ALLELOPATHIC EFFECT OF NIGER PLANT (*Guizotia abyssinica* L.) ON WEEDS AND ITS INFLUENCE ON GROWTH AND DEVELOPMENT OF BEANS (*Phaseolus vulgaris* L.)

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been submitted for a degree in any other university. No part of this thesis may reproduced without the prior written permission of the author and/or University of Eldoret.

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To my husband Mr. John Mackambo and children Becky Blessing and Precious Alvine.

ABSTRACT

Niger plant (*Guizotia abyssinica*), is an annual herbaceous plant that originated from Ethiopia. In Kenya, the plant is perceived as a weed whereas in Ethiopia and India, it is cultivated as an important oil crop. It has been observed that crops following a Niger plant infested field have fewer weeds leading to a hypothesis that Niger plant produces secondary metabolites known as allelochemicals that suppress weeds. Being environmentally friendly and with new sites of action, use of allelochemicals may be a solution to the current problems caused by herbicide use. This study was therefore carried out to determine the allelopathic effect of Niger plant on weeds and its influence on growth and development of beans in Moiben sub-county, Uasin Gishu, county. The study involved assessing the genetic diversity of Niger plant from Moiben sub-county using ISSR markers, determining secondary metabolites in Niger plant and evaluating the influence of Niger plant on growth and development of beans and weed abundance. Laboratory experiments included identifying genetic diversity of Niger plant and analysis for metabolites in Niger plant collected within Moiben sub-county. For genetic diversity experiment, plant samples were collected from every administrative ward within Moiben sub-county, DNA was extracted and Inter Simple Sequence Repeat (ISSR) markers used to assess genetic diversity. Assessment of secondary metabolites involved extraction and quantification of alkaloids, flavonoids, phenols, saponins and tannins from Niger plant samples. The field experiment involved four weed regimes and three bean varieties with three replicates. The experiment was a 4 x 3 factorial arranged in a Randomized Complete Block Design (RCBD). The four weed regimes were weed free, weedy, Niger plant intercrop and all weeds but no Niger plant. Bean varieties were Rosecoco, Mwitemania and Mwezi Mbili. Data were collected on genetic diversity of Niger plant, amount of secondary metabolites in Niger plant, stand count of beans at two weeks, plant height at 50% flowering, number of pods per plant, stand count at harvesting, number of seeds per plant and number of Field mustard (*Brassica rapa*), Broom weed (*Guiterrezia sorothrae*), Double thorn (*Oxygonium sinuatum*) Niger plant (*Guizotia abyssinica*) and couch grass (*Cynodon dactylon*). Quantitative data were subjected to ANOVA by Genstat version 14 and means separated by Duncan's Multiple Range Test (DMRT). ISSR data were used to calculate the Squared Euclidean Distance matrix. Genetic analysis resulted into a dendogram with four main clusters hence showing that the Niger plant is genetically diverse. Alkaloids, flavonoids, phenols, tannins and saponins were found to occur in Niger plant in varying concentrations depending on the site of collection. Niger plant showed a negative allelopathy to the studied weeds. Bean growth and development was enhanced by the Niger plant. In conclusion therefore, Niger plant within Moiben sub-county is genetically diverse with varying levels of secondary metabolites. Niger plant can be used to control specific weeds without compromising on bean growth and development. It is recommended that further research be carried out on the agronomic issues surrounding allelopathy for proper understanding of this upcoming technology for weed management.

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LIST OF ABBREVIATIONS

UPGMA:	Unweighted Pair Group Method with Arithmetic Mean
UPGM:	Unweighted Pair Group Method
SAHN:	Sequential Agglomerative Hierarchical Nested
NTSYS-pc:	Numerical Taxonomy Multivariate Analysis System Package
ISSR:	Inter Simple Sequence Repeat
PIC:	Polymorphic information content
CO_2:	Carbon dioxide
ATP:	Adenosine triphosate
GA:	Gibberellic acid
IAA:	Indole acetic acid
NADH:	Nicotinamide adenine dinucleotide
DNA:	Deoxyribonucleic acid

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Weeds are plants, parasitic or not, that occur in areas they are not required and at the time they should not occur. Being plants, they compete with crops for space, nutrients, water and pollinators. Due to this competition, weeds contribute to loss of crop yields, loss of biodiversity and promote loss of habitats. In the tropics, crop losses have been attributed to weeds more than diseases and pests combined. The economic value of weeds is low and hence they are included in plant pests (Singh *et al.*, 2012). Plant species that have less competitive power in the ecosystem are greatly affected by weeds more than others.

Crop losses due to weeds ranges from 25% to 60% but can be as high as 100%, especially in the case of parasitic weeds for example Striga species which is total crop failure. Losses from weeds depend on the weed pressure, weed control methods, cost of weed control and the level of weed management practices by farmers (Van Rijn, 2000).

Due to the undesired effects of weeds on crops, many approaches have been sought to try to manage them. These include manual hand weeding, biological methods, use of machines and use of chemicals. During the 20th century, pronounced attention was paid to the use of herbicides to manage the growth of weeds (Hussein *et al.*, 2001) in minimal time. However, continuous use of herbicides over the years has raised pronounced doubts about the safety of the environment and human health (El-Rokiek and Eid, 2009).

Because of the negative effects associated with most of the available weed management methods, there is need for an alternative method to control weed infestation and the accompanying losses. One of the upcoming methods is allelopathy (Ejaz *et al.*, 2015). Allelopathy refers to any direct or indirect positive and negative effects of chemical compounds of secondary metabolism of plants and microorganisms that have an influence on (Narwal and Haouala, 2013). Donor plants of allelochemicals produce different secondary metabolic components such as alkaloids and glycosides and deposit them in the soil surrounding receiver plants (Jarchow and Cook, 2009). Consequently, in use of allelopathy in weed control, the fact that allelochemicals produced by some plants act against other plants inhibiting their growth and reproduction, this is put into use in this natural method (Jarchow and Cook, 2009).

Some of the compounds which are known as allelochemicals alter the growth or physiological functions of receiving plant species. The most commonly found allelochemicals, cinnamic and benzoic acids, flavonoids, and various terpenes (Singh *et al.,* 2012), are known to be phytotoxic (Einhellig and Souza, 2002). Allelopathy is a natural and environment friendly technique which may prove to be a tool for weed management and thereby increase crop yields while conserving the environment.

Niger plant (*Guizotia abyssinica*), belongs to the family Asteraceae. Several species of family *Asteraceae* have allelopathic effects on other species, reducing seed germination and emergence of subsequent small-grain crops when grown in rotation (Muehlchen *et al.,* 1990). It is reported that Niger plant is a good forerunner for other. This is because in the following season, there is low weed abundance thus higher yields (Adarsh *et al.*, 2014). This points to the possibility that allelochemicals from plants are potential bio-herbicides.

Plate 1: Picture of Niger plant

Source: Author (2019)

1.2 Statement of the problem

A number of approaches have been put up in management of weeds including manual weeding, use of machines and use of herbicides. For faster weed management, use of chemicals stands out as the first method of choice (Tang *et al.*, 2010). Use of chemicals over a long period of time leads to chemical resistance which requires that the dosage be increased for their efficiency. However, increase of dosage leads to a public health concern since many chemicals have been linked to many lifestyle diseases (Ejaz *et al.*, 2015).

Wide spread use of synthetic chemicals in agriculture has raised concern on how agriculture can be sustainable leading to research on how farmers can lower their dependence on these chemicals by coming up with reliable substitutes to control weeds. Utilization of phytochemical properties of locally available plants holds a promising future in management of weeds. While there is evidence on the use of Niger plant allelopathy in weed management, such study has not been done in Kenya to show the crop-crop interaction and whether the plant and its metabolites can be harnessed as a potential bioherbicide. This research will therefore look into the occurance of allelochemicals in Niger plant and their allelopathic effects on both weeds and common beans. This will enlighten scientists, policy makers and other stakeholders on the potential of alternative weed control that can be achieved through use of allelopathy.

1.3 Justification

Weed management by manual weeding especially during the peak weeding season is very labour-intensive and most crops therefore pass their critical period of weed control resulting in poor or no yield. The introduction of synthetic herbicides was a new dawn in weed control. However, due to their persistent nature in the environment coupled with the fact that they are toxic, there is a serious danger to humans, animals, other non-target plants and microorganisms. This has necessitated research into alternative methods of weed management that conserve the integrity of the environment. Due to their natural effect, use of allelopathy has drawn the interest of many scholars and is the subject of this study.

1.4 Objectives

1.4.1 General objective

To evaluate the genetic diversity and allelopathic effect of Niger plant (*Guizotia abyssinica*) on weeds and its influence on growth and development of beans in Moiben sub-county, Uasin Gishu county, Kenya.

1.4.2 Specific objectives

a) To assess the genetic diversity of Niger plant from Moiben sub-county using Inter Simple Sequence Repeat (ISSR) markers.

b) To determine the types and quantities of secondary metabolites in Niger plant.

c) To evaluate the effect of Niger plant on weed abundance and bean growth and development.

1.5 Hypotheses

H_a: There exists genetic diversity in Niger plant from Moiben sub-county.

H_a: Niger plant has different secondary metabolites at varying concentrations.

H_a: Niger plant influences weed abundance and bean growth and development.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to weeds

Weeds, are stressors in crop production though they have been in existence as long as crop production. They are unwanted plants growing without human intervention, reducing biodiversity while promoting crop yield loss and for this reason they are considered crop pests.

Weed infestation is of supreme importance among biotic factors that are responsible for low crop yields. Generally, weeds reduce crop yield by interfering with different metabolic processes (Hajizadeh and Mirshekari, 2011). Yield loss due to weeds varies from one crop to another depending on weed density, weed type, duration of competition, management practices and weather conditions (Zohaib *et al.*, 2016).

For weeds to be able to out-compete crops and survive, they have adapted unique characteristics (Zimdahl, 1999). In light of these characteristics of weeds and their hazards, it becomes imperative to manage them. The best weed management system will be one that is low-cost and environmentally friendly.

Different methods have been used to manage weeds but mechanical and chemical methods are more frequently used than any other management methods. Mechanical methods like hand weeding are still useful but are getting expensive, labour intensive and time-consuming (Chaudhary, *et al.*, 2008).

Use of synthetic chemicals to manage weeds is an important alternative to other forms because it is less costly, time saving and more efficient. Since their discovery, chemical weed control agents have created a niche for themselves (Marwat, *et al.*, 2008). Herbicides have made it possible to realize high crop yields with low labour cost. Nevertheless, the indiscriminate use of herbicides has provoked an increasing incidence

of resistance in weeds to some herbicides, changes in weed population to species more related to the crop, environmental pollution and potential health hazards (Macias, *et al.*, 2014). Zohaib *et al.*, (2016), reported that herbicides significantly increase maize yield and decrease weed density. However, continuous application of herbicides cause changes in weed flora, poor controlling and evolution of some herbicide resistant weed biotypes. This necessitates the introduction of some other new herbicide options with different modes of action (Amare, *et al.*, 2014).

There is a lot of importance placed on sustainable agriculture due to the negative impacts of synthetic herbicides. Currently, research attention has shifted to reducing reliance on herbicides and looking for an alternative strategy for weed management. One of the promising research focus in weed management is to reduce over-dependence on synthetic herbicides and to move towards low-input sustainable agriculture (LISA) as a part of integrated weed management (Nikneshan *et al.*, 2014).

2.2 Allelopathy in weed management

One of the factors enhancing the success of weeds in the ecosystem is by use of allelopathy. Through this, the weeds are able to suppress native plants and thus be able to colonize an area. This ability determines the species distribution and abundance within a plant community and confer success of many invasive species.

Given the prevailing negative impacts of synthetic herbicides on the environment and human health, there is need to maintain optimal crop production with low external inputs, reducing reliance on commercial inputs and substituting them with natural products. These natural products maintain high crop productivity over a long period with minimum or no effects to the environment.

Allelopathy is the negative or positive effect of chemicals released by one plant species on the growth or reproduction of another (Callaway and Aschehoug, 2000). The plant

that produces the allelochemicals, by use of the allelochemicals, may either hinder or enhance physiological processes in the other plants in the surrounding rhizosphere (Ankita & Chabbi, 2012). Since allelochemicals re natural plant products, their use enhances crop growth and productivity as well as maintaining environmental integrity (Hegab *et al.,* 2008).

Allelopathy can be induced to plants by planting allelopathic plants either together with other crops or by rotation in the field (Khanh *et al.,* 2005). Examples of plants associated with allelopathy include alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), clovers (*Trifolium* spp., *Melilotus* spp.), oats (*Avena sativa*), pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghums (*Sorghum* spp.), sunflower (*Helianthus annuus*), sweet potato (*Ipomoea batatas*) and wheat (*Triticum aestivum*) (Weston *et al.,* 2013). The compounds that confer allelopathy are produced by growing or decomposing plants.

The readily visible effects of allelochemicals on the growth and development of plants are hindrance on germination or lower germination rate, the radicle and shoot becomes reduced, root tips become swollen to an extent that they cannot absort water and nutrients, discolouration and reduced root hair (Amb *et al.*,2016). These gross morphological effects may be secondary manifestations of primary events, caused by a variety of more specific effects acting at the cellular or molecular level in the receiver plants (Bhadoria *et al.,* 2010).

In contrast to synthetic herbicides, allelochemicals do not have toxic effects. However, their efficacy is low and their specificity low (Bhadoria, 2011). Allelopathic plants can be utilized in crop rotations, as cover crops, as intercrops or as green manure (Haider *et al.,* 2015).

2. 3 Methods of allelochemical release from donor plants

The concentration of allelochemicals in the soil differs from place to place, time to time and from different parts of the plant (Bertin et al., 2003). Plants release allelochemicals to the environment in different ways as follows:

2.3.1

This involves the exudation of volatile compounds from living green parts of the plant through stomata. Volatile allelochemicals are released to the environment from donor plants and the receiver plants absorb the allelochemicals when they mix with atmospheric moisture (Obaid and Qasem, 2005). Allelochemicals that are released by this method have to be volatile and water soluble so as to be dissolved in water. Compounds released through this manner include camphor, caphene, cineole,and dipentine.

2.3.2 Leaching

This method is for water soluble allelochemicals for example phenolics, alkaloids and terpenoids. These allelochemicals are released from aerial parts of the plant and are washed to the soil by rain water (Das *et al.*, 2012).

2.3.3 Decomposition

Decay of plant residues by action of microorganisms adds up allelochemicals in the soil (Matloob *et al.*, 2010). This is the most common mode of release of allelochemicals (Sing *et al*., 2005) and is the basis of incorporation of plant residues in the soil aimed at gaining allelopathic effects. Decomposition of rice straws in the field leads to suppression of several subsequent weeds through release of phytotoxic chemicals (Inderjit and Dakshini, 1999).

2.3.4 Exudation from roots

In this method, exudates are discharged from plant roots. While in the soil, the exudates infiltrate the surrounding soil and affect plants around them (Senarathne *et al.*, 2010). Sorghum spp. produces large quantities of root exudates containing potent allelochemicals. Sorgoleone and other related hydroquinones are produced solely by living root hairs and are exuded as golden-coloured droplets from the tips of root hairs (Czarnota *et al*., 2003). Small globules of cytoplasmic exudate are thought to be transported across the cell and deposited between the cell wall and the plasma membrane. They merge to form larger globules, which pass through the cell wall and appear as droplets on or near the tip of living root hairs (Dayan *et al*., 2009).

2.4 Mechanisms underlying allelopathy

2.4.1 Changes in cell structure

Allelochemicals have been known to distort the shape and structure of plant cells. Volatile monoterpenes, eucalyptol and camphor can widen and shorten root cells, in addition to inducing nuclear abnormalities and increasing vacuole numbers (Pawlowski *et al.*, 2012). Likewise, cinnamic acid significantly deformed the ultra-structure of cucumber chloroplasts and mitochondria (Wu *et al.*, 2015). After treatment with benzoic acid, mustard (*Brassica juncea* L.) roots displayed irregularly shaped cells arranged in a disorganized manner and disruption of cell organelles (Kaur *et al.*, 2005).

