13).

Table 13: Effect of phosphorus application on the number of branches.

Field experiment 1 (July-October 2013)			Field experiment 2(Nov.2013-March 2014)		
<i>C. brevidens</i>		<i>C. ochroleuca</i>	<i>C. brevidens</i>	<i>C. ochroleuca</i>	
P ₂ O ₅ kg/ha ¹	Number of branches	Number of branches	Number of branches	Number of branches	
0	9.47 ± 1.02a	11.96±1.32 ^a	8.44±0.83 ^a	12.72±1.52 ^a	
15	13.40±3.15 ^a	12.80±1.69 ^a	8.56 ± 0.65 ^a	11.81±1.35 ^a	
30	10.39±0.65 ^a	11.17±0.75 ^a	9.44 ± 0.71 ^a	11.39±1.28 ^a	
45	10.64±0.74 ^a	10.51±0.64 ^a	9.56 ± 0.64 ^a	13.25±1.57 ^a	
60	10.58±0.72 ^a	10.82±0.66 ^a	10.05±0.87 ^a	11.33±1.31 ^a	
75	7.83 ± 0.79 ^a	10.42±0.82 ^a	9.72 ± 0.85 ^a	16.39±3.81 ^a	
90	8.33 ± 0.64 ^a	8.64 ± 0.55 ^a	10.56±1.81 ^a	12.64±1.38 ^a	
105	7.36 ± 0.77 ^a	9.60 ± 0.73 ^a	7.67 ± 0.62 ^a	12.42±1.62 ^a	

Means with the same letter along the column are not significantly different at p≤0.05 by Student Newman Keuls

4.2.4 Number of pods

In the two field experiments, the effect of phosphorus on the number of pods was not significant (p>0.05) neither was there any interaction between phosphorus and the slender leaf species. The maximum number of pods for *Crotalaria brevidens* was 32.67 at 30 kg P₂O₅ ha⁻¹ while the minimum number of pods was 11.21 recorded at 90 kg P₂O₅

ha⁻¹. When the experiment was repeated, maximum number of pods for *Crotalaria brevidens* 28.33 was observed at 30 kg P₂O₅ ha⁻¹ while the minimum number of pods 12.58 were observed at the control (Table 14). In the first field experiment, maximum number of pods 20.62 for *Crotalaria ochroleuca* was observed at 45 kg P₂O₅ ha⁻¹. When the experiment was repeated, maximum number of pods 16.38 for *Crotalaria ochroleuca* was observed at 30 kg P₂O₅ ha⁻¹ while the minimum number of pods 11.25 was observed at 105 kg P₂O₅ ha⁻¹(Table 14).

Table 14: Effect of phosphorus on the number of pods.

P ₂ O ₅ kg ha ⁻¹	Field experiment 1 (July-October 2013)		Field experiment 2 (Nov.-March 2014)	
	<i>C. brevidens</i>	<i>C. ochroleuca</i>	<i>C. brevidens</i>	<i>C. ochroleuca</i>
	No. of pods	No. of pods	No. of pods	No. of pods
0	15.29±3.49 ^a	10.13±2.9 ^a	12.58± 0.47 ^a	14.25±4.04 ^a
15	21.08 ± 4.85 ^a	14.00±3.2 ^a	22.88±5.6 ^a	13.92±4.80 ^a
30	32.67 ± 10.81 ^a	19.50±4.2 ^a	28.33±7.6 ^a	16.38±4.18 ^a
45	13.25 ± 3.48 ^a	20.62±4.9 ^a	25.88±7.5 ^a	12.58±3.57 ^a
60	15.79 ± 4.20 ^a	16.67±4.0 ^a	23.42±6.2 ^a	15.25±4.44 ^a
75	29.29 ± 7.88 ^a	14.83±4.6 ^a	17.79±4.4 ^a	13.67±3.93 ^a
90	11.21 ± 3.00 ^a	17.71±3.7 ^a	18.00±4.1 ^a	13.67±3.58 ^a
105	26.83 ± 9.54 ^a	9.67±3.48 ^a	18.54±5.8 ^a	11.25±3.50 ^a

Means with the same letter along the column are not significantly different at p≤0.05 by Student Newman Keuls

4.2.5 Seed yield (kg ha⁻¹)

In the two field experiments, no significant (p>0.05) interaction between phosphorus and species in respect to the seed yield was observed, neither was the effect of phosphorus on the seed yield significant. During the two field experiments, *Crotalaria ochroleuca*

recorded higher seed yield than *Crotalaria brevidens* for all phosphorus levels. Maximum seed yield 1958 kg for *Crotalaria brevidens* was recorded at 15 kg P₂O₅ ha⁻¹ but when the study was repeated, at the same phosphorus level, there was a reduction of 304.6 kg (Table 15). Minimum seed yield for *C. brevidens* was 937 kg ha⁻¹ was observed at the control. When the study was repeated at the control, the seed yield more than doubled to 2008 kg. The maximum seed yield for the species 23.87 kg was observed at 90 kg P₂O₅ ha⁻¹. The seed yield increased as phosphorus increased for the species during the second experiment (Table 15).

In the first experiment, maximum seed weight of 2733 kg ha⁻¹ for *Crotalaria ochloreuca* was observed at 105 kg P₂O₅ ha⁻¹. When the experiment was repeated, maximum seed yield of 3357.68 kg ha⁻¹ was observed at 30 kg P₂O₅ ha⁻¹ for the species. For *C. ochloreuca*, the second field had higher seed yield for all phosphorus levels than the first field (Table 15).

Table 15: Effect of phosphorus on seed yield (kg ha⁻¹) of two slender leaf species in the field.

P ₂ O ₅ .kg-ha ⁻¹	Field 1 (JULY-OCTOBER 2013)		Field 2 (NOVEMBER-MARCH)	
	Seed yield kg ha ⁻¹		Seed yield kg ha-1	
	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>	<i>Crotalaria brevidens</i>	<i>Crotalaria ochloreuca</i>
0	937.35±280 ^a	2396.75±432 ^a	2008.82±860 ^a	3010.32±183 ^a
15	1958.42±280 ^a	2216.32±337 ^a	1649.82±445 ^a	3219.64±98 ^a
30	1338.14±163 ^a	2224.36±459 ^a	1456.60±175 ^a	3357.68±88 ^a
45	1387.79±19 ^a	1554.96±194 ^a	1459.82±116 ^a	1983.03±651 ^a
60	1728.36±192 ^a	1937.68±267 ^a	1874.96±526 ^a	2606±512 ^a
75	1470.64±150 ^a	2581.46±207 ^a	1226.5±244 ^a	2609.07±537 ^a
90	1340.10±164 ^a	2346.36±466 ^a	2387.18±657 ^a	3182.79±678 ^a
105	1303.64±161 ^a	2733±251 ^a	1904.75±167 ^a	2886.25±462 ^a

Means with the same letter along the column are not significantly different at $p \leq 0.05$ by Student Newman Keuls

4.2.6 Seed weight (g/1000 seeds)

No significant ($p > 0.05$) interaction between phosphorus and species in respect to the seed weight was observed. The effect of phosphorus on seed weight was only significant ($p \leq 0.05$) in *Crotalaria brevidens* but not for *C.ochloreuca* during the first field experiment conducted in the month of July-October 2013. During the second field

experiment, the effect of phosphorus was not significant ($p>0.05$) for the two *Crotalaria* species (Table 16). Maximum thousand seed weight during the first experiment for the *C.brevidens* was 5.72 g observed at 105 P_2O_5 ha^{-1} . The seed weight at 105 $kg P_2O_5 ha^{-1}$ was significantly different from the seed weight at 90 $kg P_2O_5 ha^{-1}$ and the seed weight at 0-30 $kg P_2O_5 ha^{-1}$ (Table 16). In the second field experiment, maximum thousand seed weight of 5.48 g for the species was observed at 75 $kg P_2O_5 ha^{-1}$.

The maximum seed weight for *Crotalaria ochloreuca* was 6.44 g observed at 15 $kg P_2O_5 ha^{-1}$ during the first field experiment. When the experiment was repeated maximum thousand seed weight 6.53 g was observed at 60 $kg P_2O_5 ha^{-1}$. Significant ($p\leq 0.05$) species difference was observed. *C.brevidens* had a mean seed weight of 5.23 g while *C.ochloreuca* had a mean seed weight of 6.16 g. For all P levels, *C.ochloreuca* had higher seed weight than *C.brevidens* (Table 16).

