# THE USE OF SPECIES OF *Commelina* AS FEED FOR FIELD CRICKET, *Scapsipedus icipe* (ORTHOPTERA: GRYLLIDAE)

**RUNYAMBO IRAKIZA**

**A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Award of Doctor of Philosophy in Food Security and Sustainable Agriculture of Jaramogi Oginga Odinga University of Science and Technology**

**JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY**

## DECLARATION AND APPROVAL

**Declaration**

This thesis is my original work and has not been presented for an award of a diploma or conferment of degree in any other university or institution.

Signature:  Date: 20th June 2023

Runyambo Irakiza A461/4147/2019

Approval

This proposal has been submitted with our approval as the university supervisors.

Signature:  Date: 20th June 2023

Prof. Darius A. Andika

School of Agricultural and Food Sciences

Department of Plant, Animal and Food Science

Jaramogi Oginga Odinga University of Science and Technology

Signature:  Date: 20th June 2023

Prof. Arnold Watako

School of Agricultural and Food Sciences

Department of Plant, Animal and Food Science

Jaramogi Oginga Odinga University of Science and Technology

Signature:  Date: 20th June 2023

Prof. Samuel M. Mwonga

Faculty of Agriculture

Department of Crops, Horticulture and Soils

Egerton University

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## DEDICATION

To

The cherished memory of my beloved mother, Namariza Dorcas, whose unshakable support and enduring inspiration remain

## ABSTRACT

Sub-Saharan Africa faces persistent challenges in reducing malnutrition and enhancing food security. The field cricket, *Scapsipedus icipe* Hugel and Tanga (Orthoptera: Gryllidae), is edible and could be used to alleviate malnutrition. Currently, there is limited knowledge on what plants this insect feeds on as natural diets source of proteins for mass rearing. This study aimed to determine the diversity of species of *Commelina* in different agroecological zones in Western Kenya, evaluate the feeding preferences of *S. icipe* for species of *Commelina*, and determine the optimum growing conditions for *Commelina* using cricket frass as manure. To determine the diversity of species of *Commelina,* a phytosociological method was used to collect plants using quadrats, whereas soil samples from each quadrat and managements were collected to examine the relationship between species of *Commelina* and soil and management. Preferences of *S. icipe* for species of *Commelina* were evaluated through no-choice, dual choices, and multiple-choice experiments conducted in a RCBD design in controlled environment. Optimum growing conditions for *Commelina* using cricket frass as manure were determined through experiments carried out in a screenhouse and open field environments laid in a CRD and RCBD designs, respectively. The results of the diversity showed that eleven species of *Commelina* were identified, with *C. diffusa* and *C. benghalensis* var. *benghalensis* (non-hybrid variant) having higher relative density. The results also found that the distribution of species of *Commelina* was significantly influenced by soil pH, available P, TN, fertility, and crop spacing. The feeding experiments showed that *S. icipe* had a significantly higher feeding rate on *C. petersii* and *C. forskaolii* and a significantly lower feeding rate on *Commelina* sp. and *C. purpurea* in comparison to references. There were positive significant associations between leaf feeding and Ca and NDF content of leaves and a negative significant association between Ca and NDF. A high Ca/low NDF content was recorded for *C. petersii* and a low Ca/high NDF content for *C. purpurea.* Six phytochemical constituents of the leaves influenced leaf feeding: phenols, alkaloids, tannins, glycosides, saponins and anthraquinones. Phenols stimulate feeding by *S. icipe* on *C. petersii* and *C. forskaolii,* whereas the tannins and alkaloids in *Commelina* sp. and *C. purpurea,* acted as deterrents. Optimum growing conditions for *C. petersii* were determined using cricket frass as manure, and the application rate of 15 t $ha^{-1}$ of cricket frass yielded the best results. Cricket frass significantly increased the vegetative parameters (plant height, number of leaves, number of shoots, leaf area, and plant biomass) of *C. petersii* at 5, 10, and 15 t $ha^{-1}$ compared to untreated plants. Moreover, cricket frass increased the nutrient contents of *C. petersii* for CP and NDF as well as of Ca at 5, 10, and 15 t $ha^{-1}$ compared to untreated plants. The 15 t $ha^{-1}$ provided adequate levels of CP and Ca while still maintaining a reasonable level of NDF. Using local resources such as species of *Commelina* and cricket frass as manure could be a sustainable way for mass production of *S. icipe.*

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## LIST OF ACRONYMS

AAS: Atomic Absorption Spectrophotometer

ACEII-INSEFOODS: Africa Center of Excellence in Sustainable Use of Insect as food and Feeds

ADF: Acid Detergent Fiber

Adj $R^2$-value: Adjusted R-square value

ADL: Acid Detergent Lignin

AgriSysT: Agriculture System Type

AIC: Akaike Information Criterion

ANOVA: Analysis of Variance

AOAC: Association of Official Analytical Chemists methods

ASH: Ash

a.s.l.: Above Sea Level

BIC: Bayesian Information Criterion

Ca: Calcium

CCA: Canonical Correspondence Analysis

CEC: Cation Exchange Capacity

$cm^2$: Centimeter square

CostWeedM: Cost for Weed Management

CP: Crude Protein

CRD: Completely Randomized Design

CropE: Crop Establishment

CropS: Crop Spacing

DAH: Days After Hatching

DCA: Detrended Correspondence Analysis

DM: Dry Matter

E: Pielou's evenness index

EC: Electric Conductivity

EE: Extracted Ether

EPPO: European and Mediterranean Plant Protection Organization

ESP: Exchangeable Sodium Percentage

FarmM: Farming Method

Fe: Ion

$FeCl_3$: Ion Chloride

FTEA: Flora of Tropical East Africa

g: Gram

GPS: Global Positioning System

H: Shannon-Weaver diversity index

$H_2SO_4$: Sulfuric Acid

HCl: Hydrochloric Acid

JOOUST: Jaramogi Oginga Odinga Univrsity of Science and Tchnology

K: Potassium

M: Margalef index

Mg: Magnesium

mL: Milliliter

Mn: Manganese

N: Normal

Na: Sodium

NDF: Neutral Detergent Fiber

NFE: Nitrogen Free-Extract

$NH_3$: Ammonia

NMK: National Museums of Kenya

P: Available Phosphorus

PCA: Principal Correspondence Analysis

pCCA: partial Canonical Correspondence Analysis

pH: Potential of Hydrogen

RCBD: Randomized Complete Block Design

S: Species richness

SßC: Standardize Beta Coefficients of the regression model

SurV: Surrounding Vegetation

t $ha^{-1}$: tons per hectare

TAVE: Total Amount of Variation Explained

TN: Total Nitrogen

TOC: Total Organic Carbon

U: Unexplained

VIF: Variance Inflation Factors

W/V: Mass of solute (grams) per volume of solute (milliliters)

WAT: Week After Transplanting

WeedCont: Weed Control

λ1: Marginal effects

λA: Conditional effect

# CHAPTER ONE: INTRODUCTION

## 1.1 Background information

Plants resources make food for most insects, and it is estimated that plants feeders (also called herbivorous insects) approximately account 45 to 50 percent of insect species (Bernays, 1992; Strong *et al*., 1984). Insect and plants interact differently at all levels such provision of habitat, reproduction, pollination and food supply. For instance, in this interaction some herbivorous insects feeding on one type of plant are considered as specialists, whereas others feeding on several types of plants are called generalists. Generalists have an advantage over specialists due to a broader resource base. This improves their nutrient balance and reduces exposure to high levels of particular allelochemicals (Bernays & Minkenberg, 1997). Crickets of the Gryllidae family (e.g., *Acheta*, *Gryllus*, *Teleogryllus* and *Modicogryllus*) are generalists because they can feed on plants of different families and even genera (e.g., Compositae-*Bidens*, Verbenaceae-*Lanthana*, Fabaceae-*Desmodium*, Cleomaceae-*Cleome*, Commelinaceae-*Commelina*, Asclepiadaceae-*Asclepias*, and Amaranthaceae-*Amaranthus*) (e.g., Carmona *et al*., 1999; Kinyuru & Kipkoech, 2018; Miech *et al*., 2016; Ssepuuya *et al*., 2021). In Sub-Sahara Africa, it has been reported that reported that a plant in the genus *Commelina*, namely *Commelina benghalensis* can be used as natural and affordable feed for crickets (Kinyuru & Kipkoech, 2018). Plants in the genus *Commelina* commonly known as "Dayflowers" are diverse in several agricultural ecosystems, and comprises approximately 170 to 215 species worldwide (Faden, 1998; The Plant List, 2013). As the diversity of plants is essential to sustain production of living organisms in several terrestrial ecosystems (Booth & Grime, 2003), determining the diversity of species of *Commelina* in Sub Sahara Africa is crucial for identifying potential source of feeds for crickets. This study hypothesizes that there is no diversity of species of *Commelina* in different agricultural ecosystems. However, not all crickets are known to feed on species of the genus *Commelina*. For instance, the house cricket (*Acheta domesticus* L.) is known to feed on species of *C. benghalensis*, whereas the field cricket (*Scapsipedus icipe* Hugel and Tanga) feeding on the same plant has not been explored. This is due to lack of feeding experiments which are very complex as plant-food selection by insects varies greatly depending on many factors (e.g., environment, species of cricket and species of plant). Some studies suggested that odor, visual cues like color and phytochemicals are used by insects for host plant finding and acceptance (e.g. Kostal & Finch, 1996; Prokopy *et al*., 1983a, 1983b;

Stanton, 1983). In contrast, other studies indicated that plant chemical constituents and nutrient contents are more important in food discrimination and preference (Bernays, 1995; Chapman, 1995; Chapman & de Boer, 1995; Matthews & Matthews, 2010; Simpson *et al*., 1995; Ying *et al*., 2003). Nevertheless, Ying et al. (2003) demonstrated that some insects can even differentiate diverse plant-food types based on phytochemicals and nutrient contents without relying on visual or color detections. Generally, phytochemicals in plants act as repellent or attractant for herbivorous insects, while nutrient contents are important for their development and survival (Bernays & Chapman, 1994). Moreover, the presence or relative concentrations of such bio-active compounds varies across taxonomic groups of plants (Capinera, 2014; Chapman, 2009; Ward *et al*., 2003). This study hypothesizes that the field cricket (*Scapsipedus icipe*) will have similar feeding preference for different species of *Commelina*. While feeding insects with good quality plant can improve their development and nutritional value, it is equally crucial to manage waste accumulation in cricket production to prevent significant environmental issues. Proper waste management plays a vital role in maintaining a clean and healthy environment. For instance, in large-scale insect production facilities, the substantial amount of frass produced can lead to insect mortality, air pollution, and nutrient imbalances in the soil. Therefore, implementing effective waste management practices becomes essential to mitigate these potential problems and sustain a more environmentally friendly approach to insect production. Different uses for insect frass have been proposed such as biochar, solid product from biomass pyrolysis, and organic manure (Poveda, 2021). Insect frass is rich in elements such as nitrogen, phosphorus, potassium and microorganisms important for plant growth and soil improvement (e.g., de Souza Vandenberghe *et al*., 2017; Poveda, 2021; Wanjugu *et al*., 2023). This study again hypothesizes that growing conditions for production of *Commelina* using cricket frass as manure is similar.

The field cricket, *Scapsipedus icipe* Hugel and Tanga (Orthoptera: Gryllidae) (Tanga *et al*., 2018) is edible and could be used to reduce malnutrition at local and global levels. It is native of Kenya and distributed in the tropical climate of Africa (Tanga *et al*., 2018; Magara *et al*., 2021). This insect is highly nutritional in terms of protein, fat, fibre, mineral and vitamins (Murugu *et al*., 2021). The present study aims to optimize the utilization of species of *Commelina* as sustainable

feeds for crickets, thus improving cricket farming. Therefore, enhancing cricket production can contribute to food and nutrition security in Sub Sahara Africa.

### 1.2 Statement of the problem

Crickets have significant nutritional value to address malnutrition in Sub Sahara Africa. In this part of Africa, feed scarcity, food competition between human and crickets, high cost of commercial feeds formulated for chicken as well as lack of knowledge to formulate low-cost nutritionally balanced foods for crickets are key challenges for cricket farmers. Low-cost feeds such as agricultural farm weeds are available throughout the whole seasons and can be used as balanced feeds to meet cricket nutrient requirements. A plant in the genus *Commelina,* namely *C. benghalensis* have been proposed as available and affordable feed for crickets (Kinyuru & Kipkoech, 2018). However, there are several challenges that need to be addressed about the utilization of *Commelina* plants as sustainable source of proteins in cricket farming. Firstly, the diversity of species of *Commelina* needs to be determined as this genus represents 51 species in the Flora of Tropical East Africa (FTEA) (Faden, 2012). Secondly, the preferences of crickets for diverse species of *Commelina* need to be evaluated as nutrient contents and phytochemicals influence insect feeding (Bernays, 1995; Chapman, 1995; Chapman & de Boer, 1995; Matthews & Matthews, 2010; Ying *et al*., 2003; Simpson *et al*., 1995), with some insects exhibiting higher preferences for single species, genera, family, and even cultivars (Capinera, 2014; Murray *et al*., 2007; Murray & Clements, 1994). Finally, waste disposal from cricket farming such as cricket frass using as manure for optimum growing conditions for mass production of *Commelina* plants, need be determined. These challenges are interconnected and must be considered in the cricket production as the diversity, nutrient contents and phytochemicals of *Commelina* can influence the preferences of crickets, and the growing conditions of *Commelina* influenced by cricket frass as manure affecting their productivity. Addressing these challenges can contribute to sustainable use of species of *Commelina* as feed for crickets in Sub Saharan Africa.

## 1.3 Objective of the study

### 1.3.1 General objective

To investigate the use of species of *Commelina* as sustainable and affordable feed source for field cricket, *Scapsipedus icipe*

### 1.3.2 Specific objective

The specific objectives are:

1) To determine the diversity of *Commelina* species across different agroecological zones in Western Kenya
2) To evaluate the feeding preferences of *S. icipe* for species of *Commelina*
3) To determine optimum growing conditions for production of species of *Commelina* using cricket frass as manure

## 1.4 Hypothesis

I am going to test:

1) Ho – There is no diversity in *Commelina* species across different agroecological zones in Western Kenya
2) Ho – Feeding preferences of *S. icipe* for species of *Commelina* is the same
3) Ho – Growing conditions for production of species of *Commelina* using cricket frass as manure is the same

## 1.5 Rational of the study

The use of insects as a source of protein is gaining increasing attention as a sustainable and efficient alternative to traditional livestock production. The field cricket, *S. icipe*, is edible and could be used to address the issue of malnutrition due to its high nutritional contents in terms of protein, fat, fibre, mineral and vitamins. Moreover, it is more environmentally friendly and less expensive to rear this insect for food and commercial farming. However, the availability and cost of feed remain major constraints to the development of cricket farming. The species of *Commelina* are known to be diverse, abundant and nutritious in Sub-Saharan Africa, and have the potential to serve as a cost-effective and sustainable feed source of protein in cricket farming.

### 1.6 Justification of the study

The cricket farming is important to enhance food security and generate new source of income. For plant-food habit of crickets, the genus *Commelina* has not been extensively studied as sustainable feed for crickets generally, and likely the field cricket (*S. icipe*). This study will contribute to filling this knowledge gap by determining the diversity of species of *Commelina* and evaluating the feeding preference of *S. icipe* for these plants. In addition, by determining the optimum growing conditions of species of *Commelina* using cricket frass as manure, this is very important because cricket frass is a readily available and nutrient-rich waste product of cricket farming, and can be utilized as a sustainable and cost-effective fertilizer for growing species of *Commelina* and improve soil fertility. This study is relevant for enhancing food security in Sub-Saharan Africa.

### 1.6 Scope and limitation of the study

The study was carried out at Jaramogi Oginga Odinga University of Science and Technology (JOOUST)-main campus insect and crop farms. The cricket insect type proposed in this study was the field crickets, *Scapsipedus icipe* (Orthoptera: Gryllidae). This study was limited to the ecological and nutritional traits of species of *Commelina* as feed for crickets, as well as agronomic trait of using cricket waste as natural fertilizer in the growth of species of *Commelina*. It is important to keep in mind that there may be additional traits or factors that could influence the feeding preferences of *S. icipe* beyond our context. The present study is focused on optimization of species of *Commelina* as sustainable feed for *S. icipe* in Sub-Saharan Africa.

# CHAPTER TWO: LITTERATURE REVIEW

## 2.1 Introduction

The feed supply in insect production may present a challenge, as it plays a crucial role in providing proteins and essential nutrients such as vitamins, carbohydrates, and fats to support the development of insects' bodies (Van Huis *et al*., 2022). Factors such as availability of feeds, inconsistency in nutritional composition of agricultural side streams used as feed and high cost of commercial feed formulated for chickens are major food-related-challenges limiting crickets production in Sub Saharan Africa. Agricultural plants, including farm weeds, are diverse and play a vital role in supporting various forms of biological diversity. While these plants are often perceived as detrimental to agricultural food production due to potential yield losses, numerous positive properties have been identified. These encompass their contributions to the environment and food supplementation, such as improving soil health through organic matter provision and serving as beneficial habitats for insects. Additionally, they offer nutrient enhancements, including proteins, fiber, and minerals, and have applications in traditional medicine (Chikwanha *et al*., 2007; Landis *et al*., 2005; Rodenburg *et al*., 2020). For food supplementation, agricultural farm weeds of the Commelinaceae family, such as *Commelina*, have demonstrated their potential in animal feeding. They can serve as feed for ruminants, monogastric animals like pigs, and even edible insects (Akinfemi & Mako, 2012; Chikwanha *et al*., 2007; Kuo & Fisher, 2022). The role of *Commelina* species in feeding edible insects is evident, and crickets feeding on them need to be carefully evaluated. This literature review explores the preferences of crickets for agricultural farm weeds, including species of *Commelina*, by providing insight into factors affecting feeding and enhancing plant quality as feed for insects. The plants of the Commelinaceae family are poorly investigated concerning cricket nutrition.

## 2.2 Diversity and reproduction of species of *Commelina*

Plant species growing in agriculture ecosystems are known as crops and weeds. Crops are plants cultivated on large scale for profit or subsistence, whereas weeds are considered as unwanted plants that grow in competition with crops. While all plant species that interfere with crops in agricultural ecosystems are known as weeds, in natural ecosystems all non-native plants that have negative impacts on biodiversity and ecosystem functioning are also recognized as weeds (Ehrenfeld, 2010). Members of the genus *Commelina* are weed species growing in several

agricultural ecosystems and commonly known as “Dayflower” and sometimes refer to “Wandering jew” (e.g., Brazil). This genus of the family Commelinaceae is diverse and widespread in the tropical and subtropical regions, and even warm-temperate regions of the world (Faden, 1998; Wilson, 1981). The genus *Commelina* is regarded as the largest of the family (Acevedo-Rodr & Strong, 2005) containing approximately between 170 to 215 species worldwide (Faden, 1998; The Plant List, 2013), with some species (e.g., *Commelina benghalensis*) occurring with a number of unusual morphological variants (Faden, 2012). These species are herbaceous annuals or perennials that propagate both sexually (seeds) and asexually (vegetative), with vegetative propagation highly plastic and adaptable for rapid production and uniform plant growth (Budd *et al*., 1979; Ecker & Barzilay, 1993; Webster & Grey, 2008; Yang & Kim, 2016). The stems of species of *Commelina* have a high moisture content and once well rooted they can survive for long periods without moisture and produce more seeds (Wilson, 1981). For instance, it has been reported that mature aerial seeds of species of *C. benghalensis* are produced within 14 to 22 days after flower opening (Walker & Evenson, 1985). Studies in controlled environment have shown that species of *Commelina* may depend on several factors such as light, temperature, agriculture inputs, ground water level (Haroon *et al*., 2019; Isaac *et al*., 2013; Riar *et al*., 2016).

### 2.2.1 Nutrient contents of species of *Commelina*

Species of *Commelina* are reported to be utilized as a leafy vegetable, forage for ruminants, crop protection and fuel (Lanyasunya *et al*., 2008; Makokha *et al*., 2017; Orech *et al*., 2007). With a wide range of uses, they supply a large proportion of proteins, minerals, and vitamins as food supplementation, improving the livelihoods of communities in different parts of the world. Members of the genus *Commelina* are known as major contributors of micro and macronutrients in diets, being a good source of proteins, significant amounts of calcium, iron, and manganese (Lanyasunya *et al*., 2008; Orech *et al*., 2007). For instance, *C. forskaolii*, *C. africana* and *C. benghalensis* are utilized as leafy vegetable in East Africa (Johns & Kokwaro, 1991; Orech *et al*., 2007). Moreover, it has been reported that species of *Commelina* namely, *C. diffusa*, *C. benghalensis, C. erecta* and *C. communis*, exhibited rich nutritional profile with organic matter (88.0–95.30%), crude protein (14.71 - 19.50%) and fats (2.10–3.70%) (Cavichi *et al*., 2023;

Ezeabara *et al*., 2020; Peduruhewa *et al*., 2021). These species are also rich in micro-nutrients (calcium, magnesium and manganese) and vitamin C, vitamin B2 and vitamin B3 to alleviate food malnutrition. Furthermore, several studies reported that species of *Commelina* namely, *C. forskaolii, C. benghalensis, C. africana, C. diffusa and C. erecta* are regarded as good ruminant fodder for goats, sheeps, cows and other ruminants in Sub Sahara Africa (Geesing & Djibo, 2001; Ingratubun et al., 2000; Lanyasunya *et al*., 2008).

### 2.2.2 Phytochemicals of species of *Commelina*

The plants of the genus *Commelina* are widely used in medicine due to their role as a source of bioactive compounds. Although there may be no apparent morphological characteristics in their growth, they possess therapeutic properties in traditional medicine (Bussmann *et al*., 2021; Johns & Kokwaro, 1991). They can be used for the treatment of headache, constipation, leprosy, fever, snake bite and jaundice in several part of the world (Hasan *et al*., 2008). In East Africa, species of *Commelina* are used in the treatment of mouth thrush, insanity, epilepsy and psychosis (Bussmann *et al*., 2021; Tabuti *et al*., 2003). In Central and South Africa, *Commelina* are applied to treat infertility in women and in India used as bitter, laxative, anti-inflammatory, demulcent, emollient and depressant (Hong & DeFillipps, 2000). Studies on the biological activity of species of *Commelina* have demonstrated that leaf extracts contain various phytochemicals, such as alkaloids, flavonoids, steroids, terpenoids, volatile oils, saponins, and tannins, among which flavonoids are the most frequently identified (Martínez & Swain, 1985). Additionally, the chemical compositions of species of *Commelina* are highly variable due to growth characteristics, genetic variations, and environmental factors. For example, in *C. erecta,* 13 phenolic compounds were identified, with apigenin, luteolin, and quercetin derivatives being the most abundant (Cavichi *et al*., 2023), while *C. diffusa* contains 21 phytochemical components, with sterols, terpenoids, and alkanes being abundant (Rahman *et al*., 2021).