2.4.2 Inhibition of cell division and elongation

Allelochemicals interfere with the process of cell division. In an experiment, allelochemical monoterpenoids affected cell proliferation and Deoxyribonucleic acid (DNA) synthesis in plant meristems (Nishida *et al.*, 2005); and sorgoleone reduced the

number of cells in each cell division period, damaging tubulins and resulting in polyploidy nuclei (Hallak *et al.,* 1999). Following the treatment of soy bean with aqueous leaf extracts from *Datura stramonium* L., Cai and Mu (2012) found that higher concentrations of the extracts inhibited primary root elongation and lateral root development, decreased root hair length and density, inhibited cell division in root tips and increased the chromosomal aberration index and micronucleus index. All these result in poor plant growth and stunting which affect production.

2.4.3 Imbalances in the antioxidant system

After exposure to allelochemicals, the recipient plants may rapidly produce reactive oxygen species (ROS) in the contact area (Ding *et al.,* 2007), and alter the activity of antioxidant enzymes such as superoxide dismutase and peroxidase (Zeng *et al.,* 2008).

2.4.4 Increases in cell membrane permeability

Allelochemicals significantly inhibit the activity of antioxidant enzymes and increase free radical levels, resulting in greater membrane lipid peroxidation and membrane potential alteration, which diminish the scavenging effect on activated oxygen and damage the whole membrane system of plants (Harun *et al.*, 2014). The growth of wild mustard seedlings were found to be inhibited by an aqueous extract of barley aerial parts through increasing lipid peroxidation (Farhoudi and Lee, 2013).

2.4.5 Effect on the plant growth regulation system

Allelocemicals can alter the contents of plant growth regulators or induce imbalances in various phytohormones, which inhibits the growth and development of plants, for example, with respect to seed germination and seedling growth. Most phenolic allelochemicals can stimulate Indole acetic acid (IAA) oxidase activity and inhibit the

reaction of POD with IAA, bound Giberrellic acid (GA) or IAA to influence endogenous hormone levels (Yang *et al.,* 2005).

2.4.6 Effect on plant photosynthesis

The impacts of allelochemicals on plant photosynthesis mainly involve inhibition of or damage to the synthesis machinery and acceleration of the decomposition of photosynthetic pigments (Wu *et al.,* 2015). This decreases the photosynthetic pigment contents blocking energy and electron transfer and inhibiting the synthesis of adenosine triphosate (ATP) (Meazza *et al.,* 2006). Allelochemicals affect photosynthesis mainly by influencing the function of photosystem II (PS II) (Wang *et al.,* 2014).

2.4.7 Influence on respiration

Different stages of respiration such as electron transfer in the mitochondria, oxidative phosphorylation, Carbon dioxide (CO_2) generation and ATP enzyme activity can be influenced by allelochemicals (Fang and Zhihui 2016). These chemicals can reduce oxygen intake, which prevents Nicotinamide adenine dinucleotide (NADH) oxidation, inhibits ATP synthesis enzyme activity, reduces ATP formation in mitochondria, disturbs plant oxidative phosphorylation and ultimately inhibits respiration; on the other hand, they can stimulate the release of CO_2, which promotes respiration. Hejl and Koster (2014) observed that juglone could reach the mitochondria in the root cells of corn and soy bean seedlings, thereby disrupting root oxygen uptake.

2.5 Factors influencing release of allelochemicals from plants

Release of allelochemicals is largely influenced by abiotic factors. The amount of allelochemicals produced is directly proportional to the level of stress in the environment. Drought, irradiation, temperature, pH, contamination, ultraviolet light,

nutrient deficiency and diseases are the abiotic factors affecting release of allelochemicals from plants.

Biotic factors influencing the release of allelochemicals include competitors, herbivores and soil microorganisms. Allelochemicals can be metabolized by soil microorganisms and be transformed resulting in more or less toxic compounds. Because of their anti-microbial activity, some alkaloids resist microbial change (Iqbal *et al*., 2019).

Biotic factors activate the plant defenses and stimulate the plant to secrete bitter substances, or those that harden the tissues, that are toxic or give off unpleasant odours.

2.6 Fate of allelochemicals in the soil

Once released, allelochemicals enter a complex plant-soil system in which diverse factors affect their availability, and consequently their effective influence on target plants (Kruse *et al.,* 2000). Factors that affect allelochemical availability in the soil include leaching, physiochemical processes, microbial breakdown and uptake by plants (Inderjit, 2001). They can also bind to organic matter and clays in the soil. Oxidation and sorption are the primary factors involved in the disappearance of allelochemicals. Soil microbes take up the compounds released from plants and degrade them through the action of extra-cellular and intercellular microbial enzymes for their own energy building processes (Kruse *et al.,* 2000). Such microbial transformations can either detoxify the soil of these compounds or produce other more phytotoxic allelochemicals (Bhinu *et al.,* 2006).

Understanding allelopathy can be used to manage weeds in a natural way that conserves environmental integrity. Research on this area by use of Niger plant is scarce and that is the subject of this study.

In a nut shell, weeds are a major challenge in agricultural production systems dating as far as domesticated agriculture. Many approaches, including manual weeding and use of chemicals have been proposed to try and manage the weeds but so far little has been achieved. Manual weeding is not reliable especially during bad weather and at the peak of weeding when it is difficult to obtain labour. Chemical use in weed control works fast and efficiently but the cost of the herbicides is prohibitive and there is a risk to the environment in terms of pollution. All this has aggravated the need for a more environmentally sound weed control measure. Studies have shown that allelopathy provides an effective and natural alternative control of weeds.

Although there is on-going research on allelopathy, much more needs to be done for proper and in-depth understanding of the subject for its acceptability and adoption. There exist research gaps in areas of allelopathic plant-crop interactions on growth and development and this is one of the objectives that the current study tries to address.

2.7 Common beans

Common beans (*Phaseolus vulgaries* L.) are leguminous plants in the family Fabaceae. They are annual plants grown worldwide for their edible dry seeds. Beans do well in mid altitude, arid and semi - arid areas of Kenya.

2.7.1 Role of beans in nutrition and economy

Nutritionally, beans provide high levels of proteins, minerals and vitamins (Beebe et al., 2000). Together with the international market, there is a ready local market for Kenyan beans. The price depends on the season and the variety of the beans whereby some varieties have better returns than others. Common bean varieties depend on the locality. In the North Rift, Mwezi mbili, Mwitemania and Rose coco are common.

2.7.2 Effect of weeds on bean growth and development

Common beans are poor competitors against weeds. This is majorly because beans have a slower growth compared to most weeds thus suffer from successive flushes of weeds, causing significant yield losses.

As in all other crops, weeds compete with beans for nutrients, moisture and space. If uncontrolled, weeds can completely suppress beans. The weeds also harbor pests and diseases which also reduce yields.

2.7.3 Weed control in beans

To ensure high yields in beans, proper weed control is critical so as to eliminate competition for light, nutrients and moisture giving the crop the opportunity to establish well. Weed control also curtails opportunities for pest establishment in the crop.

Timing for weed control is critical. Weeds are easier to control early in the season before they are fully established. Therefore, it is important to observe the critical period of weed control. Weeding can be less effective in drought stress.

Weed control methods in beans include:

Chemical method

This is the use of chemicals to control weeds. The method id fast and easy, without mechanical damage to the crop. Through this method, weeds with similar morphological factors with the crop are effectively controlled.

Mechanical method

This involves the removal of weeds using tools and implements like hoes. It should be done carefully in order to prevent mechanical damages to the crop.

Cultural method

This includes planting early maturing bean varieties, using clean seeds, mulching, crop rotation and early planting.

Generally, an integrated weed management strategy is the most preferred since no single method has proved to be effective in weed control.

CHAPTER THREE

ASSESSMENT OF GENETIC DIVERSITY OF NIGER PLANT (*Guizotia abyssinica* L.) FROM MOIBEN SUB COUNTY, KENYA USING INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

3.1 Abstract

Niger plant (*Guizotia abyssinica*), exhibits phenotypic plasticity in different environments. In order to study Niger plant, there is need to assess its genetic diversity since *Guizotia* species has a high number of species which may be confused amongst themselves. To achieve this, Inter Simple Sequence Repeat (ISSR) markers were used to estimate genetic diversity among 12 wild populations of Niger plant from Moiben sub-county, Kenya. Total genomic DNA was extracted as per the Cetyltrimethylammonium bromide (CTAB) method proposed by Doyle (1987) and subjected to ISSR analysis using 20 primers. None of the primers produced unique banding patterns for each collection. ISSR data were used to calculate a Squared-Euclidean Distance matrix. All the twenty primers (100%) gave polymorphic bands thus they were all considered for further analysis. The allele frequency of all the primers was below 0.95 indicating that they were all polymorphic in character. Gene diversity was high ranging from 0.3550 to 0.7337 with a mean value of 0.6302. The ISSR based UPGMA clustering produced four clusters. In conclusion, Niger plant within Moiben sub-county was be genetically diverse although heterozygosity was not noticed. The study recommends further and detailed analysis of Niger plant so as to form a basis for further development of the plant.

3.2 Introduction

Niger plant (*Guizotia abyssinica*) is exclusively diploid (2n=30) and a completely outcrossing species with self-incompatibility. It is an annual plant originating from Ethiopia where it is under cultivation for edible oil. The plant has bright yellow flowers, pollinated by insects, mainly bees. Taxonomically, Niger plant belongs to the family Asteraceae (Compositae), tribe Heliantheae and sub tribe Coreopsidinae.

Niger plant exhibits a high variability of morphological characteristics as influenced by the prevailing environmental conditions such as rainfall, temperature, altitude, growing period and edaphic factors. This makes morphological identification of varieties

difficult since these characters are not discrete. DNA markers provide a powerful tool for genetic evaluation and marker-assisted breeding of crops and especially for cultivar identification (Rai *et al.,* 2010). DNA-marker technology detects more polymorphisms and is not influenced by prevailing environmental conditions. These Deoxyribonucleic acid (DNA) markers can identify many genetic loci simultaneously with an excellent coverage of the entire genome, are phenotypically neutral and can be applied at any development stage (Genet *et al.,* 2005). ISSR markers, just like any other Polymerase Chain Reaction (PCR) -based markers, are rapid and require only a small amount of the template DNA. ISSR information does not require genome sequence information but produce highly polymorphic pattern.

Genetic diversity is a product of interplay of biotic factors, physical environment, artificial and plant factors (Frankel *et al.,* 2014). It refers to the total number of genetic characteristics in the genetic makeup of a species and serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment thus those individuals are more likely to survive to produce offspring bearing that allele (NBII, 2011). Knowledge of genetic diversity and relationship among sets of germplasm is beneficial to all phases of crop improvement (Geleta *et al.,* 2007).

The purpose of the present study was to investigate, through the use of ISSR markers, the genetic diversity of randomly collected Niger plant in Moiben sub-county. This is the first attempt to estimate genetic variability among Niger plant in Kenya in an effort to provide some information as a basis for future research.

3.3 Objective

To assess the genetic diversity among the Niger plant populations in Moiben sub-county, Kenya.

3.4 Materials and methods

3.4.1 Study site

The plant material studied was collected from all the administrative wards in Moiben sub-county. Moiben sub county stands at an altitude of 2163 m above sea level and at 0°49′N - 0.82°N and 35°23′E and 35.38°E

The area has a bimodial rainfall pattern where long rains are experienced between April to July and short rains between September and November. The area dominantly falls under upper midland agro ecological zone (Jaetzold and Schmidt, 1983) with agriculture as the main economic activity. Maize and wheat are the main crops grown for both commercial and subsistence purposes. Cattle rearing is also practiced.

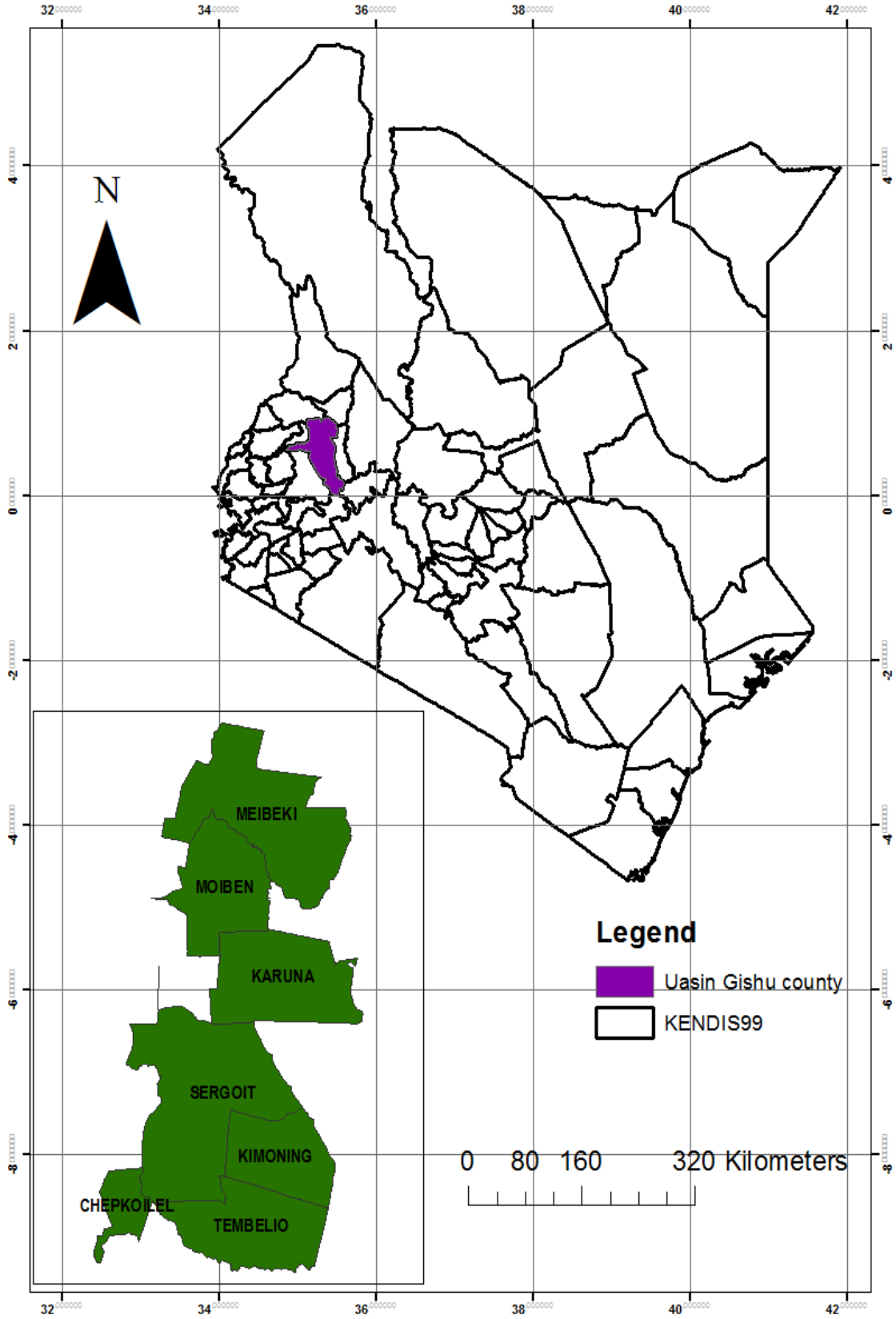


Fig. 1: Map of Kenya showing Uasin Gishu county, where Moiben sub-county is located. Source: Google (2019)

3.4.2 Plant material

Tender leaves of twelve Niger plant wild populations were collected randomly from all administrative wards within Moiben sub-county. A single collection from one site was considered a population. Since it was not possible to obtain a sample of characterized Niger plant, sunflower (*Helianthus annus*) was used as a check since the two belong to the same family. The samples were collected into zip lock bags, packed into a cool box and transported to the University of Eldoret where they were stored in a freezer until use. Certified sunflower seed was planted in University of Eldoret green house for one month. Tender leaves were harvested for genetic analysis.

3.4.3 DNA extraction

DNA extraction was done following the Cetyltrimethylammonium bromide (CTAB) method proposed by Doyle *et al*., (1987) as detailed in Appendix I (a).