Table 16: Effect of phosphorus on the seed weight (g/1000 seeds)

P_2O_5 $kg ha^{-1}$	Field experiment 1 (July-October 2013)			Field experiment 2 (Nov. 2013-March 2014)		
	<i>C. brevidens</i> Seed weight	<i>C. ochloreuca</i> Seed weight	P means	<i>C. brevidens</i> Seed weight	<i>C. ochloreuca</i> Seed weight	P means
0	4.81 ± 0.16 ^b	6.01 ± 0.22 ^a	5.41 ± 0.26 ^a	4.84 ± 0.08 ^a	6.23 ± 0.20 ^a	5.53 ± 0.28 ^a
15	5.12 ± 0.11 ^b	6.44 ± 0.10 ^a	5.78 ± 0.26 ^a	5.05 ± 0.22 ^a	6.33 ± 0.18 ^a	5.69 ± 0.28 ^a
30	5.27 ± 0.05 ^{ba}	6.15 ± 0.05 ^a	5.71 ± 0.17 ^a	4.95 ± 0.55 ^a	6.15 ± 0.14 ^a	5.55 ± 0.35 ^a
45	5.28 ± 0.10 ^{ba}	5.96 ± 0.08 ^a	5.62 ± 0.14 ^a	5.41 ± 0.34 ^a	6.20 ± 0.21 ^a	5.80 ± 0.24 ^a
60	5.21 ± 0.22 ^{ba}	6.25 ± 0.12 ^a	5.73 ± 0.23 ^a	4.94 ± 0.31 ^a	6.53 ± 0.15 ^a	5.73 ± 0.34 ^a
75	5.34 ± 0.08 ^{ba}	6.43 ± 0.05 ^a	5.88 ± 0.21 ^a	5.48 ± 0.38 ^a	6.17 ± 0.16 ^a	5.86 ± 0.23 ^a
90	5.10 ± 0.10 ^b	5.93 ± 0.24 ^a	5.51 ± 0.20 ^a	4.75 ± 0.20 ^a	6.26 ± 0.08 ^a	5.50 ± 0.30 ^a
105	5.72 ± 0.09 ^a	6.12 ± 0.05 ^a	5.92 ± 0.09 ^a	4.81 ± 0.06 ^a	6.27 ± 0.19 ^a	5.54 ± 0.29 ^a
Species means	5.23 ± 0.06 ^b	6.16 ± 0.05 ^a		5.028 ± 0.11 ^b	6.266 ± 0.06 ^a	

Means with the same letter along and across the column are not significantly different at $p\leq 0.05$ by Student Newman Keuls

4.3 Effect of phosphorus on the nutrient content of two slender leaf species in the field study.

4.3.1 Magnesium content (mg/100 g)

There was no interaction between phosphorus and species, phosphorus and stage of growth and there was no interaction between phosphorus and field. The effect of phosphorus on the magnesium content was not significant ($p>0.05$). However, the stage of growth was significant ($p\leq 0.05$). Higher significant magnesium content was observed at the maturity stage of growth. The mean magnesium content in slender leaf in the first field ranged from 0.19-0.46 mg for *C.brevi dens* while for *C.ochloreuca* the magnesium content ranged from 0.19-0.37 mg (Tables 17). During the second field experiment, the mean magnesium content for *C.brevi dens* ranged from 0.19-0.39 mg while the magnesium content in *C.ochloreuca* ranged from 0.19-0.46 mg (Table 19).

4.3.2 Calcium content (mg/100g).

No significant interaction was observed between phosphorus and species, phosphorus and stage and there was no interaction between phosphorus and field ($p>0.05$) in respect to the calcium content in slender leaf. The effect of phosphorus on the calcium content in slender leaf was not significant ($p>0.05$). However, there was significant ($p\leq 0.05$) differences in the fields where the slender leaf was grown. Higher calcium content was observed in Field 2. There was significantly ($p\leq 0.05$) higher calcium content in at the maturity stage of growth. The effect of phosphorus application on the calcium content in the leaves of slender leaf was not significant ($p>0.05$). The calcium content in leaves of slender leaf in Field 1 ranged from 0.01-0.13 mg for *C.brevi dens* while calcium levels in *C.ochloreuca* ranged from 0.01-0.17 mg (Table 17). In the second field, the calcium levels ranged from 0.03-0.65 mg for *C. brevi dens* while the calcium levels for *C.brevi dens* ranged from 0.03-0.56 for *C.ochloreuca* (Tables 19).

4.3.3 Iron content (mg/100g)

There was no significant interaction between phosphorus and species, phosphorus and stage of growth and there was no significant interaction between phosphorus and field in respect to the iron content in slender leaf. The effect of phosphorus was not significant ($p>0.05$) on the iron content. However there was significant ($p\leq 0.05$) interaction between stage and field. In the first field experiment, the iron content in slender leaf ranged from 0.23-1.33 mg for *C.brevioidens* and 0.23-4.88 mg for *C.ochlorella* (Table 17). In the second field experiment, the iron levels for *C.brevioidens* ranged from 0.15-1.98 mg while in *C.ochlorella*, the iron levels ranged from 0.27-1.98 mg/100g of leaves (Tables 19).

4.3.4 Zinc content (mg /100 g)

No significant interaction between phosphorus and species, phosphorus and stage of growth or stage of growth and field was observed in respect to the zinc content in slender leaf. The effect of phosphorus on the zinc content in the leaves of slender leaf was not significant ($p>0.05$). The zinc content in the first field for *C.brevioidens* ranged from 6.5-14.4 mg while the zinc content in *C.ochlorella* ranged from 5.2-18.8 mg (Table 17). Results from the second field showed that the zinc content for *C.brevioidens* ranged from 4.6-15.3 while for *C.ochlorella* the zinc content ranged from 4.8-12.3 mg (Tables 19).

4.3.5 Potassium content (%)

There was no significant interaction between phosphorus and species, phosphorus and stage of growth and no significant interaction between species and stage was observed in respect to the potassium content in slender leaf. The stage of growth was significantly different ($p\leq 0.05$). The highest K content was found at the vegetative stage of growth. The effect of phosphorus on the K content was not significant ($p>0.05$). The mean K

content in leaves of slender leaf in the first field ranged from 1.3-3.3 % for *Crotalaria brevidens* while the K content for *Crotalaria ochroleuca* ranged from 1.4-3.0 %. In the second field, the mean K content ranged from 1.0-3.5 for *C. brevidens* while the K content in *C. ochroleuca* ranged from 1.3-3.4% (Tables 17 and 19).

Table 17: Effect of P on nutrient content in two slender leaf species at different stages of growth.

FIELD EXPERIMENT 1(JUNE-OCTOBER 2013)														
	P ₂ O ₅ kgha ⁻¹	Mg	Ca	Fe	Zn	K		P ₂ O ₅ kgha ⁻¹	Mg	Ca	Fe	Zn	K	
<i>C. brevidens</i>	0	0.36	0.05	0.26	7.20	1.90	<i>C. ochroleuca</i>	0	0.34	0.06	0.44	9.70	2.10	
	15	0.29	0.05	0.37	7.30	2.00		Vegetative	15	0.25	0.09	0.90	10.30	2.40
	30	0.29	0.07	0.43	10.10	2.30			30	0.25	0.04	0.40	8.00	2.10
Vegetative	45	0.46	0.09	0.44	8.40	3.30		45	0.32	0.05	0.48	9.00	1.90	
	60	0.15	0.05	0.18	8.30	2.10		60	0.20	0.07	1.10	10.70	2.60	
	75	0.25	0.08	0.49	6.80	1.30		75	0.25	0.08	0.54	8.60	2.30	
	90	0.32	0.01	0.48	6.50	1.70		90	0.24	0.06	0.57	13.80	1.80	
	105	0.28	0.05	0.23	6.60	2.20		105	0.32	0.06	0.28	8.00	2.30	
Reproductive	0	0.19	0.07	0.62	7.10	1.90	Reproductive	0	0.33	0.01	0.32	4.40	1.40	
	15	0.22	0.02	0.73	7.10	1.90		15	0.19	0.11	1.09	11.90	2.10	
	30	0.30	0.01	0.73	6.00	1.50		30	0.22	0.10	1.23	7.60	1.80	
	45	0.30	0.11	0.72	9.20	1.60		45	0.33	0.11	0.92	15.50	2.04	
	60	0.19	0.08	0.35	6.70	1.30		60	0.28	0.10	0.48	6.80	1.80	
	75	0.23	0.11	0.96	11.10	2.10		75	0.32	0.12	0.29	8.30	1.80	
	90	0.20	0.17	0.65	11.20	2.60		90	0.28	0.05	0.43	8.30	2.00	
	105	0.18	0.08	0.56	9.90	2.10		105	0.21	0.05	0.33	6.40	2.10	
Maturity	0	0.34	0.09	1.03	12.40	2.60		0	0.30	0.01	1.21	8.50	2.20	
	15	0.30	0.11	1.33	13.80	2.70		15	0.40	0.10	0.48	9.40	2.70	
	30	0.30	0.06	0.48	8.60	2.30	Maturity	30	0.35	0.18	4.88	18.80	3.00	
	45	0.31	0.09	1.19	14.40	2.90		45	0.37	0.03	0.36	5.20	2.50	
	60	0.26	0.09	0.37	8.80	2.30		60	0.33	0.04	0.53	12.60	2.50	
	75	0.38	0.07	0.58	13.80	2.60		75	0.32	0.13	1.28	10.80	2.40	
	90	0.36	0.05	0.57	9.30	2.30		90	0.27	0.06	0.61	10.60	2.50	
	105	0.34	0.10	0.82	8.40	2.60		105	0.20	0.05	0.33	6.40	2.10	
S.Em		±0.044	±0.053	±0.383	±3.039	±0.335	S.Em		±0.044	±0.053	±0.383	±3.039	±0.335	

Table 18: Effect of P on nutrient content of two slender leaf species at vegetative, reproductive and maturity stages of growth.