Some phytochemicals produced by various plants act as antifeedants or phagostimulant to many herbivorous insects. Antifeedants or repellents are a class of compounds that inhibit insect feeding, although they do not directly kill insects. Most plants that show antifeedant activity are classified

into the following four categories: sesquiterpene lactones, heterogeneous flavonoids, quassins, and limonoids (Pan *et al*., 2016). Some of these agents can either show only relative antifeedant activity within a certain period or show a long period antifeedant activity, by affecting insect host selection. As for feeding phagostimulants, these compounds serve as important host recognition cues for herbivorous insects. Such cue compounds can occur as volatile attractants or as contact stimulants that insects only perceive after biting the tissue. For instance, sucrose alone and some phenols are known to play a role of phagostimulant stimulating cells in the lateral and epipharyngeal sensilla of many herbivorous insects (Chapman, 2003). Hence, feeding by herbivorous insects is governed by the balance of phagostimulatory and deterrent inputs, and this might be the sole determinant of acceptance or rejection.

## 2.3 Cricket insects

### 2.3.1 Morphological structure and Ecology

Crickets account approximately 2400 species belonging in the order of Orthoptera and Gryllidae family. Crickets have hard shell called an exoskeleton covered the body with a morphological structure characterized by hind jumping legs, long thread-like antennae, two slender tactual abdominal cerci, three tarsal segments, and some bulbous sensory setae basally on the insides of the cerci. Cricket has three stages in their life cycle: egg, nymph, and adult. At maturity, a female cricket has a long tube called ovipositor extending from the end of the abdomen that easily lay approximately 100 to 200 eggs.

The humidity, land-use management and ecosystem disturbance regulate the range, population and density of crickets (McCluney & Date, 2008). For instance, a female cricket requires warm and moisture sites for oviposition and immatures have difficulties to survive in cold environments (Ferreira & Ferguson, 2010). Nevertheless, crickets occur over a wide geographical range and live in trees, shrubs, grass and even underground. Crickets are omnivorous, feeding on varieties type of foods. They eat dried organic materials, fresh plant matter, fruits, seeds, and both living and dead organisms. In nature, crickets play important role in the decomposition of communities of many ecosystems.

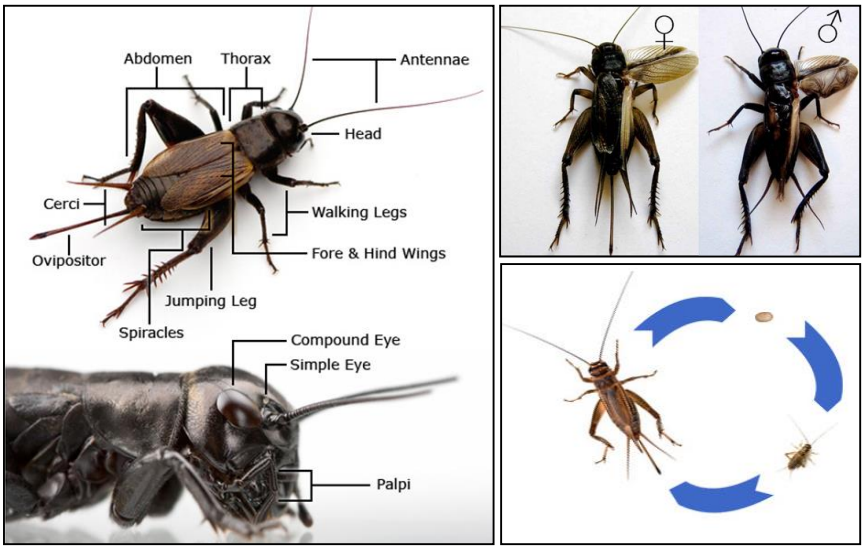



**Figure 1. Morphological structure and life cycle of a field cricket**

### 2.3.2 Domestication and mass rearing

The cricket production system is expected to grow significantly as an increasingly affluent global population reaches over 9 billion by 2050 due to the demand for protein. Crickets farming are considered has the most common species farmed. For instance, it was recently estimated that over 20 000 cricket farms have been successfully recognized in Thailand, whereas in Texas about 22 million crickets raised every month at Aspire Food Group's indoor (Engelking, 2017; Udomsil *et al*., 2019). Edible insects are harvested from their natural habitats (Van Huis *et al*., 2013), with some species domesticated for home consumption purposes as well as for business supply at small and large scales. However, successful in domestication of cricket requires knowledge of proper technics regarding housekeeping, adequate tools and materials, good food quality and best practice of environmental conditions. Crickets have a short life span and can be easily reared with plant materials.

### 2.3.3 Nutritional composition of crickets

There are several species of cricket world widely consumed. Specific analyses of different nutritional aspects of crickets have been done on several species, such as the field cricket (e.g., *Gryllus* and *Teleogryllus* genera) and the house cricket (*Acheta* genus). An investigation carried

by Wang *et al*. (2004) on the nutritional content of the adult crickets (*Gryllus testaceus* Walker) indicated that the insect is rich in crude protein 58.3 %; fat 10.3 %, chitin 8.7 % and ash 2.96 % on dry matter basis respectively. In the same study, it was concluded that the total percentage of oleic acid, linolic acid and linolenic acid was 77.51%. In another study by Bawa *et al*. (2020) on nutritional content of the house cricket (*Acheta domesticus*), it has been shown that the insect contain high mineral contents for sodium, calcium, potassium, phosphorus and iron as well as vitamin B content for B2, B3 and B12. It is important to note that different feeds have an effect on the nutritional composition of cricket for crude proteins, carbohydrates, fats and macro/micronutrients (Bawa *et al*., 2020; Miech *et al*., 2016; Oloo *et al*., 2020; Orinda *et al*., 2017). Furthermore, crickets can be processed into foods such as biscuits, bread and flour without losing their micronutrient value (Dobermann *et al*., 2019).

### 2.4 Insect behavior and food-plant selection

Agricultural plants such as weed farms are considered as primary source of food for many insects (Capinera, 2005). It has been shown that many insects are able to feed on many of them to be known as generalists, while few of them can feed on specific plants and are known as specialists. This involves a multifaceted selection criterion mostly related to selection and behavior habits, mouthpart morphology, allelochemical-based compounds preference, feed on plants within certain families and feed on plants part such as inflorescence, stems or leaves. For instance, Opoke *et al*. (2019) conducted a study on the preference plants for the edible grasshopper (*Ruspolia differens*) and the insect was found to feed frequently on two grasses (*Panicum maximum*, and *Sporobolis pyramidalis*), but could also accept a wide range of species of the same family namely, *Brachiaria ruziziensis*, *Chloris gayana*, *Hyparrhenia rufa*, *Cynodon dactylon* and *Pennisetum purpureum*. Furthermore, it was reported in the same study that the most predominantly part of these plants eaten by the insect were the inflorescences. Another study conducted on the edible crickets (*Gryllus pennsylvanicus* L.) reported that the insect eat average of 223 redroot pigweed (*Amaranthus retroflexus* L.) seeds per day (Carmona *et al*., 1999), while the house cricket, *Acheta domesticus* can feed well on several species including species of *Commelina benghalensis*, *Bidens pilosa, Gallisonga parviflora, Lantana camara* and *Demodium spp.* (Kinyuru & Kipkoech, 2018). However, it is important to note that on some occasion, most insects over-run their food supply,

and may be forced to feed on nonpreferred or unacceptable plants. In this case, behavior orientation of insects plays an important role in the choice of resources (Finch & Collier, 2000).

### 2.5 Farming systems

Farming system is a method of farming which aimed to cultivating land and raising crops in such a way as to keep the soil alive and healthy by use of various kind of organic wastes such as crop, animal, farm waste and other biological material along with biofertilizers. It basically brings together the plant development, impact on growth of soil, water, nutrients, disease and pests and the influence of management processes. Good farming system meet the growing demand for food, focus on closing yield gaps, and take into account the minimization of environmental stress (Balmford *et al*., 2005; Pradhan *et al*., 2015). There are different types of agricultural farming systems depending on the purpose of the plant use under a given social, economic and environmental conditions. In this case, the type of crop grown may depends on the traditional, organic or conventional management systems available. However, crop production for phytomass purpose can be grown in different types of environments from open fields, greenhouses with or without environmental control units, to indoor system.

The open field system is characterized by stable crops in a naturally environment with very low natural and artificial controllability of the root zone and aerial environment (weather), whereas the indoor system is generally characterized by high artificial controllability of both the aerial and root zone environment. As for the greenhouses, it shows intermediate stability and controllability between open fields and indoor systems (Kozai & Niu, 2016). Additionally, in many urban landscapes of the world with scarcity of arable lands, the practice of growing crops on top of residential, commercial, and industrial buildings has been successfully adopted as a mean to maximize underutilize spaces. The system is well known as Rooftop system (RS), with building design with green roof (Mandel, 2013; Sabeh, 2016; Whittinghill & Starry, 2016), recognized as mitigation strategies to alleviate urban particulate pollution (Speak *et al*., 2012). Sometimes RS is an integration part of indoor system when plants are produced following a vertical farming system. Vertical farming is the practice of producing food on vertically inclined surfaces.

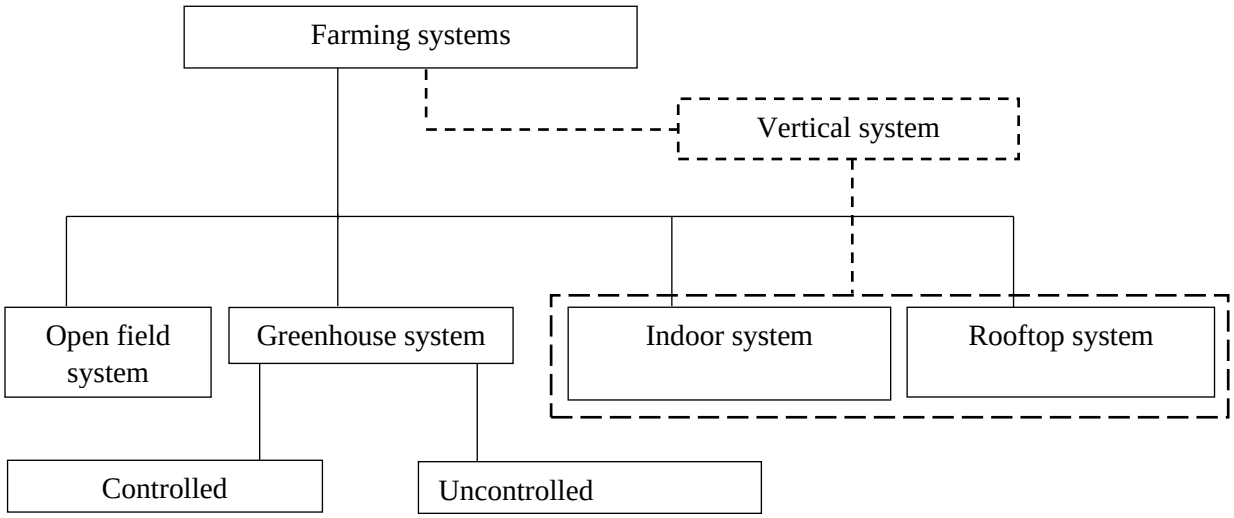



**Figure 2. Type of farming systems**

### 2.5.1 Role of fertilizer in farming system

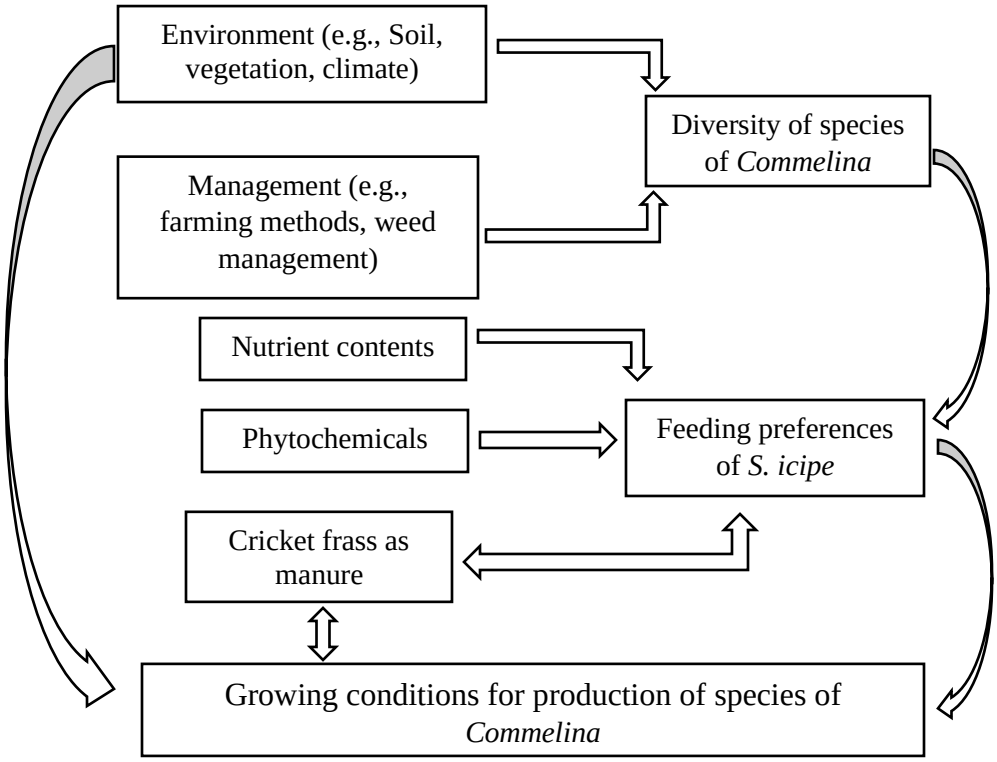
Farming systems rely on fertilizers (both synthetic fertilizers and animal manure) for crop production. Fertilizer has been defined as any substance, solid or liquid, inorganic, natural or synthetic, single or combination of materials that is applied to soil or to a plant to provide one or more of the essential elements for plant nutrition, growth yield, or quality, or for producing a chemical change in the soil that contributes to improved plant nutrition and growth (Kozai & Niu, 2016). Fertilization is successfully achieved when the soil or any substrate is supplied with essential nutrient in adequate amount and proportion throughout the plant growing season. Plant nutrition and soil fertility can be adapted to specific site by combining nutrients from organic, mineral and biofertilizer sources to serve needs of food production and economic, environmental and social viability. Different plants need specific nutrients to meet their requirements to sustaining nutrient level in the soil or substrate. Hence, nutrient is classified in three groups based on plant grow need. Most plant needs macronutrients or primary nutrient including nitrogen (N), phosphorus (P), potassium (K), secondary nutrients such as calcium (Ca), magnesium (Mg) and sulphur (S) and micronutrients or trace elements such as Chlorine (Cl), Iron (Fe), Manganese (Mn), boron (B), selenium (Se), zinc (Zn), copper (Cu), molybdenum (Mo). Macronutrients are the

elements that the plant needs in high concentrations, whereas secondary nutrients are required in smaller amounts in comparison to macronutrient (Kihara *et al*., 2017). Micronutrient are also needed in small amount and plays important role in the growth and defense of plants (Römheld & Marschner, 2018). The main three sources of fertilizers are organic, inorganic and biofertilizers. Organic fertilizers are made from natural and organic materials mainly composed of peat moss, compost, or other animal manure and plant products degradable, whereas inorganic fertilizers are made of chemical components that contain necessary nutrients usually in the form of liquid, powdered or granular available in bags. The biofertilizer fertilizer are valuable microorganisms such as bacteria, algae and fungi alone or combination which play a vital role in plant development by helping them reach and absorb nutrients.

### 2.5.2 Role of water, temperature and light in farming system

The three most important variable affecting plant growth are water, light exposure, and temperature in any farming system. The requirement or amount of these variables for a plant to be produced is important. The concept to understand when growing plants is the rule of limiting factors, which determines the quality of the plant and determine optimum growing conditions of these variables. Water movements (water-table fluctuations) have particularly drastic consequences on plants (Bornette & Puijalon, 2009). Hence, applying too much water can suffocate plant roots and too little water causes growth to become erratic and stunted. Watering frequency will depend on the conditions under which the plants are growing. As for temperature and light, these two are linked through the processes of photosynthesis and respiration. For instance, reductions in light intensity could affect carbon balance of plant (Lichtenthaler *et al*., 1981; Su *et al*., 2014), while high temperature than needed speeds up respiration leaving the plant with little to none of resources for growth. Therefore, under insufficient light and above or below temperature that is needed the plants do not grow.

## 2.6 Conceptual framework of the study




Notes
One-way arrows indicate that one variable influence another in a unidirectional manner.
Two-way arrows indicate that two variables influence each other in a bidirectional or feedback loop manner.

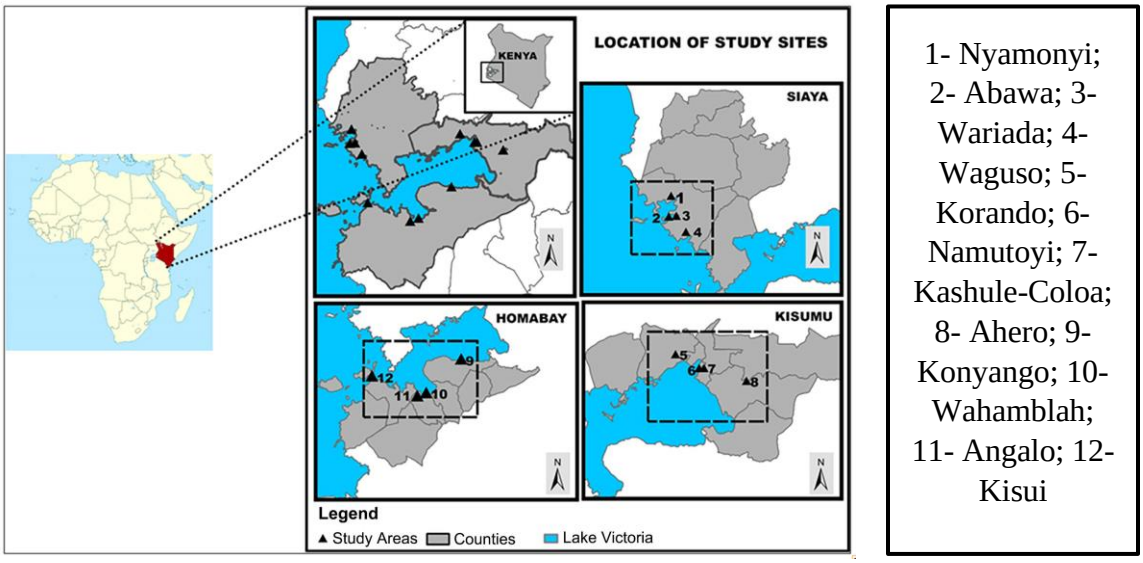
**Figure 3. Conceptual framework**

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 To determine the diversity of species of *Commelina* across different agroecological zones in Western Kenya

### 3.1.1 Study site

This study was carried out between October-December 2020 in three Kenyan counties (Siaya, Kisumu and Homabay) surrounding Lake Victoria in Western Kenya. Kenya is mostly divided in seven agroecological zones, with the western Kenya classified between zones I and III characteristics with humid to sub-humid climate (Sombroek *et al*., 1982; Jaetzold & Schmidt, 1982). The vegetation surrounding the Lake Victoria varies from woodlands, wetlands, and even sometimes croplands. The annual precipitation in Siaya county range between 1100 – 1600 mm, whereas in Kisumu it varies between 1200 – 1700 mm. As for Homabay county, the precipitation ranges between 1200 – 1800 mm per annum. The main soils types in cultivated land in Western Kenya are mostly a mixture of acrisols, nitrosols, ferralsols and cambisols (Ngome *et al*., 2011a; Omuto, 2013) suitable for agriculture (Recha, 2018). In the study site, several crops are grown by smallholder farmers. This includes maize, sorghum, rice, bean, vervet bean, kale, tomato, spinach, cabbage, nightshade, spider flower, onions, sugarcane, citrus, orange, mango, avocado, papaya, sweet potato, cucumber, groundnut and cotton. Predominance of conventional system is adopted and applied by farmers as cultural practice, but can also follow certain techniques/methods such as crop spacing, crop establishment, farming methods, manure inputs and control of weed. The farm lands in the three counties comprised 12 production sites under two agriculture system types, the rainfed and irrigation systems. These production sites adjacent Lake Victoria were chosen due to accessibility and area where agriculture activities were still taking place (Figure 4). Geographically, these sites undulate at an altitude ranging between 1121.9 m and 1174.4 m above sea level.




1- Nyamonyi; 2- Abawa; 3- Wariada; 4- Waguso; 5- Korando; 6- Namutoyi; 7- Kashule-Coloa; 8- Ahero; 9- Konyango; 10- Wahamblah; 11- Angalo; 12- Kisui

**Figure 4. Map indicating the location of the study area in Western Kenya (production sites located in Siaya, Kisumu and Homabay counties)**

### 3.1.2 Data collection

#### 3.1.2.1 Plant Sampling

Purposive sampling technique was employed to collect the species of *Commelina* in farmer fields using quadrat of 1 × 1 m size. Since farmer fields comprise small hectarage with irregular shape, the number of sampled fields differed between production sites. Hence, three quadrats per field were sampled to maintain the filed uniformity corresponding to 180 quadrats recorded in total of our study area. The species of *Commelina* and associated weed species were recorded following phytosociological method determined by Mueller-Dombois and Ellenberg (1974) that consist of counting all individual species in a quadrat. Identification of weed species was captured using field guides (Agyakwa & Akobundu, 1987; Ivens, 1967), regional flora for comprehensive identification of Commelinaceae family (Agnew, 2013; Faden, 2012), and AFROweeds identification tool (Rodenburg *et al*., 2016). Weed species difficult to identify in the field were collected and pressed for later determination at the East African Herbarium (EAH) of the National Museums of Kenya. Correct species names were verified using The Plant List (2013) and Faden (2012) for *Commelina* plants. Life-cycle of weed species were classified in five groups (annuals, perennials, short-lived perennial, parasitic and unknown).