3.4.4 DNA quantification

A detailed procedure for DNA quantification is given in Appendix I (b) (Allagher, 2011).

3.4.5 PCR amplification

PCR amplification was done as per the method of Sharma *et al*., (2016) in Appendix I (c).

3.4.6 Polymerase Chain Reaction (PCR) and polyacrylamide3 electrophoresis

The ISSR primers used for polymerase chain reaction are indicated in Table 1.

Table 1. List of primers and annealing temperatures

No.	Primer name	Number of polymorphic bands	Primer sequence	Annealing temp (^{0} C)
1	2903	14	ACACACACACACACACYT	43
2	2904	5	BDBCACACACACACACA	39
3	2906	5	HVHTGTGTGTGTGTGTG	40
4	2909	15	AGAGAGAGAGAGAGAGAGC	44
5	2910	8	GAGAGAGAGAGAGAGAT	41
6	2911	13	AGAGAGAGAGAGAGAGC	44
7	2922	9	AGAGAGAGAGAGAGAGC	44
8	2923	8	AGAGAGAGAGAGAGAGG	44
9	2924	5	GAGAGAGAGAGAGAGAT	41
10	2934	5	GTGTGTGTGTGTGTGTGC	44
11	2939	5	ACACACACACACACACT	41
12	2941	10	ACACACACACACACACACG	44
13	2955	18	GAGAGAGAGAGAGAGAYC	47
14	2956	7	GAGAGAGAGAGAGAGAYG	47
15	2961	9	CACACACACACACACARCG	48
16	2964	9	GTGTGTGTGTGTGTGTYC	45
17	2976	11	AGCAGCAGCAGCAGCAGC	52
18	2998	4	HBHAGAGAGAGAGAGAG	39
19	2999	5	BHBGAGAGAGAGAGAGA	39
20	3013	8	ACTTCCCCACAGGTTAACACA	48

3.5 Data scoring and statistical analysis

Each ISSR fragment was considered as a simple bi-allelic locus with one amplifiable and one null allele. PCR amplification profiles of the 12 Niger plant genotypes and 1 sunflower genotype that was used as a check from the same family were scored by visual observation. The presence of amplified bands at each position was recorded as 1 (one) and its absence as 0 (zero). The pair-wise genetic similarities were computed using Jaccard's similarity coefficient and a corresponding dendogram of genetic relatedness was constructed by applying Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm.

3.6 Determination of genetic similarities

Similarity matrices for ISSR were subjected to Unweighted Pair Group Method with Arithmetic Mean (UPGMA). A dendogram was constructed from molecular data by the Unweighted Pair Group Method (UPGM) and clustering using sequential agglomerative hierarchical nested (SAHN) program and tree plot of Numerical Taxonomy Multivariate Analysis System Package (NTSYS-pc) software, version 2.1.

3.7 Results and discussion

3.7.1 Microsatellite (ISSR) analysis

The study involved the use of twenty primer sets to amplify DNA extracts from 12 wild Niger plant populations and 1 sunflower genotype. All the twenty primers (100%) gave polymorphic bands thus they were all considered for further analysis. This percentage was higher than that reported by Yohhanes *et al.*, (2007) who obtained 89.83%. A total of 73 alleles were detected. The number of alleles detected per locus ranged from 2 to 5 with an average of 3.65 (Table 2).

3.7.2 Polymorphic information content (PIC) of markers

The markers with high polymorphic information content of more than 0.5 were P2976, P2941, P2903, P2955, P2906, P2961, P2934, P2923, P2922, P2904, P2964, P3013, P2998, P2956 and P2911 with 0.6841, 0.6731, 0.6731, 0.6727, 0.6727, 0.6580, 0.6580, 0.6560, 0.6409, 0.6197, 0.6130, 0.6039, 0.5361, 0.5361 and 0.5353 PIC respectively. Markers P2999, P2924, P2939 and P2909 had a PIC of 0.4836, 0.4836, 0.4652 and 0.2920 respectively (Table 2).

The polymorphic information content (PIC) value was calculated to characterize the capacity of each primer to detect polymorphic loci which ranged from 0.2920 to 0.6841 with a mean of 0.5725. The result showed that most of the primers were highly informative and can be used to study phylogenetic relationship and genetic diversity in future. The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character (Asare *et al.*, 2011). Gene diversity was high ranging from 0.3550 to 0.7337 with a mean value of 0.6302.

Table 2: Polymorphism, diversity and frequency results

Marker	Major allele frequency	Sample size	Allele no.	Gene diversity	PIC
P2903	0.4615	13	5	0.7101	0.6731
P2904	0.4615	13	4	0.6746	0.6197
P2906	0.3846	13	4	0.7219	0.6727
P2909	0.7692	13	2	0.3550	0.2920
P2910	0.7692	13	2	0.3550	0.2920
P2911	0.4615	13	3	0.6154	0.5353
P2922	0.3846	13	4	0.6982	0.6409
P2923	0.4615	13	5	0.6982	0.6560
P2924	0.6154	13	3	0.5444	0.4836
P2934	0.3846	13	4	0.7101	0.6580
P2939	0.5385	13	3	0.5562	0.4652
P2941	0.4615	13	5	0.7101	0.6731
P2955	0.3846	13	4	0.7219	0.6727
P2956	0.5385	13	3	0.6036	0.5361
P2961	0.3846	13	4	0.7101	0.6580
P2964	0.3846	13	4	0.6746	0.6130
P2976	0.3077	13	4	0.7337	0.6841
P2998	0.5385	13	3	0.6036	0.5361
P2999	0.6154	13	3	0.5444	0.4836
P3013	0.4615	13	4	0.6627	0.6039
Mean	**0.4885**	**13**	**3.6500**	**0.6302**	**0.5725**

An Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendogram based on pair wise comparison of genetic distance of 12 Niger plant genotypes based on ISSR data was as shown in Figure 2. Populations from Chepkoilel, Kimoning, Kaptuktuk, Merewet, Tembelio, Kapsaos and Kimumu were grouped together in one cluster, while Sergoit formed its own distinct cluster. Three populations (Huruma, Moiben and Sigot), were in the same cluster leaving out Cheplaskei with its own cluster. The genetic distance revealed that the closest genotypes were Sunflower, Merewet and Tembelio (Fig. 2).

The high genetic diversity exhibited by the Niger plant can be ascribed to the outcrossing mode of pollination exhibited by the plant. Niger plant is pollinated by

insects, mainly bees (Getinet and Sharma 1996). There was no heterozygosity observed from the results. This is in contrast with Zakir *et al.,* (2015) who did a study in Ethiopia using ISSR and obtained a heterozygosity ranging from 0.245 (in primer 2976) – 0.497 (in 2939 and 2904). Lack of heterozygosity is a subject of further research with different primers to ascertain the true position. Since Niger plant grows wild in the area of study, breeding is not controlled and the plant relies on natural pollination. Growing in diverse environmental conditions within Moiben sub-county, the plant might have been forced to evolve differently for its survival thus the high genetic diversity.

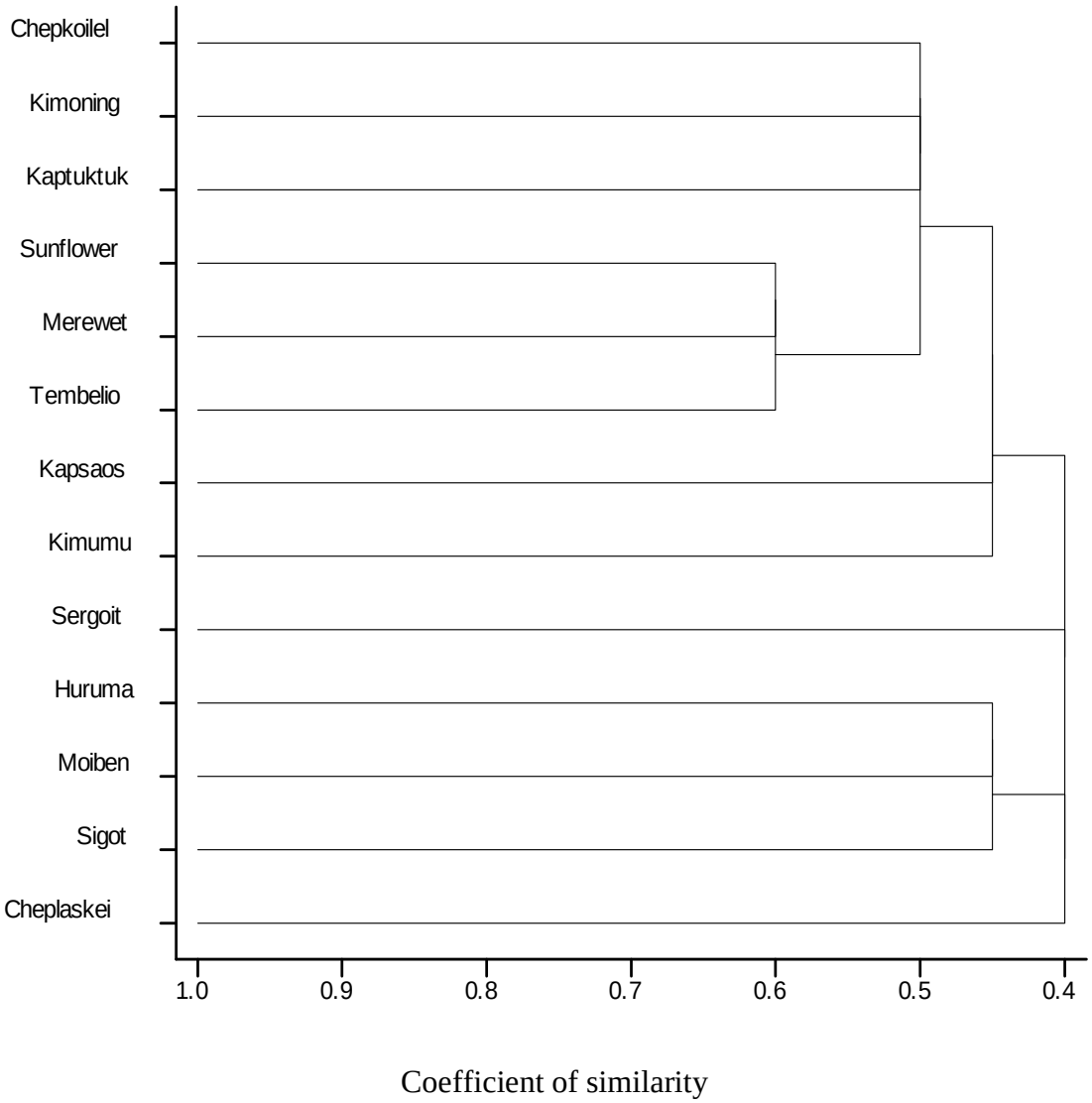


Fig. 2: Clustering by region for Niger plant from Moiben sub-county

Sunflower* is not a site but is a plant that was used as a check because it is in the same family with Niger plant.

While the general trend in the UPGMA clustering is that of grouping populations by region of origin and proximity of geographic location of the collection sites, not all populations, however, belonging to the same region were grouped together in the same cluster (Fig. 2). This observation was also made by Yohannes *et al.*, (2007). This could be because of the continuity of the Niger growing areas which makes the transfer of seed materials from one region to the other possible. Being perceived as a weed, Niger plant growth in the region remains uncontrolled. The fact that Niger plant is strictly cross pollinated aggravates the situation.

The genetic distance between Cheplaskei population and those from other areas is large indicating some sort of genetic isolation of the Cheplaskei population from those of other regions. This led to Cheplaskei populations forming a separate cluster in the dendogram clustering.

3.8 Conclusions and recommendations

3.8.1 Conclusions

1. Niger plant populations within Moiben sub-county are genetically diverse.
2. There was no heterozygosity in the Niger plant populations studied.

3.8.2 Recommendation

Further study should be carried out in other areas with different agro-ecological zones.

CHAPTER FOUR

DETERMINATION OF TYPES AND QUANTITIES OF SECONDARY METABOLITES IN NIGER PLANT (*Guizotia abyssinica* L.) FOUND WITHIN MOIBEN SUB – COUNTY, KENYA

4.1 Abstract

Plants are a major source of active chemical constituents that can be used against other plants. From natural ecosystems, it has been found that exudates from some plants influence the growth and development of other plants. Niger plant (*Guizotia abyssinica*) exudes secondary metabolites phenols, flavonoids, tannins and saponins that influence plant growth either growing together or in the succeeding season. The aim of this experiment was to extract, identify and quantify the secondary metabolites in Niger plant collected from within Moiben sub-county, Uasin Gishu county, Kenya where it grows wild. Plant materials were collected from all administrative wards in Moiben sub-county and secondary metabolites extracted by standard procedures. The extracts were made from the whole plant, roots and shoots. Data were collected on the levels of alkaloids, flavonoids, phenols, saponins and tannins. Data were analyzed by ANOVA in Genstat and Duncan's Multiple Range Test (DMRT) used to separate means. All the five metabolites; alkaloids, flavonoids, phenols, saponins and tannins were present in the plant samples at different quantities. Niger plant contains many metabolites thus further studies should be done for better understanding and application of their allelopathic effect for weed control.

4.2 Introduction

Plants produce chemical compounds as a result of secondary metabolism. The chemical compounds cannot be directly linked with plant growth and development but can influence several processes in the ecosystem. These compounds are known as secondary metabolites or allelochemicals. Secondary metabolites are non-nutritional thus are produced in stressful conditions for defense (Khan *et al.*, 2008).

Due to this, plants can regulate the microbial community in their immediate vicinity, endure herbivores, encourage symbiotic improvement, change the physical and chemical properties of the surrounding environment and inhibit the growth of plant competitive species (Pedrol and Salguero 2012). Allelopathy can either be negative

where the activities of the recipient plant are hindered, or positive where the activities of the recipient plant are enhanced (Eichenberg *et al.* 2014). Allolopathy can affect many plant processes thus dictating the use of a specific piece of land (Chon *et al.*, 2006). Due to their suppressive effect, allelochemicals can be used for weed control and thus in the synthesis of new herbicides.

Wise exploitation of allelopathy in cropping systems may be an effective, economical and natural method of weed management, and a substitute for heavy use of herbicides. Allelochemicals have a great potential as bio-herbicides since they have a shorter half -life and thus can be rapidly biodegraded. Their mode of action is different from synthetic herbicides owing to comparatively fewer halogen substituents and no unnatural ring structures (Roth *et al.*, 2000). Allelochemicals, also known as phytochemicals, are environmentally friendly because they have low or no toxicity to animals and beneficial insects, possess an array of activity with varying and diverse sites of action and have a comparatively high degradation rate (Cloyd, 2004).

Plants in the family Asteracea have been noted to be highly allelopathic (Roth *et al.*, 2000). It has been observed that, Niger plant, an Asteracea, is a good precursor for cereals, pulses and oil seeds, because crops following Niger plant have less weed infestation (Adarsh *et al.*, 2014). This means that a field previously infested with Niger plant when put under production of cereals, pulses and oil crops have fewer weeds. This study was therefore carried out to determine the presence and amount of secondary metabolites in Niger plant within Moiben sub-county.

4.3 Objectives

i. To determine the presence and quantity of major secondary metabolites in whole Niger plant from various sites within Moiben sub-county.

ii. To determine the presence and quantity of secondary metabolites in the shoots and roots of Niger plant from various sites within Moiben sub-county.

4.4 Materials and methods

A laboratory experiment was conducted in the department of Chemistry at University of Eldoret. The metabolites evaluated were phenols, saponins, tannins, flavonoids and alkaloids since they are among the major metabolites in plants. Selection of the metabolites was because they are the most abundant in Niger plant that are linked to allelopathy.

4.4.1 Sample collection and extract preparation

Complete Niger plant samples were harvested at flowering stage from the six administrative wards in Moiben sub-county where they grow wild as weeds. The samples were kept in zip lock bags and carried in a cooler box to University of Eldoret Chemistry laboratory. The samples were divided into two whereby in one half, the shoots were separated from the roots. In the other half, the samples were treated as whole plants. The plant samples were washed to remove dirt and oven dried at 72^0 C till constant weight. Using a hand held electric grinder, the samples were separately ground into fine powder. The powder was transferred into labelled 500 ml conical flasks into which distilled water was added. The mixture was stirred then left to stand for 2 hours after which it was filtered using a sieve then by Whatman filter paper (No. 1). The filtrate was collected in a beaker for further analysis.