Field experiment 1(JUNE-OCTOBER 2013)											
	P ₂ O ₅ kg ha ⁻¹	P	N	β carotene	Protein		P ₂ O ₅ kg ha ⁻¹	P	N	β carotene	Protein
<i>C. brevidens</i>	0	0.58	1.50	0.00	9.38	<i>C. ochroleuca</i>	0	0.60	1.00	0.00	6.25
	15	0.53	0.50	0.00	3.13		15	0.59	1.50	0.00	9.38
	30	0.59	2.00	0.00	12.50		30	1.60	1.00	0.00	6.25
	45	1.50	2.00	0.00	12.50		45	2.10	2.50	0.00	15.63
	60	1.80	2.50	0.00	15.63		60	1.60	1.00	0.00	6.25
	75	1.50	1.50	0.00	9.38		75	1.60	1.00	0.00	6.25
	90	1.20	1.50	0.00	9.38		90	1.90	2.00	0.00	12.50
Vegetative	105	1.80	0.50	0.00	3.13	105	1.50	2.50	0.00	15.63	
	0	0.59	3.00	2.93	18.75	0	0.48	2.50	3.13	15.63	
	15	0.59	2.00	3.73	12.50	15	0.69	3.50	3.06	21.88	
	30	0.56	4.00	3.34	25.00	30	1.20	2.50	3.74	15.63	
	45	1.60	4.00	2.67	25.00	45	1.40	1.50	3.10	9.38	
	60	1.10	3.00	3.06	18.75	60	1.20	1.50	3.44	9.38	
	75	1.90	2.50	2.96	15.63	75	0.90	1.00	3.68	6.25	
Reproductive	90	1.10	3.50	2.61	21.88	90	1.20	2.00	1.72	12.50	
	105	1.20	2.50	2.04	15.63	105	1.30	4.50	3.91	28.13	
	0	0.82	1.50	0.00	9.38	0	0.68	2.00	0.00	12.50	
	15	0.75	1.50	0.00	9.38	15	0.80	1.00	0.00	6.25	
	30	0.63	2.00	0.00	12.50	30	0.84	2.00	0.00	12.50	
	45	0.47	1.50	0.00	9.38	45	0.38	2.50	0.00	15.63	
	60	0.72	1.00	0.00	6.25	60	0.55	2.50	0.00	15.63	
Maturity	75	0.68	1.00	0.00	6.25	75	0.31	1.50	0.00	9.38	
	90	0.57	2.00	0.00	12.50	90	0.89	2.00	0.00	12.50	
	105	0.25	2.00	0.00	12.50	105	0.53	4.50	3.91	28.13	
	S.Em	±0.129	0.464	±0.221	±2.899	S.Em	±0.129	0.464	±0.221	±2.899	

N.B (1) Effect of phosphorus treatment on the nutrients was only significant for beta carotene ($p \leq 0.05$).

N.B (2). P (%), N (%), Protein (%), Beta carotene (mg/100 g)

4.3.6 Phosphorus content (%)

During the two field experiments, no significant interaction between phosphorus and species and between phosphorus and stage was observed. The effect of phosphorus on the phosphorus content in slender leaf was not significant ($p > 0.05$). There were significant ($p \leq 0.05$) differences in the stage of growth and the field where the slender leaf was grown. Higher significant P content was found during vegetative stage. The

phosphorus levels in *C.brevidens* in field 1 ranged from, 0.25-1.8 % while in *C.ochloreuca*, the level of P ranged from 0.31-2.1 (Table 18). During the second field experiment, the P content for *C.brevidens* ranged from 0.4-2.4 % while the P levels in *C.ochloreuca* ranged from 0.39-2.0 % (Tables 20).

4.3.7 Nitrogen content (%)

No significant interaction between phosphorus and stage, stage and field and no interaction between phosphorus and species in respect to the nitrogen content in slender leaf was observed (Appendix 2). Although the effect of phosphorus application was not significant ($p>0.05$) on the nitrogen content, the stage of growth was significantly different ($p\leq 0.05$). The highest nitrogen content was observed at the maturity stage of growth. The nitrogen levels in Field 1 ranged from 0.5-4% for *Crotalaria brevidens* and 1.0-4.5 for *Crotalaria ochloreuca*. In Field 2, the nitrogen content in *C.brevidens* ranged from 0.5-3.5% while in *C.ochloreuca* the N content ranged from 0.39-2.0% respectively (Table 18 and 20).

4.3.8 Protein content (%)

There was no interaction between phosphorus treatment and species, phosphorus and stage of growth and no interaction between phosphorus and field in respect to the protein content in slender leaf. The effect of phosphorus on the protein content in slender leaf was not significant ($p>0.05$). Significant ($p\leq 0.05$) stage differences in the protein content of slender leaf was observed. The highest mean protein content 16.19 % was found in the maturity stage of growth. During the first field experiment for *C.brevidens*, the protein content ranged from 3.13-25 % while in *C.ochloreuca* the protein content ranged from 6.25-28.13 % (Table 18). In the second field experiment, the protein content for *C.brevidens* ranged from 3.13-21.88 while the ranges of protein for *C.ochloreuca* were 6.25-25 0% (Table 20).

4.3.9 Beta carotene (mg/100g)

Significant ($p \leq 0.05$) interaction between field and stage and between stage and species in respect to the beta carotene content was observed. The effect of phosphorus on the beta carotene content of slender leaf was significant ($p \leq 0.05$). In the first field experiment, no beta carotene was observed at the vegetative and maturity stages of growth. The maximum beta carotene content 3.73 mg for *Crotalaria brevidens* was observed at 15 kg P₂O₅ ha⁻¹ while 3.91mg of beta carotene was observed for *Crotalaria ochroleuca* at 105 kg P₂O₅ ha⁻¹(Table 18). During the second field experiment, no beta carotene was observed at the vegetative stage of growth but was observed at the reproductive and maturity stages of growth. At the reproductive stage, *Crotalaria brevidens* recorded a maximum of 3.58 mg of beta carotene at 15 kg P₂O₅ ha⁻¹while *Crotalaria ochroleuca* recorded a maximum of 3.94 mg of beta carotene. At the maturity stage, 4.67 mg of beta carotene was observed at 45 kg P₂O₅ ha⁻¹ for *Crotalaria brevidens* while a maximum 4.08 mg beta carotene was observed at the same phosphorus level for *Crotalaria ochroleuca* (Table 20). The level of beta carotene (2.304 mg) in Field 2 was more than double the mean level in Field 1 (1.023 mg) (Appendix 5).

Table 19. Effect of phosphorus on the nutrient content of two slender leaf species in (mg/100 g).