**Picture 1: Plant sampling using quadrats followed by identification at the East African Herbarium (EAH) of the National Museums of Kenya**

### 3.1.2.2 Environment and management variables

In the study area, 60 soil samples were used to explain consistency of environment nutrient variables background on species of *Commelina*. Soils were sampled at a depth of 0 - 20 cm (Cline, 1944). After sampling, the soils were labelled, air-dried, sieved with 2-mm aperture and placed in plastic bags. Later, about 200 grams of each soil sample from same production site were combined in a composite sample to be submitted for laboratory analysis at Kenya Agriculture and Livestock Research Organization (KALRO) in Nairobi. The analysis included soil pH, electric conductivity (EC), total organic carbon (TOC), total nitrogen (TN), available phosphorus (P), cation exchange capacity (CEC), exchangeable Ca, Mg, K, Na, exchangeable sodium percentage (ESP) and soil texture (silt, sand and clay). The concentration of TN, TOC, CEC, Exchangeable Ca, Mg, K, Na, ESP and soil texture class (silt, sand and clay) were expressed in percentage, whereas available P was expressed in ppm (party per million) and Electric conductivity (EC) expressed in mS/cm (millisiemens per centimeter). The Soil pH and EC were determined in a 1:2.5 (w/v) soil-water suspension with a pH meter and conductivity meter, respectively (Varley, 1972). For the determination of Ca, Mg, K, Na and CEC, soil samples were leached with 1N ammonium acetate buffered at pH 7. The leachates were analyzed for exchangeable Ca, Mg, K and Na. Furthermore, samples were leached with 1 N KCl, and the leachate was used for the determination of the CEC. The determination of Na and K elements were done with a flame photometer, whereas Ca and Mg

elements were determined with AAS (atomic absorption spectrophotometer). The CEC was determined by distillation followed by titration with 0.01 N HCl (Page, 1982). Conventional routine methods were used to determine TOC (Nelson & Sommers, 1996), available P (Mehlich, 1984) and TN (Bremner, 1965). The soil texture (proportion of silt, clay and sand) was determined by the Hydrometer method (Bouyoucos, 1951).

For field management, 60 farmers were interviewed using semi-structured questions about farming methods, crop establishment, crop spacing, weed control, cost of weed control, fertility, and agriculture system type to understand the background of these variables on species of *Commelina*. Soil sampling was conducted in their fields, and information on surrounding vegetation was recorded through field observations. The data were captured using an open questionnaire pre-installed in ODK tools (ODK Collect v1.28.2). The derivation of surrounding vegetation and management variables is presented in Appendix 1.

### 3.1.3 Data analysis

To assess the relative density of weed species in quadrats, absolute density was measured as total number of individual species per total number of quadrats studied. The relative value was obtained by dividing absolute value by total value for all species multiply by 100 percent, calculated in Microsoft Office Excel 2021® program.

The diversity indices of species of *Commelina* among production sites was evaluated as Shannon-Weaver (H) diversity index (Shannon & Weaver, 1949), Pielou's evenness (E) index (Pielou, 1966) and Margalef (M) index (Magurran, 1988). These indexes were calculated following the equations:

$$H = -\sum_{i=1}^{s}\left(P_i\right)\left(\ln P_i\right) \qquad (1)$$

$$E = \frac{H}{\mathrm{Ln}S} \qquad (2)$$

$$M = \frac{S-1}{\mathrm{Ln}N} \qquad (3)$$

where $P_i$ is the proportion of all observations in the $i^{\text{th}}$ species, $N$ the total number of individuals of all species in the sample, Ln = $\log\text{base}_n$ and $S$ the number of unique species per quadrat. Higher

value of these indices indicates high diversity and lower value a low diversity, a value of indices equals to 0, indicates community dominated with only one species.

Differences in environment variables among production sites were assessed by the analysis of variance (ANOVA). At all analysis, pairwise comparison evaluated significance of means for any difference in variable among production sites using Turkey's Honest Significant Difference test ($P < 0.05$). All 14 environment variables were normalized by logarithmic [$\log(x + 1)$] transformation to meet the assumptions of normality because one unit variation in nutrient concentration is considered as much more important at low than it is at high concentrations (Palmer, 1993).

The relationship between diversity of species of *Commelina*, environment and management variables were evaluated using multiple linear regression analysis. Prior to analysis, the diversity indices and environment variables were standardized. Shannon-Wiener diversity index (H'), Pielou's evenness index (E) and Margalef index (M) were considered as responses, whereas environment and management variables were predictors. Selection of best model depended on statistic methods for Adjusted $R^2$ values and difference between models for Akaike's information criterion and Bayesian information criterion. The standardized beta coefficients, ranked the order of predictors in term of their contribution to the model. In multiple linear regression, significant ($P < 0.05$) environment variables with variance inflation factor (VIF < 20) were used as dropping threshold. This procedure resulted in elimination of two variables: calcium and cation exchange capacity due to high multicollinearity (Appendix 1). Analysis of variance and multiple linear regressions were employed using STATA 14.2 statistical software (Stata Corp LLC, Texas, USA).

To explain the relationship of weed species—explanatory variables, multivariate statistical analysis as ordination technique was employed (Hanzlik & Gerowitt, 2016). Prior to multivariate analysis, we prepared three matrices in form of tables: 1) weed count with r rows and c columns (r = 180 quadrats; c = 115 species); 2) weed count with r rows and s columns for species of *Commelina* (s = 11 species); 3) an environment and management matrix with r rows and v columns (v = 22 variables) of which 14 quantitative environment variables: electric conductivity (EC), soil pH, total organic carbon (TOC), total nitrogen (TN), available phosphorus (P), cation exchange

capacity (CEC), Exchangeable Ca, Mg, K, Na, exchangeable sodium percentage (ESP), soil texture (silt, sand and clay) and seven qualitative variables, management recorded into "binary dummy" variables (farming method, crop establishment, crop spacing, weed control, cost of weed control, fertility and agriculture system type). Description of surrounding vegetation as environment variable was also recorded as "binary dummy". To achieve the assumptions of normality, weed count data were square-root transformed, which is considered the most appropriate for count data in quadrat (Lepš & Šmilauer, 2003), while quantitative environment nutrient variables follow a logarithmic [log(x + 1)] transformation. The scientific names of all recorded weed species were replaced by their five-character EPPO codes (European and Mediterranean Plant Protection Organization (EPPO, 2021).

*Detrended Correspondence Analysis (DCA)*

A Detrended Correspondence Analysis (DCA) was run on the entire data set (180 quadrats by pattern of 115 species) to detect ecological conditions of species of *Commelina* and composition of associated weed species. Because some species of *Commelina* were recorded with low counting, rare species were not down-weighted or selected following a specific criterion to allow maximum differentiation among species.

*Canonical Correspondence Analysis (CCA)*

At first, a Canonical Correspondence Analysis (CCA) was performed to link the relationship between the distribution of *Commelina* and environment and management variables. The data set of 180 quadrats by patterns of 11 species of *Commelina* revealed a unimodal rather than linear ordination technique checked by DCA depending on the gradient length (SD units > 3) (Ter Braak & Verdonschot, 1995). Hence, we subjected our data to a Canonical Correspondence Analysis (CCA, assuming unimodal response) using methods recommended by Lepš & Šmilauer (2003). All multicollinearity issues among explanatory variables were checked by discarding variables with variance inflation factor (VIF = 0 or VIF > 20; Ter Braak & Verdonschot, 1995). We analyzed marginal and conditional effect using forward selection to rank importance of environment and management variables that build our minimal significant model. Only significant ($P < 0.05$) variables were used for CCA ordination to improve explanation of variables in the

diagram, and variables with non-significance ($P > 0.05$) were excluded (Borcard *et al*., 1992).

Secondly, a partitioning variation of the two sets of explanatory variables ("environment" and "management") was assessed using *Commelina* data set (180 quadrats by patterns of 11 species of *Commelina*). Partitioning variation was helpful to quantify fraction of variation explained of each single effect of explanatory set ("environment" and "management", respectively) and "shared" effect (environment × management). This was resulting in the summation of all fractions ("environment" + "management" + "shared" effect + U, with U being the unexplained variation). To achieve this approach, a series of CCAs and partial CCAs (pCCAs) were carried out following Borcard *et al.* (2012) steps: 1) a CCA with all two variable sets (environment and management) initiated for quantification of fraction of total amount of variation explained (TAVE), no covariable was included; 2) a pCCA with one of the two variable sets as environment variable and the other as covariable to get single effect for each set of variable; 3) variation of shared effect interaction between the two variable sets was calculated; 4) unexplained proportion of variation was calculated (100-TAVE). Analyses of DCA, CCAs and pCCAs ordinations were performed using CANOCO program (version 4.56; Ter Braak & Smilauer, 2002) and CanoDraw for Windows (version 4.12; Ter Braak & Smilauer, 2002) to visualize the graphs generated by DCA and CCA.

### 3.2 To evaluate the feeding preference of *Scapsipedus icipe* for species of *Commelina*

#### 3.2.1 *Scapsipedus icipe*

Colonies of *Scapsipedus icipe* were obtained from Jaramogi Oginga Odinga University of Sciences and Technology (JOOUST) insect farm in Bondo, Kenya since 2014. The crickets are reared in different screenhouses under optimal temperature range between 28$^{o}$C to 36$^{o}$C, 59-77% relative humidity (RH), and scotophase of 12 hours. They are feed on commercial diet (chicken mash) from Unga Farm Care (E. A.) Ltd FUNGO® Grower Mash, Nairobi, Kenya. Field cricket, *S. icipe* is a native species of Kenya and it is adapted to diverse climatic zones and different food types (Tanga *et al*., 2018).

Three batches of eggs of *S. icipe* were incubated in three 100-litre plastic buckets (950 eggs/buckets). The eggs were placed on humid cotton wool in the buckets and covered with 1 mm mesh net to prevent predators eating the crickets and their escaping. To provide a refuge, egg trays (29 cm × 29.5 cm) were placed vertically in the buckets. Drinking water was provided ad libitum in the form of moist cotton wool in a 16 cm saucer, which was changed every two days. Other conditions, such as, cleaning, disinfection and control of predators was monitored every day following the procedure in the cricket rearing handbook of Orinda *et al*. (2021). Crickets were supplied with commercial diet for a period of 30 days, starting from day 14 Post-Hatching (PH). One-month old crickets with same body size were used for preference experiments. Crickets were weighted at the beginning (1$^{st}$ day - initial weight) and end (5$^{th}$ day - final weight) in no-choice experiment. Mortality of crickets was recorded daily in each treatment and dead insects replaced immediately by live ones from backup buckets, which each contained 15 crickets reared in the same food-plant/treatment as those selected for the preference experiments. Prior to providing the crickets with leaves of *Commelina* they were deprived of food for 16 h to increase their hunger.

**Picture 2: Collection of cricket eggs and cricket maintenance at JOOUST insect**

### 3.2.2 Source and management of species of *Commelina*

A total of 11 of species *Commelina* were obtained from different agroecological zones of Western Kenya (Appendix 4). Each of these species was grown in a plot of 1 m × 1 m, replicated three times and watered every day for a month before use in the feeding experiments. No pesticides or fertilizers were used. Fresh leaves were cut from the first to third nodes in the apex of each *Commelina* plant for feeding to the crickets. In addition, fresh leaves of each of the 11 species were harvested for analysis of their nutrient content and phytochemicals. Prior to feeding, leaves of each species were rinsed with clean water and then left to dry for 10 min. During the experiments, the crickets were provided with fresh leaves every day. It is important to note that only leaves of *Commelina* plants were supplied as food in this experiment because they were considered to contain the nutrients essential for the development of herbivorous insects (Dethier, 1954).

Calculation of total leaf area ($cm^2$), consumed leaf area ($cm^2$) and percentage of leaf consumed (%) were measured using LeafByte: mobile application (version 1.3.0; Getman-Pickering *et al*., 2020) on Apple iPad mini-3 tablet. The leaves were measured flat before and after feeding using a transparent glass protector, model iPad mini tablet. In order to obtain accurate pictures taken at an angle, a white background scale with 4 black dots arranged in a square (10 cm spacing) was used.

### 3.2.3 Experiment design

To evaluate feeding preferences of crickets on different species of *Commelina*, three experiments were carried out. A no-choice, dual-choice and multiple-choice experiments were conducted over a period of five days. The no-choice involved 11 species of *Commelina* as treatments, whereas dual-choice experiments involved 11 species of *Commelina* from which 10 treatments were made. The multiple-choice experiment comprised four *Commelina* as treatments. Each experiment was conducted in over a period of 5 days from 18$^{th}$ April to 1$^{st}$ May, 2021. A randomized complete block design with three replicates was involved in each experiment.

#### 3.2.3.1 Leaf feeding

*No-choice experiment*

The purpose of this experiment was to evaluate the rate of feeding of the cricket on the leaves of the most and least preferred species of *Commelina*. Preferences of crickets were assessed relative to two reference species, COMBE1 and COMBE2. In this experiment, crickets were fed 1.5 grams, which was based on a preliminary experiment.

*Dual-choice experiments*

The dual experiments were carried out in similar manner to no-choice experiment, however two *Commelina* species were compared as the experimental unit. Two dual-choice tests were carried out—the first test evaluated each species of *Commelina* in comparison to COMBE 1 and the second test compared to COMBE 2. A preliminary experiment indicated that 1 gram should be fed to the crickets in this experiment.

*Multiple-choice experiment*

This experiment aimed to rank the most preferred species identified in no-choice and dual-choice experiments. Hence, it was done in a similar manner as the no-choice experiment, but there was no reference species. The four most preferred species of *Commelina* were compared with one another. A preliminary experiment indicated that 0.8 grams should be fed to the crickets in this experiment.

### 3.2.3.2 Nutrient contents analysis

Fresh tender leaves of each species of *Commelina* were separately harvested then oven dried at 65$^{o}$C for 24 hours and airdried for 24 hours, and finally crushed using a blander (Sinbo SHB 3090 Turbo Blender, Made in P.R.C.). The powder from each species was passed through a 45 mm aperture sieve. Approximately 25 grams of fine powders of each species of *Commelina* were placed in polyethene bags and kept airtight for nutrient analysis at the nutritional laboratory, faculty of veterinary medicine, department of animal production, University of Nairobi. Nutrient analysis was done involving proximate components and Van soest system for fiber fractions as well as mineral content.

### 3.2.3.3 Proximate components and Van soest system for fiber fractions

Proximate analysis was done using 5 grams of the powder of each plant (Kirk & Sawyer, 1980). The analysis included determination of moisture content (Mc) conversion to dry matter (DM), ash (ASH) content, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen free extract (NFE) according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1998). Moisture content was determined by heating the sample in an oven at 105°C for 12 h, cooled in desiccants at 60°C and weighed. Ash content, which indicates the mineral content, was determined by incinerating samples at 550°C in a muffle furnace, which were then cooled and weighed. Crude protein was determined using the Kjeldahl method and the values multiplied by 6.25. Ether extract of the samples were also obtained. Crude fibre content was obtained by successive digestion of defatted samples using 1.25% sulphuric acid and 1.25% sodium hydroxide solutions (Kirk & Sawyer, 1980). Nitrogen free extract (NFE) was obtained by subtracting the percentage of the above determinations from 100%.

Van Soest analysis was used to determine values of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), levels of cellulose and hemicellulose. NDF, ADF and ADL were determined using the Van Soest & Robertson (1985) method.

### 3.2.3.4 Mineral composition

One gram of leaves powders was placed in muffle furnace at $550^0$C for 1 h. The ashed sample was dissolved in hot 10% of hydrochloric acid and nitric acid (ratio 3:1) and diluted to 100 mL standard flask with distilled water. Content of the various minerals (Iron, Zinc, Calcium, magnesium, sodium, potassium, manganese and copper) were determined using atomic absorption spectrometry (AAS) (Shimadzu, AA-6300, Tokyo, Japan) according to Association of Official Analytical Chemists methods (AOAC, 1998).

### 3.2.3.5 Extraction and analysis of phytochemicals

Leaves of 11 species of *Commelina* were harvested and then left to dry for 7 days to induce the production of bio-active compounds. The dried leaves were powdered using a blender (Sinbo SHB 3090 Turbo Blender) and passed through a 45 mm sieve. The powders were kept in air-tight polythene bags in cool-dry place until required for the laboratory analysis at the nutritional laboratory, faculty of veterinary medicine, department of animal production, University of Nairobi. Confirmatory qualitative tests for nine phytochemicals (phenols, alkaloids, glycosides, tannins, steroids, flavonoids, terpenoids, saponins and anthraquinones) were done using standard methods Yadav & Agarwala (2011), Evans (2009), Sofowora (1993) and Harborne (1973).

*Test for Phenols*

*Ferric chloride test*: a mixture of 2 mL plant extract and 2 mL distilled water followed by 10 % $FeCl_3$ solution were mixed. The bluish black color appearance was an indication of the presence of phenol.

*Test for Alkaloids*

*Mayer's test:* Two mL of water extract was mixed with 2 mL of 1 % HCl and heated gently, then two drops of Mayer's reagent and Dragendorff's reagent were added to the mixture. The presence of yellow precipitation or turbidity confirmed the presence of alkaloids.

*Test for Glucosides*

*Libermann's Test:* Two mL of chloroform and 2 mL of acetic acid were added on 2 mL of crude extract. The solution was ice cooled followed by addition of concentration of $H_2SO_4$. Color change from blue to green indicates the presence of glycosides.

*Test for Tannins*

*Ferric chloride Test:* Two mL of 5 % $FeCl_3$ solution was added to 2 mL of plant extract. Appearance of dark blue of greenish black color indicated the presence of tannins.

*Test for Steroids*

*Sulphuric acid Test:* two mL of extract was mixed with 2 mL of chloroform and concentrated $H_2SO_4$ was added by sides of the test tube and observed for red color at the lower chloroform layer indicated the presence of steroids.

*Test for Flavonoids*

Four mL of extract was taken and 2 mL of 50% methanol added. The solution was warmed and magnesium was added. This was followed by addition of 5 to 6 drops of concentrated HCl. Red coloration confirmed the presence of flavonoids.

*Test for Terpenoids*

*Sulphuric acid Test:* Four mL of extract was dissolved in 3 mL of chloroform. This was than evaporated to dryness and 2 mL of concentration. $H_2SO_4$ was added and heated for about 3minutes. A grayish color indicated the presence of terpenoids.

*Test for Saponins*

*Foam Test:* two mL of extract was taken in a test tube and 10 mL of distilled water was added and shaken vigorously. Formation of foams confirmed the presence of saponin.

*Test for Anthraquinones*

*Borntrager's Test:* A total 0.5 mL of extract was boiled with 2 mL of 10% HCl for how many minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% $NH_3$ solution was added to the mixture and heated. Formation of rose-pink color indicated the presence of anthraquinones in the extracts.

(a) Lab work (b) Foams, presence of saponins (c) blue-dark, presence of phenols

**Picture 3: Extraction and analysis of phytochemicals of species of *Commelina* at the University of Nairobi**

### 3.2.4 Data analyses

Collected data on consumed leaf area, total leaf area, percentage of leaf consumed (%), initial insect weight, final insect weight and mortality were entered and recorded in Microsoft Excel 2021©. Data were checked for normality and homoscedacity using Shapiro-Wilk and Bartlett tests. A logarithmic [log(x+1)] transformation was applied to data where necessary. Non-parametric tests were used in case of nonnormal distribution and no homoscedastic data after transformation.

To differentiate the rate at which the cricket fed on the leaves, comparisons of leaf nutrient concentrations and mortality of crickets were tested using ANOVA, paired t-test and Kruskal-Wallis tests at different critical values of alpha ($\alpha$ =0.0001, $\alpha$ = 0.001, $\alpha$ = 0.01 and $\alpha$ = 0.05).

To determine the relationship between insect final gained weight and percentage of leaf consumed (%) in no choice experiment based on linearity assumption, a simple linear regression model at critical values of alpha ($\alpha$ = 0.05).

Spearman correlation was used to determine associations between the percentage of the leaves consumed and leaf nutrient content at a critical value of $\alpha$= 0.05, whereas Principal

Component Analysis (PCA) using standardized variables (factor loading < .28) revealed patterns of relationship between species and nutrient contents.

Beta regression model was used to determine the phytochemicals that influence leaf feeding at a critical value ($\alpha = 0.05$). This regression is an extension of the generalized linear model and most suitable for situations in which the dependent variable, proportion of the leaves eaten, is positive and recorded at intervals, 0 to 1 and the endpoints 0 and 1 excluded (Cribari-Neto & Zeileis, 2010; Ferrari & Cribari-Neto, 2004). Seven independent variables: phytochemicals either present or absent, and dependent ones: proportions of the leaves eaten were computed. Flavonoids and terpenoids were not included in the analysis as both were only recorded once. Probit functional link for conditional means and log functional link for conditional scales using OIM (Observation Information Matrix) of standard error type was applied. The marginal effect assessed the relative importance of each independent variable in explaining the variation in the dependent variable. A biplot was used to display the relationship between significant phytochemicals from the regression model and species.

The beta regression is parametric and assumes beta distribution with the dependent variable follows a density distribution

$$g(\mu_t) = \sum_{i=1}^{k} \quad x_{ti}\,\beta_i = \eta_t$$

where $\beta = (\beta_{1,\ldots}\beta_k)^{\mathrm{T}}$ is a vector of unknown regression parameters ($\beta \in R^k$) and assumed to $x_{i1,\ldots\ldots\ldots,}\, x_{tk}$ are observations on $k$ covariates ($k < n$), which are assumed fixed and known. Finally, $g(.)$ is strictly monotonic and twice differentiable link function that maps (0,1). The probit function $g(\mu)$ was used $g(\mu) = \varphi^{-1}(\mu)$, where $\varphi$ (.) is the cumulative distribution function of standard random variable.

ANOVA, Kruskal-Wallis tests and paired t-tests were performed using Prism Software (version 8.0.2, GraphPad Inc., San Diego, CA), Spearman correlations, principal component analysis, simple and beta regression model were employed using STATA 14.2 software (Stata Corp LLC, Texas, USA).

### 3.3 To determine optimum growing conditions for production of species of *Commelina* using cricket frass as manure

#### 3.3.1 Experimental site

The experiments were carried out at the Jaramogi Oginga Odinga University of Science and Technology (JOOUST) crop farm from March to June 2022 cropping seasons, with an annual rainfall of 900-1600 mm. The experimental site was located at N 0° 5' 29.328" latitude and E 34° 15' 27.2874" longitude, with an average altitude of 1,200 m a.s.l. (above sea level). The most prominent soil types in the studied area are loamy-sandy. Additional information regarding the characteristics of the soil and cricket frass can be found in Table 1 and Table 2.