4.4.2 Phytochemical tests

Equal amount of each extract was dispensed into six test tubes for screening. The evaluation tests were done according to standard protocol of each metabolite as

explained in Appendix II. The procedure used was suggested by Makkar & Goodchild (1996).

4.4.3 Quantification of metabolites

The various procedures followed to determine the amount of secondary metabolites are as explained in Appendix III by procedures proposed by Bray and Thorpe (1954), Harborne (1973) and Jia *et al*. (1999).

4.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Genstat version 14. Means were separated by Duncan's Multiple Range Test (DMRT) at 5 % level of probability.

4.6 Results and discussion

The results of the study presented in Tables 3, 4 and 5 below show the amount of secondary metabolites studied in extracts of the whole Niger plant, shoots and roots from different study sites within Moiben sub-county.

Table 3. Results of phytochemical analysis of Niger plant extracts from various sites (whole plant).

Collection site	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	1.60a	1.27a	2.07bc	0.57a	2.17a
Kimoning	1.53a	1.07a	1.90ab	0.53a	2.33a
Kaptuktuk	1.50a	1.03a	1.83a	0.60a	2.47a
Merewet	1.60a	1.17a	2.03abc	0.57a	2.57a
Tembelio	1.57a	1.07a	2.00abc	0.57a	2.47a
Kapsaos	1.50a	1.30a	2.17c	0.57a	2.30a
Kimumu	1.50a	1.23a	2.13bc	0.60a	2.57a
Sergoit	1.53a	1.10a	2.07abc	0.53a	2.63a
Huruma	1.50a	1.10a	2.13bc	0.57a	2.43a
Moiben	1.60a	1.17a	2.07abc	0.63a	2.67a
Sigot	1.57a	1.20a	1.97abc	0.63a	2.43a
Cheplaskei `	1.47a	1.13a	1.90ab	0.57a	2.27a
Mean	**1.54**	**1.15**	**2.02**	**0.58**	**2.44**
DMRT	0.09	0.14	0.10	0.08	0.24
CV%	6.9	15.2	6.0	17.8	12.2

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

Table 4. Results of phytochemical analysis of Niger plant extracts from various parts of Moiben sub county (Roots)

Collection site	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	1.00a	1.07b	1.77b	0.37a	1.67a
Kimoning	1.13a	0.80ab	1.53ab	0.27a	2.07a
Kaptuktuk	0.97a	0.83ab	1.30a	0.40ab	1.80a
Merewet	1.03a	0.90ab	1.53ab	0.43ab	1.83a
Tembelio	1.03a	0.90ab	1.70b	0.27a	2.07a
Kapsaos	1.07a	1.03ab	1.80b	0.30a	1.93a
Kimumu	1.03a	0.90ab	1.60ab	0.40ab	2.03a
Sergoit	1.20a	0.77a	1.57ab	0.27a	2.23a
Huruma	1.03a	0.90ab	1.60ab	0.40ab	1.97a
Moiben	1.03a	0.93ab	1.57ab	1.23b	2.17a
Sigot	1.07a	0.97ab	1.44ab	0.30a	1.80a
Cheplaskei	0.97a	0.80ab	1.30a	0.30a	1.80a
Mean	**1.05**	**0.9**	**1.56**	**0.41**	**1.95**
DMRT	0.13	0.12	0.16	0.37	0.26
CV%	15.80	15.90	12.60	31.40	16.60

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

Table 5. Results of phytochemical analysis of Niger plant extracts from various parts of Moiben sub county (Shoots)

Collection site	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	0.67a	0.37a	0.50a	0.47ab	0.60a
Kimoning	0.43a	0.40a	0.47a	0.43ab	0.53a
Kaptuktuk	0.50a	0.37a	0.53a	0.40ab	0.80a
Merewet	0.53a	0.40a	0.57a	0.37ab	0.77a
Tembelio	0.50a	0.37a	0.53a	0.47ab	0.50a
Kapsaos	0.43a	0.57a	0.67a	0.33a	0.53a
Kimumu	0.60a	0.43a	0.47a	0.47ab	0.70a
Sergoit	0.50a	0.47a	0.53a	0.40ab	0.57a
Huruma	0.50a	0.40a	0.60a	0.33a	0.63a
Moiben	0.63a	0.33a	0.50a	0.43ab	0.57a
Sigot	0.53a	0.43a	0.63a	0.50b	0.73a
Cheplaskei	0.60a	0.47a	0.50a	0.33a	0.70a
Mean	**0.54**	**0.42**	**0.54**	**0.41**	**0.64**
DMRT	0.12	0.11	0.15	0.07	0.13
CV%	27.7	32.1	34.8	20.1	24.6

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

4.6.1 Phenols

Results showed that the whole plant, shoot and root extracts contained phenols though at varying levels. The level of phenols was highest in the whole plant extract, root and

shoot extracts respectively (Table 3, Table 4 and Table 5). As pertains to site, there were significant differences in the level of phenols where Chepkoilel, Kimumu and Huruma were not significantly different from one another. Levels in Kimoning and Cheplaskei did not show any significant differences amongst themselves whereas Merewet,Tembelio, Sergoit, Moiben and Sigot did not have any significant differences. In Kaptuktuk and Kapsaos, alkaloid levels were significantly different from one another and distinct from all the rest of the sites. The highest levels of phenols were recorded in Chepkoilel, Sergoit and Moiben at 2.07 mg/g while the lowest amount was in Kaptuktuk (1.83 mg/g).

The levels of phenols in the roots showed significant differences among the sites. Chepkoilel, Tembelio and Kapsaos samples had no significant differences amongst them whereas there were no significant differences between Kimoning, Kaptuktuk, Kimumu, Sergoit, Huruma, Moiben and Sigot sites. Kaptuktuk and Cheplaskei sites did not have significant differences amongst them. There were no significant differences in the amount of secondary metabolites in the shoots.

4.6.2 Tannins

Tannins are natural products of secondary metabolism. They are found distributed in all plant parts. Samples collected from Moiben site had the highest mean of all metabolites (1.63 mg/g) followed by Sergoit site (1.61 mg/g). The least mean for all the metabolites was recorded in samples from Cheplaskei (1.47 mg/g).

4.6.3 Saponins

Saponin levels were significantly different amongst the sites both in the shoots and roots. In the root samples, Chepkoilel, Kimoning, Tembelio, Kapsaos, Sergoit, Sigot and Cheplaskei did not have significant differences among them. Kaptuktuk samples had significant differences from all the rest whereas Merewet, Kimumu and Huruma

did not have significant differences amongst them but were significantly different from the rest. In shoot samples, there were three clusters of similarity: Chepkoilel, Kimoning, Kaptuktuk, Merewet, Tembelio, Kimumu, Sergoit and Moiben were not significantly different from one another. Kapsaos, Huruma and Cheplaskei samples were significantly different from the rest of the sites. Samples from Sigot showed unique results that were significantly different from all the rest.

4.6.4 Alkaloids

There were no significant differences in the level of alkaloids from all the sites both in the roots and shoots.

4.6.5 Flavonoids

There were significant differences in the level of flavonoids in the roots. Here, Chepkoilel and Sergoit sites were distinctly different from one another and from all the rest of the sites. However, there were no significant differences in the levels of flavonoids in the shoots.

Results of the study revealed that Niger plant contains secondary metabolites at varying levels in the whole plant, roots and shoots.

Extracts from the whole plant had significant differences only in phenols. Here, Kaptuktuk had significant differences from all the other sites. The highest levels of phenols were recorded in Kapsaos while the lowest were in Kaptuktuk. All the other sites had intermediate levels between the two ranges.

In the root extract, there were no significant differences in the level of alkaloids and tannins. Flavonoid levels were not significantly different in Kimoning, Kaptuktuk, Merewet, Tembelio, Kapsaos, Kimumu, Huruma, Moiben, Sigot and Cheplaskei.

However, there were significant differences between all the above sites, Chepkoilel and Sergoit. On levels of phenols, Kaptuktuk and Cheplaskei were significantly different from Chepkoilel, Tembelio and Kapsaos which were in turn significantly different from Kimoning, Merewet, Kimumu, Sergoit, Huruma, Moiben and Sigot. Saponins revealed Chepkoilel, Kimoning, Tembelio, Kapsaos, Sergoit, Sigot and Cheplaskei sites were significantly different from Kaptuktuk, Merewet, Kimumu and Huruma sites. Sergoit site was significantly different from all the other sites too.

In shoots, significant differences were recorded only in saponins. The highest levels of saponins were in Sigot (0.50 mg/g) and he lowest was in Kapsaos, Huruma and Cheplaskei all with 0.33 mg/g. The remaining sites had between 0.37 mg/g to 0.47 mg/g and were not significantly different from one another.

There were differences in the levels of secondary metabolites in different parts of the plant. However in some, the levels in the roots were the same as those in the shoots. This can be attributed to the natural distribution of the metabolites in the plant parts where they are produced.

Different sites also revealed differences in the level of some metabolites in Niger plant. According to Ncube *et al.,* (2012), ecological factors play a big role in affecting plant secondary metabolites. It is an inherent trait in plants that they resist biological, physical and chemical environmental stresses by regulating the accumulation of secondary metabolites in long periods of adaptation to the environment (Ferreyra *et al.,* 2012). Thus the present results can be attributed to the environmental conditions in the area that the Niger plants were collected from. Some factors have been singled out as having an effect on metabolite concentration in plants. Studies by Zobayed *et al.* (2005) have shown that heat stress is an important environmental factor in promoting accumulation

of secondary metabolites for *Hypericum* (*Hypericum perforatum*). This means that same plants exposed to different temperature levels can have different levels of metabolites.

Differences in soil conditions may contribute to the differences in plant metabolite levels in different sites. Inorganic elements in the soil have a major impact on the accumulation of secondary metabolites. It has been shown that different kinds of ecological factors have different extents of effects on different secondary metabolites of the root of *S. baicalensis* (Abdul *et al.*, 2009). Inorganic elements in the soil including Mg, Mn, Cr and Fe affect different secondary metabolites (Zhao and Guo, 2010).

4.7 Conclusions and recommendations

4.8.1 Conclusions

1. Niger plant contains phenols in both the roots and shoots at varying concentrations.
2. Tannins were the least secondary metabolites in Niger plant among those that were studied.
3. Saponis were present in Niger plant but the levels were significantly different amongst the sites both in the shoots and roots.
4. Niger plant contains alkaloids though there were no significant differences from all the sites both in the roots and shoots.
5. There were significant differences in flavonoid levels in the roots but the levels were not significantly different in the shoots.

4.8.2 Recommendation

Follow-up studies should aim to extrapolate these results and extend to the phytochemistry and biological activity of secondary metabolites.

CHAPTER FIVE

INFLUENCE OF NIGER PLANT (*Guizotia abyssinica* L.) ON WEED ABUNDANCE AND GROWTH AND DEVELOPMENT OF COMMON BEANS (*Phaseolus vulgaris* L.)

5.1 Abstract

Plants release many secondary metabolites to the environment that can be harnessed for important uses. These secondary metabolites are known as allelochemicals. The current worldwide demand for cheaper, more environmentally-friendly weed management technologies has motivated a number of studies on the allelopathic interaction between crops and weeds. Niger plant has been observed to have allelopathic effects on certain weeds. In order to evaluate the influence of Niger plant on common beans and selected weeds, a 4 x 3 experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates. Treatments included weedy check (no weed control measure, having all weeds including Niger plant), weed free, Niger plant intercrop and all weeds except Niger plant. Three cultivars of beans (Rosecoco, Mwitemania and Mwezi Mbili) were used. Data collection on beans included stand count at two weeks, plant height at 50% flowering, number of pods per plant, stand count at harvesting and number of seeds per plant. On weed species, data collected included the total number of four prominent weeds over a span of four weeks. A 50 x 50 cm quadrat was laid on the same spot in all the treatments and the weeds enclosed within it were counted separately. Data analysis was done by ANOVA in Genstat and results presented using graphs and tables. Results showed that Niger plant enhanced bean growth and development whereas it inhibited the germination and growth of some weeds i.e. field mustard, broom weed, double thorn and couch grass. It was concluded that Niger plant exhibited negative allelopathy on the weeds that were studied and positive allelopathy on all the bean cultivars. From the results it is recommended that further research be carried out on more crops and more weeds so as to have an in-depth understanding of this subject.

5.2 Introduction

Niger plant (*Guizotia abyssinica*), is a herbaceous green plant with bright yellow flowers in the Family Asteraceae. In Kenya, Niger plant is considered a weed especially in the highlands of the North Rift Valley where cereals are extensively grown. However, in Ethiopia and India, Niger plant is cultivated for production of edible oil at 51% and 20% respectively of all edible oil used (Patil *et al.,* 2013).

Weed infestation is a major concern to crop production especially in the tropics where much time and labour are devoted to weed control. It is estimated that about 50-70% of the labuor in crop production is spent weeding (Chikoye, *et al.,* 2007). In Africa, yield losses due to weeds range from 25% to total crop failure. Weeds cause yield loss in crops through both competition for water, light and nutrients and by allelopathy (Zohaib *et al,* 2016). Coexistence with weeds can modify plant morphology biomass accumulation, plant growth and, successively, the yield of crops of interest by interfering with different metabolic processes (Wandscheer *et al.,* 2013).

In light of the losses caused by weeds, and given the fact that human population is ever increasing and thus stretching the demand for food, weed control is a major concern thus weed- mediated decline in crop production needs urgent intervention so as to attain high yields and achieve food security. For economic purposes, weed control techniques attempt to achieve a balance between cost of control and crop yield lost. Herbicide discovery in the 1950s was a major boost to crop production. However, the indiscriminate use of herbicides worsens the quality of soil, water, other life support systems, human health and food coupled with herbicide resistance. As a result of the increasing awareness of the adverse toxicological effects of synthetic herbicides, one of the recent trends in weed management is to reduce heavy reliance on synthetic herbicides and to move towards low input sustainable agriculture (LISA) (Nikneshan *et al.,* 2014). One of the promising alternatives to herbicide use is allelopathy.

Allelopathy is a phenomenon of growth interference of one plant on another through the release of chemicals from another plant into the environment (Inderjit & Callaway 2003). The chemicals released are known as allelochemicals. Plants with allelopathic potential help to reduce weed intensity, and hence improve crop productivity when intercropped with other plants (Saudy, 2015). The allelochemicals, also known as

secondary metabolites, are liberated from plants and affect the germination and growth of recipient plants (Asaduzzaman *et al.,* 2013). According to Gallandt *et al.,* (1999), allelochemicals affect weed dynamics by reducing and delaying seed germination and establishment, in addition to suppressing individual plant growth resulting in an overall decline in the density and vigor of the weed community. Allelopathy can be exploited for weed suppression, and can thus be helpful in reducing reliance on herbicides (Weston *et al.,* 2013).

Allelochemicals released by plants include phenolics, flavonoids or terpenoids (Macías *et al.,* 2007). Wise exploitation of allelopathy in cropping systems may be an effective, economical and natural method of weed management, and a substitute for heavy use of herbicides. Allelochemicals usually have a mode of action different from synthetic herbicides, being more easily and rapidly degradable owing to a shorter half-life with comparatively fewer halogen substituents and no unnatural ring structures (Roth *et al.,* 2000). Because of this, allelochemicals have low or no toxicity to animals, have different sites of action and degrade faster in the environment (Cloyd, 2004). To exert effect on the recipient plants, allelochemicals may influence vital physiological processes such as respiration, photosynthesis, cell division and elongation, membrane fluidity, protein biosynthesis and activity of many enzymes, and may also affect tissue water regime (Field *et al.,* 2006).

Plants in the family Asteracea have been noted to be highly allelopathic. It has been observed that, Niger plant, a plant in this family, is a good precursor for cereals, pulses and oil seeds, because crops following Niger plant in a rotation have less weed infestation (Adarsh *et al.,* 2014) implying that the previous crop of Niger plant exudes some chemicals into the rhizosphere that affect growth of other plants. Niger plant also

contributes to conservation of soil health and land rehabilitation because of its mycorrhizal relationship and its potential as a bio-fertilizer.