Field experiment 2 (NOVEMBER 2013-MARCH 2014)													
	P ₂ O ₅ kg ha ⁻¹	Mg	Ca	Fe	Zn	K		P ₂ O ₅ kg ha ⁻¹	Mg	Ca	Fe	Zn	K
<i>C.brevidens</i>	0	0.25	0.10	1.06	8.00	2.60	<i>C.ochroleuca</i>	0	0.38	0.03	0.47	7.10	2.20
Vegetative	15	0.29	0.05	0.39	4.60	2.10	Vegetative	15	0.28	0.16	0.43	6.30	2.30
	30	0.33	0.32	0.92	12.00	2.30		30	0.30	0.03	0.84	8.10	1.70
	45	0.23	0.24	1.64	6.60	1.90		45	0.36	0.16	0.27	5.10	1.50
	60	0.27	0.06	0.69	5.50	1.50		60	0.23	0.27	0.91	7.40	3.20
	75	0.24	0.41	0.45	11.40	2.80		75	0.23	0.09	0.37	7.50	2.10
	90	0.26	0.06	0.69	9.90	2.50		90	0.34	0.28	1.00	12.30	2.70
	105	0.34	0.06	0.29	5.70	2.00		105	0.26	0.14	0.37	6.30	2.30
	0	0.33	0.43	0.83	7.90	2.10		0	0.31	0.46	1.17	11.50	2.30
	15	0.19	0.65	1.98	7.40	1.80		15	0.36	0.48	0.99	9.90	1.70
Reproductive	30	0.22	0.20	0.60	9.20	1.90	Reproductive	30	0.19	0.30	0.85	11.70	2.40
	45	0.33	0.42	0.58	15.30	2.50		45	0.31	0.32	0.40	6.30	1.60
	60	0.28	0.41	0.77	10.00	2.90		60	0.37	0.34	0.56	9.20	1.90
	75	0.32	0.42	0.79	6.30	1.00		75	0.31	0.30	0.27	4.80	1.30
	90	0.28	0.36	0.18	8.20	1.50		90	0.29	0.50	0.48	12.00	1.90
	105	0.21	0.20	0.55	10.00	3.20		105	0.35	0.56	1.98	11.60	1.70
	0	0.38	0.03	0.48	8.50	3.30		0	0.40	0.07	0.48	6.90	3.20
	15	0.39	0.05	0.46	6.80	2.80		15	0.36	0.09	0.62	11.90	3.40
Maturity	30	0.24	0.03	0.50	10.50	2.90	Maturity	30	0.41	0.04	0.51	6.40	2.50
	45	0.36	0.13	0.53	8.50	3.60		45	0.28	0.06	0.43	6.20	3.50
	60	0.38	0.04	0.38	12.20	2.80		60	0.28	0.03	0.27	8.00	2.50
	75	0.27	0.04	0.41	9.10	2.80		75	0.45	0.05	0.56	8.50	3.30
	90	0.33	0.10	0.57	15.30	3.50		90	0.44	0.07	0.55	8.80	3.30
	105	0.29	0.04	0.15	7.20	2.10		105	0.46	0.56	1.98	11.60	1.70
S.Em		±0.044	±0.053	±0.383	±3.039	±0.335	S.Em		±0.044	±0.053	±0.383	±3.039	±0.335

N.B The effect of phosphorus was not significant on the nutrient content (p≤0.05)

Table 20: Effect of phosphorus on the nutrient content of two slender leaf species

Field experiment 2 (NOVEMBER 2013-MARCH 2014)											
	P ₂ O ₅ kg ha ⁻¹	P	N	β carotene	Protein		P ₂ O ₅ kg ha ⁻¹	P	N	β caroten	Protein
<i>C. brevidens</i>	0	1.40	0.50	0.00	3.13	<i>C. ochroleuca</i>	0	1.20	2.00	0.00	12.50
Vegetative	15	1.10	1.50	0.00	9.38	Vegetative	15	1.10	2.00	0.00	12.50
	30	1.50	2.00	0.00	12.50		30	0.51	1.50	0.00	9.38
	45	0.56	0.50	0.00	3.13		45	0.66	2.00	0.00	12.50
	60	0.52	0.50	0.00	3.13		60	0.59	2.00	0.00	12.50
	75	0.34	1.00	0.00	6.25		75	0.62	2.00	0.00	12.50
	90	0.43	1.00	0.00	6.25		90	0.64	1.50	0.00	9.38
	105	0.63	1.50	0.00	9.38		105	0.67	2.50	0.00	15.63
	0	0.41	3.50	2.35	21.88		0	0.53	2.00	3.58	12.50
	15	0.73	1.50	3.58	9.38		15	0.39	4.00	3.36	25.00
Reproductive	30	0.95	1.50	2.21	9.38	Reproductive	30	0.69	2.50	3.75	15.63
	45	0.57	2.00	2.04	12.50		45	0.57	2.50	3.09	15.63
	60	0.40	2.00	2.94	12.50		60	0.62	3.00	3.94	18.75
	75	0.61	2.50	2.80	15.63		75	0.65	2.00	3.52	12.50
	90	0.76	2.00	2.91	12.50		90	0.69	1.00	3.06	6.25
	105	0.77	2.00	2.92	12.50		105	0.53	1.50	3.31	9.38
	0	2.40	1.50	3.51	9.38		0	2.00	2.00	3.55	12.50
	15	1.50	1.50	3.91	9.38		15	1.70	1.00	3.57	6.25
Maturity	30	0.40	2.00	4.41	12.50	Maturity	30	0.58	2.00	3.59	12.50
	45	0.54	1.50	4.67	9.38		45	0.53	2.50	4.08	15.63
	60	0.72	1.00	3.55	6.25		60	0.62	2.50	3.97	15.63
	75	0.41	1.00	4.30	6.25		75	0.31	1.50	2.78	9.38
	90	0.51	2.00	3.38	12.50		90	0.38	2.00	4.04	12.50
	105	0.54	2.00	3.98	12.50		105	0.60	1.50	3.31	9.38
S.Em		±0.129	±0.464	±0.221	±2.899	S.Em		±0.129	±0.464	±0.221	±2.899

N.B (1). Effect of phosphorus treatment on the nutrients was only significant for beta carotene ($p \leq 0.05$).

N.B (2). P (%), N (%), Protein (%), Beta carotene (mg/100 g)

CHAPTER FIVE

DISCUSSION

5.1 Effect of phosphorus on growth of two slender leaf species in the greenhouse pot study and the field.

5.1.1 Plant height

Significant interaction ($p \leq 0.05$) between phosphorus and species in respect to the plant height was observed in the first greenhouse experiment. As P increased the plant height increased above the control (Table 5). The results showed the importance of phosphorus in cell division leading to increased plant height. However, the two *Crotalaria* species responded differently to the different phosphorus levels applied, indicating species differences and this agrees with the findings by Grant et al. (2005) that plant species differ in their P requirement, in the methods used to access available P and also in their response to P fertilizer application. During the second season, even when lower levels of P were applied higher plant height was recorded (Table 5). These results showed that other factors other than fertilizers influenced the plant height. Such increase could probably be attributed to weather factors since the season was much cooler with mean temperatures of 19°C and cloudy thus the reduced light intensity encouraged competition for light resulting to the higher height (Table 3a). Hussien et al. (2013) reported maximum plant height in Chick pea after application of 60 kg P₂O ha⁻¹ results which are in agreement with those observed in *Crotalaria brevidens* first season.

Phosphorus application did not have a significant ($p > 0.05$) effect on plant height for the two *Crotalaria* species in the field studies (Table 9). This was probably because *Crotalaria* species like other legumes form a symbiotic relationship with rhizobia

bacteria present in the soil. Immediately the legume seeds germinate the root hairs are infected by the rhizobia bacteria which multiply rapidly to form nodules that would compete for carbohydrates formed by the plants through the photosynthesis process. Thus even when P was applied to the slender leaf plants, there was no significant effect on the plant height. The results of this study agree with the findings by Pramanik et al. (2010) that phosphorus fertilization did not have a significant effect on plant height in the growth of seven green manure crops that included *Crotalaria juncea*. Similarly, Turuko and Mohammed, (2014a) found that application of phosphorus did not have a significant effect on the plant height in the growth of common bean (*Phaseolus vulgaris L.*).

5.1.2 Number of leaves

Significant interaction between phosphorus and species was observed in respect to the number of leaves in the greenhouse experiments. During the two of greenhouse experiments, the response of slender leaf to applied P was significantly different (Table 6). Higher phosphorus levels influenced the higher number of leaves during the first experiment when it was warm throughout with an average temperature of 20°celsius (Table 3a). Increased temperature have been reported to increase growth rate due to higher P absorption and solubility as earlier reported by Ercoli et al. (1996). During the second experiment, conducted in the months of June –November 2013, the average temperatures were 18.3°celsius in the first three months after planting (Table 3a). Lower P levels recorded higher number of leaves. These results indicated that other factors other than phosphorus could have contributed to a higher number of leaves which agrees with the findings by Grant et al.(2001) that in P deficient leaves chlorophyll content is increased This increase could have caused higher photosynthesis rate resulting to increased number of leaves.

However, the effect of phosphorus application on the number of leaves for the two slender leaf species during the two field studies was not significant (Table 10). In the

first field experiment conducted in the months of July-October, optimum phosphorus levels for leaf formation was 30 kg P₂O₅ ha⁻¹ for both *Crotalaria* species. These results are in agreement with the findings by Oyewale et al.(2013) in the growth of snake plants where the number of leaves and leaf length increased at 0-30 kg ha⁻¹ of P, and beyond that level there was a decline in the number of leaves. However, during the second field experiment conducted in the months of Nov-March 2014 season, 60 kg P was optimum for leaf formation (Table 10). The different optimum phosphorus levels for the two seasons showed during that during the warm months, there was higher absorption and uptake of phosphorus than the cooler season of June –October (Table 3a and 3b).