**Table 1. Chemical properties of soil before the experiment presented as mean (±SE)**

| Parameters | Mean |
|---|---|
| pH | 6.4±0.2 |
| Ec (dS/m) | 0.25±0.01 |
| Total carbon (%) | 1.32±0.04 |
| Organic carbon (%) | 1.01±0.03 |
| Total Nitrogen (%) | 0.09±0.03 |
| Available Phosphorus (%) | 0.0019±0.0001 |
| Calcium (%) | 0.03±0.01 |
| Magnesium (%) | 0.0032±0.0002 |
| Potassium (%) | 0.0156±0.0002 |

**Table 2. Chemical composition of cricket frass used in the experiment presented as mean (±SE)**

| Parameters | Mean |
|---|---|
| pH | 7.98±0.00 |
| Ec (dS/m) | 2.20±0.04 |
| Organic carbon (%) | 25.4±0.3 |
| Nitrogen (%) | 2.16±0.05 |
| Phosphorus (%) | 0.79±0.02 |
| Calcium (%) | 0.38±0.01 |
| Magnesium (%) | 0.15±0.02 |
| Potassium (%) | 0.98±0.03 |
| Sodium (%) | 0.016±0.002 |
| Manganese (%) | 0.031±0.002 |
| Zinc (%) | 0.0050±0.0002 |
| Copper (%) | 0.0010±0.0002 |
| Iron (%) | 0.0090±0.0001 |

### 3.3.2 Experimental design

#### 3.3.2.1 Pot experiment

The pot experiment was conducted using different application rates of cricket frass manure on *C. petersii* plants. Cricket frass treatments were applied at four rates: 0, 5, 10, and 15 tons per hectare (t $ha^{-1}$**)** (with 0 t $ha^{-1}$ used as the reference) in a Completely Randomized Design (CRD) replicated four times. Sixteen pots with small holes at the bottom were utilized in the experiment, each having a capacity of 15 L and a diameter of 40cm. The pots were filled with a mixture of sand and loam soil in a 2:1 v/v ratio. Two weeks after cricket frass application, ten plantlets at the three-leaf stage were transplanted into each pot.

#### 3.3.2.2 Field experiment

The layout of the field experiment was a Randomized Complete Block Design (RCBD) with four replications. The species *C. petersii* were transplanted vegetatively at the three-leaf stage, similar to the pot experiment. After land preparation, 36 plants were transplanted in plots of 9 $m^2$ with a spacing of 50 cm within rows and 5 cm before the first hill (Appendix 9). The distance between treatments was 1 m, while the spacing between replications was 2 m. Cricket frass underwent a composting process for three months and was applied to the soil two weeks before transplanting.

Two weeks were given for the manure to naturally breakdown and release nutrients, making them available to the plants at the time of transplanting. Irrigation of the plants after transplanting was carried out twice a day, maintaining field capacity soil moisture. Weed control was performed through hand weeding on a weekly basis. Plant protection measures, such as pest control, were consistently maintained throughout the experiment.

### 3.3.3 Data collection

The growth parameters and foliar nutrient contents of *C. petersii* were collected from the sampling area at central harvest to assess the plant's response to the manure. Data were collected for 12 plants (Appendix 9). The growth parameters included plant height, number of leaves, number of shoots, leaf area, fresh leaf weight, and fresh dry weight. The nutrient contents were analyzed for proximate analysis, calcium (Ca), phosphorus ($P^{3+}$), and neutral detergent fiber (NDF).

#### 3.3.3.1 Plant height (cm)

Plant height was taken as the length from the bases of the plant to the tip. Plant height was recorded using a plastic ruler in an interval of two weeks starting two weeks after transplanting (WAT).

#### 3.3.3.2 Number of leaves

Number of leaves were counted by calculating all leaves of plants in an interval of two weeks starting two weeks after transplanting (WAT).

#### 3.3.3.3 Number of shoots

Number of shoots were counted by calculating all young shoots in an interval of two weeks starting from two weeks after transplanting (WAT).

#### 3.3.3.4 Leaf area (cm2)

The leaf area was calculated by multiplying the leaf height to the leaf width. The Leaf height and leaf width were measured on a fully emerged leaf from sampling area in an interval of two weeks starting two weeks after transplanting (WAT).

#### 3.3.3.5 Fresh weight (g)

The weight of fresh leaves was harvested on 12 plants from the central harvested area and taken on electrical balance separately for each replication.

#### 3.3.3.6 Dry weight (g)

The fresh weight leaves were oven dried at 65°C for 24 hours, then dry weighted, and finally measured on electrical balance for each treatment separately.

#### 3.3.3.7 Proximate analysis

Proximate analysis was determined using 5 g of each plant powder (Kirk & Sawyer, 1980). The analysis included the determination in fine powders of moisture content, ash content, crude protein,

ether extract, crude fiber and nitrogen free extract (NFE). Briefly, moisture content was determined by heating the sample in an oven at 105ºC for 12 hours to remove its water contents, then cooled in desiccants at 60°C and weighted. Ash content used as indication of minerals was determined by incineration of the samples at 550◦C in a muffle furnace until completely ashes. It was cooled in desiccants and weighed. The crude protein was determined by the Kjeldahl method and the values multiplied by the factor of 6.25 (Kirk & Sawyer, 1980). Ether extract (EE) was detected by solvent extraction of diethyl ether. The nitrogen free-extract (NFE) is found by subtracting the percent of the above determinations from 100%, thus giving the percent NFE. The recommended methods according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1998) were used for the determination of dry matter (DM), ash content (ASH), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract (NFE).

#### 3.3.3.8 Determination of Ca, $P^{3+}$, NDF (%)

The leaves powder was ashed and digested in 6N HCl. Content of minerals Calcium (Ca) and Phosphorus ($P^{3+}$) were determined using atomic absorption spectrometry (AAS) (Shimadzu, AA-6300, Tokyo, Japan) according to Association of Official Analytical Chemists methods (AOAC, 1998). The Neutral Detergent Fiber (NDF) was determined by standard method established by Van soest & Robertson (1985).

### 3.3.4 Data analysis

Data collected were subjected to analysis of variance (ANOVA) in CRD for pot and RCBD for field experiments using GraphPad Prism Software (version 8.0.2, Inc., San Diego, California) and STATA 14.2 software (Stata Corp LLC, Texas, USA). Means were compared using Turkey's Honest Significant Difference (TSD) at a critical value ($\alpha = 0.05$) (Gomez & Gomez, 1984).

# CHAPTER FOUR: RESULTS

## 4.1 To determine the diversity of species of *Commelina* across different agroecological zones in Western Kenya

### 4.1.1 Species diversity

In total, 115 weed species representing 80 genera from 30 families were recorded. Members of five families constituted 66 species (57.3%) of the total flora, Asteraceae (18 species), Poaceae (15 species), Commelinaceae and Fabaceae (12 species) and Cyperaceae (9 species) (Figure 5). Families (e.g., Amaranthaceae, Malvacae, Euphorbiaceae, Solanaceae) constituted six, five and four species, respectively. The remaining flora were composed by monogeneric families (14) represented by single species (e.g., Apiaceae, Molluginaceae, Onagraceae, Orobanchaceae, Pondeteriaceae). The genera with the highest number of species were *Commelina* (11 species) followed by *Cyperus* and *Amaranthus* (4 species) and finally *Desmodium, Euphorbia* and *Crotalaria* (3 species) (Appendix 2). Rank of ten weed species with high density in our study area was: *Cynodon dactylon*, *Parthenium hysterophorus*, *Commelina diffusa*, *Cyperus rotundus*, *Xanthium strumarium*, *Echinochloa colona*, *Stachytarpheta jamaicensis*, *Portulaca oleracea*, *Bidens Pilosa* and *Digitaria abyssinica*. The list of all recorded plants species is presented in Appendix 2. Regarding species of *Commelina*, 11 species were recorded (Appendix 2) which *C. diffusa* and *Commelina benghalensis var*. *benghalensis* (non-Hybrid variant) were the two species with high relative density (8.87% and 2.17%, respectively). In term of diversity of species of *Commelina* per production site, Abawa had greater diversity and Ahero presented a low diversity (Appendix 1).

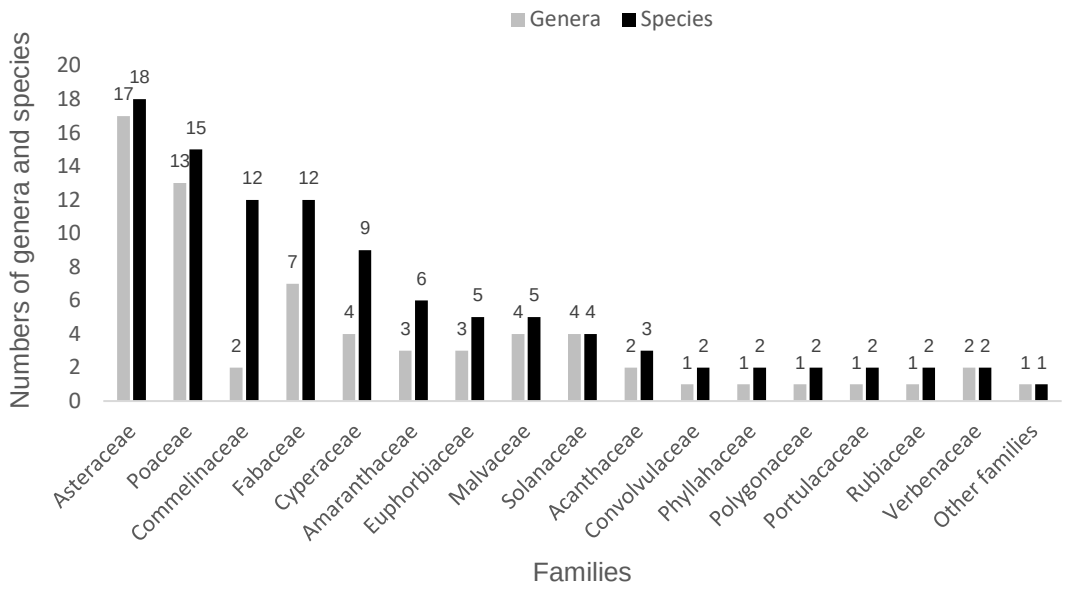



**Figure 5. Representation of numbers of genera and species per family**

Note: Only families with two species are shown, whereas other families (14) are reported with only one genus and one species

### 4.1.2 Effect of environment and management variables on the diversity of *Commelina*

The analysis of variance (ANOVA) indicated that eight environment variables (TN, available P, pH, EC, CEC, Ca, Mg and ESP) were significantly ($P < 0.05$) different between production sites, whereas TOC, K, Na and soil textures (sand, silt and clay) did not show significant ($P > 0.05$) differences (Appendix 1).

According to the model comparison methods, Multiple Linear Regression analysis showed the Margalef index (M) fitting the best model with significant 10 variables that combine ESP, Mg, soil pH, TN, agriculture system type, crop spacing, weed control, EC, crop establishment and available P. The Shannon-Weaver (H) diversity index and Pielou's evenness index (E) were also significantly related to 9 predictors. To rank the most important predictor in the best model, high value of standardized beta coefficient for ESP, Mg, soil pH, TN, agriculture system type showed stronger effect on the diversity of species *Commelina* (Table 3).

**Table 3. Multiple Linear Regression Analysis between diversity indices of *Commelina*—Environment and Management in Western Kenya**

| | | Variables | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Environment | | | | | | | Management | | | | | | | | | |
| | | pH | TN | P | EC | Mg | ESP | SurV | FarmM | CropS | Fertility | AgriSysT | WeedCont | CropE | AIC | BIC | Adj $R^2$ | *P*-value |
| Indices | H | 0.001 | 0.068 | 0.001 | 0.09 | 0.001 | 0.001 | 0.027 | 0.082 | 0.001 | 0.022 | 0.002 | 0.003 | 0.001 | −23.83 | 20.86 | 0.65 | <0.0001 |
| | SßC | 0.334 | −0.122 | −0.338 | −0.128 | 0.77 | 0.6 | 0.252 | −0.097 | 0.201 | 0.138 | 0.356 | 0.161 | −0.586 | | | | |
| | E | 0.001 | 0.068 | 0.001 | 0.09 | 0.001 | 0.001 | 0.027 | 0.082 | 0.001 | 0.022 | 0.002 | 0.003 | 0.001 | −141.42 | −96.72 | 0.65 | <0.0001 |
| | SßC | 0.334 | −0.122 | −0.338 | −0.128 | 0.77 | 0.6 | 0.252 | −0.097 | 0.201 | 0.138 | 0.356 | 0.161 | −0.586 | | | | |
| | M | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.087 | 0.7 | 0.001 | 0.422 | 0.001 | 0.001 | 0.001 | −305.18 | −260.47 | 0.79 | <0.0001 |
| | SßC | 0.532 | 0.348 | −0.663 | −0.294 | 0.689 | 0.767 | 0.068 | −0.016 | 0.217 | 0.036 | 0.343 | 0.132 | −0.497 | | | | |

Note: Akaike's information criterion (AIC), Bayesian information criterion (BIC), Adjusted R-square (Adj $R^2$), *P*-value and standardize beta coefficients (SßC) of regression model are shown. TN = total nitrogen, P = available phosphorus, EC = electric conductivity, Mg = magnesium, ESP = exchangeable sodium percentage, SurV = surrounding vegetation, FarmM = Farming method, CropS = Crop spacing, AgriSysT = Agriculture System Type, WeedCont = weed control, CropE = Crop establishment

### 4.1.3 Detrended Correspondence Analysis

The Detrended Correspondence Analysis run for the entire 115 weed species (Figure 6) detect three clusters indicating difference ecological conditions for species of *Commelina* and composition of weed species. The first cluster comprises *Commelina erecta* subsp. *livingstonii* and *Commelina africana* set to the right part of the graph together accompanied with eight weed species typical for cultivated upland fields under rainfed system. This included the species *Withania somnifera, Athroisma stuhlmannii, Sida cordifolia, Setaria verticillata, Crotalaria retusa, Solanum incanum, Achyranthes aspera* and *Striga hermonthica*. As the field condition increases with the degree of water level, species mostly related to the irrigated system occurred. The second cluster is positioned at the center of the diagram occurring in both rainfed and irrigated systems and containing seven species of *Commelina* namely, *C. diffusa, C. benghalensis* var. *benghanlensis* (non-hybrid variant), *Commelina petersii*, *Commelina forskaolii, Commelina bengalensis* (hybrid variant), *Commelina kotschyi* and *Commelina sp.*). Weed species associated with *C. diffusa* included *Echinochloa colona, Eleusine indica, P. oleraceae, Leonotis nepetifolia, Galinsoga parviflora* and *Stephania abyssinica,* whereas *Gomphrena celosioide, Euphorbia heterophylla, Senna obtusifolia, Dactyloctenium aegyptium, Desmodium incanum* and *Acanthospermum hispidum* accompanied *C. benghalensis* var. *benghanlensis* (non-hybrid variant). The *Commelina* plants (*C. petersii*, *C. forskaolii* and *C. bengalensis*-hybrid variant) were all together associated with five weed species (*Desmodium tortuosum, Ischaemum rugosum, Sida acuta, Boerhavia diffusa*. and *Malvastrum coromandelianum*, whereas *C. kotschyi* was associated with three species (*Euphorbia hirta*, *Amaranthus spinosus* and *Parthenium hysterophorus*). The species *Commelina sp*. was related with *Crotalaria brevidens, Pycreus lanceolatus, Sporobolus pyramidalis* and *Asystasia gangetica*. As for the third cluster that includes *Commelina latifolia* var. *latifolia* and *Commelina purpurea* located to the left part of the graph, are exclusive under irrigated system mostly inundated by water. For instance, *C. latifolia* var. *latifolia* was accompanied with three semi-aquatic weed species (*Phragmites australis, Typha domingensis, Mimosa pigra* and *Echinochloa pyramidalis*) preferring prolonged water supply, whereas *C. purpurea* was mostly accompanied with an aquatic species *Centela asiatica*.

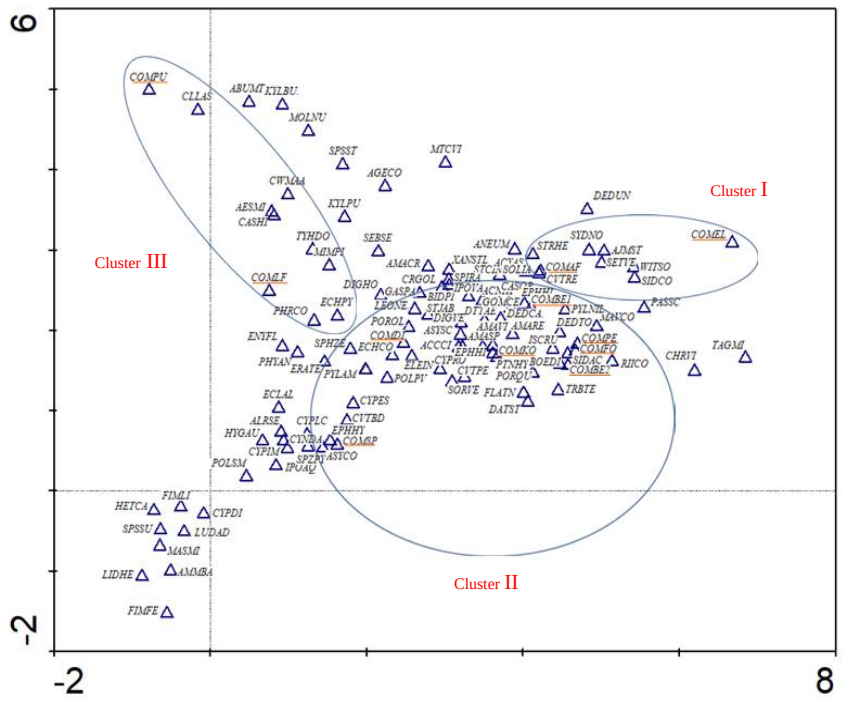



**Figure 6. Ordination diagram showing results of Detrended Correspondence Analysis (DCA). The species of *Commelina* (underlined in red) with their associated weed species.**

Note: Abbreviations of the 11 species of *Commelina* and associated weed species are presented in Appendix 2

### 4.1.4 Canonical Correspondence Analysis

#### 4.1.4.1 Variance Partitioning

Results from CCA and pCCA analyses identify the total amount of variation explained (TAVE) with single effect of "environment" and "management", and shared effect (environment × management). The single effect of "environment" explains 10.57% of the total variance in the *Commelina* data set, not explained by "management". The single effect of "management" explains 5.97% of the total variance not accounted for "environment". The total shared variance of environment × management was −0.4%, indicating that variance explained by this interaction was minor than single variance explained by the environment and management, individually. The total amount of variation explained (TAVE) was 16.14%, whereas 83.86% remained unexplained (*U*) (Table 4).

**Table 4. Partitioning variation of the *Commelina* data matrix**

| Effect | Variation explained (%) |
|---|---|
| Pure effect: Environment | 10.57 |
| Pure effect: Management | 5.97 |
| Shared effect: Environment x management | −0.4 |
| Unexplained | 83.86 |
| Total variance | 100 |
| Total Amount of Variation Explained | 16.14 |

#### 4.1.4.2 Variables Ranking

The marginal effect indicates variance explained if only single variable is used and pH is the most important variable followed by crop establishment, fertility, agriculture system type, K and available P. In this context, the remaining variables plays secondary role. After the pH variable is selected and all the variables are included in the ordination model, crop establishment, K and agriculture system type decrease dramatically, whereas TN, crop spacing and available P increase. During the forward selection with the set of Monte Carlo tests (999 permutations), the conditional effect indicates highly significant ($P < 0.01$) increases for pH and available P. The variables TN, fertility and crop spacing conferred significant ($P < 0.05$) (Table 5). All other variables remained not significant. Important variables that construct our minimal model were pH, available P, TN, fertility and crop spacing. The Variance Inflation Factors (VIFs) were all below 10 (Table 5), indicating low collinearity, and hence little redundancy among variables.

**Table 5. Variable explaining *Commelina* data set obtained from summary of forward selection and inter-set correlations of the environment and management variables with the first two ordination axes from the Canonical Correspondence Analysis (CCA)**

| Canonical Correspondence Analysis (CCA) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Set | Variables | Inter-set correlation | | Marginal | Conditional | | | VIF |
| | | Axis 1 | Axis 2 | $\lambda 1$ | $\lambda A$ | F-ratio | *P*-value | |
| "*Environment*" | pH | 0.594 | 0.020 | 0.31 | 0.31 | 7.47 | 0.001** | 4.36 |
| | P | 0.293 | −0.106 | 0.09 | 0.14 | 3.47 | 0.008** | 4.34 |
| | TN | −0.044 | 0.230 | 0.07 | 0.09 | 2.08 | 0.022* | 3.02 |
| | K | 0.230 | −0.097 | 0.10 | 0.07 | 1.64 | 0.12ns | 2.24 |
| | EC | 0.225 | −0.074 | 0.06 | 0.03 | 0.68 | 0.547ns | 2.28 |
| | Na | −0.175 | −0.042 | 0.04 | 0.06 | 1.4 | 0.215ns | 1.98 |
| | Mg | −0.018 | −0.026 | 0.02 | 0.04 | 1.15 | 0.313ns | 2.91 |
| | Surrounding vegetation | −0.149 | −0.015 | 0.05 | 0.04 | 1.2 | 0.288ns | 2.09 |
| "*Management*" | Crop establishment | 0.254 | −0.111 | 0.13 | 0.07 | 1.73 | 0.113ns | 8.93 |
| | Fertility | 0.331 | 0.035 | 0.13 | 0.09 | 2.52 | 0.038* | 2.16 |
| | Irrigation | 0.265 | −0.087 | 0.11 | 0.07 | 1.86 | 0.141ns | 6.09 |
| | Weed Control | 0.071 | 0.231 | 0.07 | 0.07 | 1.15 | 0.121ns | 1.53 |
| | Farming methods | 0.137 | −0.054 | 0.07 | 0.03 | 1.61 | 0.516ns | 2.05 |
| | Crop spacing | −0.151 | −0.057 | 0.06 | 0.08 | 2.01 | 0.032* | 2.04 |
| | Cost for weed management | 0.168 | −0.014 | 0.04 | 0.03 | 0.72 | 0.674ns | 1.70 |

Note: **Highly significant, $P < 0.01$; *Significant, $P < 0.05$; ns = not significant, $P > 0.05$

#### 4.1.4.3 The species of *Commelina*—Environment and management variables relationship

Results from this relationship are presented in Table 5, Figure 7 and Appendix 3. The Monte Carlo permutation test shows first canonical axis and all canonical axes highly significantly ($P < 0.002$, $F$-ratio = 10.501; $P < 0.001$, $F$-ratio = 2.091; 999 permutations under reduced model). Significant canonical axes indicate strong relationship between *Commelina* data set and explanatory variables. Moreover, CCA showed strong ecological relationship between *Commelina* data set and the considered explanatory variables, with species-environment correlations of 0.74 and 0.65 on the first and second axes, respectively. Only the first two canonical axes (75.2%) were used because of the high explained variability in *Commelina* data set. The total inertia stated by the CCA model was 7.564 (Appendix 3). The projection of significant environmental variables on axis 1 reveals

positive correlation with soil pH and *available P* content and negative correlation with TN as indicated by the interset correlations (0.594 and 0.292, −0.044, respectively) (Table 5). Axis 2 was positively correlated with *TN*, but negatively correlated with soil pH and available P content. The position of *Commelina forskaolii* is closely related to soil pH and soil rich in available P content. Similarly, *Commelina benghalensis 2* (hybrid variant) is predicted to have it optimum with respect to soil type rich in available P content. *Commelina latifolia* var. *latifolia* and *Commelina sp*. confounded on same position are also predicted to occur in soil rich in *available P* content, although not strongly linked as it is for the two previous species of *Commelina*. The species *Commelina purpurea* and *Commelina petersii* corresponds to a soil rich in TN content, whereas *Commelina africana* and *Commelina erecta* subsp. *livingstonii* in an opposite direction refers to soil poor in TN. The position of *Commelina benghalensis* var. *benghanlensis 1* (non-hybrid variant) and *Commelina diffusa* near the origin of the ordination diagram is an indication of these species to thrive in wide ecological field conditions. The two dummy management variables (fertility and crop spacing) having also their centroid near the origin, indicate major effect on species of *Commelina*. However, it is suggested that fertility might have higher effect on the species of *Commelina* than crop spacing (interset correlation 0.331 and −0.151 with axis 1; Table 5) regarding agricultural inputs.