Even with the ongoing advances in research on allelopathy, the knowledge gap is still vast. The effect of Niger plant secondary metabolites on crops has not been studied to combine its effects on both crops and weeds. This study was therefore carried out to evaluate the influence of Niger plant on bean growth and development and specific weed abundance over time. There is a need to expand on the knowledge of interference mechanisms of Niger plant in order to better understand its success as a weed, and to seek ways to harness its success in improving crop production.

5.3 Objectives

i. To evaluate the allelopathic effect of Niger plant on growth and development of beans.
ii. To determine the weed suppressive activity of Niger plant in the field.

5.4 Hypotheses

H_a: Niger plant has an allelopathic effect on growth and development of beans.

H_a: Niger plant has a suppressive ability on weeds in the field.

5.5 Materials and methods

5.5.1 Study site

Field experiments were conducted at the University of Eldoret Research farm for two seasons from September – December 2017 and December – February, 2018. The area lies at an altitude of 2100 m above sea level and a longitude of 35^o 18’ E and 0^o 30’ N

latitude. Rainfall is relatively high at 730 mm with an annual temperature range between 9.5^0 C and 23.5^0 C respectively.

5.5.2 Experimental treatment, design and plot lay out

Experimental treatments

The experiment involved growing three cultivars of beans under four different weed regimes. The weed regimes included a weedy treatment (W) where there were all weeds including Niger plant, a treatment with only Niger plant growing amongst the beans (NP), weed free treatment, (WF) and a treatment that had all weeds growing except Niger plant (All – N.P). A weedy treatment was achieved by letting all the weeds, including Niger plant, that could germinate to grow together with the beans for the entire period. In a Niger plant intercrop treatment, all weeds were removed except Niger plant which was allowed to grow with the beans. Since the Niger plant germinated on its own, its distribution did not follow any pattern. The study area lies within Kimumu sub location so the Niger plant here as studied earlier had alkaloids 1.50 mg/g, flavonoids 1.23 mg/g, phenols 2.13 mg/g, tannins 0.60 mg/g and saponins 2.57 mg/g. From the dendogram clustering, Niger plant in Kimumu sub location was closest with that of Kapsaos and in the same cluster with that of Tembelio, Merewet, Kaptuktuk, Kimoning, Chepkoilel and the sunflower check.

Weed free treatments had all the weeds removed as soon as they were spotted. In treatments with all weeds except Niger plant, only Niger plant was weeded out leaving all the other weeds to grow with beans. Hand weeding was done by uprooting by. In the field layout, these were represented as T1, T2, T3 and T4 respectively.

The three bean cultivars included Rosecoco (V1), Mwitemania (V2) and Mwezi Mbili (V3) commonly grown by farmers in the region. The choice of the study site was guided by presence of Niger plant weeds in the earlier cropping season.

5.5.2 Experimental design and field lay out

The experimental design for the experiment was a 3x4 factorial arranged in Randomized Complete Block Design (RCBD) and replicated three times. The dimensions of each plot were 1.05 by 0.7 metres, separated by a 0.5 metres path. The beans were spaced at 15 x 10 cm giving a total of 49 plants per plot separated by a 0.5 m path. This is shown in Figure 3 below.

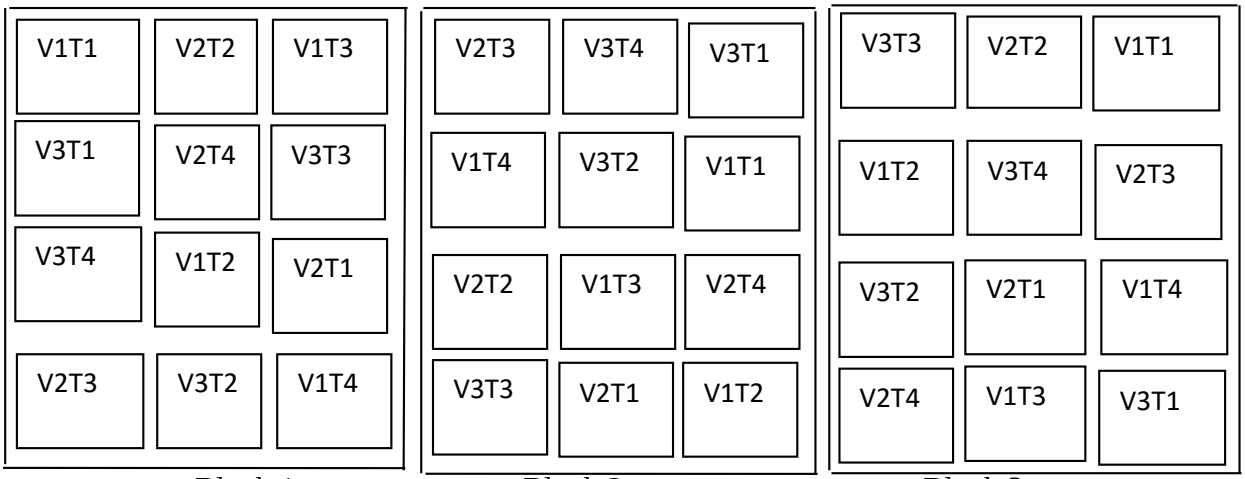


Fig. 3: Field lay out.

Where: **T1** -Weedy

T2- Niger plant intercrop

T3- Weed free

T4- All weeds except Niger plant

V1- Rosecoco

V2- Mwitemania

V3- Mwezi Mbili

5.5.3 Establishment of field experiment and management

The field was dug to a fine tilth targeting 15 cm of the top soil. Animal manure was broadcast on the soil surface until planting time when it was incorporated into the soil. Following the pre-planned design shown in Fig. 3, the field was marked ready for planting. Three cultivars of beans were planted at a uniform spacing of 15 cm by 10 cm.

5.5.4 Parameters measured

Data were collected on the following parameters:

Weed abundance

A 50 x 50 cm quadrat was laid at the center of each plot the specific weeds within the quadrat counted. This was done for four consecutive weeks. The quadrat was laid on the same spot each time. The weed species on which data were collected were:

i. Field mustard (*Brassica rapa*)
ii. Broom weed (*Gutierrezia sarothrae*).
iii. Double thorn (*Oxygonum sinuatum*)
iv. Niger plant (*Guizotia abyssinica*)
v. Couch grass (*Cynodon dactylon*)

Stand count at two weeks post emergence

This was done by physically counting the number of the emerged bean seedlings two weeks after planting. It involved all the seedlings per plot in the field.

Plant height at 50% flowering

To determine the plant height, a ruler was placed at the base of the soil and the highest point of the plants on the ruler was recorded in centimeters. A sample of nine plants, three per row from the middle rows was taken and used for determination of height.

Number of pods per plant

The number of pods per plant was done at the time of maturity when podding was completed. The pods were physically counted from a sample of beans comprising of nine plants, three per row from three middle rows that were selected randomly.

Stand count at harvesting

The number of standing plants in each plot was taken by counting all the plants at the time of harvesting.

Number of seeds per plant

After harvesting when all the pods were dry, a sample of bean plants was taken and threshed separately. The samples were all counted and those with even numbers selected. The seeds were counted and their number recorded.

5.6 Statistical model

$Y_{ijk} = \mu + \beta_i + W_j + V_k + W*V_{jk} + \Sigma_{ijk}$

Where: Y_{ijk} = Observations on experimental units due to ijk^{th} factor

μ = General mean

β= effect due to blocks

W = effect due to j^{th} weed regime

V= effect due to k^{th} cultivar

W*V = Interaction effect of weed regime and cultivar

Σ = Experimental error

5.7 Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using Genstat version 14 and means separated by Duncan's Multiple Range Test (DMRT) at 5% level of probability.

5.8 Results and discussion

5.8.1 Effect of Niger plant on abundance of selected weeds

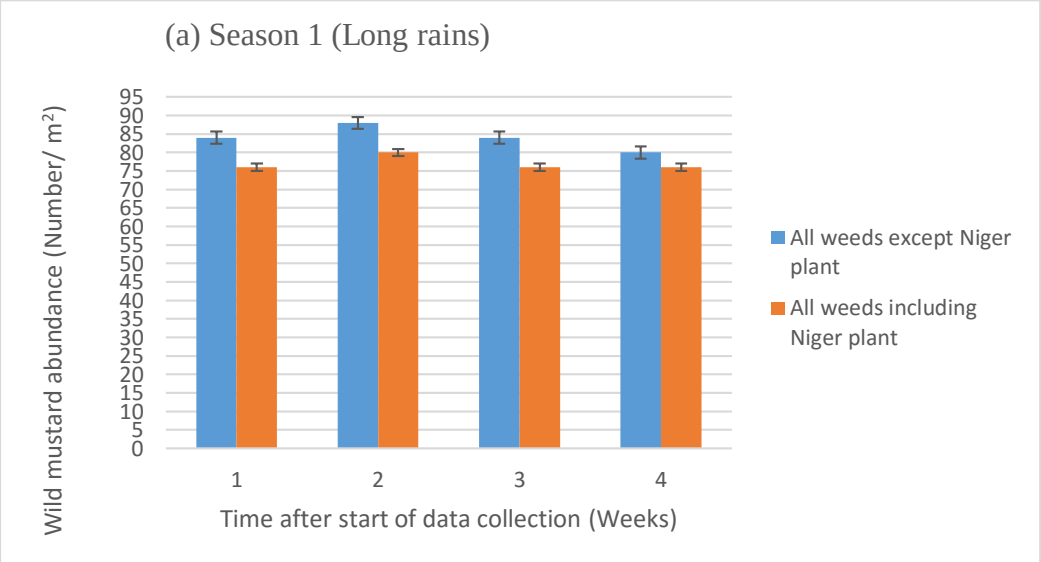
Effect of Niger plant on abundance of Wild mustard

Results of the study showed that in season 1, there were significant differences between the two weed regimes from which data were collected on the abundance of wild mustard (Fig. 4). In the course of the four-week period through which data was collected, amongst the specific weed regimes, there were variations in weed abundance though the variations were not statistically significant. In all weeds except Niger plant regime, weed abundance started at 84 in week 1, rose to 88 in week 2 before falling back to 84 in week 3. In week 4, the weed abundance was 80. In weedy regime, week 1 had 76 weeds which rose to 80 in week 2 before settling at 76 in both week 3 and 4.

From the results, it is clear that all weeds except Niger plant regime had higher abundance of Wild mustard than the abundance in weedy regime. This can be because in weedy regime, the presence of Niger plant suppresses the germination and growth of wild mustard. In all weeds except Niger plant regime, there was no Niger plant effect thus

higher abundance of Wild mustard. El- Rokiek *et al*., (2010) in their study illustrated that mango leaves induced significant reduction in the growth of mother tubers in purple nut sedge. Growth inhibition in weeds recorded by many allelopathic plants is in response to accumulation of phenolic compounds indicating allelopathic stress (El-Rokiek, 2007).

In season 2, there were no significant differences in the first three weeks. Significant differences were noted in week four where all weeds except Niger plant regime was significantly higher than in weedy regime. This can be attributed to the accumulating effect of the Niger plant allelopathy in the soil to levels that were injurious to other weeds. Sisodia and Siddiqui (2010) conducted a study on the allelopathic effects of *Croton bonplandianum* on germination and seedling growth and concluded that effect was found to increase with increasing concentrations of different aqueous extracts.



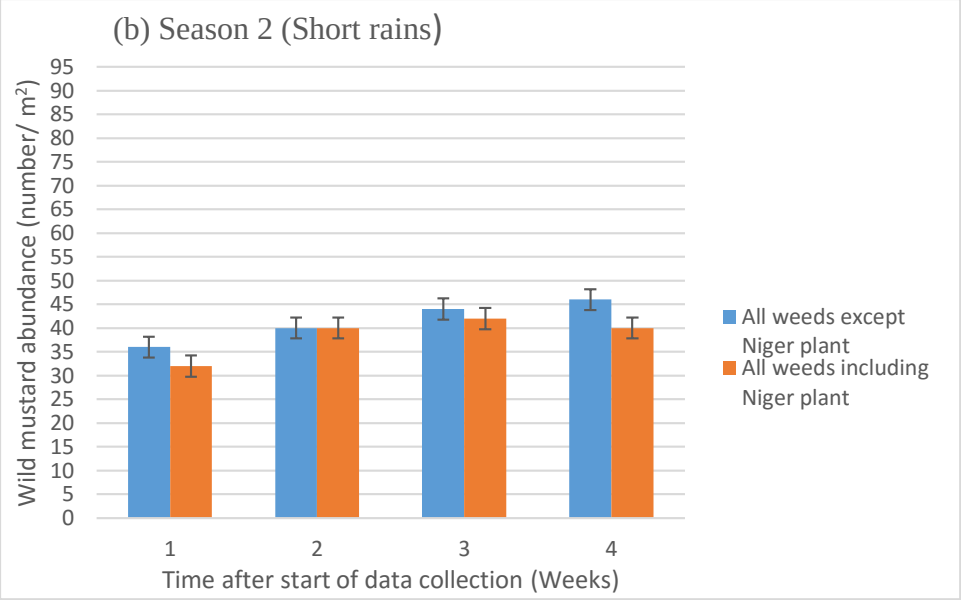


Fig. 4: Effect of Niger plant on abundance of Wild mustard

Effect of Niger plant on abundance of Broom weed

The results of the study shown in Figure 5 below revealed that there were significant differences between the two weed regimes in all the four weeks of season 1 and the first week of season 2. Season 1 had high weed abundance in all weeds except Niger plant regime and this can also be due to the absence of Niger plant in the immediate vicinity to exert allelopathic effects. Presence of Niger plant in the weedy regime may have led to the introduction of allelochemicals to the soil that suppressed weed germination and growth. These results are in agreement with Ejaz *et al.*, (2015), who found out that allelopathic chemicals in Tobacco and Eucalyptus significantly suppressed weeds by reducing weed density.

In season 2, there was lower abundance of Broom weed in week 1 than in week 2. This observation may have been due to delayed germination occasioned by Niger plant allelochemicals in the soil. A study by Herro & Callaway (2003) showed in some plant species, allelochemicals cause delayed germination and reduction in in seedling growth. Since the Broom weed delayed to germinate, the

seedlings may have faced stiff competition for resources from the already established ones. This may have led to the reduction in the number of Broom weed observed in week 3 and 4.