5.1.3 Number of branches

Significant ($p \leq 0.05$) interaction between phosphorus and species was observed. The effect of phosphorus on the number of braches was significant ($p \leq 0.05$). In the first greenhouse experiment, more branches were observed at higher phosphorus in both slender leaf species. This showed the importance of the adequate amount of phosphorus in order to enhance branch formation (Table 7). Similar reports by Turuko and Mohammed, (2014b) found that higher rates of phosphorus increased the number of branches per plant in Okra and beans. Since phosphorus plays a major role in cell division, higher levels enhanced growth resulting to a higher number of branches. However, 90 kg P₂O₅ ha⁻¹ seemed the optimum level for *Crotalaria brevidens* and 105 kg P₂O₅ ha⁻¹ was optimum for *C.ochloreuca*.

The effect of phosphorus during the second greenhouse experiment was not significant ($p > 0.05$). However, lower levels of phosphorus recorded a higher number of branches (Table 7). This could have been contributed by the prevailing weather during the season of growth as it was cooler with temperatures below 20°C (Table 3). The low temperature

would have slowed solubility and absorption of phosphorus thus reducing the uptake but this did not reduce growth rate since more braches were observed at lower P.

During the two field studies, the effect of phosphorus on the number of branches for the two slender leaf species was not significant ($p>0.05$). The study showed that during the second season which was warmer more phosphorus was utilized for branch formation than the first season, but that did not necessarily give a higher number of branches (Table 3 and 4). Different results were reported in the growth of chick pea where Hussen et al. (2013) found the maximum number of branches at 60 kg P₂O₅ ha⁻¹ in the growth of chick pea unlike what was reported in this study (Table 13).

5.1.4 Number of pods

Significant ($p\leq 0.05$) interaction between phosphorus and species was observed in respect to the number of pods. This showed species difference as each species responded differently to the applied P. The effect of phosphorus on the number of pods was significantly ($p\leq 0.05$) different. Lower levels of phosphorus, 45 kg P₂O₅ ha⁻¹ significantly influenced the number of pods during the first greenhouse experiment for *C.brevidens*, while higher P levels, 105 kg P₂O₅ kg resulted to higher number of pods for *C.ochloreuca* (Table 8). Similarly, although the effect of phosphorus was not significant during the field study, maximum number of pods for both species was recorded at 30-45 kg P₂O₅ kg ha⁻¹. These lower phosphorus levels recorded higher number of pods for the species over the control (Table 8). This response indicated that the slender leaf species differed in their capacity to absorb and utilize fertilizer P. However, increased number of pods after phosphorus application shows the role of P fertilizers in and pod formation especially in legumes as earlier noted by Marschner, (1995).

In the two field experiments, no interaction was observed between phosphorus and the species and phosphorus application did not have significant ($p>0.05$) effect on the number of pods (Table 14). For the two slender leaf species lower phosphorus levels

recorded higher number of pods similar to the greenhouse experiments. The results show that legumes such as slender leaf have the ability to utilize low phosphorus to produce pods. The findings of the study differ from the findings by Ali et al. (2013) who found the highest number of filled pods in soy bean recorded at 80 kg P₂O₅ kg ha⁻¹ while Turuko and Mohammed, (2014 a) reported maximum number of pods at 20 kg P₂O₅ ha⁻¹ in the growth of common bean.

5.1.5 Seed yield (g/plant)

Significant ($p \leq 0.05$) interaction between phosphorus and species was observed in the first greenhouse experiment but not in the second experiment. The effect of phosphorus application was significant ($p \leq 0.05$) in the first greenhouse experiment. Higher seed yield for the two slender leaf species was recorded during the first greenhouse experiment conducted in the months of January-May when the weather was warmer than during the second experiment conducted in the months of June-November 2013 (Table 9 and Table 15). These results could be due to the different weather conditions that were prevailed during the study (Table 3 and 4). The second season was much cooler, and powdery mildew appeared as severe fungus infection on the shoots, and the upper leaf surfaces. This problem greatly limited photosynthesis and plant growth resulting in lower yields. Reports of powdery mildew in *Crotalaria* species have been made and the disease has the potential of affecting the health and quality of the crop (Baiswar et al., 2013a). Yield losses due to powdery mildew in *Crotalaria spp* have been reported of upto 14 percent , especially when the temperatures were lower than normal (Geuens & Maia, 2009).

Results of the study showed that both slender leaf species required high phosphorus levels for maximum seed weight confirming the role of phosphorus in seed production. Similar results by (Ibrahim et al., 2014) were reported where maximum grain weight was observed at 90 kg P₂O₅ ha⁻¹ in sesame seeds. Rahman et al.,(2008) reported

different findings where maximum seed weight was attained in Mung bean after P application of 40 kg P₂O₅ ha⁻¹ while the maximum seed yield was reported at 60 kg P₂O₅ ha⁻¹ in the growth of linseed (Aii et al., 2002). However during the two field studies (Table 15), the effect of P on seed yield was not significant yet higher seed yield was recorded during the warmer months which was similar to the greenhouse study (Table 3 and 4). Lower P levels recorded higher grain weight. These results indicate that higher temperatures influences absorption and solubility of least available P for higher grain production.

5.1.6 Thousand seed weight

There was significant interaction between phosphorus and species was observed during the first greenhouse experiment in respect to the seed weight (Table 10). *Crotalaria ochloreuca* had higher 1000-seed weight than *C.brevidens* in almost all phosphorus levels apart from at 90 kg P₂O₅ ha⁻¹. Species differences were observed in the way each responded to different phosphorus levels (Table 10 and Table 16). Although phosphorus application did not have significant ($p>0.05$) effect on the seed weight during the second greenhouse experiment and the second field experiment, addition of higher levels of phosphorus contributed to high grain quality. Similar findings were reported by Ibrahim et al. (2014) where maximum seed weight was recorded at 90 kg P₂O₅ kg ha⁻¹ in the sesame seed, and these results were similar to those reported for *Crotalaria brevidens* in this study. Different results were reported by Jan et al.(2014) who found maximum seed weight in Sesame at P application of 100 kg ha⁻¹.

5.2 Effect of phosphorus on nutrient content of two slender leaf species

5.2.1 Magnesium content (mg/100 g)

No interaction between phosphorus and stage of growth or interaction between phosphorus and field was observed. However, the stage of growth was significant ($p \leq 0.05$). The effect of phosphorus on the magnesium content in slender leaf was not significant ($p > 0.05$). Mg content decreased as phosphorus increased (Table 17 and 19). The level of magnesium in the study was within the optimal range 0.15-0.35% that is found in leaves (Marschner, 1995). There was significantly higher Mg levels as plant matured than at vegetative stages of growth for the two slender leaf species, results that confirmed reports by (Harry and Jones, 1996) that in legumes magnesium content in leaves increased with age .

The first field experiment recorded significantly higher Mg level than the second field. The different levels of magnesium in the two fields could have been caused by magnesium leaching and unbalanced NPK application together with the high competition of competing ions in the soil solution as suggested by (Wiesler et al, 2001a). Application of phosphorus fertilizers reduced magnesium content in tomato and spinach leaves growing in sandy soils but increased magnesium content in apple leaves (Harry & Jones, 1996)

5.2.2 Calcium content (mg/100 g)

No significant interaction between phosphorus and species and between phosphorus and stage was observed. However, significant interaction between stage and field was observed in respect to the calcium content in slender leaf. The effect of phosphorus was not significant on the calcium content in slender leaf. However, in acid conditions,

phosphorus favors Ca uptake (Harry and Jones, 1996) unlike what was observed in this study. According to (Marschner (1995), calcium content in plants varies between 0.1-5.0% of dry weight, but this again depends on growing conditions, plant species and plant organ. The calcium levels in this study 0.5-1.5% were within the range found mature leaves. In leafy vegetables, an increase of calcium concentration in the external solution may lead to its increase in the leaves as reported by Wiesler et al.(2001). In this study calcium was not added but the soil had sufficient levels (Table 2). However, calcium content in this study increased as plant matured. Reports have been made that calcium content in leaves increased as temperature increased in the growth of Amaranth leaves (Modi, 2007).

5.2.3 Iron content (mg/100g)

No interaction between phosphorus and species or between phosphorus and stage of growth was observed in respect to the iron content. However significant interaction ($p \leq 0.05$) was observed between stage and the field in respect to the iron content in slender leaf (Appendix 2). The effect of phosphorus on the iron content was not significant ($p > 0.05$). However, as P increased to 45 kg the amount of Fe decreased (Table 17 and 19). The maximum iron content (1.98 mg) found in this study was higher than the level 0.8 mg reported by Abukutsa Onyango,(2004) but differ significantly from other reports by the same author (Abukutsa -Onyango, 2010) where slender leaf species from the lake Victoria region of Kenya recorded 6.4 mg of iron when boiled and 5.8 mg when fried . Although application of phosphorus enhances plant growth excess amount has been found to depress growth by decreasing the uptake and translocation of iron. Excess application of P has been reported to decrease the solubility of iron in plants (Marschner, 1995). However in this study no growth depression was noted but the iron content in the soil was below the optimum levels in the soil (Table 2).