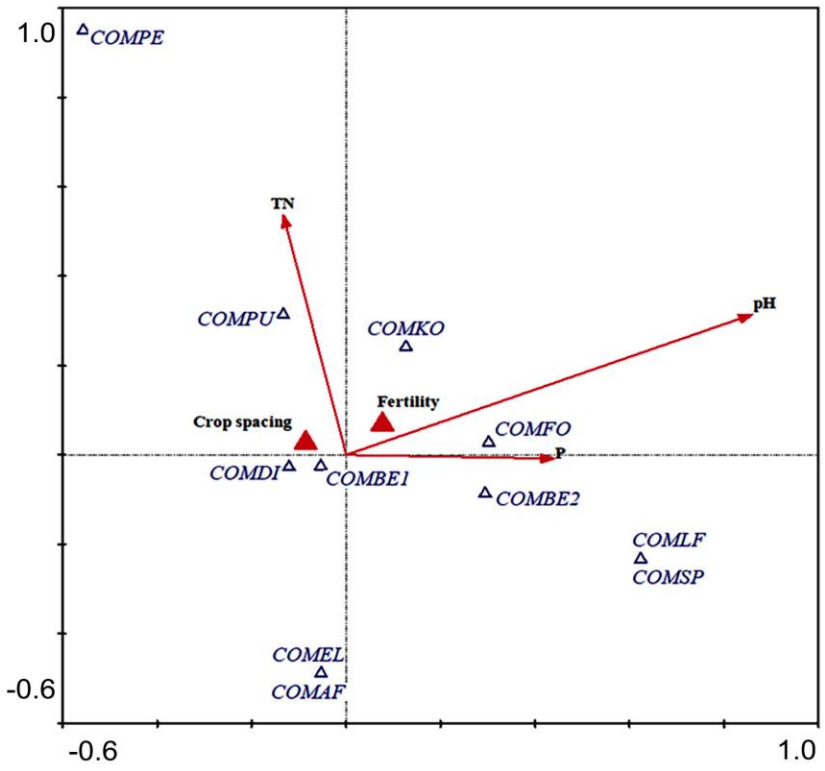



**Figure 7. Ordination diagram showing the Canonical Correspondence Analysis (CCA)**

Note: The length of the vector is linked to its importance. The angle between two vectors reflects the degree of correlation between variables, and the angle between a vector and each axis reflects its correlation with the axes. Abbreviations of the 11 species of *Commelina* and associated species are presented in Appendix 2

## 4.2 To evaluate the feeding preferences of *Scapsipedus icipe* for species of *Commelina*

### 4.2.1 Leaf feeding

The response of *Scapsipedus icipe* to the leaves of the different species of *Commelina* in no-choice, dual choice and multiple-choice experiments differed.

#### 4.2.1.1 No-choice

Of the 11 species of *Commelina* tested, a highly significant ($F$ = 8.316, df = 10, $P$ = 0.0002; $F$ = 8.316, df = 10, $P$ = 0.0082) higher rate of feeding was recorded for COMPE when the crickets were provided with only one species of *Commelina*, compared to the rates recorded for COMBE1 and COMBE2 (Table 6). Similarly, a significantly higher ($F$ = 8.316, df = 10, $P$ = 0.0010; $F$ = 8.316, df = 10, $P$ = 0.0404) feeding rate was recorded for COMFO than COMBE1 and COMBE2. Unlike the suitable species, highly significant ($F$ = 8.316, df = 10, $P$ < 0.0001; $F$ = 8.316, df = 10, $P$ = 0.0284) low rates of feeding were recorded for COMPU and COMSP, compared with that recorded for the two-reference species. Other species of *Commelin*a did not differ significantly ($P$ > 0.05) when compared with the two-reference species. Moreover, this experiment showed a positive significant ($R^2$ = 0.5377; $P$ = 0.0102) relationship between final insect gained weight and percentage of leaf consumed (%) showing a linear increasing of insect gained weight, with an increasing in the percent leaf consumed (Appendix 5). The mean weight gain of crickets fed with COMPE was 0.423 g ± 0.007, which was higher than mean weight gain of crickets fed with COMBE1 (0.294 g ± 0.009) and COMBE2 (0.240 g ± 0.005).

**Table 6. Variation in the consumption of different species of *Commelina* by *S. icipe* in no choice experiments compared with that recorded in the two reference species (COMBE 1 and COMBE 2, individually)**

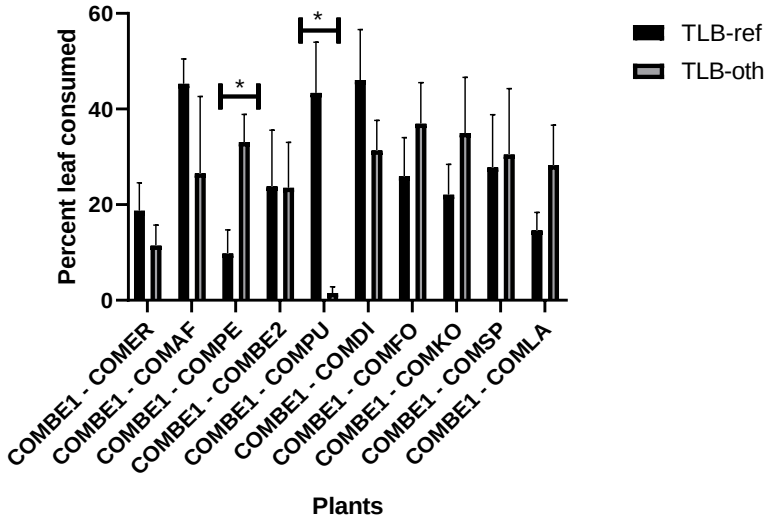
| Dunnett's multiple comparisons test | Mean | Dunnett's multiple comparisons test | Mean |
|---|---|---|---|
| COMBE 1 as reference | | COMBE 2 as reference | |
| COMER | 30.53ns | COMER | 30.53ns |
| COMAF | 33.81ns | COMAF | 33.81ns |
| COMPE | 38.76*** | COMPE | 38.76** |
| COMPU | 19.30**** | COMPU | 19.30**** |
| COMDI | 34.11ns | COMDI | 34.11ns |
| COMBE 2 | 32.35ns | COMFO | 37.52* |
| COMFO | 37.52*** | COMKO | 31.62ns |
| COMKO | 31.62ns | COMSP | 26.93* |
| COMSP | 26.93ns | COMLA | 29.74ns |
| COMLA | 29.74ns | COMBE 1 | 29.98ns |
| *COMBE1* | *29.98* | *COMBE 2* | *32.35* |

Note: Comparisons of means using Dunnett's test at different significant levels: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, ns – not significant

#### 4.2.1.2 Dual-choice

When insects were feeding on two *Commelina* plants, COMPE showed significant (t = 8.509, df = 2, $P = 0.0135$; t = 8.755, df = 2, $P = 0.0128$) feeding rate for dual choice experiment-1 with COMBE 1 and dual choice experiment-2 with COMBE 2 as references, respectively (Figure 8). Significant (t = 7.549, df = 2, $P = 0.0171$; t = 6.128, df = 2, $P = 0.0256$) low feeding rates were found on COMPU and COMER in comparison to COMBE 1 and COMBE 2 as references for experiment-1 and experiement-2, respectively.

(a) Dual choice experiment-1




(b) Dual choice experiment-2

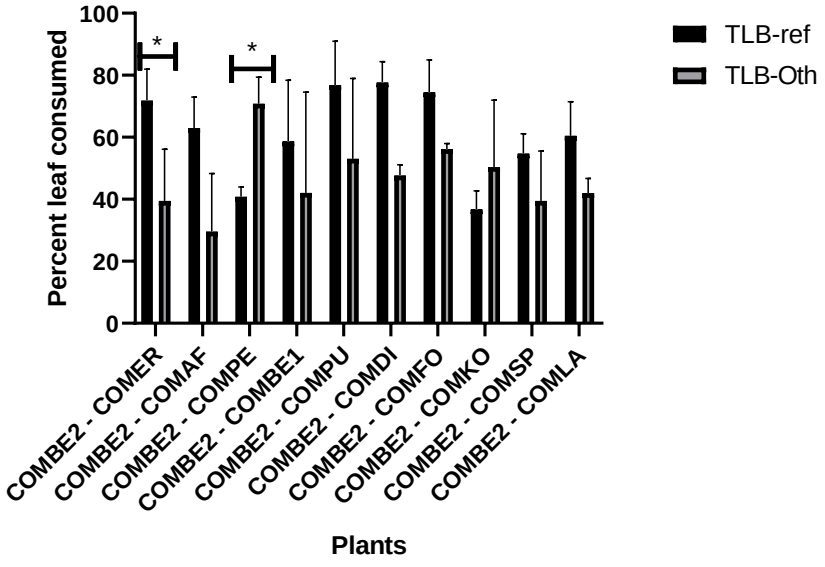



**Figure 8. Area of leaf of species of *Commelina* consumed by the cricket relative to the total leaf area provided in (a) dual choice experiment 1 and (b) dual choice experiment 2. Paired t-tests were used to compare the means of all species of *Commelina* relative to references with the *P*-values. * $P < 0.05$, ns – not significant.**

Note: TLB-ref (percentage of leaf consumed for reference) and TLB-Oth (percentage of leaf consumed for another species)

#### 4.2.1.3 Multiple-choice

When crickets were given a choice of the four most preferred species of *Commelina* in the no-choice experiment (COMPE, COMFO, COMBE1 and COMBE2), the feeding rate recorded for COMPE was highly significantly the highest ($F$ = 37.87, df = 3, $P$ < 0.0001) (Figure 9). Based on this result, COMPE was ranked as the most suitable species followed by COMFO, then COMBE1 and COMBE2.

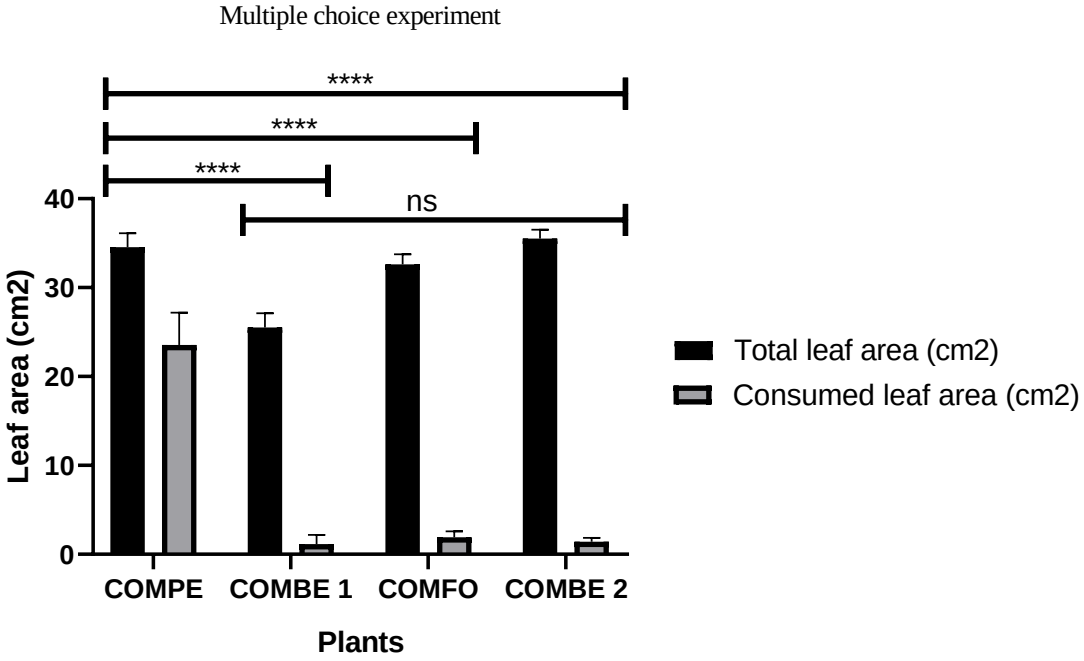



**Figure 9. Area of leaf of four species of *Commelina* consumed by the cricket relative to the total leaf area provided.**

Note: Turkey's Honest Significant Difference test (THD) was used to compare the means with the *P*-values. **** $P$ < 0.0001, ns – not significant

#### 4.2.1.4 Insect mortality

Mortality of the crickets in the no-choice experiment was very low (9.69%) (Kruskal-Wallis test, Kruskal-Wallis statistic = 32.33, $P$ = 0.450, Table 7) and no deaths were recorded in the dual and multiple-choice experiments.

**Table 7. Insect mortality in no-choice experiment**

| Kruskal-Wallis test | |
|---|---|
| *P*-value | 0.45 |
| Number of groups | 33 |
| Kruskal-Wallis statistic | 32.33 |

### 4.2.2 Nutrient contents across species

The nutrient contents of the species differed significantly ($P < 0.05$), except for the minerals magnesium, copper and zinc (Appendix 6, Appendix 7). Five Principal Components (PC1, PC2, PC3, PC4 and PC5) are greater than one in the scree plot [eigenvalue with (Rho) > 1; Appendix 8], and together explained 88.83% of the variability. These five components accounted for 35.46%, 18.25%, 14.75%, 11.78% and 8.59%, respectively. Five major nutrient groups were identified: (NDF, Mn and Fe) in PC1, (K, Na, EE and CP) in PC2, (DM, NFE and cellulose) in PC3, (ash and Ca) in PC4 and (ADF and ADL) in PC5 (Table 8). The distribution of the species in the biplots of the PCA indicate that COMPE and COMLA contain more CP and COMPE more Ca. However, these two species were low in Fe, Mn and cellulose, ADL and NDF. COMKO had an intermediate level of CP, Ca and EE. COMBE1 had an intermediate level of EE, cellulose and ADL. COMEL, COMBE2 and COMFO had intermediate levels of EE, Na and K. As for COMPU, it had high levels of cellulose, ADF, ADL, NDF and DM. COMDI had high values for NFE. COMSP and COMAF were far from one another and the other species in terms of the level of nutrients (Figure 10).

**Table 8 . Principal Component Analysis (PCA), showing different position of nutrient contents of *Commelina* plants with standard deviation (SD), percentage of explained variation and cumulative percentage related to each principal component eigenvectors**

| Nutrient content | Principal Component Eigenvectors | | | | |
|---|---|---|---|---|---|
| Variable | PC1 | PC2 | PC3 | PC4 | PC5 |
| DM | 0.2412 | 0.0472 | 0.3387 | 0.2204 | -0.336 |
| ASH | 0.1869 | 0.0277 | -0.076 | 0.6385 | -0.1065 |
| EE | 0.0257 | 0.4529 | 0.2877 | 0.0948 | 0.381 |
| CP | -0.2675 | 0.2879 | -0.2684 | 0.0011 | -0.0347 |
| NFE | -0.1688 | -0.2703 | 0.3419 | -0.4464 | 0.1368 |
| NDF | 0.375 | 0.1976 | -0.1717 | -0.1724 | 0.0626 |
| ADF | 0.2953 | -0.0163 | -0.3282 | 0.0056 | 0.3803 |
| ADL | 0.2654 | 0.239 | 0.2397 | -0.0907 | -0.4632 |
| Hemicellulose | 0.2752 | 0.2485 | 0.071 | -0.2715 | -0.2091 |
| Cellulose | 0.278 | 0.2415 | -0.325 | -0.2374 | 0.1527 |
| Na | 0.1347 | -0.3429 | 0.2174 | 0.1525 | 0.2797 |
| K | 0.1388 | -0.4283 | -0.3158 | 0.1293 | -0.2075 |
| Ca | -0.2735 | 0.2916 | 0.2019 | 0.297 | 0.2156 |
| Mn | 0.3158 | -0.1689 | 0.3105 | -0.122 | 0.1255 |
| Fe | 0.3781 | -0.0696 | 0.1357 | 0.1561 | 0.3114 |
| | Principal Component Eigenvalues | | | | |
| SD | 0.22 | 0.26 | 0.26 | 0.27 | 0.26 |
| % of Variance | 35.46 | 18.25 | 14.75 | 11.78 | 8.59 |
| Cumulative % | 35.46 | 53.7 | 68.45 | 80.24 | 88.83 |

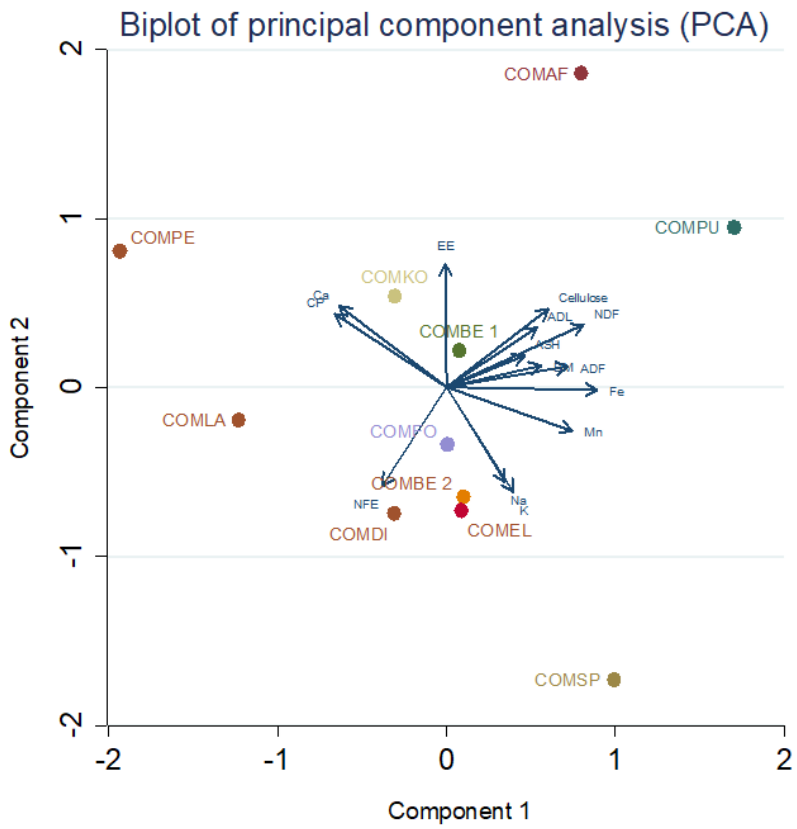



**Figure 10. Biplot of the first two Principal Component Analysis (Component 1, explained variation 35.46%) and (component 2, explained variation 18.25%), representing the distribution of the variation in the nutrient content of different species of *Commelina***

Note: Nutrient contents are dry matter (DM), neutral detergent fiber (NDF), crude protein (CP), ash (ASH), cellulose, acid detergent fiber (ADF), extracted ether (EE), nitrogen free-extract (NFE), acid detergent lignin (ADL), manganese (Mn), calcium (Ca), potassium (K), sodium (Na) and ion (Fe) according to the factor loadings (eigenvector value > 0.28) of components. Scientific names of observations are presented in Appendix 2

There was positive correlation (Spearman's *rho* = 0.6364, $P < 0.05$) between the percentages of the leaves consumed and their calcium content was significant. In addition, there was a significant positive correlation (Spearman's *rho* = 0.6273, $P < 0.05$) between the percentages of the leaves consumed and their neutral detergent fibre content. There was, however, a significant negative correlation (Spearman's *rho* = –0.6091, $P < 0.05$) between the calcium and neutral detergent fibre content and significant positive correlation between the calcium and protein content of the leaves (Spearman's *rho* = 0.6455, $P < 0.05$) (Table 9).

**Table 9. Results of the Spearman's correlation assessing the relationships between different nutrient contents and percentage of the leaves consumed**

| | DM | ASH | EE | CP | NFE | NDF | ADF | ADL | Cellulose | Na | K | Ca | Mn | Fe | % leaf consumed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM | 1.0000 | | | | | | | | | | | | | | |
| ASH | **0.6105*** | 1.0000 | | | | | | | | | | | | | |
| EE | 0.0319 | 0.2273 | 1.0000 | | | | | | | | | | | | |
| CP | -0.4009 | -0.2273 | 0.1545 | 1.0000 | | | | | | | | | | | |
| NFE | -0.3007 | **-0.6636*** | -0.1364 | -0.3091 | 1.0000 | | | | | | | | | | |
| NDF | 0.2916 | 0.1818 | 0.1545 | -0.2909 | -0.3455 | 1.0000 | | | | | | | | | |
| ADF | 0.3371 | 0.3545 | -0.1545 | -0.2727 | -0.4455 | 0.5455 | 1.0000 | | | | | | | | |
| ADL | **0.7153*** | 0.2636 | 0.1545 | -0.2909 | -0.1636 | 0.5364 | 0.0273 | 1.0000 | | | | | | | |
| Cellulose | 0.1549 | -0.0273 | 0.0273 | 0.0636 | -0.4636 | **0.7727*** | 0.5818 | 0.3818 | 1.0000 | | | | | | |
| Na | 0.0868 | 0.4419 | 0.164 | -0.5695 | 0.2096 | -0.123 | -0.082 | -0.018 | -0.5604 | 1.0000 | | | | | |
| K | 0.2484 | 0.3519 | **-0.6791*** | -0.4659 | -0.0198 | 0.0248 | 0.3569 | -0.02 | -0.0397 | 0.3378 | 1.0000 | | | | |
| Ca | 0.0364 | 0.0364 | 0.3273 | **0.6455*** | -0.1455 | **-0.6091*** | -0.2636 | -0.164 | -0.3273 | -0.2642 | -0.5106 | 1.0000 | | | |
| Mn | 0.5571 | 0.2415 | 0.0364 | **-0.6378*** | 0.2597 | 0.3462 | 0.4237 | 0.3645 | 0.0638 | 0.2078 | 0.3031 | -0.2323 | 1.0000 | | |
| Fe | 0.589 | **0.6697*** | 0.2005 | **-0.6378*** | -0.205 | 0.4647 | **0.6333*** | 0.2825 | 0.1731 | 0.3813 | 0.3825 | -0.287 | **0.8174*** | 1.0000 | |
| % leaf consumed | 0.2141 | 0.2818 | 0.2636 | 0.0727 | -0.1455 | **0.6273*** | -0.1818 | -0.155 | -0.3818 | 0.123 | -0.2131 | **0.6364*** | -0.2278 | -0.0547 | 1.0000 |

Note: Significant difference assessed at *P*-value, *: $P < 0.05$, significant variables are in bold

### 4.2.3 Phytochemicals

Flavonoids and terpenoids were present in all plant samples. Phenols, glycosides, alkaloids and anthraquinones were absent in COMAF and COMLA. Tannins and phenols were absent in samples of COMBE1 and COMBE2. Saponins and anthraquinones were both absents in the sample of COMPE, whereas COMFO only lacked saponins. The other plants didn't differ much in either presence or absence of different phytochemicals (Table 10).