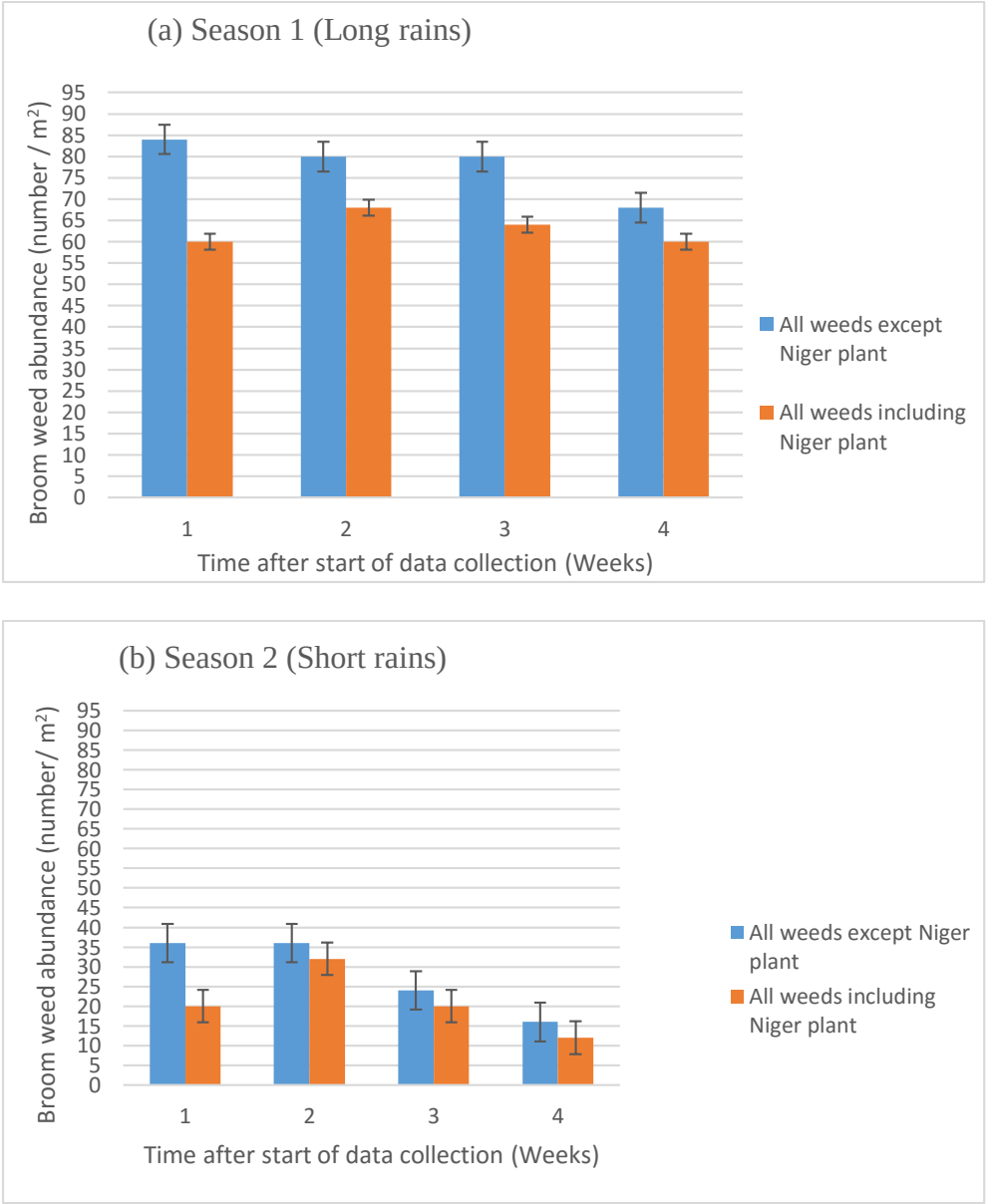


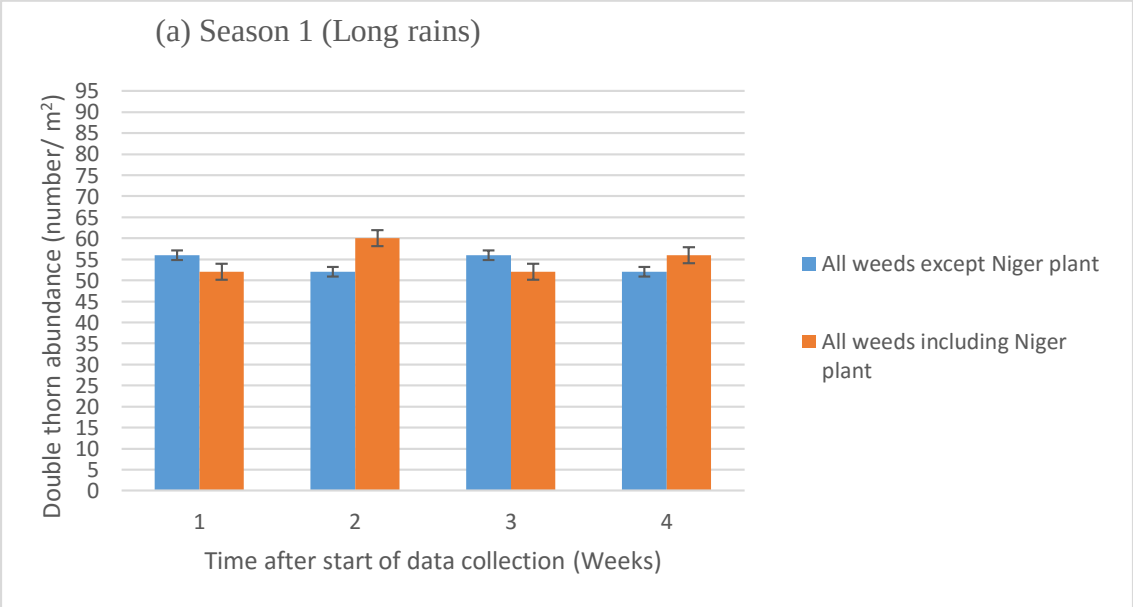
Fig. 5: Effect of Niger plant on abundance of Broom weed

Effect of Niger plant on abundance of Double thorn

In double thorn, there were significant differences in season 1. In season 2, significant differences were in week one. There was low germination percentage in weedy regime in season 1. High abundance was recorded in season 2 but the newly emerged seedlings

could not survive. This led to numbers decreasing sharply from week 2 to week 4. In all weeds except Niger plant regime, germination was high and the number was maintained to the second week when sharp increases were noticed. Low germination percentage could be as a result of enzyme and hormone interference in the receiver plants. Turk and Tawaha (2003), studying the allelopathic effect of black mustard (*Brassica nigra*) on germination and seedling growth of wild oat (*Avena fatua*) observed that protease enzyme activity was suppressed causing reduced water uptake which led to poor seed germination thus low stand count.

The sharp decrease in double thorn abundance can be attributed to a low threshold to allelochemicals. A study by Hussein (2014), revealed that different plants differ in their allelochemical threshold thus some are affected more by the same concentration of allelochemicals than others.



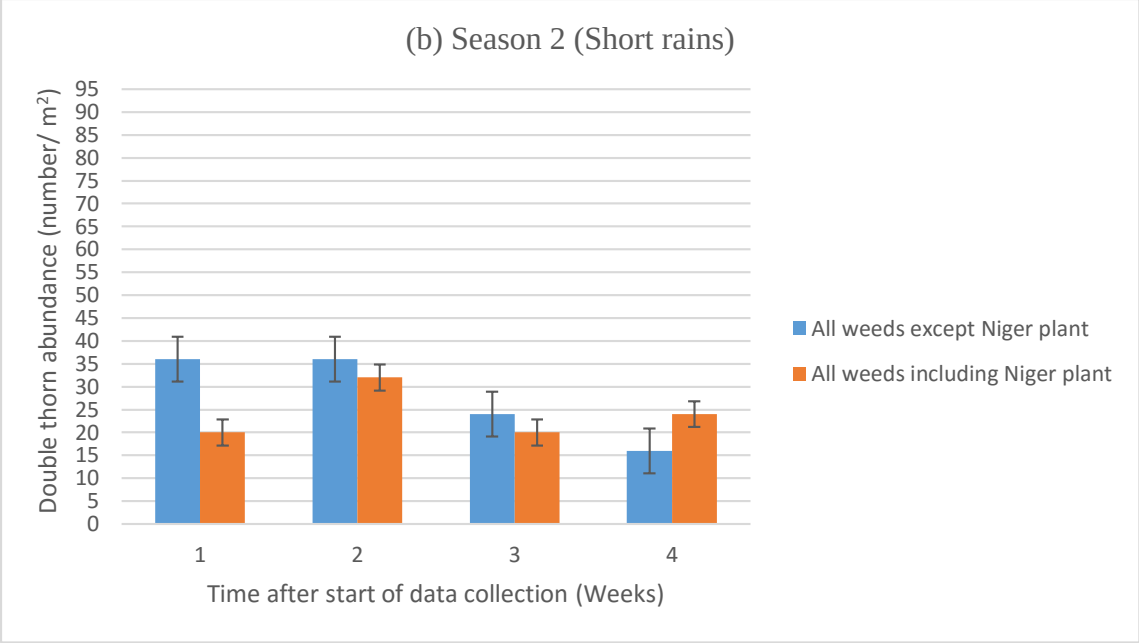


Fig. 6: Effect of Niger plant on abundance of Double thorn

Effect of Niger plant on abundance of Couch grass

Couch grass abundance in season 1 showed significant differences in week 1 and 2 (Fig. 7). In week 1, there was a higher number of emerged weeds in all weeds except Niger plant regime. The number of sprouted weeds reduced in week 2 through withering and death. A few more weeds sprouted in week 3 but the number reduced in week 4. The dynamics observed can be partly attributed to allelopathy and competition. Chon *et al.*, (2006) attributed the highly allelopathic herbicidal potential of some plant extracts to the presence of allelopathic substances for example coumarin, benzoic acid and cinnamic acid. This is in agreement with a study by Kumbhar and Patel, (2016) which concluded that Niger plant, just like other members of the Asteraceae family, has many different kinds of allelochemicals, chief among them being phenolics. It was also seen that weeds that emerged later that the second week do not survive but rather wither and die off. This can be attributed to the direct effect of competition for space to take foot

on and also competition for nutrients and water given that the newly germinated seedlings are not competitive enough in acquiring these resources.

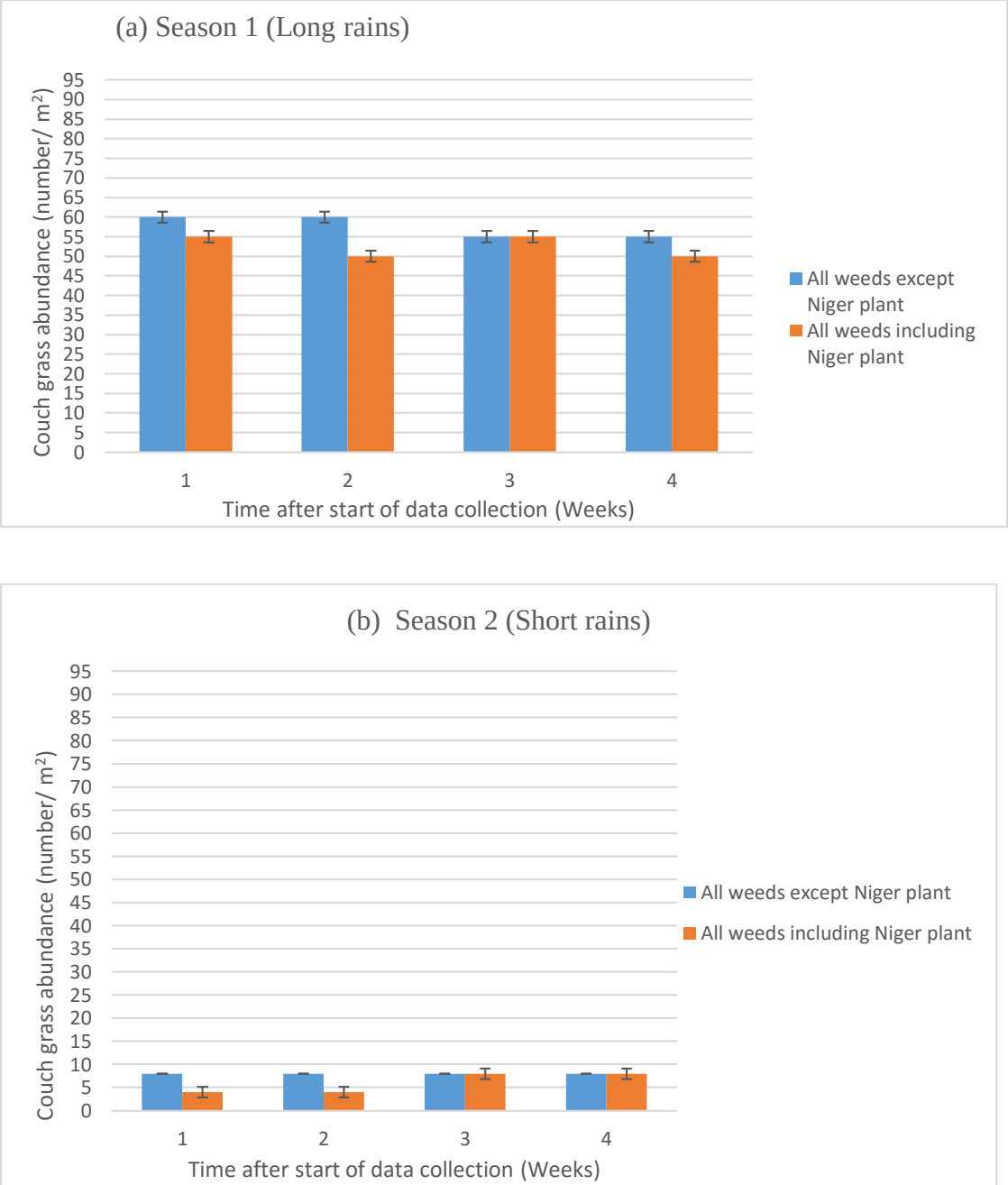


Fig. 7: Effect of Niger plant on abundance of Couch grass

5.8.2 Effect of weed regime and bean cultivar on bean growth and development

Effect of bean cultivar on bean stand count at two weeks

As pertains to bean stand count at two weeks, there was no significant differences in the various bean cultivars used in either season 1 or 2 (Table 6 a and b and Appendix VII a and b)). There was a higher mean of bean stand count in season 2 (43) whereas the mean was 38 in season 1.

Table 6: Effect of bean cultivar on growth and development of beans

(a) Season 1 (Long rains)

Cultivar	S. count at two weeks	Height (cm)	No. of pods per plant	No. of seeds per pod	Stand count at harvesting
Rosecoco	38a	30a	4a	3a	18a
Mwitemania	38a	30a	6b	3a	22b
Mwezi mbili	37a	31a	7c	3a	19a
DMRT	0.62	1.01	0.65	0.30	1.62
CV%	3.6	8.1	29.4	17.0	20.1

(b) Season 2 (Short rains)

Cultivar	S. count at two weeks	Height (cm)	No. of pods per plant	No. of seeds per pod	Stand count at harvesting
Rosecoco	42a	32a	5a	5a	25a
Mwitemania	43a	32a	8b	5a	28a
Mwezi mbili	43a	45b	8b	5a	27a
DMRT	1.55	1.55	0.66	0.60	1.55
CV%	14.4	10.5	22.3	20.1	14.4

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

Table 7: Effect of weed regime on bean growth and development

(a) Season 1 (Long rains)

Weed regime	Stand count at two weeks	Height (cm)	No. of pods per plant	No. of seeds per pod	Stand count at harvesting
All weeds including Niger plant	36a	21a	3a	2a	10a
All weeds except Niger plant	36a	22a	3a	2a	11a
Niger plant intercrop	37a	39b	9c	4c	34c
Weed free	36a	39b	7b	3b	24b
DMRT	0.71	1.01	0.75	0.34	1.87
CV%	3.6	8.1	29.4	27.0	20.1

(b) Season 2 (Short rains)

Weed regime	Stand count at two weeks	Height (cm)	No. of pods per plant	No. of seeds per pod	Stand count at harvesting
All weeds including Niger plant	33a	32a	5a	4a	13a
All weeds except Niger plant	37b	33a	5a	4a	21b
Niger plant intercrop	43d	40b	11c	6b	40d
Weed free	40c	40b	7b	5ab	33c
DMRT	1.79	1.79	0.77	0.70	1.79
CV%	14.4	10.5	22.3	30.1	14.4

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

Effect of weed regime on bean stand count at two weeks

There were significant differences in stand count at two weeks due to weed regime in season 2 (Table 7 a and b and Appendix VII b). Weedy regime was the most sensitive of all, recording the lowest stand count at 32 (65%), its highest being 34 (71%) plants per plot.

Niger plant intercropped treatment recorded an impressive stand count at 43 (88%) and 42 (86%) plants per plot. It was in weed free treatment that the highest mean was recorded in Mwitemania (90%) though there were no significant differences between Niger plant and weed free treatments. Effects on treatments with all weeds except Niger plant were the same as those of weedy treatments though significantly different from Niger plant intercropped treatment and weed free treatment.

Stand count at two weeks is mostly a reflection of germination percentage. Poor stand count at two weeks can be attributed to the effect of weed exudates on the germinating seeds. This finding is in agreement with that of Shao *et al.,* 2005, who reported that some weeds release allelochemicals into the soil, which affect the germination and stand establishment of associated as well as succeeding crops. Inhibition of germination occurs through interruption of respiratory enzymes and enzymes involved in oxidative pentose phosphate pathway which lead to inhibition of respiration in the germinating seed (Muscolo *et al.,* 2001) resulting in delayed or suppressed germination. An experiment by Tanveer *et al.,* (2015) revealed that *E. helioscopia* allelochemicals in the soil hindered emergence of chick pea, wheat and lentil.