5.2.4 Zinc (mg /100g)

No interaction between phosphorus and species, stage of growth and field was observed. Application of phosphorus in the growth of slender leaf did not have any significant ($p>0.05$) effect on the zinc content in the leaves (Table 17 and 19). The level of zinc in the *Crotalaria* leaves was below the 15-50 mg kg⁻¹ that is considered as the sufficient range in leaves (Marschner, 1995). Addition of high levels of phosphorus to crops has been reported to induce zinc deficiency in plants growing in soils that are low in zinc even when the leaves show adequate levels of zinc the growth of such plants is depressed as reported by Gianquinto et al, (2000). However, this addition might sometimes alter the soil or plant factors. The zinc level in the soil where these plants were grown was 0.11 mg kg⁻¹ during the first season and 0.07 mg kg⁻¹ during the second season (Table 2). Zinc is known to enhance the uptake rate of phosphorus by roots and shoots resulting in its high levels in leaves as was the case in this study and also reported by (Marschner, 1995). Even when higher levels of phosphorus were observed in the leaves no signs of toxicity or growth depression was noted.

5.2.5 Potassium content (%)

Interaction between phosphorus and species, or phosphorus and stage of growth or between phosphorus and field was not observed. However, the stage of growth was significant ($p\leq 0.05$). In this study, phosphorus treatment did not have any significant effect on the K content (Table 17 and 19) probably because phosphorus is an anion and may not compete with K for uptake. These results differ from those reported by Kruczek, (2005) that application of P fertilization in rows of young developing maize plants increased leaf nitrogen but decreased the content of potassium and calcium when ammonium phosphate was used but not when super phosphate was used. The K content

decreased with age of the leaves in this study. This observation has been made in legumes where K decreases with age and may temporarily increase in case of its availability in the substrate (Harry and Jones, 1996). The function of K in the loading of sucrose and mass flow of solute transport in the sieve tubes may be high at a reproductive stage where it is required in higher quantities than at maturity hence the reduction as the plant matures.

5.2.6 Phosphorus content (%)

No interaction between phosphorus and species was observed, nor was there any interaction between phosphorus and stage of growth in respect to the phosphorus levels in slender leaf. Results of this study showed that application of phosphorus fertilization had no significant ($p>0.05$) effect on the phosphorus levels in the leaves of the slender leaf (Table 18 and 20). However, the stage of growth and the field where the plants were grown were significant ($p\leq 0.05$). High phosphorus content was found at vegetative stages thereby acting as a source leaf for nutrients and this agrees with reports by Harry and Jones, (1996) that phosphorus decreases with the age of the tissue. Kruczek, (2005) and Shaikh et al.(2008) reported that increasing phosphorus fertilization during the developmental stages of the growth for maize and fodder limbo grass increased phosphorus content at maturity. However, in the present study no significant increase in leaf phosphorus was noted when the phosphorus fertilization was added.

5.2.7 Nitrogen content (%)

In this study, no interaction between phosphorus and species in respect to the nitrogen content in slender leaf was observed. The effect of phosphorus on the nitrogen content was not significant ($p>0.05$) for the two slender leaf species during the two seasons of growth in the field. However, the stage of growth was significant ($p\leq 0.05$). Higher

nitrogen content was observed at the maturity stage of growth (Table 18 and 20). This is because the nitrogen content in plants is mobilized in leaves especially when there is inadequate supply as was the case in this study, no nitrogen was supplied. The nitrogen level found in the leaves was within the critical range in plants of between 1-6% of the dry weight. Although the application of P did not have significance on the nitrogen content, Rotaru et al. (2015) reported that nitrogen assimilation and chlorophyll contents in crops depended on P supply.

5.2.8 Protein content

No interaction between phosphorus and species, phosphorus and stage of growth and no interaction was noted between stage of growth and field. The effect of phosphorus application on the protein content in the leaves of slender leaf was not significant ($p>0.05$). However, the stage of growth was significant ($p\leq 0.05$) (Appendix 2). Although application of phosphorus was not significant, addition of different phosphorus rates enhanced protein synthesis as higher protein content was found in the leaves of both slender leaf species (Table 18 and 20). This could have been contributed by the adequate N levels in the soil before application of phosphorus in the field contributing to the high protein levels (Table 2). The stage of development had a significant effect on the protein content with the highest content recorded at vegetative and the lowest recorded at reproductive stage. This could be due to the source to sink translocation of nutrients where protein level increased as the plant matured. Modi, (2007 reported that maximum quality can be obtained either before or after maximum dry or fresh matter yield has been achieved.

5.2.9 Beta carotene content (mg/100 g)

Significant ($p\leq 0.05$) interaction between stage and field, species and stage of growth was observed in respect to the beta carotene content. Phosphorus application had significant ($p\leq 0.05$) effect on the content of beta carotene in this study (Appendix 2).

Significantly higher beta carotene content was observed in the first field than the second field. These results that could have been contributed to the different climatic conditions in agreement with the findings by Amaya-Rodrigues, (2000) that the beta carotene level in plants is influenced by many factors such as maturity stage, climatic conditions, soil type, effect of agrochemicals, differences in distribution among distinct parts of the plant, cultivation conditions, damage to the plant structure, exposure to light together with storage and processing conditions. Higher beta carotene content was recorded at maturity stage of growth (Table 18 and 20). Similar findings were reported by Lisiewska et al, (2006) who found higher carotenoids content during maturity stage of iceberg and butter heads varieties of lettuce. These findings differed from those by Khuantrairong and Traichaiyaporn, (2012) who found positive correlation between phosphorus and beta-carotene in the cultivation of *Cladosphora sp.* However, Dumas et al., (2003) found that increasing P from 0 to 100 mg L⁻¹nutrient solution under hydroponics growth conditions greatly improved color and lycopene content but not beta carotene content in tomatoes.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND FURTHER RESEARCH

6.1 CONCLUSION

Phosphorus had a significant effect ($p \leq 0.05$) on plant height, the number of leaves, the number of branches, the number of pods, seed yield and seed weight of the two slender leaf species in the greenhouse but not under field conditions. However, the two *Crotalaria* species had different response to phosphorus applied for the different agronomic aspects studied. There were seasonal differences in the response of phosphorus to different agronomic aspects. Phosphorus effect in the warm season of the greenhouse study had higher values for all parameters studied than during the cooler season in the greenhouse.

Phosphorus had significant ($p \leq 0.05$) effect only on the beta carotene content in slender leaf but not on all other nutrients studied in this experiment. However, there was significantly higher in the levels of beta carotene in Field 2 than in Field 1. Significantly higher levels of magnesium, calcium, nitrogen, protein and beta carotene were observed at maturity stage of growth while significantly higher levels of potassium and phosphorus were observed at the vegetative stage of growth. *Crotalaria ochloreuca* had significantly higher levels of beta carotene and seed weight than *Crotalaria brevidens*.

6.2 RECOMMENDATIONS

For higher leaf production in slender leaf species application of 60 kg, P₂O₅ kg ha⁻¹ is recommended.

For higher grain weight in slender leaf, application of 90 kg P₂O₅ kg ha⁻¹ is recommend.

Crotalaria ochloreuca had higher grain weight than *Crotalaria brevidens* thus would be recommended for grain production.

The growth of slender leaf species should be done during the warm months to avoid yield reduction caused by powdery mildew that would cause economic damage.

6.3 SUGGESTIONS FOR FURTHER STUDIES

Further studies should be done on the Rhizobium strains in *Crotalaria* species that have the capacity to solubilize phosphorus enabling its availability to even other subsequent crops.

Further studies should be done on the effect of phosphorus on the anti-nutrients and phytochemicals in *Crotalaria* species.

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APPENDICES

Appendix 1: Effect of phosphorus on growth parameters of slender leaf species.