**Table 10. Phytochemicals present in leaf extracts of species of *Commelina* (number of samples n = 2)**

| Species codes | Phenols | Glycosides | Steroids | Alkaloids | Flavonoids | Tannins | Terpenoids | Saponins | Anthraquinones |
|---|---|---|---|---|---|---|---|---|---|
| COMPU | - | + | - | + | + | + | + | + | - |
| COMLA | - | - | + | - | + | + | + | - | + |
| COMDI | + | + | + | + | + | + | + | + | - |
| COMFO | + | + | + | + | + | + | + | - | + |
| COMPE | + | + | + | + | + | + | + | - | - |
| COMEL | + | + | + | + | + | + | + | + | + |
| COMKO | + | - | - | - | + | + | + | + | + |
| COMBE 1 | - | + | + | + | + | - | + | + | + |
| COMBE 2 | - | + | + | + | + | - | + | + | + |
| COMSP | + | + | + | - | + | + | + | + | - |
| COMAF | - | - | + | - | + | + | + | + | - |

Note: (+), Present; (-), Absent

The likelihood ratio test statistic of the beta regression analysis was statistically significant ($P < 0.05$). This indicates that this model fits the results well and that phenols have a highly significant positive ($P < 0.001$) influence on leaf feeding and steroids not significant positive ($P > 0.05$) effect. Glycosides, alkaloids and tannins had highly significant negative ($P < 0.001$) effects on leaf feeding, whereas saponins and anthraquinones had only significant negative ($P < 0.05$) effects. Marginal effects indicate an increase or decrease in the magnitude of the dependent variable with a-unit increase in each of the independent variables. Hence, an increase in phenols is associated with an increase in the percentage of the leaves consumed, whereas increases in alkaloids, glycosides, tannins, saponins and anthraquinones were associated with decreases in the percentage of the leaves consumed. For example, a 1% change in the independent variable is associated with an increase in the percentage of the leaves consumed of 0.10% when there is an increase of 1% in phenol content, whereas 1% increase in alkaloids decreased leaf feeding by 0.13% (Table 11).

**Table 11. Results of the Beta regression used to determine the phytochemicals influencing leaf feeding**

| Variables | Coeff. | Std. Err. | z | *P*-value | 95% Conf. Interval | |
|---|---|---|---|---|---|---|
| Leaf feeding | | | | | Lower | Upper |
| Phenols | 0.5070 | 0.1160 | 4.33 | 0.001 | 0.277 | 0.7362 |
| Glycosides | -0.7541 | 0.1994 | -3.77 | 0.001 | -1.146 | -0.3622 |
| Steroids | 0.2041 | 0.1202 | 1.70 | 0.090 | -0.0315 | 0.0439 |
| Alkaloids | -0.6231 | 0.1579 | -3.95 | 0.001 | -1.0278 | -0.2908 |
| Tannins | -0.6593 | 0.1880 | -3.51 | 0.001 | -0.4085 | -0.005 |
| Saponins | -0.2067 | 0.1029 | -2.01 | 0.045 | -0.4085 | -0.005 |
| Anthraquinones | -0.2571 | 0.1032 | -2.49 | 0.013 | -0.4594 | -0.0549 |
| Cons | -0.1154 | 0.3008 | -0.38 | 0.701 | -0.7051 | 0.4742 |
| Scale | | | | | | |
| Cons | 4.8249 | 0.4251 | 11.35 | 0.001 | 3.9916 | 5.6582 |

Average Marginal effects: Model OIM

| Variables | dy/ex | Std. Err. | z | *P*-value | 95% Conf. Interval | |
|---|---|---|---|---|---|---|
| Phenols | 0.0913 | 0.0220 | 4.14 | 0.001 | 0.0481 | 0.1345 |
| Glycosides | -0.1778 | 0.0450 | -3.94 | 0.001 | -0.2662 | -0.0894 |
| Alkaloids | -0.1335 | 0.0346 | -3.85 | 0.001 | -0.0655 | -0.2015 |
| Tannins | -0.1663 | 0.0459 | -3.62 | 0.001 | -0.2564 | -0.0021 |
| Saponins | -0.0459 | 0.0223 | -2.05 | 0.040 | -0.0897 | -0.0021 |
| Anthraquinones | -0.0461 | 0.0178 | -2.58 | 0.010 | -0.0811 | -0.0110 |

Note: Regression diagnostics: number of observations, 11; likelihood ratio $\chi 2$ (*P*-value), 18.99 (0.0082); log likelihood, 20.36.

The biplot shows the association between phytochemicals and species of *Commelina*. The arrows indicate the extent of the variation in the phytochemical content. The longer the arrow and the direction, the greater the variation. Based on the significant phytochemicals identified by the beta regression analysis, phenol in COMPE and COMDI was strongly associated and moderately associated in COMFO. Moreover, the tannins, glycosides and alkaloids, respectively, in COMSP, COMEL and COMPU were strongly associated. The species COMBE1 and COMBE2 were

associated by both saponins and anthraquinones. As for COMLA, COMAF and COMKO, they were not associated with any of the significant phytochemicals (Figure 11).

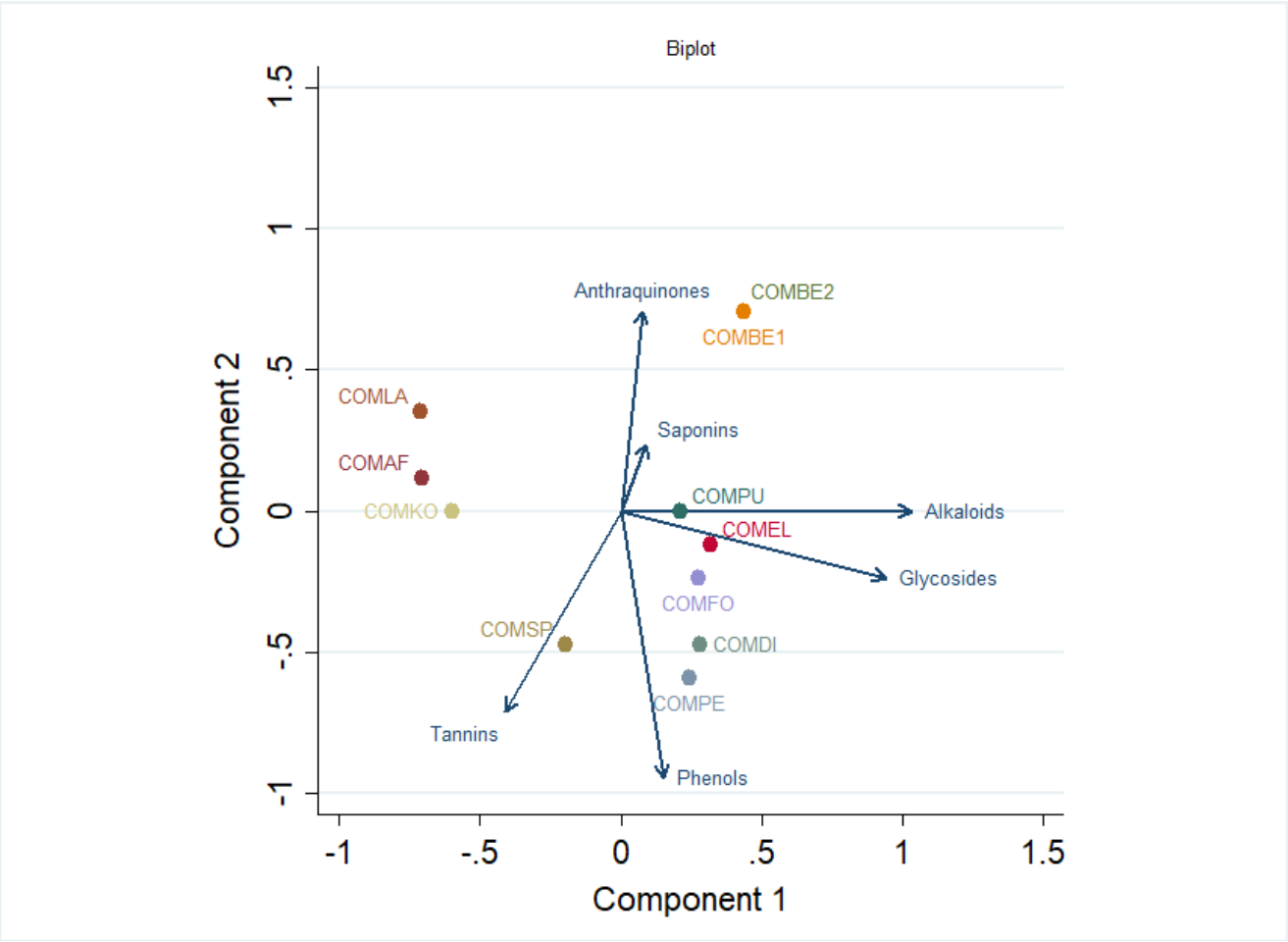



**Figure 11. Biplot of the relationships between phytochemicals, results of beta regression analysis and species of *Commelina* (component 1, explained variance 32.90%; component 2, explained variance 28.57% and total explained variance 61.47%). Scientific names of species are listed in Appendix 1**

## 4.3 To determine optimum growing conditions for production of species of *Commelina* using cricket frass as manure

### 4.3.1 Soil analysis

The results of the soil analysis taken before the experiment is presented in Table 1. The soil used in this study was a loam-sandy type. Most of soil elements were low, except for organic carbon that were adequate, and hence the soil required fertility improvement.

### 4.3.2 Cricket frass analysis

The results of the cricket manure analysis taken before the application are presented in Table 2. The pH was neutral, indicating that there was no need to apply lime if the soil had been highly acidic. The manure showed a high percentage of organic carbon and nitrogen, while calcium (Ca), phosphorus (P), magnesium (Mg), and potassium (K) were available in lower percentages. Other elements, such as iron (Fe), manganese (Mn), zinc (Zn), sodium (Na), and copper (Cu), were available in trace amounts.

### 4.3.3 Pot experiment

#### 4.3.3.1 Plant height

There were significant ($P < 0.05$, as shown in Table 12) differences between the treatments of 0 t $ha^{-1}$, 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ at Week 4, Week 6 and Week 8. However, there were no significant ($P > 0.05$) differences between the treatments at the early stage of the experiment (Week 2).

**Table 12. Effect of cricket frass on plant height of *C. petersii*, indicating the pot experiment recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | week 6 | Week 8 |
|---|---|---|---|---|
| 0 t $ha^{-1}$ | $14.5 \pm 1.07^{a}$ | $40.75 \pm 1.85^{a}$ | $64.00 \pm 3.69^{ab}$ | $110 \pm 3.98$ |
| 5 t $ha^{-1}$ | $16.42 \pm 0.96^{a}$ | $46.66 \pm 1.65^{ab}$ | $62.00 \pm 3.30^{a}$ | $120 \pm 3.56$ |
| 10 t $ha^{-1}$ | $17.70 \pm 1.24^{a}$ | $52.60 \pm 2.14^{bc}$ | $65.33 \pm 4.26^{ab}$ | $123 \pm 4.60$ |
| 15 t $ha^{-1}$ | $17.90 \pm 1.07^{a}$ | $58.10 \pm 1.85^{c}$ | $77.50 \pm 3.69^{b}$ | $127.50 \pm 3.98$ |
| *P*-value | > 0.05 | < 0.05 | < 0.05 | < 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.3.2 Number of leaves

Significant ($P < 0.05$, as indicated in Table 13) differences between treatments were observed at Week 8, but not at the early weeks (Week 2, Week 4, and Week 6). Cricket frass manure had a significant effect on the number of leaves of *C. petersii*.

**Table 13. Effect of cricket frass on number of leaves of *C. petersii*, recorded between an interval of two weeks.**

| Treatment | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|
| 0 t ha$^{-1}$ | 5.75 ± 0.47$^{a}$ | 21.50 ± 1.84$^{a}$ | 54.25 ± 8.18$^{a}$ | 71.25 ± 1.03$^{ab}$ |
| 5 t ha$^{-1}$ | 5.25 ± 0.47$^{a}$ | 22.75 ± 4.81$^{a}$ | 52.5 ± 4.17$^{a}$ | 68.25 ± 2.05$^{a}$ |
| 10 t ha$^{-1}$ | 5.00 ± 0.40$^{a}$ | 24.00 ± 2.48$^{a}$ | 54.75 ± 8.10$^{a}$ | 74.50 ± 1.84$^{ab}$ |
| 15 t ha$^{-1}$ | 6.00 ± 1.00$^{a}$ | 24.25 ± 3.01$^{a}$ | 63.25 ± 5.52$^{a}$ | 77.25 ± 0.75$^{b}$ |
| *P*-value | > 0.05 | > 0.05 | > 0.05 | < 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.3.3 Number of shoots

The pot experiment did not show any differences across treatments ($P > 0.05$, as indicated in Table 14). Plants grown with a cricket frass application rate of 15 t ha$^{-1}$ did not differ significantly from plants grown with no frass application (0 t ha$^{-1}$).

**Table 14. Effect of cricket frass manure on number of shoots of *C. petersii*, recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|
| 0 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 1.50 ± 0.28$^{a}$ | 2.25 ± 0.25$^{a}$ | 3.00 ± 0.40$^{a}$ |
| 5 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 1.50 ± 0.28$^{a}$ | 2.00 ± 0.40$^{a}$ | 3.00 ± 0.40$^{a}$ |
| 10 t ha$^{-1}$ | 0.50 ± 0.28$^{a}$ | 1.50 ± 0.50$^{a}$ | 2.00 ± 0.40$^{a}$ | 2.75 ± 0.47$^{a}$ |
| 15 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 1.50 ± 0.28$^{a}$ | 2.25 ± 0.25$^{a}$ | 2.75 ± 0.47$^{a}$ |
| *P*-value | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.3.4 Leaf area

There were significant ($P < 0.05$, as shown in Table 15) differences between the treatments of 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ in the pot experiment at Week 4, Week 6 and Week 8. However, at the early stage of the experiment (Week 2), the leaf area between treatments was not significant.

**Table 15. Effect of cricket frass on leaf area of *C. petersii*, recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | week 6 | Week 8 |
|---|---|---|---|---|
| 0 t $ha^{-1}$ | $21.14 \pm 2.64^{a}$ | $29.87 \pm 0.32$ | $34.07 \pm 0.53^{b}$ | $36.60 \pm 0.58$ |
| 5 t $ha^{-1}$ | $22.00 \pm 3.95^{a}$ | $32.24 \pm 0.45^{a}$ | $38.82 \pm 0.74^{ab}$ | $40.62 \pm 0.30^{a}$ |
| 10 t $ha^{-1}$ | $22.10 \pm 1.64^{a}$ | $33.37 \pm 0.38^{ab}$ | $39.77 \pm 1.17^{a}$ | $41.55 \pm 0.94^{a}$ |
| 15 t $ha^{-1}$ | $23.25 \pm 2.77^{a}$ | $34.43 \pm 0.50^{b}$ | $40.20 \pm 2.00^{a}$ | $41.83 \pm 1.50^{a}$ |
| *P*-value | > 0.05 | < 0.05 | < 0.05 | < 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.3.5 Fresh/dry weights at harvesting

There were highly significant ($P < 0.0001$) differences in the leaves of *C. petersii* as affected by the rates of cricket frass application at harvesting (Figure 12). The yield of fresh weight increased as the rate of application increased. There was a substantial difference between the highest fresh mass yield and the lowest fresh weight leaves.

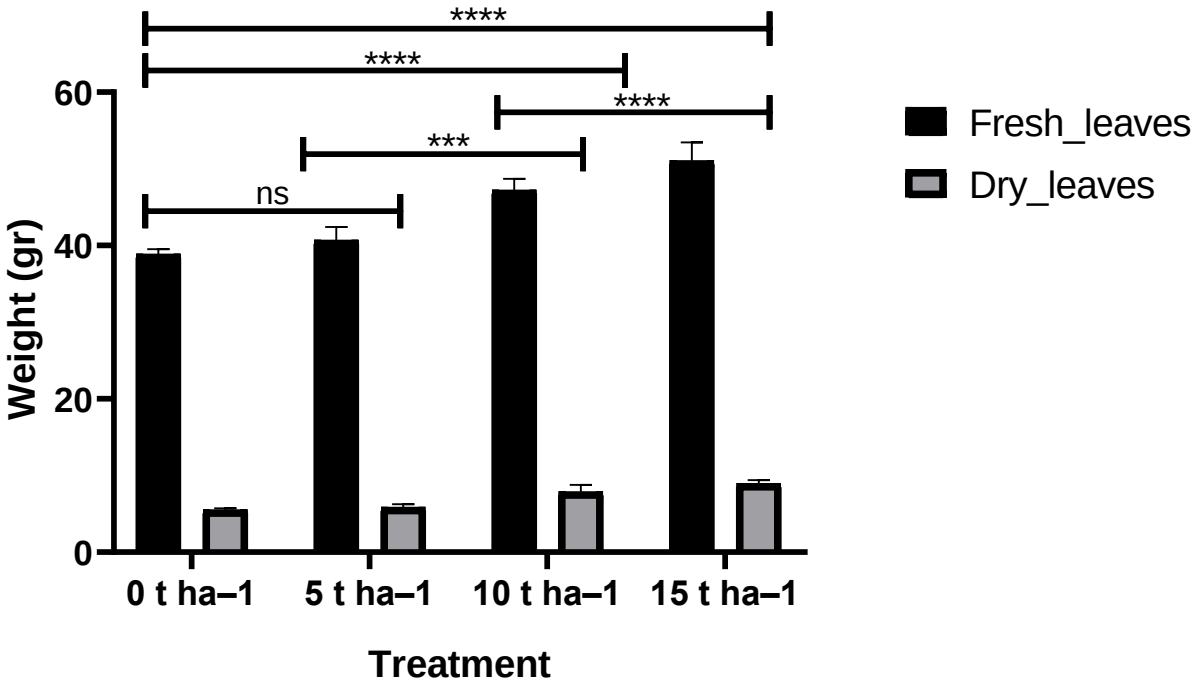



**Figure 12. Relative fresh/dry weights of *C. petersii* at harvesting in pot experiment**

Note: Significant levels assessed with different *P*-value, ****: $P < 0.0001$; ***: $P < 0.001$; and ns: not significant ($P > 0.05$)

#### 4.3.5.6 Proximate composition, calcium, neutral detergent fiber and phosphorus

The results of the pot experiment, as presented in Table 16, indicate that most of the parameters for proximate analysis, calcium, neutral detergent fiber, and phosphorus were highly significant ($P < 0.0001$; $P < 0.01$) with the application of cricket frass at different rates, except for dry matter (DM). However, the application of cricket frass manure at 10 t $ha^{-1}$ revealed an increase in crude protein (CP), neutral free extract (NFE), neutral detergent fiber (NDF), and ether extract (EE), whereas calcium (Ca), crude fiber (CF), and ash (ASH) were favored at the application rate of 15 t $ha^{-1}$. The phosphorus ($P^{3+}$) content increased at the application rates of cricket frass at 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ in comparison to the reference rate (0 t $ha^{-1}$).

**Table 16. Effect of different rates of cricket frass manure on proximate composition, neutral detergent fibre, calcium and phosphorus for pot experiment. Data are presented as mean (± SE) and coefficient of variation (CV)**

| Treatment | DM (%) | ASH (%) | EE (%) | CP (%) | CF (%) | NFE (%) | NDF (%) | Ca (%) | $P^{3+}$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0 t $ha^{-1}$ | 89.92±0.02[a] | 14.41±0.00 | 2.22±0.02 | 23.11±0.01 | 13.56±0.00 | 46.7±0.09 | 50.65±0.53[a] | 5.14±0.02[a] | 0.13±0.02 |
| 5 t $ha^{-1}$ | 89.40±0.02[a] | 15.17±0.00 | 3.34±0.03 | 25.15±0.07 | 13.98±0.05 | 42.36±0.06 | 54.60±0.29[b] | 5.18±0.0[a] | 0.30±0.00[a] |
| 10 t $ha^{-1}$ | 89.74±0.02[a] | 16.25±0.00 | 4.05±0.03 | 26.71±0.15 | 14.61±0.00[a] | 38.38±0.00[a] | 59.06±1.05 | 5.68±0.49[a] | 0.36±0.01[a] |
| 15 t $ha^{-1}$ | 89.88±0.01[a] | 17.71±0.00 | 3.70±0.01 | 26.22±0.01 | 14.65±0.01[a] | 37.72±0.03[a] | 53.63±0.27[ab] | 9.14±0.03 | 0.36±0.02[a] |
| %CV | 0.26 | 3.23 | 23.83 | 6.31 | 3.63 | 3.58 | 5.40 | 30.53 | 37.73 |
| *P*-value | 0.36 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.003 | 0.0009 | 0.001 |

Note: Means with different letters in the same column for treatment rates are significantly different at $P < 0.05$.

### 4.3.4 Field experiment

#### 4.3.4.1 Plant height

The field experiment demonstrated significant ($P < 0.05$) differences between treatments at various weeks (as shown in Table 17). Plant height increased with the higher application rates of cricket frass, and the 15 t $ha^{-1}$ rate exhibited the greatest plant height.