Effect of cultivar on plant height at 50% flowering

Differences due to cultivar were significant only in season 2 where cultivar 3 (Mwezi Mbili) had an average height of 45 cm as shown in Table 6a and b and Appendix VII b. Both cultivar 1 (Rosecoco) and cultivar 2 (Mwitemania) had means of 32 cm. This could be attributed to the morphology of the bean cultivars. Mwezi Mbili grows tendrils that could support themselves on any available vegetation thus its longer height. Mwitemenia had shorter tendrils while Rosecoco did not have any.

Effect of weed regime on plant height at 50% flowering

There were significant differences ($P \leq 0.05$) in bean height both in weed free treatment and those that had all weeds except Niger plant in both season 1 and season 2 (Table 7 a and b and appendix VII a and b). Weedy treatment where there were all weeds including Niger plant and those that had all weeds except Niger plant were not significantly different from one another though different from the other two treatments.

Whereas some crops are negatively affected by allelopathy, in some, the effects are favourable and the plants are said to exhibit positive allelopathy. A large number of allelochemicals such as ferulic acid, chlorogenic acid, caffeic acid, p-coumaric acid and gallic acid have been observed to cause hindrance of plant growth (Muzaffar *et al.*, 2012). This hindrance can be the reason why in weedy treatment and treatment that had all weeds except Niger plant, there were low plant height means at 32 and 33 cm. Results from a study by Hussain *et al.*, (2007) on allelopathic effect on wheat showed a promotion in wheat height though it was reduced in other crops. The results of this study agree with these earlier findings.

Through disruption of vital physiological processes, allelochemicals released from plants trigger a decrease in growth and development in some plants (Shao-Lin *et al.*,

2004) but not in others. This specificity in reaction can be the result of genetic differences and how well the plant is able to adapt to the stress it is subjected to. Reduction in growth may also be attributed to water stress that reduces cell expansion or due to structural changes in membranes of the cells including alteration in membrane portions (Einhellig, 2004), or due to the suppression of cell division as a result of crop-weed competition.

Effect of bean cultivar on the number of pods per plant

There were significant differences between pods of different bean cultivars in both seasons (Table 6 a and b and Appendix IX a and b). Cultivar 3 (Mwezi Mbili), had the highest mean at 7 pods per plant. This can also be attributed to the morphology of the different cultivars. Cultivar 3 grows long apical tendrils that support themselves on other plants in close proximity to them and due to this they have a larger surface to grow pods. Additionally, due to the tendrils, this cultivar was able to compete effectively with weeds for sunlight. Cultivar 1 (Rose coco) having no tendrils was chocked by weeds and suffered the effects of lack of sunlight. Cultivar 2 (Mwitemania) had medium length tendrils that have enabled it to produce average results.

Effect of weed regime on the number of pods per plant

Results from this study showed that there were significant differences ($P \leq 0.05$) in the number of pods per plant in weed free and Niger plant intercrop treatments. This is shown in Table 7 a and b and appendix VIII a and b. In both season 1 and season 2, Niger plant intercropped treatment had the highest means at 9 and 11 pods per plant respectively. Weed free treatment recorded the same number of pods in both season 1 and season 2 (7 pods per plant). Weedy treatment and all weeds except Niger plant treatment had higher means in season 2 though not significantly different from one another. In both season 1 and season 2, cultivar 1 (Rosecoco) had the lowest mean at 4

and 5 pods per plant respectively whereas cultivar 2 (Mwitemania) and cultivar 3 (Mwezi Mbili) means were not significantly different from one another in both seasons.

Some allelochemicals produced by plants act to stimulate pod formation. In a study on sorghum allelopathy, Tesfamariam *et al.*, (2014) found out that sorgoleone, a sorghum allelochemical, influenced the biogeochemical cycles of nutrients, by reducing the activity of *Nitrosomonas* bacteria and consequently increased the ammonium content in the soil leading to higher crop yields. This finding agrees with that of the present experiment. Another likely reason for maximum numbers of seeds per pod is the hindrance of weed germination and growth, which lowered the competitive pressure between beans and weeds.

Weedy treatment and those that had all weeds except Niger plant showed low numbers of pods per plant. This can be attributed to weed-crop competition for nutrients, water and space. In addition, there was a higher incident of pest (aphid) attack on the beans in weedy treatment which were thought to have been encouraged due to the presence of weeds of *Brassica* family. There were few aphids in weed free treatment and none in Niger plant treatment. Since beans growing in weedy treatments and those with all weeds except Niger plant had compromised height, the number of pods per plant was also directly affected.

Interaction between cultivar and weed regime on number of pods per plant

Interactions between bean cultivar and weed regime resulted in significant differences in number of pods in both season 1 and 2 as shown in Table 8 a and a and Appendix X a and b. There were significant differences in the various weed regimes. In weedy regime, Mwezi mbili had the highest number of pods per plant and was significantly different from both Mwitemania and Rose coco. In all weeds except Niger plant regime,

there were significant differences where Mwitemania was significantly different from the other two cultivars. There were no significant differences between Mwezi mbili and Rose coco in all weeds except Niger plant regime. In Niger plant intercrop, there were significant differences in all the bean cultivars. Mwezi mbili had the highest number of pods per plant (14). Followed by Mwitemenia (9) and Rose coco (4). There were significant differences in weed free regime where Mwezi mbili was significantly different from the rest.

Within the bean cultivars significant differences were noted. In Mwezi mbili cultivar, the highest number of pods per plant were in Niger plant intercrop (14). Significant differences were also noted in weed free regime where Mwezi mbili had 8 pods per plant as opposed to 4 and 3 in weedy and all weeds except Niger plant regimes respectively. There were no significant differences between weedy regime and all weeds except Niger plant regime.

In Mwitemenia cultivar, there were significant differences in all the weed regimes with Niger plant intercrop regime having the highest number of pods and weedy regime having the lowest number of pods.

There were significant differences in Rose coco cultivar. Whereas weedy and all weeds except Niger plant regimes were not significantly different from one another, Niger plant intercrop regime, with 4 pods per plant was statistically different from the weed free regime at 6 pods per plant.

In season 2 weedy regime, Mwezi mbili was significantly different from Mwitemania and Rose coco. There were no significant differences across all the three bean cultivars in all weeds except Niger plant regime. In Niger plant intercrop regime, there were significant differences in all the bean cultivars with 16, 13 and 5 pods per plant in

Mwezi mbili, Mwitemania and Rose coco respectively. In weed free regime, there were significant differences in all the bean cultivars.

Within the bean cultivars in season 2, Mwezi mbili had 16 pods in Niger plant intercrop regime which was the highest. The number of pods in Mwezi mbili in both weedy and weed free regimes was not significantly different but was significantly different from the number of pods in all weeds except Niger plant regime.

In Mwitemania cultivar, there were significant differences in all the different weed regimes. In ascending order, the number of pods per plant were 4, 6, 9 and 13 in weedy, all weeds except Niger plant, weed free and Niger plant intercrop respectively. In Rose coco cultivar, there were no significant differences in all the four weed regimes.

Table 8: Interaction between cultivar and weed regime on number of pods per plant

(a) Season 1 (Long rains)

	Bean cultivars		
Weed regime	Mwezi mbili	Mwitemania	Rose coco
All weeds including Niger plant	4ab	2a	2a
All weeds except Niger plant	3a	4ab	2a
Niger plant intercrop	14d	9c	4ab
Weed free	8c	7b	6b
DMRT	1.28		
CV%	28.7		

(b) Season 2 (Short rains)

Weed regime	Bean cultivars		
	Mwezi mbili	Mwitemania	Rosecoco
All weeds including Niger plant	7b	4a	5a
All weeds except Niger plant	5a	6ab	5a
Niger plant intercrop	16e	13d	5a
Weed free	7b	9c	5a
DMRT	1.27		
CV %	21.5		

Means followed by different letter(s) in a column and row are statistically significant at 5 % level of probability.

Effect of cultivar on the number of seeds per pod

In both seasons, there were no significant differences in the number of seeds per pod as shown in Table 6 a and b and Appendix XI a and b.

Effect of weed regime on the number of seeds per pod

Weedy treatment recorded the lowest count at 2 and 4 seeds per pod in season 1 and season 2 respectively. The highest number of seeds per pod was counted in Niger plant treatment in season 2 (Table 7 and appendix XI a and b). In both seasons, there were significant differences.

In weedy treatment and in all weeds except Niger plant treatment, competition for light, among other resources may have contributed to the low pod counts. The best results were obtained from Niger plant-bean intercrop pointing to possibilities of Niger plant having a stimulatory effect on bean development hence more seeds per pod. Similar results were recorded by Ejaz *et al.*, (2015) who found out that there was a higher

number of tillers and spikes in wheat intercropped with sunflower. Majeed *at al*., (2012) reported that extracts of *Chenopodium album* promoted the number of tillers and grains in wheat. Another likely reason for a higher number of seeds per pod in Niger plant treatment could be the hindrance of weed germination and growth, which lowered the competitive pressure between weeds and beans. Average results in weed free treatment were attributed to lack of competition for resources. Weed – crop competition may have been responsible for low counts in weedy treatment and those with all weeds except Niger plant.

Effect of cultivar on stand count at harvesting

There were no significant differences in cultivar on stand count at harvesting in either season.

Effect of weed regime on stand count at harvesting

Effects of weed regime on stand count of beans at harvesting is shown in Table 7 a and b above and appendix XII a and b. In season 1, results of weedy treatment and those with all weeds except Niger plant were not significantly different ($P \leq 0.05$) from one another. However, there were significant differences between Niger plant and weed free treatments. Allelochemicals liberated from the weeds in the crop vicinity affect seed germination, seedling growth and stand establishment, ultimately leading to a reduced number of plants per unit area.

5.9 Conclusions and recommendations

5.9.1 Conclusions

1. Niger plant enhanced the growth and development of beans.

2. Niger plant exhibited negative allelopathy on the weeds that were studied i.e. field mustard, broom weed, double thorn and couch grass.

5.9.2 Recommendations

1. Further research should be carried out for means on how Niger plant secondary metabolites can be extracted.
2. A wide study should be done on more weeds so as to widen the understanding on the effectiveness of Niger plant in suppressing weeds.
3. Mechanisms by which Niger plant promotes growth and development of beans.

CHAPTER SIX

GENERAL RESULTS, CONCLUSIONS AND RECOMMENDATIONS

6.1 summary of results

Results obtained from the study established that the Niger plant that occurs within Moiben sub-county is genetically diverse. From the constructed dendogram, the Niger plant was separated into four main clusters. Of the four clusters, two sites (Cheplaskei and Sergoit) were distinct from all the rest. The diversity was attributed to different evolutionary mechanisms given that breeding is not controlled and the fact that Niger plant is a purely outcrossing plant. As opposed to earlier studies, there was no heterozygosity in the analyzed Niger plant and this is a point of concern. The probable reasons may be the markers that were used or that the Niger plants could have had a common origin.

Analysis of secondary metabolites showed that there were varied levels of phenols, alkaloids, flavonoids, tannins and saponins in Niger plant collected within the study site as pertains both the part of the plant being analyzed and the site the plant was collected from. These are not the only secondary metabolites in Niger plant but for the sake of this study, the five secondary metabolites were chosen since they are indicated to be the most abundant from literature review. In all cases, secondary metabolite levels in the whole plant were higher than in either the shoot or the root and this may have been because of the differences in compartmentalization of metabolites within the plant depending on plant needs. The differences in levels of secondary metabolites in Niger plant in different sites was attributed to the differences in environmental conditions in the specific sites. The secondary metabolites are meant to enable the plant producing them adapt to the prevailing environmental conditions therefore the higher the stress

faced by the plant, the higher the level of the specific metabolite meant to counter the stress.

The differences in the levels of metabolites in Niger plant could also be attributed to the genetic diversity noted earlier. From the results, sites with closely related Niger plant recorded almost similar levels of secondary metabolites. This means that the genetic makeup of the Niger plant determines the level of secondary metabolites the specific Niger plant yields. This may go on to affect the efficiency of the said Niger plant in allelopathy.

There was enhanced growth and development in all the three cultivars of beans used. All the five parameters that were measured i.e. stand count at two weeks post emergence, plant height at 50 % flowering, number of pods per plant, stand count at harvesting and number of seeds per plant were improved in Niger plant intercrop compared to the controls. This was attributed to the positive effects of allelochemicals on the beans. Poor results were obtained in weedy treatments as a result of competition between the weeds and the beans for moisture, nutrients and space.

There were significant differences in growth of beans amongst the three bean cultivars owing to the diverse morphology of the cultivars. Mwezi mbili cultivar develops long tendrils that were used to support the plant by twinning on surrounding weeds and thus access sunlight for photosynthesis. Due to this, there was a higher number of pods as compared to the other cultivars.

There were significant differences in specific weed abundance pertaining the four weed regimes studied i.e. weedy, Niger plant intercrop, weed free and all weeds except Niger plant. The weeds studied were Field mustard, Broom weed, Double thorn, Niger plant and Couch grass since they were the most abundant weeds in the study area and

occurred in random distribution. Results showed that there were reduced numbers of weeds in Niger plant intercrop regime than in the rest of the weed regimes. Incidences of delayed germination of specific weed seeds were also seen where weeds could germinate towards the end of the second week especially in the second season.

Major changes in specific weed abundance were seen in season two where the number of weeds was at almost half of that in the first season. This could be due to the accumulation of Niger plant allelochemicals in the soil that affected weed seed germination, seedling growth and development in the second season. In the first season, not much of the secondary metabolites had accumulated. This shows that allelochemicals are effective at a given concentration which has to be reached and maintained for better results. Specific weeds were noted to have germinated but could wither, yellow and die out. This observation may have been brought about as a result of the effect of secondary metabolites which interfered with vital physiological processes of the weeds such as respiration, photosynthesis, cell division and elongation.

Generally, the research achieved all its objectives of analyzing the genetic diversity in Niger plant, identifying the types and quantities of secondary metabolites in Niger plant and assessing the effect of intercropping Niger plant with beans and weed abundance.

6.1.1 Conclusions

1. Niger plant that occurs within Moiben sub-county is genetically diverse.
2. All the secondary metabolites analyzed in Niger plant i.e. alkaloids, phenols, flavonoids, saponins and tannins were positively identified and their quantities determined as being different from one another depending on the site of collection of the Niger plant.

3. Intercropping Niger plant with beans results in better growth and development of beans.
4. Niger plant intercrop leads to suppressed abundance of specific weeds.

6.1.2 Recommendations

1. The Niger plant used in the study was genetically diverse and therefore there is need for more research with known pure lines of Niger plant to establish the differences if any, in allelopathy.
2. Since the study zeroed in on only five metabolites, there is need for further research on more secondary metabolites in Niger plant that may be involved in allelopathy.
3. A study should be done on the persistence of Niger plant allelochemicals in the soil to be able to predict the effect that can be exerted on the crops and weeds in the following cropping season.
4. Since the experiment made use of Niger plant in the wild set up with no determined spacing, more research needs to be done on the right spacing for intercropping Niger plant in different crops so as to have maximum effect.
5. Further study on Niger plant allelopathy should be carried out in other agro ecological zones to find out whether the genetic diversity can be maintained.
6. There is need for the same research on a controlled environment to find out the effect of the interactions between Niger plant secondary metabolites and the soil biotic and abiotic factors that may interfere with allelopathy.

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APPENDICES

APPENDIX I: (a) DNA extraction procedure

Tender leaves that had been kept in the freezer were ground into fine powder using a motor and pestle under an extraction buffer. The extracts were transferred to appendorf tubes each with 500 µl of 2 x CTAB mecaptoethanol extraction buffer and the samples transferred to an ice-cold chamber. This followed incubation in a water bath at 65^0 C for 1 hour. The tubes were shaken and inverted after fifteen minutes. After this, 500 µl of chloroform-isoamyl alcohol (24:1) was added and the mixture inverted for 5 minutes at a temperature of 25^0 C in order to mix well. Using a centrifuge, the mixture was mixed at 14000 rounds per minute for 10 minutes. A clear top layer of 400 µl was pipetted into labelled appendorf tubes and 250 µl of isopropanol added. The mixture was inverted to mix then placed in an incubator for 10 minutes.