SUMMARY ANALYSIS OF VARIANCE FOR GROWTH BOTH IN THE GREENHOUSE AND FIELD							
Greenhouse experiment 1							
	Plant height	Branch	Leaves	Pods	Seed yield	Thousand seed weight	
Effect							
P	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0005	<0.0001 ***	<0.0001 ***	
Species	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0067 **	0.0002 **	<0.0001 ***	
Intrac-tion P*Species	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0149 *	0.0023**	ns	
Greenhouse experiment 2							
	Plant height	Branch	Leaves	Pods	Seed yield	Thousand seed weight	
Effect							
P	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0005 **	ns	ns	
Species	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0067**	0.0439 *	0.0021 **	
Intrac-tion P*Species	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0149 *	ns	ns	
Field experiment 1							
	Plant height	Branch	Leaves	Pods	Seed yield	Thousand seed weight	
Effect							
P	ns	ns	ns		ns	0.0014 **	
Species	ns	ns	ns		<0.0001 ***	<0.0001 ***	
Intrac-tion P*Species	ns	ns	ns		ns	ns	
Field experiment 2							
	Plant height	Branch	Leaves	Pods	Seed yield	Thousand seed weight	
Effect							
P	ns	ns	ns	ns	ns	ns	
Species	ns	ns	ns	ns	<0.0001 ***	0.0051**	
Intrac-tion P*Species	ns	ns	ns	ns	ns	0.0159*	

N.B:*** Significant $p \leq 0.0001$, ******Significant $p \leq 0.01$, *****Significant $p \leq 0.05$, ns non-significance.

Appendix 2 Summary analysis of variance for mineral and nutrients for slender leaf in the field studies

Effect of P treatment	Mg	Ca	Fe	Zn	K	P	N	Protein	Beta carotene
Treatment(P)	ns	ns	ns	ns	ns	ns	ns	ns	0.0466*
Species	ns	ns	ns	ns	ns	ns	ns	ns	0.0013**
Stage of growth	0.0002**	<0.0001***	ns	ns	<0.0001**	<0.0001***	<0.0001***	<0.0001***	<0.0001***
Field	0.0414*	<0.0001***	ns	ns	0.0294*	<0.0001***	ns	ns	<0.0001***
P*Stage	ns	ns	ns	ns	ns	ns	ns	ns	ns
Stage *Field	ns	<0.0001***	0.0036**	ns	ns	<0.0001***	ns	ns	<0.0001***
P*Species	ns	ns	ns	ns	ns	ns	ns	ns	ns
Species*Stage	ns	ns	ns	ns	ns	ns	ns	ns	0.0049**
P*Species*Stage	ns	ns	ns	ns	ns	ns	0.0460*	0.0460*	ns

N.B. n s -Non significance * Significant $p \leq 0.05$ ** Significant $p \leq 0.01$

*** Significant $p \leq 0.0001$.

Appendix 3: Comparison of nutrients in slender leaf at the different stages of growth.

SNK GROUPING FOR STAGES OF GROWTH				
		Means at the different stages of growth		
NUTRIENT		Vegetative	Reproductive	Maturity
Mg		0.28 ^b	0.20 ^a	0.31 ^a
Ca		0.07 ^b	0.11 ^b	0.24 ^a
Fe		0.79 ^a	0.57 ^a	0.24 ^a
Zn		10.05 ^a	8.22 ^a	9.72 ^a
K		2.79 ^a	2.187 ^b	1.93 ^c
P		1.13 ^a	0.92 ^b	0.59 ^c
N		1.69 ^b	1.52 ^b	2.47 ^a
Protein		10.55 ^b	9.47 ^b	15.43 ^a
Beta carotene		1.91 ^b	0.00 ^c	3.07 ^a

Appendix 4: Comparison of nutrient levels in slender leaf species

SNK GROUPING	MEANS FOR SPECIES	
	Species means	
NUTRIENT	<i>C.brevidens</i>	<i>C.ochloreuca</i>
Mg	0.29 ^a	0.31 ^a
Ca	0.14 ^a	0.14 ^a
Fe	0.75 ^a	0.65 ^a
Zn	9.24 ^a	9.42 ^a
K	2.33 ^a	2.28 ^a
P	0.87 ^a	0.89 ^a
N	1.90 ^a	1.89 ^a
Protein	11.78 ^a	11.85 ^a
Beta carotene	1.55 ^b	1.77 ^a

Appendix 5: Comparison of the nutrient in slender leaf in different fields

SNK GROUPING FOR	MEANS FOR FIELD	
	Field means	
NUTRIENT	FIELD 1	FIELD 2
Mg	0.28 ^b	0.31 ^a
Ca	0.08 ^b	0.20 ^a
Fe	0.75 ^a	0.65 ^a
Zn	9.49 ^a	9.17 ^a
K	2.19 ^b	2.41 ^a
P	0.58 ^b	1.18 ^a
N	1.98 ^a	1.80 ^a
Protein	12.37 ^a	11.26 ^a
Beta carotene	1.02 ^b	2.30 ^a

Appendix 6: Effect of phosphorus on the agronomic performance of two *Crotalaria* species in Kenya. Published manuscript.



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RESEARCH PAPER

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Effect of phosphorus on the agronomic performance of two *Crotalaria* species in Kenya

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Key words: Phosphorous, Indigenous vegetables, Slender leaf.

Abstract

Crotalaria brevidens Benth. and *Crotalaria ochroleuca* G. Don. are two promising indigenous vegetables grown and consumed in Kenya. Yields of 2 to 3 ton ha⁻¹ have been reported without application of fertilizers compared with a potential of 10 -12 tons ha⁻¹. Phosphorus is one of the most limiting nutrients in many tropical soils causing low production of food crops including African indigenous vegetables. A greenhouse experiment was set to evaluate the effect of different phosphorus levels on growth of two *Crotalaria* species. Seeds of two *Crotalaria* species were sown in 2kg plastic pots filled with a mixture red soil and sand in the ratio of 5:1. Eight equivalent levels of phosphorous; 0, 15, 30, 45, 60, 75, 90, 105 kg P₂O₅ ha⁻¹ were applied at planting in form of Triple super phosphate. The experiment was laid out in completely randomized design replicated four times. Data on plant height, number of leaves, branches and pods, 1000 seed weight were recorded and analyzed using SAS 9.1.3 Software. Analysis of variance and significant means were separated using Student Newman Keuls test at p≤0.05. There was significant interaction (p≤0.0001) between phosphorous levels and species in mean plant height, number of branches and number of leaves. Number of leaves increased as phosphorus levels increased for both varieties. There was clear species difference in their response to different phosphorus levels. This research work is relevant to farmers growing slender leaf without addition of fertilizers to realize that additional of higher levels of phosphorus will enhance various parameters of its production.

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Introduction

Slender leaf is a legume in the family Fabaceae. The family contains 600 species that grow wild in the tropical and subtropical areas (Abukutsa-Onyango, 2004). The genus *Crotalaria* has 500 species of herbs and shrubs found in Africa. It originated from Northern Nigeria then spread to Ethiopia and Southern Tanzania. Among the slender leaf species growing in Africa, *Crotalaria brevidens* and *Crotalaria ochroleuca* are used as vegetables (Abukutsa-Onyango, 2007). They are commonly cultivated and consumed throughout East Africa and to a limited extent in West Africa (Abukutsa-Onyango, 2004). The young leaves and shoots that are consumed have been reported to be a good dietary source of provitamin A, carotenoids, vitamin C, iron, calcium and proteins (Abukutsa-Onyango, 2004). The species have also been reported to have medicinal applications (Uiso and Johns, 1996).

Although slender leaf is commercially produced in Kenya, only 2-4 tons per hectare have been harvested, yet it has potential of producing 10-12 tons per hectare (Abukutsa-Onyango, 2007). The major constraints in slender leaf production have been poor quality seed, lack of technical production and utilization packages (Abukutsa-Onyango, 2007). Continuous cropping of land with little return of nutrients leads to low soil fertility characterized by low amounts of soil organic carbon, nitrogen, and phosphorus (Okalebo *et al.*, 1990). Both phosphorus and nitrogen (N) deficiencies are extensive in sub-Saharan African agricultural soils and this causes low crop productivity, especially in smallholder agriculture (Buresh *et al.*, 1997). Phosphorus fertilization stimulates growth (Singh and Sale, 2000) and is required for photosynthesis (Freedman *et al.*, 1989). It also increases hydraulic conductivity of roots (Radin and Eidenbock, 1984). Crops require great amounts of phosphorus for the development of meristematic tissues, which divide and grow rapidly as observed by Brady and Weil, 2000. Application of 26.4 kg phosphorus ha⁻¹ improved seed yield,

nitrogenase activity and nodulation in pea (*Pisum sativum* L.) (Kasturi, 1995). In mung bean (*Vigna radiate* L.), application of 26 kg P ha⁻¹ increased the nodulation and yield (Sarkar and Banik, 1991). Shenoy and Kalagudi, 2005 observed yields reduction of 10-15% due to phosphorus deficiency in the production of sunflower (*Helianthus annuus* L.).