**Table 17. Effect of cricket frass on plant height of *C. petersii*, recorded between an interval of two weeks.**

| Treatment | Week 2 | Week 4 | week 6 | Week 8 |
|---|---|---|---|---|
| 0 t $ha^{-1}$ | 15.65 ± 0.57[ab] | 34.525 ± 2.26[a] | 53.5 ± 3.22[a] | 85.75 ± 1.71[a] |
| 5 t $ha^{-1}$ | 15.37 ± 1.51[a] | 35.67 ± 2.51[a] | 51.75 ± 2.95a | 83.25 ± 2.89[a] |
| 10 t $ha^{-1}$ | 17.45 ± 1.41[ab] | 38.32 ± 2.46[a] | 59.00 ± 3.18[a] | 90.00 ± 2.48[a] |
| 15 t $ha^{-1}$ | 22.47 ± 2.49[b] | 58.27 ± 1.90 | 83.5 ± 3.22 | 111.25 ± 5.02 |
| *P*-value | < 0.05 | < 0.05 | < 0.05 | < 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.4.2 Number of leaves

There was a significant ($P < 0.05$, as indicated in Table 18) effect of cricket frass on the number of leaves of *C. petersii* at Week 4.

**Table 18. Effect of cricket frass on number of leaves of *C. petersii*, recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|
| 0 t ha$^{-1}$ | 10.75 ± 0.47$^{a}$ | 45.5 ± 1.25 | 131 ± 1.22$^{a}$ | 173 ± 2.48$^{a}$ |
| 5 t ha$^{-1}$ | 10.75 ± 0.47$^{a}$ | 42.75 ± 3.06 | 134 ± 1.47$^{a}$ | 174.25 ± 1.75$^{a}$ |
| 10 t ha$^{-1}$ | 12.5 ± 0.86$^{a}$ | 52.00 ± 2.41$^{a}$ | 131.25 ± 1.88$^{a}$ | 178.75 ± 2.62$^{a}$ |
| 15 t ha$^{-1}$ | 12.5 ± 0.86$^{a}$ | 52.00 ± 2.41$^{a}$ | 131.25 ± 1.88$^{a}$ | 178.75 ± 2.62$^{a}$ |
| *P*-value | > 0.05 | < 0.05 | > 0.05 | > 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.4.3 Number of shoots

The application of cricket frass had an effect on the number of shoots of *C. petersii*. Significant differences ($P < 0.05$, as shown in Table 19) were observed between treatments at Week 6 and Week 8, indicating that the impact of the different manure application rates on the number of shoots became evident during these later weeks of the experiment. However, at the early stages of the experiment (Week 2 and Week 4), there were no significant differences in the number of shoots among the treatments. Notably, plants grown with a cricket frass application rate of 15 t ha$^{-1}$ exhibited the largest number of shoots.

**Table 19. Effect of cricket frass on number of shoots of *C. petersii*, recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|
| 0 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 3.00 ± 0.40$^{a}$ | 10.25 ± 0.62$^{a}$ | 18.25 ± 1.10$^{a}$ |
| 5 t ha$^{-1}$ | 1.00 ± 0.00$^{a}$ | 2.75 ± 0.25$^{a}$ | 11.25 ± 0.62$^{a}$ | 17.00 ± 1.77$^{a}$ |
| 10 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 3.00 ± 0.40$^{a}$ | 11.00 ± 0.40$^{a}$ | 20.75 ± 1.65$^{ab}$ |
| 15 t ha$^{-1}$ | 0.75 ± 0.25$^{a}$ | 3.50 ± 0.64$^{a}$ | 13.75 ± 0.25 | 25.5 ± 0.64$^{b}$ |
| P-value | > 0.05 | > 0.05 | < 0.05 | < 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.4.4 Leaf area

Significant ($P < 0.05$) differences were observed between treatments at Week 2 and Week 6. Leaf area in all treatments increased with the application of cricket frass, as shown in Table 20. Notably, the 15 t ha$^{-1}$ frass manure application rate resulted in the largest leaf area.

**Table 20. Effect of leaf area of *C. petersii* to cricket frass, recorded between an interval of two weeks**

| Treatment | Week 2 | Week 4 | week 6 | Week 8 |
|---|---|---|---|---|
| 0 t ha$^{-1}$ | 10.09 ± 2.89$^{a}$ | 24.02 ± 1.02$^{a}$ | 29.67 ± 1.18$^{a}$ | 32.52 ± 0.93$^{a}$ |
| 5 t ha$^{-1}$ | 17.7 ± 2.44$^{ab}$ | 27.96 ± 2.73$^{a}$ | 32.76 ± 0.34$^{ab}$ | 37.19 ± 0.36$^{a}$ |
| 10 t ha$^{-1}$ | 20.27 ± 3.39$^{ab}$ | 28.61 ± 3.10$^{a}$ | 35.13 ± 2.51$^{ab}$ | 38.71 ± 3.66$^{a}$ |
| 15 t ha$^{-1}$ | 21.18 ± 0.98$^{b}$ | 30.48 ± 1.64$^{a}$ | 36.98 ± 1.38$^{b}$ | 39.99 ± 2.08$^{a}$ |
| *P*-value | < 0.05 | > 0.05 | < 0.05 | > 0.05 |

Note: Means for a treatment sharing the same superscript letter are not significantly different at $P < 0.05$

#### 4.3.4.5 Fresh/dry weight at harvesting

There were highly significant ($P < 0.0001$; $P < 0.01$) differences in the leaves of *C. petersii* as affected by the rates of cricket frass application at harvesting, as shown in Figure 13. The yield of fresh weight increased as the rate of application increased. There was a substantial difference between the highest fresh mass yield and the lowest fresh weight leaves. Plants grown with an application of cricket frass at 15 t ha$^{-1}$ had the highest fresh/dry leaf weights.

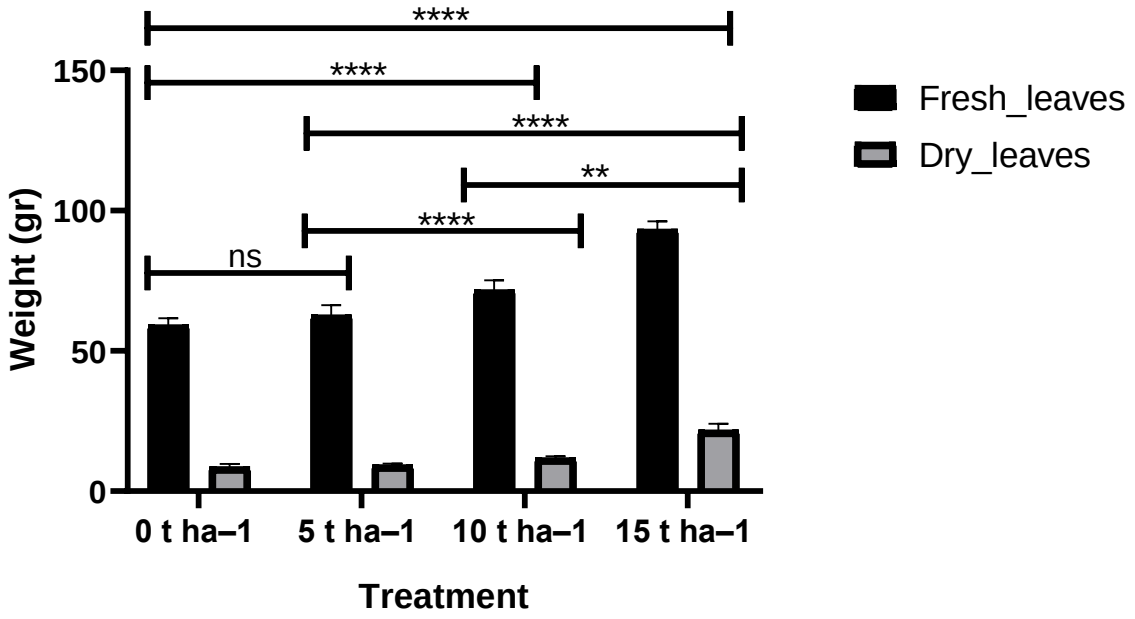



**Figure 13. Relative fresh/dry weights of *C. petersii* at harvesting in field experiment.**

Note: Significant levels assessed with different *P*-value, ****: $P < 0.0001$; ***: **: $P < 0.01$; *: $P < 0.05$ and ns: not significant ($P > 0.05$)

#### 4.3.4.6 Proximate composition, calcium, neutral detergent fiber and phosphorus

The application of different rates of cricket frass on *C. petersii* at the field level significantly ($P < 0.0001$, $P < 0.05$) influenced the ash (ASH), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), calcium (Ca), and phosphorus ($P^{3+}$), but not the dry matter (DM), crude fat (CF), and neutral free extract (NFE) (as shown in Table 21). The CP, EE, NDF, and $P^{3+}$ increased with the application of cricket frass manure at 10 t $ha^{-1}$, whereas Ca increased with the application of cricket frass at 15 t $ha^{-1}$. The NFE content was higher at 0 t $ha^{-1}$ compared to 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$.

**Table 21. Effect of different rates of cricket frass on proximate composition, neutral detergent fibre, calcium and phosphorus in field experiment. Data are presented as mean (± SE) and coefficient of variation (CV)**

| Treatment | DM (%) | ASH (%) | EE (%) | CP (%) | CF (%) | NFE (%) | NDF (%) | Ca (%) | $P^{3+}$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0 t $ha^{-1}$ | 91.17±0.60$^{a}$ | 15.38±0.01$^{a}$ | 4.22±0.02$^{ab}$ | 24.44±0.42 | 15.22±0.10 | 40.74±0.37 | 52.89±0.50 | 6.14±0.02$^{a}$ | 0.15±0.00 |
| 5 t $ha^{-1}$ | 90.93±0.44$^{a}$ | 15.97±0.25$^{a}$ | 5.21±0.1b$^{c}$ | 26.31±0.04$^{a}$ | 16.44±0.3 | 36.07±0.4 | 58.77±0.12$^{a}$ | 6.90±0.01$^{a}$ | 0.42±0.01$^{a}$ |
| 10 t $ha^{-1}$ | 90.49±0.26$^{a}$ | 16.44±0.10 | 5.31±033$^{c}$ | 26.83±0.03$^{a}$ | 16.50±0.16 | 34.92±0.08 | 59.08±0.04$^{a}$ | 8.49±0.37$^{b}$ | 0.47±0.01$^{a}$ |
| 15 t $ha^{-1}$ | 90.37±0.30$^{a}$ | 17.96±0.14 | 3.89±0.10$^{a}$ | 26.82±0.03$^{a}$ | 15.57±0.34 | 35.76±1.10 | 55.37±0.18 | 8.55±0.01$^{b}$ | 0.46±0.02$^{a}$ |
| %CV | 0.41 | 5.31 | 15.24 | 4.34 | 4.01 | 1.15 | 4.43 | 15.89 | 39.77 |
| *P*-value | 0.33 | 0.0006 | 0.01 | 0.003 | 0.055 | 0.0004 | 0.0003 | 0.0018 | 0.0002 |

Note: Means with different letters in the same column for treatment rates are significantly different at $P < 0.05$.

# CHAPTER FIVE: DISCUSSION

## 5.1 To determine the diversity of species of *Commelina* across different agro-ecological zones in Western Kenya

### 5.1.1 Species diversity

The floristic analysis of our study area showed that the majority (57.3%) of the recorded flora were composed with five important families, Asteraceae, Poaceae, Commelinaceae, Fabaceae and Cyperaceae. This result was consistent with the finding in adjacent agro-ecological zone in Kiisi County (Charles *et al*., 2019). The families of Asteraceae, Poaceae, Cyperaceae and Fabaceae have been previously considered among the common pattern in the riparian zones and adjacent of the Lake Victoria basin (Sayer *et al*., 2018). Additionally, surrounding vegetation adjacent to our study area proven the establishment of heliophylic families.

The current investigation showed that species with high relative density were predominant. Four annual species (grasses, *E. colona and D. abyssinica*; broadleaves, *B. pilosa* and *P. oleracea*) and two perennial species (sedge *C. rotundus* and grass *C. dactylon*) were the most dominant with the highest relative densities. This confirmed earlier report in western part of Kenya, reviewed by Odhiambo *et al*. (2015) and Ngome *et al*. (2013b). Additionally, *E. colona*, *C. rotundus*, *C. dactylon* and *B. pilosa* were documented as world's worst weeds of many crops (Holm *et al*., 1977). The potential of these weed species to infest and grow fast in many cropping systems is explained through seed dispersal mechanism (for *E. colona* and *B. pilosa*) and persistent from bulbs, tubers and stolons (for *C. rotundus* and *C. dactylon*). As for *C. diffusa* and *P. oleraceae*, they are more aggressive and grow in moist soil with a wide range of agricultural inputs. The high density of annual species (*P. hysterophorus* and *X. strumarium*) is explained by their invasiveness affecting many countries world widely, including Kenya (Beale *et al*., 2020). Similarly, the perennial *S. jamaicensis* have also recently been recorded as invasive (Witt & Luke, 2017).

### 5.1.2 Effect of variables on the diversity of species of *Commelina*

We found that the diversity of species of *Commelina* was significantly related to nutrients (ESP, Mg, pH, TN, EC and available P) and management variables (agriculture system type, crop

spacing, weed control, crop establishment). One of the reasons for the environment nutrients to affect the diversity of *Commelina* could be attributed to greater accumulation of these elements at the topsoil near the Lake as discussed by Fungo *et al*. (2011), mostly beneficial to plants species with low rooting systems. For instance, the ability of soil sodicity known as exchangeable sodium percentage (*ESP*) to affect the diversity of *Commelina* can be attributed to the soil irrigated by water containing residual of sodium carbonate. According to Orina *et al*. (2020) and Ogutu-Ohwayo *et al*. (1997), the water body of Lake Victoria has experienced several changes regarding physico-chemical properties in the last past decades caused by human activities increasing toxic pollution from inappropriate application of fertilizers, industrial and domestic waste discharge considered as secondary source of sodicity. Another possible reason for these nutrients to affect the diversity of species of *Commelina* is perhaps that, our study area is predominant with hand hoe tillage in a perennial cropping system. A report by Steenwerth *et al*. (2002) suggested that in a perennial system where hand hoeing tillage is the main land preparation there is limited change in vegetation leading to less leaching of base cations in comparison to annual cropping systems. Nevertheless, agriculture system type among management variables exerts important effect on the diversity of species of *Commelina* as these plants showed some preferences regarding water degree in either irrigated or rainfed systems. Furthermore, the occurrence of *Commelina* plants in farmer fields have been related to high proliferation of these species through both asexually (or vegetatively) and sexually (aerial and subterranean seeds) mostly coinciding with agricultural inputs (Isaac *et al*., 2013).

### 5.1.3 Ecological conditions of species of *Commelina* and composition of weed species

We detected that various weed species were connected with different species of *Commelina*. Composition of weed species strongly linked might provide a description of field conditions (Cáceres & Legendre, 2009). Hence, weed species such as *S. hermonthica, S. verticillata, S. incanum* and *A. aspera* associated with *C. erecta* subsp. *livingstonii* and *C. africana* are considered as makers of cultivated upland field previously reported in East Africa (Ivens, 1967). Preference of irrigated to flooded system for weed species (e.g., *T. domingensis, M. pigra, E. pyramidalis, C. asiatica*) associated with *C. latifolia* var. *latifolia* and *C. purpurea* have been previously reported in lowland irrigated system of East Africa (Irakiza *et al*., 2021). The observation of higher number

of weed species associated with *C. diffusa* and *C. benghalensis* var. *bengalensis* (non-hybrid variant) among other species of *Commelina* is explained by the fact that these two species are cosmopolitan plants being able to infest a large number of crops. For instance, *C. benghalensis* itself have been reported to infest 25 different crops (Holm *et al*., 1977). Similarly, weed species associated with *C. benghalensis* have also extended a broad ecological range in infesting several cropping systems (Akobundu, 1987; Rao *et al*., 2017). It is important to mention that *C. benghalensis* var. *benghalensis* with the identity of non-hybrid variant in this investigation is diploid (chromosome count number 2n = 22), and hence most world widely distributed. Its counterpart *C. benghalensis* (hybrid variant) refers to any compatibility in hybridization within *C. bengalensis* variants. According to Faden *et al*. (2012), variants of *C. bengalensis* are more diverse morphologically (diploid, tetraploid, and even higher ploidies) in Kenya and need further taxonomic studies.

### 5.1.4 Effect of environment and management on the distribution of species of *Commelina*

The results of forward selection suggest that the distribution of C*ommelina* species is driven by five important explanatory variables. The species of *Commelina* responded primary to soil pH followed by available P, then with TN, fertility and crop spacing. The forward selection procedure selects "best" explanatory variable in which the order selected offers ranking in their importance (Ter Braak & Verdonschot, 1995). The role of soil pH on weed communities have been previously noted by several works of other authors (Fried *et al*., 2008; Pinke *et al*., 2012). Soil pH can be a restraining factor for many weed species including species of *Commelina* (Erviö *et al*., 1994). Some species might occur within a narrow soil pH range, while others will occur in a wide soil pH range. The range value of soil pH between 5.8 - 8.0 in the current investigation indicates that the species of *Commelina* can thrive in both acidic and saline soils. Species of *Commelina* tolerate different soil types and have been successfully introduced in several habitat of East Africa (Faden, 2012). The second highly significant nutrient variable on species of *Commelina* was the available phosphorous (P). Phosphorous elements are considered vital for plants in metabolism, cell division, photosynthesis and other many physiological and development processes. A study by Urich *et al*. (2003) demonstrated that the roots, leaves and total plant biomass of some species of *Commelina* responded significantly in high than low phosphorus concentration. The third

significant nutrient variable was the total nitrogen (TN). One possible raison for nitrogen to affect the species of *Commelina* could be attributed to different dosages and types of nitrogen that farmer applies in their fields having a direct effect on weed vegetation. Quantifying nitrogen level (i.e. manures) that farmers apply in their field was not possible as this was beyond the scope of the current study. Finally, fertility and crop spacing were also found to be significant management variables. The position of the two variables at the centroid of CCA diagram indicates their key role for species of *Commelina* in our study area. Singh & Sharma, (1989) showed that soil fertility and crop spacing affect weed vegetation. In their findings, it was observed that weed species captures high amount of nutrient in wide row spacing (>50 cm) than it is in narrow spacing (30 cm). In consideration to the physiological growth habit of plants of the genus *Commelina* at field level, it is possible to assume that nutrient uptake can be enhanced under wide spacing than narrow spacing, however this need to be confirmed by extra studies.

### 5.1.5 Partitioning variation

Using partitioning variation, we disentangled the influence of environment and management on species of *Commelina*. Our results showed small amount of variation explained (16.14%), but higher in comparison to some other studies conducted in Europe and Asia ranging between 2% and 11.5% (Fanfarillo *et al*., 2020; Lososová & Cimalová, 2009; Nowak *et al*., 2015; Šilc *et al*., 2009). The main discrepancy between the aforementioned studies and our investigation was the inclusion of climatic and crop type variables on a large number of data set. Our study did not include climatic variables due to difficulties in accessing meteorological data and crop type for the raison that farmers were practicing subsistent agriculture in small hectarage. However, we focused on factors such as environment and management to capture different agronomic features affecting the species of *Commelina*. Decomposition of the explained variation revealed great importance of environment than management. This observation is consistent to study conducted by Dale *et al*. (1992). The small fraction of shared effect suggests that environment and management factors have more individualistic nature than interactive nature in our study area. Nevertheless, negative value of shared effect has been stressed as theoretically, but unlikely to occur in real ecology arena (Borcard *et al*., 1992). Furthermore, it has been discussed that negative variance of two variables acts as suppressor between each other (Olea *et al*., 2010). The fairly high unexplained fraction can

be attributed to stochastic variation or unmeasured local abiotic and biotic factors that we missed to be described. In this regard, unrepresented factors such as particular classes of nutrients, macro-organism (e.g., insects) and micro-organism (e.g., bacteria) as well as micro-climatic and meso-climatic conditions could influence the local distribution of *Commelina* species.

### 5.2 To evaluate the feeding preferences of *Scapsipedus icipe* for species of *Commelina*

This study evaluates the preferences of *S. icipe* for feeding on different species of *Commelina* in a screenhouse. It also reports the link between the nutrient content and chemical constituents of leaves with this cricket's preference for feeding on the leaves of particular species of *Commelina*.

#### 5.2.1 Leaf feeding

Based on rates feeding COMPE is the most suitable plant for rearing *S. icipe* followed by COMFO, and COMPU was the least suitable. The other species that were less consumed than the two-reference species, COMBE1 and COMBE2, however, are not inedible. The rate of feeding recorded for these species indicated that when suitable plants are scarce and the crickets are hungry, they are likely feed on many of them. In addition, the low mortality of crickets fed on leaves of species of *Commelina* in this study, indicates they are a good quality food. Nevertheless, food selection by crickets is complex and involves visual, olfactory, habitat, intraspecific, celestial (sun and sky), magnetic of the field and leaf nutrient cues (Horch et al., 2017; Kuo & Fisher, 2022; Tyree et al., 1976; Ugolini, 2021; Vaga et al., 2021).

#### 5.2.2 Relationship between leaf feeding and nutrient contents

The effect on the growth and survival of crickets of feeding on weeds is well studied (e.g., Tyree *et al*., 1976; Miech *et al*., 2016; Choo *et al*., 2017; Kinyuru & Kipkoech, 2018; Ng'ang'a *et al*., 2020; Vaga *et al*., 2020, 2021; Kuo & Fisher, 2022), whereas the relationship between feeding and nutrient content is less investigated. Nevertheless, there is some information on the components of some plants provided as food or incorporated in mixed diets for crickets (Miech et al. (2016) and Vaga et al. (2020, 2021), which indicates nutrient content is important. The results presented reveal a significant positive association between the percentage of the leaves eaten and Ca and NDF, and significant negative association between Ca and NDF, which indicates the key roles of these two nutrients for *S. icipe.* Moreover, the PCA confirmed these results as it revealed an inverse relationship between the concentrations of NDF and Ca in COMPE and COMPU.