In order to pellet the DNA, the mixture was then centrifuged at 14000 rpm for 10. The upper portion was then discarded by use of yellow tips followed by addition of 320 µl of 1 x TE and the samples placed on ice. A further 40 µl of magnesium chloride was added and the contents incubated on ice for 10 minutes followed by a centrifugation to 14000 rpm for 10 minutes and the supernatant discarded. The pellet was then vacuum dried for 5 minutes before adding 5 µl of R-nase enzyme and placed on a water bath set at 37^0 C for 2 hours. 40 µl of sodium acetate was added followed by 250 µl of isopropanol and the contents incubated for 15 minutes at room temperature. This was followed by a 10 minute centrifugation of 14000 so as to re-pellet the DNA and the supernatant was discarded.

A 1 ml aliquot of 70 % ethanol was then added to the pellet followed by another centrifuge of 14000 rpm for 5 minutes. The supernatant was discarded followed by a quick spin for 2 minutes. The supernatant was then gently discarded and any liquid

from the tube drained off using a clean tissue paper. The pellet was dried to remove the remaining liquid then the DNA pellet was again suspended in 50 µl of 1 x TE, left to stand for 10 minutes before it was stored at 4^0 C.

(b) DNA quantification

The quality and quantity of the DNA was verified by electrophoresis on a 0.8 % (w/v) agarose gel, for 45 minutes at 80 volts. Lambda phage DNA was used as the standard. After electrophoresis, the gel was stained in ethidium bromide (10 mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. The concentrations of the samples were determined by comparing band sizes and intensities of the test DNA with those of standard lambda DNA. Between 0.5 µl and 1 µl of high quality DNA was obtained and was diluted to 0.02 µg/µl with TE buffer water for the PCR amplification.

(c) PCR amplification

PCR reactions were performed in a Mastercycler (Eppendorf ®) using in a final volume of 20 µl BioneerAccuPower® containing 4 µl premix (1U Top DNA, 250 µM each dNTP, 10 mM Tris-Hcl pH 9.0, 30 mM KCL, 1.5 mM MgCl, stabilizer and tracking dye, 0.0025 ng/ µl of each primer 0.5 ng of template DNA and 6 µl of double distilled water (ddH_2O). The PCR cycles consisted of 94^0 C for 3 minutes for initial denaturation, 94^0 C for 3 minutes for actual denaturation, annealing at 56^0 C for 30 seconds, extension at 72^0 C for 1 minute followed by 34 cycles of 30 seconds at 94^0 C, 1 minute at 56^0 C, for 1 minute at 72^0 C and a final extension step of 5 minutes at 72^0C. The DNA fragments were separated on 4 % agarose gel run at 100 volts (V) for 2 hours using 0.5 M TBE buffer. The DNA fragments in gel were visualized by staining in 0.5 µl/mg ethidium bromide for 30 minutes and rinsed in distilled water for 20 minutes,

visualized and photographed on ultraviolet trans-illuminator at 312 nm. Allele sizes were scored using a 1000 base pairs (bp) molecular size ladder.

APPENDIX II: Procedure for phytochemical test

The following tests were done following a standard procedure adopted by Makkar & Goodchild (1996).

a) Tannins

Into a test tube containing 100 mg plant powder, 3 ml of butanol-HCl reagent (95 ml of n-butanol and 5 ml of concentrated HCl) was added. Cotton wool was used plug the test tube. This was followed by heating the test tube at 70 ^{0}C on a water bath for one hour. The presence of tannins was shown by formation of a pink color.

b) Phenols

Into a test tube containing 1 ml of extract, 2 drops of 5 % w/v ferric chloride were added. A greenish precipitate indicated the presence of phenols.

c) Flavonoids

To 5 ml of the extract, 2 ml of 10 % w/v sodium hydroxide (0.01 M) was added. There was formation of a yellow color. This was followed by addition of dilute hydrochloric acid. There was a change in color from yellow to colorless indicating the presence of flavonoids.

d) **Alkaloids**

Niger plant powder was used to prepare an extract. To do this, 500 mg oven dried plant powder was placed into a conical flask. To the powder, 3 ml of methanol containing 10% acetic acid was added. Ammonium hydroxide was added to it drop wise. A positive result was shown by formation of precipitates.

e) **Saponins**

To test for saponins, Niger plant powder extract measuring 500 mg was introduced into a conical flask. Into the flask, 50 % aqueous methanol was added and allowed to stand for six hours. The extract was decanted into a test tube and vigorously by hand. The test is positive if a persistent foam is formed.

APPENDIX III: Quantitative analysis of compounds

a) Determination of total phenols

Total phenol content was estimated using the Folin–Ciocalteau method as described by Bray and Thorpe (1954). A measure of 0.5 ml Folin–Ciocalteau reagent was added to 1 ml of sample. It was then incubated for three minutes at 35^0 C. This was followed by addition of two ml of 20% sodium bicarbonate. The mixture was homogenized and incubated in a water bath at 100^0 C for one minute. Using cold running water, the mixture was rapidly cooled and read at 650 nm absorbance against a reagent blank using UV spectrophotometer. The results were expressed as mg/g sample.

b) Determination of flavonoids

The determination of flavonoids was as given by Jia *et al*. (1999). A 1 ml extract was taken from the sample and mixed with 0.075 ml of 5% sodium nitrite solution then incubated at room temperature for five minutes. After this, 10% aluminum chloride was added and incubated at room temperature for six minutes and 1 N NaOH added. Absorbance was read at 510 nm against a reagent blank.

c) Determination of tannins

Tannins were determined by a method suggested by Bray and Thorpe (1954). A sample extract measuring 1 ml was mixed with 5 ml of vannilin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as catechin equivalents.

d) Determination of alkaloids

Determination of alkaloids was done by the procedure given by Harborne (1973). Using a motor and pestle, 10 mg of the plant sample was homogenized. Into the motor and

pestle, 20 ml of methanol: ammonia was added at the ratio of 68:2. The mixture was decanted into a conical flask and left to stand for 24 hours. This was followed by addition of fresh methanol and ammonia at the same ratio as before. The procedure was repeated thrice and the extracts pooled together then evaporated using a flash evaporator. The residue was treated with 1 N Hcl and kept overnight. The acidic solution was extracted with 20 ml of chloroform thrice, pooled together and the organic layers evaporated to dryness. The acidic layer was basified with concentrated sodium hydroxide to pH 12 and extracted with chloroform (20 ml) thrice. The chloroform layers were dried over absorbent cotton and evaporated to dryness. This was weighed and the fraction that contains alkaloids expressed as mg/100 g.

e) Determination of saponins

Standard saponin solution was prepared by dissolving 10 mg of diosgenin, add 16 ml methanol and 4 ml distilled water. To the aliquots for each tube, 0.25 ml of 8 % vanillin reagent and 2.5 ml of 72 % v/v added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 ^{0}C water bath. After incubating for 10 minutes, the tubes were cooled in ice cold water bath for 4 min. A 0.1 g of freeze dried sample was dissolved in aqueous methanol (80%, 0.1 ml) and 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm. This method was suggested by Harborne (1973).

APPENDIX IV: ANOVA for secondary metabolites in the whole plant

(a) Alkaloids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02389	0.01194	1.05	
Site	11	0.07222	0.00657	0.58	0.825
Residual	22	0.24944	0.01134		
Total	35	0.34556			

R-Square	C V (%)	Root MSE	Mean
0.278	6.9	0.1065	1.539

(b) Flavonoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02056	0.01028	0.34	
Site	11	0.23639	0.02149	0.70	0.723
Residual	22	0.67278	0.03058		
Total	35	0.92972			

R-Square	C V (%)	Root MSE	Mean
0.276	15.2	0.1748	1.153

(c) Phenols

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.05722	0.02861	1.95	
Site	11	0.36222	0.03293	2.24	0.061
Residual	22	0.32278	0.01467		
Total	35	0.74222			

R-Square	C V (%)	Root MSE	Mean
0.565	**6.0**	**0.1211**	**2.022**

(d) Saponins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Rep stratum	2	0.01389	0.00694	0.66	
Site	11	0.03556	0.00323	0.31	0.977
Residual	22	0.23278	0.01058		
Total	35	0.28222			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.175	17.8	0.1028	0.578

(e) Tannins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.07167	0.03583	0.40	
Site	11	0.73667	0.06697	0.75	0.681
Residual	22	1.96167	0.08917		
Total	35	2.77000			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.291	12.2	0.2986	2.450

APPENDIX V: ANOVA for secondary metabolites in Niger plant shoots

a) Shoot alkaloids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.01389	0.00694	0.31	
Site	11	0.18306	0.01664	0.75	0.680
Residual	22	0.48611	0.02210		
Total	35	0.68306			

R-Square	C V (%)	Root MSE	Mean
0.29	**27.7**	**0.15**	**0.536**

b) Shoot flavonoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00167	0.00083	0.05	
Site	11	0.06750	0.00614	0.36	0.960
Residual	22	0.37833	0.01720		
Total	35	0.44750			

R-Square	C V (%)	Root MSE	Mean
0.15	**32.1**	**0.13**	**0.408**

c) Shoot phenols

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.07167	0.03583	1.01	
Site	11	0.13417	0.01220	0.34	0.965
Residual	22	0.78167	0.03553		
Total	35	0.98750			

R-Square	C V (%)	Root MSE	Mean
0.21	**34.8**	**0.19**	**0.542**

d) Shoot saponins

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	0.010556	0.005278	0.78	
Site	11	0.115556	0.010505	1.55	0.185
Residual	22	0.149444	0.006793		
Total	35	0.275556			

R-Square	C V (%)	Root MSE	Mean
0.46	**20.0**	**0.08**	**0.411**

e) Shoot tannins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00722	0.00361	0.15	
Site	11	0.33639	0.03058	1.25	0.316
Residual	22	0.53944	0.02452		
Total	35	0.88306			

R-Square	C V (%)	Root MSE	Mean
0.39	**24.7**	**0.16**	**0.636**

APPENDIX VI: ANOVA for secondary metabolites in Niger plant roots

a) Root alkaloids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02722	0.01361	0.50	
Site	11	0.14306	0.01301	0.48	0.898
Residual	22	0.59944	0.02725		
Total	35	0.76972			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.22	15.8	0.17	1.047

b) Root flavonoids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02667	0.01333	0.65	
Site	11	0.28000	0.02545	1.24	0.322
Residual	22	0.45333	0.02061		
Total	35	0.76000			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.40	15.9	0.14	0.90

c) Root phenols

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	0.09056	0.04528	1.17	
Site	11	0.80556	0.07323	1.90	0.097
Residual	22	0.84944	0.03861		
Total	35	1.74556			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.51	12.6	0.20	1.567

d) Root saponins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.4006	0.2003	0.97	
Site	11	2.3356	0.2123	1.03	0.452
Residual	22	4.5194	0.2054		
Total	35	7.2556			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.38	**31.4**	**0.45**	**0.411**

e) Root tannins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0972	0.0486	0.46	
Site	11	0.9697	0.0882	0.84	0.603
Residual	22	2.3028	0.1047		
Total			35	3.3697	

R-Square	C V (%)	Root MSE	Mean
0.32	16.6	0.32	1.947

APPENDIX VII: (a) Stand count at two weeks season 1 (Long rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.167	0.083	0.04	
Weed regime	3	7.667	2.556	1.11	0.365
Cultivar	2	0.667	0.333	0.15	0.866
Weed regime.cultivar	6	28.000	4.667	2.03	0.104
Residual	22	50.500	2.295		
Total	35	87.000			

R-Square	C V (%)	Root MSE	Mean
0.42	3.6	1.51	38

(b) Stand count at two weeks season 2 (Short rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	16.222	8.111	2.08	
Weed regime	3	739.639	246.546	63.23	<.001
Cultivar	2	20.056	10.028	2.57	0.099
Weed regime.cultivar	6	35.944	5.991	1.54	0.213
Residual	22	85.778	3.899		
Total	35	897.639			

R-Square	C V (%)	Root MSE	Mean
0.9	5.2	1.9	43

APPENDIX VIII: (a) Plant height at 50% flowering season 1 (Long rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.389	2.694	0.44	
Cultivar	2	12.389	6.194	1.02	0.378
Weed regime	3	2782.000	927.333	152.31	<.001
Weed regime.cultivar	6	21.167	3.528	0.58	0.743
Residual	22	133.944	6.088		
Total	35	2954.889			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.95	8.1	2.47	31

(b) Plant height at 50% flowering season 2 (Short rains)

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	9.056	4.528	0.80	
Weed regime	3	1895.861	631.954	111.87	<.001
Cultivar	2	1.722	0.861	0.15	<.001
Weed regime.cultivar	6	26.722	4.454	0.79	0.208
Residual	22	124.278	5.649		
Total	35	2057.639			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.94	10.5	2.38	35

APPENDIX IX: (a) Number of pods season 1 (Long rains)

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	0.722	0.361	0.19	
Weed regime	3	225.000	75.000	38.72	<.001
Cultivar	2	93.056	46.528	24.02	<.001
Weed regime.cultivar	6	113.833	18.972	9.80	<.001
Residual	22	42.611	1.937		
Total	35	475.222			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.91	29.4	1.39	5

(b) Number of pods season 2 (Short rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.722	0.361	0.14	
Cultivar	2	80.889	40.444	15.36	<.001
Weed regime	3	225.000	75.000	28.48	<.001
Weed regime.cultivar	6	134.667	22.444	8.52	<.001
Residual	22	57.944	2.634		
Total	35	499.222			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.88	22.3	1.62	7

APPENDIX X: (a) Interactions between cultivar and weed regime season 1 (Long rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	70.389	35.194	14.40	<.001
Treatment	3	278.222	92.741	37.94	<.001
Cultivar.treatment	6	87.611	14.602	5.97	<.001
Residual	24	58.667	2.444		
Total	35	494.889			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.88	23.7	1.56	5.44

(b) Interactions between cultivar and weed regime season 2 (Short rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	80.889	40.444	16.55	<.001
Treatment	3	225.000	75.000	30.68	<.001
Cultivar.treatment	6	134.667	22.444	9.18	<.001
Residual	24	58.667	2.444		
Total	35	499.222			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.88	21.5	1.56	7.28

APPENDIX XI: (a) Number of seeds per pod season 1 (Long rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3889	0.1944	0.37	
Cultivar	2	1.0556	0.5278	1.00	0.384
Weed regime	3	28.0833	9.3611	17.74	<.001
Weed regime.cultivar	6	2.5000	0.4167	0.79	0.588
Residual	22	11.6111	0.5278		
Total	35	43.6389			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.73	17.0	0.73	3

(b) Number of seeds per pod season 2 (Short rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.167	0.583	0.27	
Cultivar	2	0.167	0.083	0.04	0.963
Weed regime	3	28.750	9.583	4.38	0.015
Weed regime.cultivar	6	16.500	2.750	1.26	0.317
Residual	22	48.167	2.189		
Total	35	94.750			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.49	20.1	1.48	5

APPENDIX XII: (a) Stand count at harvesting season 1 (Long rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	31.50	15.75	1.00	
Cultivar	2	81.50	40.75	2.59	0.098
Weed regime	3	3568.31	1189.44	75.67	<.001
Weed regime.cultivar	6	135.61	22.60	1.44	0.245
Residual	22	345.83	15.72		
Total	35	4162.75			

R-Square	**C V (%)**	**Root MSE**	**Mean**
0.92	20.1	3.96	20

(b) Stand count at harvesting season 2 (Short rains)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.06	1.03	0.05	
Cultivar	2	35.06	17.53	0.92	0.414
Weed regime	3	2415.22	805.07	42.18	<.001
Cultivar.weed regime	6	123.61	20.60	1.08	0.405
Residual	22	419.94	19.09		
Total	35	2995.89			

R-Square	C V (%)	Root MSE	Mean
0.86	15.6	4.37	28