The effect of nitrogen levels between 0-100 kg ha⁻¹ on the growth, leaf and seed yield has been investigated in slender leaf (Abukutsa-Onyango, 2007). However, the effect of phosphorus on the growth of slender leaf has not been investigated in Kenya. The objective of this study was therefore to evaluate the effect of phosphorus on the growth of two varieties of slender leaf.

Materials and methods

Experimental site

The study was carried out at Jomo Kenyatta University of Agriculture and Technology (JKUAT) demonstration farm situated 36 km North-East of Nairobi along the Thika-Nairobi highway. Juja is in the Upper midland zone 4 which is semi-humid to semi-arid, situated at 1530 meters above sea level. The area receives an annual rainfall of 1074 mm with annual mean temperature of 20.5 °C (Batjes, 2006).

Experimental design and materials

A composite sample of red soil (Humic nitisols) and sand mixture in the ratio of 5:1 was taken to the laboratory for analysis of nitrogen, phosphorus and potassium (NPK) using the procedures described by Okalebo, 1986. The soil mixture was then put in 2kg plastic pots which were set up in a greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in the month of January-May 2013. These were then arranged in complete randomized design (CRD) and replicated four times.

Seeds of *C. brevidens* and *C. ochroleuca*, obtained from Indigenous vegetable multiplication center at JKUAT were sown in 2 kg plastic containers consisting analyzed soil. The seeds germinated in five days. Eight equivalent levels of 0, 15, 30, 45, 60, 75, 90 and 105kg of Triple super phosphate fertilizer

(TSP) supplied the required phosphorus and was applied at planting. The plants were kept weed free and watering was done three times per week. Data on plant height, number of branches, number of leaves and number of pods was collected from the second week after germination up to maturity at the 15th week. 1000 seed weight was collected was collected after the seed had dried at the 17th week. Data was analyzed using SAS 9.1.3. Software. Analysis of variance (ANOVA) was done and means were

separated using student Newman Keuls (SNK) test at $p \leq 0.05$.

Results and discussion

The analysis of soil mixture used in the study showed that the phosphorus level was 0.0001%. At phosphorus level below 0.2% plants show phosphorus deficiency (Mills and Jones, 1995). The phosphorus level in the study soil was far below the phosphorus deficiency level in plants of 0.2 % and could not sustain the growth of the slender leaf as shown in Table 1.

Table 1. Soil composition before addition of phosphorous.

Chemical	Amount
Nitrogen	0.05%
Potassium	0.4%
Phosphorus	0.0001%(1ppm)
EC	0.09 ds/m
pH	5.69

Plant height

There was significant interaction between phosphorus levels and *Crotalaria* species on the plant height $p \leq 0.0001$ (Table 2). *Crotalaria ochroleuca* recorded the highest mean plant height of 70.8 cm at 15 kg P_2O_5 ha^{-1} while the lowest plant height 56.1cm was recorded at 60 kg P_2O_5 ha^{-1} . *Crotalaria brevidens* recorded highest mean plant height of 37.5cm after application of 60 kg P_2O_5 ha^{-1} with lowest mean plant height 28.4cm obtained in the control with zero phosphorus. The optimum fertilizer level for *C.ochroleuca* was 15 kg P_2O_5 ha^{-1} for enhanced plant height above which there was no more increase. For *C.brevidens* the optimum phosphorus level for plant height was 60 kg P_2O_5 ha^{-1} above which the plant height would not increase. This different response to different phosphorus levels for the two *Crotalaria* species could probably be due to their species

highest height achieved after application of 96 kg P_2O_5 ha^{-1} .

Number of leaves

The mean numbers of leaves per plant at different phosphorus levels were significantly different ($p \leq 0.0001$), with the number of leaves increasing with increase in phosphorus levels (Table 2). *Crotalaria brevidens* had a higher mean number of leaves 115.9 leaves per plant than *C. ochroleuca* with 49.7 mean number of leaves during the twelve weeks of growth. The highest mean number of leaves was obtained at 105kg P_2O_5 ha^{-1} for both species. Lowest number of leaves was found when no phosphorus was applied for both *Crotalaria* species. Observations from this study show that higher levels of phosphorus are required for production of higher number of leaves resulting in a larger leaf area for

difference. These results differed from those of Pramanik *et al.*, 2009 who found phosphorus fertilization did not have significant effect when applied in the growth of seven green manure crops that included *Crotalaria juncea*. However, the effects of phosphorus fertilization on stem height in beans was found significant by Rahini *et al.*, 2012 with Nduhiu *et al.*

photosynthesis. When the nutrient supply is suboptimal leaf growth is limited as the net photosynthesis becomes low resulting to less number of leaves. Different results were reported by Oyewale, 2013 which showed that the leaves of snake plant (*Trichosanthes cucumerina* L.) increased between 15-30 kg P₂O₅ ha⁻¹ beyond which there was reduction.

Table 2. Effect of phosphorus levels on some growth parameters of the two slender leaf species.

P Levels (kg/ha)	Crotalaria brevicens			Crotalaria ochroleuca		
	plant height(cm)	Number of leaves	Number of branches	plant height(cm)	Number of leaves	Number of branches
0	28.4±3.6 ^c	71.3 ±2.3 ^c	8.0 ± 1.0 ^e	63.6 ± 9.2 ^{abc}	27.9 ± 5.1 ^d	7.1 ± 1.0 ^e
15	34.0 ±4.3 ^{ab}	84.5 ±3.3 ^c	8.9± 1.0 ^d	70.8 ± 9.2 ^a	40.1 ± 7.7 ^c	9.8 ± 1.2 ^b
30	31.0 ±3.7 ^{bc}	106.5±7.2 ^b	9.8 ±1.0 ^{bcd}	59.7± 7.9 ^{bc}	49.0 ± 9.1 ^b	9.7 ± 1.1 ^b
45	31.3±3.8 ^{bc}	122.3±19.7 ^b	9.6 ± 1.0 ^{cd}	59.5±7.8 ^{bc}	45.4 ± 7.6 ^{bc}	9.7± 1.1 ^b
60	37.5 ± 4.2 ^a	135.2±20.6 ^a	10.3±1.1 ^{bc}	56.1 ± 6.7 ^c	60.1 ± 10.5 ^a	12.0 ± 1.2 ^a
75	31.2±3.7 ^{bc}	127.9±21.4 ^b	9.4± 1.0 ^{cd}	56.6 ± 6.7 ^{bc}	52.0 ± 9.3 ^a	11.9 ± 1.3 ^a
90	35.4 ± 3.9 ^a	137.5±23.5 ^a	11.3 ±1.2 ^a	66.4 ± 7.8 ^{ab}	58.8 ± 9.8 ^a	11.7 ± 1.2 ^a
105	35.1 ± 4.0 ^a	141.9±25.1 ^a	10.8±1.2 ^{ab}	58.4 ±6.8 ^{bc}	64.3 ± 11.8 ^a	12.3 ± 1.1 ^a

Mean numbers followed by same letter are not significantly different $p \leq 0.05$ by SNK.

Number of pods

Application of 45 kg P₂O₅ ha⁻¹ gave the highest mean number of pods 18.4 for *C. brevidens* and this was significantly different from other levels while the least mean pod number was 7.4 attained after application of 60 kg P₂O₅ ha⁻¹ at maturity. This response was different for *C. ochroleuca* where the highest number of pods 18.9 was attained at 105 kg P₂O₅ ha⁻¹ (Table 3). The increment in number of pods at highest phosphorus level confirms the role of phosphorus in promoting formation of nodes and pods in legumes. However, the two *Crotalaria* species responded differently to phosphorus application probably due to their genetic potential. These results differ from those of Basu *et al.*, 2003 where maximum number of pods was achieved at application of 60 kg P₂O₅ ha⁻¹ in ground nut (*Arachis hypogea*). 45 kg P₂O₅ ha⁻¹ was optimum in forming pods for *C. brevidens* while *C. ochroleuca* produced highest number of pods at application of 105 kg P₂O₅ ha⁻¹.

Thousand Seed weight

For *C. brevidens* seed weight at zero phosphorus was significantly different from 90 kg P₂O₅ Ha⁻¹ but not different from other levels (Table 3). The highest seed weight 6.04 g/1000 was found at 90 kg P₂O₅ ha⁻¹ while the lowest 4.3 g/1000 was obtained without phosphorus. These results were different from those of *C. ochroleuca* where highest thousand seed weight of 6.9 g was obtained at 30 kg P₂O₅ ha⁻¹ and the lowest 5.1g, at 90 kg P₂O₅ ha⁻¹. The results for *C. brevidens* were similar to those obtained by Turuko and Mohammed, 2014 who found the highest 1000 seed weight at 90 kg P₂O₅ ha⁻¹ in common bean (*Phaseolus vulgaris*).

Conclusion

The results of this experiment showed that there was significant interaction between phosphorus and the

parameters.

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