Indeed, the most consumed species (COMPE) contained a high concentration of Ca and low NDF, whereas the least consumed (COMPU) contained a low concentration of Ca and high NDF. These results are in accordance with the results of Vaga et al. (2021) in which the house cricket *A. domesticus* preferred *L. album* that has a low NDF. In addition, *Acheta* prefers fresh-cut *T. pratense* with a low NDF to late-cut *T. pratense* with high NDF (Vaga *et al*., 2020). In contrast, Miech et al. (2016) report that the Cambodian field cricket *T. testaceus* is tolerant of the high fibre contents of its most preferred species, *Cleome rutidosperma*. Furthermore, the results on the role of fibre are inconsistent, with some studies reporting a high fibre content associated with high feeding and high performance (Tyree *et al*., 1976; Veenenbos & Oonincx, 2017) and others high fibre contents and low feeding and low performance (Nakagaki & Defoliart, 1991; Orinda *et al*., 2017). Hence, the effect of fibre content on the rate of feeding in crickets is not clearly understood, and more studies are needed. In this study, the low level of feeding on COMPU, could be attributed to *S. icipe* having to spend more time chewing its more fibrous leaves (Faden, 2012). The current study also showed that the nutritional profile of species of *Commelina* is rich in CP, DM, NFE and minerals (Ca and Mn). Magara et al. (2019) and Murugu et al. (2021) report that these nutrients are important for the growth and development of the field cricket *S. icipe*. The crude protein contents of COMPE and COMBE1 were higher than that reported for *C. rutidosperma* (22.2 %) fed to *Teleogryllus* crickets (Miech *et al*., 2016) and *L. album* (22 %) + *T. pratense* (19.9 %) in mixed diets for *Acheta* crickets (Vaga *et al*., 2021). According to Bawa et al. (2020), the protein content of cricket diets is crucial for their growth and development despite low concentrations of nutrient such as EE and minerals (Fe, Zn, Mg, and Cu). The house cricket, *A. domesticus* can be successfully reared on a *Commelina* diet (known as COMBE1) (Kinyuru & Kipkoech, 2018). In the preference tests, crickets preferred COMPE, which has higher calcium content than the other species. With respect to the Ca content of crickets, Murugu et al. (2021) compare Ca content of *S. icipe* to that of plants (e.g., sorghum, maize, wheat, kidney bean) and animal (e.g., beef, goat, chicken, eggs) sources and conclude that Ca content of *S. icipe* is, with the exception of kidney beans and eggs, higher. Hence, consumption of *S. icipe* reared on diets such as COMPE rich in Ca could increase the availability of calcium, especially for children, and reduce the effects of calcium deficiency in low-income countries in Sub-Saharan Africa. It should be noted that the *Commelina*

plants used in the present study were harvested from various agro-ecological zones in Western Kenya and cultivated at the JOOUST crop farm. Thus, it cannot be excluded that their nutritional content will differ if grown at other geographic locations with different soil profiles.

### 5.2.3 Relationship between leaf feeding and phytochemical constituents

*Commelina* plants are widely used in medicine as they are a source of bioactive compounds. Leaf extracts of species of *Commelina* contain alkaloids, flavonoids, steroids, terpenoids, volatile oils, saponins and tannins of which flavonoids are the most frequent and abundant (e.g., Ghosh *et al*., 2019; Kansagara & Pandya, 2019; Ezeabara *et al*., 2020; Bussmann *et al*., 2021; Islam *et al*., 2021). In the current study, flavonoids and terpenoids were detected in all species, indicating that crickets fed *Commelina* could be a good source for humans of some important antioxidants and antibacterial substances (Grabmann, 2005; Panche *et al.*, 2016). In addition, the chemical constituents of the leaves of *Commelina* had a crucial role in the feeding of *S. icipe*. The relationship between phytochemicals and feeding preferences of crickets are poorly investigated compared to those of Orthoptera, such as, Acrididae. This is possibly because most Acrididae are more devastating pests than Gryllidae. For Orthoptera, there are several examples of the chemical constituents of leaves being important in determining their feeding preferences. Bernarys & Chapman (1994) and Sanjayan & Ananthakrishnan (1987) report examples of chemical constituents of plants acting as stimulants or deterrents for feeding in Orthoptera. The results of the current study indicate that phenols are likely to increase leaf feeding and alkaloids, glucosides, tannins, anthraquinones and saponins decrease leaf feeding, that is, phenols acted as stimulants and alkaloids and tannins as deterrents. These results are consistent with those reported for Acrididae (e.g., Harley & Thorsteinson, 1967; Mole & Joern, 1994; Dini & Owen-Smith, 1995; Wallace, 2013). For example, phenols stimulate feeding in the grasshopper, *Melanoplus bivitattus* (Harley & Thorsteinson, 1967; Wallace, 2013) and alkaloids and tannins deter feeding in the locust, *Locustana pardalina* and two grasshoppers, *Ageneotettix deorum* and *Phoetaloites nebrascensis* (Dini & Owen-Smith, 1995; Mole & Joern, 1994). While substances that stimulate feeding can be specific, at high concentrations they can act as deterrents (Chapman, 2009). In the present study only the phytochemicals that influenced the feeding of crickets are reported. There

is a need for more quantitative data on the chemicals in *Commelina* plants as these plants were not completely rejected by *S. icipe* despite containing some deleterious chemicals. The low mortality of the crickets indicates they are well adapted to deal with deleterious chemicals. Herbivorous insects in general are well adapted to deal with phytochemicals in their diet, e.g., by rapid excretion, detoxification and avoiding ingesting toxins (Brattsten, 1988; Schoonhoven, Van Loon, & Dicke, 2005).

## 5.3 To determine optimum growing conditions for production of species of *Commelina* using cricket frass as manure

The application of different rates of cricket frass on *C. petersii* in pot and field experiments significantly affected most of the vegetative parameters. Additionally, laboratory analysis of the nutrient contents contained in the leaves of *C. petersii* revealed different responses depending on the application rates. The findings highlight the potential for using cricket frass at different rates to enhance *C. petersii* plant production in both small and large-scale agriculture. This sustainable approach aligns with the principles of organic farming, which aims to reduce environmental impacts, promote plant health, and address climate change (Meemken & Qaim, 2018; Mondelaers *et al*., 2009).

### 5.3.1 Effect of cricket frass on the vegetative parameters of *C. petersii*

The application rate of cricket frass significantly influenced the vegetative parameters of *C. petersii*. Plants treated with the manure showed higher plant height, number of shoots, number of leaves, leaf area, and plant biomass compared to untreated plants. This enhancement in growth can be attributed to the contribution of cricket frass to soil fertility, especially in soils with low organic carbon contents. Xu *et al*. (2002) reported that organic manure applied before planting can promote vegetative growth in leafy plants. Notably, under restricted root growth conditions in the control environment, there was no significant growth in the number of shoots for *C. petersii*. However, in open field conditions, *Commelina* plants tend to prioritize root growth to propagate both asexually (vegetatively) and sexually (aerial and subterranean seeds) (Isaac *et al*., 2013).

In the current study, cricket frass applied at a higher rate resulted in improved vegetative parameters for *C. petersii*. Similar observations were reported by Riar *et al*. (2016) in their study on *Commelina benghalensis*, where increased nutrition positively influenced vegetative growth. Generally, different species of *Commelina* respond well to increased agricultural inputs (Isaac *et al*., 2013). However, discrepancies were observed in previous studies. For instance, Bukari *et al*. (2021) found that cricket frass affected the leaves of green mustard, *Brassica junceae* (L.) Czern, but not of water spinach, *Ipomoea aquatica* Forssk. Further investigations are needed to elucidate

the effect of cricket frass on specific leaves' physiological processes, as the observations suggest that some leaves may be affected while others may not be influenced.

### 5.3.2 Effect of cricket frass on the nutrients of *C. petersii*

The increase in crude protein, neutral detergent protein, calcium, phosphorus, and other proximate contents due to the application of different cricket frass rates is consistent with the findings of Bukari *et al*. (2021) on different plants. In their studies, it was demonstrated that plants treated with cricket frass showed increased nitrogen uptake and protein contents compared to those treated with other manure such as chicken dung. The nitrogen application from cricket frass in the soil enhanced plant uptake, leading to increased crude protein content. Nitrogen plays a vital role in various physiological processes of plants, including protein and nucleic acid synthesis. Moreover, insect frass serves as a substantial soil amendment that boosts nitrogen uptake in plants (Hartz & Johnstone, 2006; Tanga *et al*., 2022).

Regarding neutral detergent fiber (NDF) content, it is used to evaluate fiber content, which may influence digestibility of forage crops and feed quality for animal feeding. The variation in NDF content at different application rates highlights the importance of considering nitrogen rates in optimizing fiber quality. Cricket frass application at rates of 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ exhibited higher NDF content, indicating lower forage quality compared to untreated plants (0 t $ha^{-1}$). This difference can be attributed to nitrogen rates that might alter the quality of fiber contents. However, NDF content of cricket frass application at rates of 5 t $ha^{-1}$ and 10 t $ha^{-1}$ showed lower forage quality in comparison to the cricket frass application at rate of 15 t $ha^{-1}$. This suggests that nitrogen rates may influence fiber quality differently. Previous studies have discussed how fiber contents in plants decrease significantly with an increase in nitrogen fertilization rate in plant tissues (Cherney & Cox, 1992; Johnson *et al*., 2001).

As for calcium and phosphorus minerals, they were significantly influenced by cricket frass, consistent with the findings of Bukari *et al*. (2021). The 15 t $ha^{-1}$ treatment provided adequate levels of crude protein (CP) and calcium (Ca) while maintaining a reasonable level of NDF.

# CHAPTER SIX: CONCLUSION AND RECOMMANDATIONS

The investigation of using species of *Commelina* as sustainable and affordable feed source for field cricket, *Scapsipedus icipe* was conducted between the period of October 2020 and June 2022. Emphasis was placed on determining the diversity of species of *Commelina* across different agroecological zones in Western Kenya, evaluating the feeding preferences of *S. icipe* on species of *Commelina*, and determine optimum growing conditions for production of species of *Commelina* using cricket frass as manure. The conclusion and recommendations can be summarized as follows:

Firstly, species of *Commelina* are diverse in Western Kenya and prefer different ecological conditions. In the survey, 11 species of *Commelina* were identified of which *Commelina diffusa* and *Commelina benghalensis var. benghalensis* (non-hybrid variant) have high relative density and the high number of associated weed species. The diversity of species *Commelina* is influencing by the environment (exchangeable sodium percentage, magnesium, soil pH, and total nitrogen) and management (agriculture system type). The distribution of species of *Commelina* responded to soil pH, available phosphorous, total nitrogen, fertility, and crop spacing. The identified five important variables affecting the distribution of species of *Commelina* will certainly contribute to the prioritization of ecological aspects leading to the growth condition of species of *Commelina*. The environment is strong explanatory factor of species of *Commelina* than management. This is the first investigation of environmental and management factors affecting the species of *Commelina* locally and regionally, although there is clearly a need for large-scale studies that include other factors.

Secondly, this study provides an insight into the importance of nutrients and phytochemicals in determining the suitability of species of *Commelina* as food for the field cricket *S. icipe*. The cricket shows a strong preference for particular species of *Commelina*, with *C. petersii* and *C. forskaolii* the most suitable followed by the two reference species, *C. benghalensi* var. *benghalensis* (nonhybrid variant) and *C. benghalensis* (hybrid variant), and the least preferred *Commelina* sp. and *C. purpurea.* There were positive associations between leaf feeding, Ca and NDF, and negative associations between Ca and NDF. The species *C. petersii* has a high Ca/low NDF content, whereas *C. purpurea* has a low Ca/high NDF content. Six phytochemicals (phenols,

alkaloids, tannins, glycosides, saponins and anthraquinones) influenced the leaf feeding of *S. icipe*, with the phenols in *C. petersii* and *C. forskaolii* acting as stimulants, and the tannins, glycosides and alkaloids in *Commelina* sp., *C. erecta* L. var. *livingistonii* and *C. purpurea* acting as deterrents. The low mortality of the cricket recorded in this study indicate this insect thrives on *Commelina*-based diets, which are a good source of CP for their growth and development. For the mass rearing of this cricket the leaves of *C. petersii* are highly recommended.

Finally, it was revealed that *C. petersii*, the most preferred species by *S. icipe*, responded positively to different application rates of cricket frass as manure. The rates (5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$) of cricket frass increased vegetative growth (plant height, number of shoots at field level, number of leaves, leaf area, and plant biomass) compared to untreated rate (0 t $ha^{-1}$). Additionally, cricket frass as manure increased nutrient contents for CP and Ca at the application rates of 5 t/ha, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ in pot and field experiments. As for NDF content, cricket frass application at rates of 5 t $ha^{-1}$, 10 t $ha^{-1}$, and 15 t $ha^{-1}$ exhibited higher content compared to untreated rate (0 t $ha^{-1}$). The 15 t $ha^{-1}$ rate provided adequate levels of CP and Ca while still maintaining a reasonable level of NDF content. Therefore, the recommended rate for increasing vegetative growth and nutrient contents of *C. petersii* as feed for the field cricket, *S. icipe*, is 15 t $ha^{-1}$ of cricket frass. Encouraging cricket farmers to adopt sustainable farming with cricket frass waste as organic manure can optimize the growing conditions of *C. petersii*. Extensionists should train farmers on good agricultural practices and correct application of the recommended rate of cricket frass as organic manure in the cultivation of *C. petersii*. To validate the recommended rate, further studies should be conducted to explore higher levels of cricket frass and analyze phytochemicals in both pot and field experiments, as cricket frass may impact their levels.

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# APPENDINCES

## Appendix 1. Environment and management variables used for the analysis of *Commelina* data set in selected agro-ecological zones in Western Kenya. Environment variables were normalized by logarithmic transformation and presented as Mean (± SE).

| Explanatory variables | Production sites | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Quantitative* | Waguso | Wariada | Nyamonyi | Abawa | Kasule coloa | Korando | Ahero | Namutoyi | Konyango | Angalo | Wahamblah | Kisui |
| *Total Nitrogen %* | $0.14^{abc}$±0.01 | $0.13^{ab}$±0.00 | $0.12^{ab}$±0.01 | $0.21^{d}$±0.01 | $0.13^{ab}$±0.00 | $0.18^{bcd}$±0.01 | $0.11^{a}$±0.01 | $0.12^{ab}$±0.01 | $0.22^{d}$±0.01 | $0.20^{cd}$±0.02 | $0.16^{abcd}$±0.00 | $0.14^{abc}$±0.00 |
| *Phosphorus (Mehlich) ppm* | $62.2^{ef}$±2.00 | $36.8^{cd}$±0.15 | $44.8^{de}$±0.95 | $25.9^{a}$±1.05 | 16.6±2.90 | $147.6^{g}$±4.60 | $28.1^{ac}$±2.00 | $25.3^{a}$±1.80 | $235.8^{b}$±1.10 | $177.0^{bg}$±1.00 | $64.5^{f}$±1.60 | $231^{b}$±4.00 |
| *Total Org. Carbon %* | $1.47^{a}$±0.04 | $1.04^{a}$±0.05 | $1.39^{a}$±0.07 | $2.38^{a}$±0.07 | $1.42^{a}$±0.31 | $2.04^{a}$±0.03 | $1.04^{a}$±0.97 | $1.36^{a}$±0.37 | $2.29^{a}$±0.42 | $2.22^{a}$±0.44 | $1.81^{a}$±0.17 | $1.63^{a}$±0.99 |
| *Soil pH-H2O (1:2.5)* | 7.0±0.02 | $6.8^{a}$±0.01 | $6.7^{a}$±0.02 | $6.7^{a}$±0.03 | 5.8±0.03 | 6.3±0.01 | $6.8^{a}$±0.01 | 6.1±0.01 | $6.7^{a}$±0.03 | $8.0^{c}$±0.00 | $7.8^{b}$±0.01 | $7.9^{bc}$±0.00 |
| *Elect. Cond. mS/cm* | 0.83±0.01 | $0.36^{a}$±0.05 | $0.30^{a}$±0.01 | $0.32^{a}$±0.02 | $0.17^{a}$±0.07 | $0.43^{a}$±0.08 | $0.17^{a}$±0.07 | $0.16^{a}$±0.06 | $0.27^{a}$±0.08 | $0.38^{a}$±0.03 | $0.37^{a}$±0.06 | $0.40^{a}$±0.06 |
| *Cat. Exch. Cap. meq%* | $15.1^{abd}$±0.90 | $14.9^{abd}$±0.10 | $10.8^{d}$±1.00 | $20.4^{abc}$±2.50 | $23.1^{abc}$±3.00 | $33.7^{c}$±1.88 | $13.1^{ad}$±0.70 | $14.4^{abd}$±2.55 | $20.4^{abc}$±1.55 | $32.2^{c}$±3.90 | $25.4^{bc}$±2.50 | $34.2^{c}$±4.90 |
| *Calcium meq%* | $46.3^{abd}$±0.90 | $31.9^{cd}$±1.20 | $28.6^{c}$±0.30 | $54.1^{bg}$±0.20 | $49.8^{ab}$±7.10 | $76.2^{eg}$±0.90 | $36.6^{acd}$±5.50 | $40.6^{abcd}$±0.70 | $51.3^{ab}$±2.80 | $118.8^{f}$±4.60 | $82.4^{ef}$±1.20 | $104.8^{ef}$±5.60 |
| *Magnesium meq%* | $5.9^{abc}$ ±0.80 | $4.2^{abc}$±0.10 | $2.9^{ac}$±0.80 | $9.7^{ab}$±0.40 | $6.6^{abc}$±1.20 | $9.9^{ab}$±1.50 | $2.5^{c}$±1.80 | $4.5^{abc}$±0.40 | $4.5^{abc}$±1.30 | $6.6^{abc}$±0.70 | $10.9^{ab}$±1.00 | $13.5^{b}$±0.80 |
| *Potassium meq%* | $1.0^{a}$±0.15 | $1.2^{a}$±0.10 | $1.0^{a}$±0.20 | $1.8^{a}$±0.90 | $1.2^{a}$±0.90 | $1.9^{a}$±1.20 | $1.3^{a}$±0.20 | $0.7^{a}$±0.20 | $1.6^{a}$±0.60 | $2.5^{a}$±1.50 | $1.8^{a}$±0.60 | $3.6^{a}$±0.30 |
| *Sodium meq%* | $1.6^{a}$±0.10 | $0.8^{a}$±0.70 | $0.5^{a}$±0.40 | $0.2^{a}$±0.10 | $0.9^{a}$±0.30 | $0.7^{a}$±0.50 | $0.1^{a}$±0.05 | $0.01^{a}$±0.00 | $1.2^{a}$±0.10 | $0.6^{a}$±0.35 | $1.0^{a}$±0.30 | $0.4^{a}$±0.30 |
| *ESP* | $10.6^{d}$±0.20 | $5.4^{abd}$±0.50 | $4.6^{abd}$±0.30 | $1.5^{abc}$±0.20 | $3.9^{abd}$±1.00 | $2.1^{abc}$±1.10 | $0.8^{ac}$±0.60 | $0.1^{c}$±0.09 | $5.9^{bd}$±2.00 | $1.9^{abc}$±1.20 | $3.9^{abd}$±0.70 | $1.2^{abc}$±0.50 |
| *Sand %* | $60^{a}$±10.00 | $60^{a}$±4.00 | $66^{a}$±7.00 | $54^{a}$±6.00 | $56^{a}$±7.00 | $52^{a}$±5.00 | $72^{a}$±17.00 | $52^{a}$±11.00 | $56^{a}$±10.00 | $60^{a}$±9.00 | 54±9.00 | $60^{a}$±15.00 |
| *Silt %* | $12^{a}$±4.00 | $6^{a}$±2.00 | $12^{a}$±3.00 | $16^{a}$±6.00 | $10^{a}$±4.00 | $6^{a}$±4.00 | $10^{a}$±4.00 | $20^{a}$±9.00 | $14^{a}$±4.00 | $8^{a}$±3.00 | $10^{a}$±5.00 | $10^{a}$±6.00 |
| *Clay %* | $28^{a}$±8.00 | $34^{a}$±6.00 | $22^{a}$±2.00 | $30^{a}$±9.00 | $34^{a}$±8.00 | $42^{a}$±11.00 | $18^{a}$±7.00 | $28^{a}$±7.00 | $30^{a}$±9.00 | $32^{a}$±7.00 | $36^{a}$±5.00 | $30^{a}$±8.00 |
| *Qualitative (binary dummy)* | | | | | | | | | | | | |
| *Surrounding Vegetation* | P-v/c-W | P-v/c-W | P-v/c-W | P-v/c-W | P-v/c-F | P-v/c-F | P-v/c-F | P-v/c-F | P-v/c-F | P-v/c-F | P-v/c-F | P-v/c-F |
| *Farming methods* | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI | PM/MI |
| *Crop spacing* | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm | 1-50cm/>50cm |
| *Crop establishment* | T/D | T/D | T/D | T/D | T/D | T/D | T/D | T/D | T/D | T/D | T/D | T/D |
| *Fertility* | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA | A/nA |
| *Agriculture system type* | I/R | I/R | I/R | I/R | I/R | I/R | I/R | I/R | I/R | I/R | I/R | I/R |

| *Weed Control* | Hw/Cr | Hw/Cr | Hw/Cr | Hw/Cr | Hw | Hw | Hw | Hw | Hw/Cr | Hw/Cr | Hw/Cr | Hw/Cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cost of weed control* | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost | None/Cost |
| *Diversity indexes* | | | | | | | | | | | | |
| *H* | 0.992 | 0.928 | 1.003 | 1.508 | 1.252 | 0.600 | 0.000 | 0.289 | 0.405 | 0.870 | 0.721 | 1.191 |
| *E* | 0.715 | 0.669 | 0.723 | 1.087 | 0.903 | 0.432 | 0.000 | 0.208 | 0.292 | 0.627 | 0.520 | 0.859 |
| *M* | 0.601 | 0.494 | 0.573 | 1.116 | 0.673 | 0.321 | 0.000 | 0.164 | 0.481 | 0.570 | 0.666 | 0.609 |

| **Variable** | **VIF** |
|---|---|
| *Magnesium meq%* | 10.77 |
| *Soil pH-H2O (1:2.5)* | 10.05 |
| *Crop establishment* | 9.57 |
| *Agriculture system type* | 7.35 |
| *Phosphorus (Mehlich) ppm* | 6.73 |
| ESP | 4.77 |
| *Elect. Cond. mS/cm* | 4.23 |
| *Total Nitrogen %* | 3.21 |
| *Surrounding Vegetation* | 2.83 |
| Fertility | 2.06 |
| *Farming methods* | 1.86 |
| *Crop spacing* | 1.76 |
| *Weed Control* | 1.56 |
| *Mean VIF* | 11.57 |

Note: Diversity values per production site are presented as Shannon-Weaver (H) diversity index, Pielou evenness index (E) and Margalef index (M). Variables and their derivation, surrounding vegetation, (P-v/c-W): Papyrus vegetation/cleared woodland; Farming method, (PM/MI): Pure monoculture/Mixed intercropping; Crop spacing, 1-50cm/above 50cm; Crop establishment, (T/D): Transplanting/Direct sowing; Fertility, (A/nA): Applied/not Applied; Agriculture system type, (I/R): Irrigated system/Rainfed system; Weed control, (Hw/Cr): Hand weeding/Crop rotation; Cost of weed control, (None/Cost): None/100-1000Ksh. Also, VIF (Variance Inflation factor) of environment nutrient variables used in multiple linear regression are mentioned. Means with different letters in the same row for environment variables are significantly different ($P < 0.05$).

**Appendix 2. List of 115 weed species recorded in selected agroecological zones in Western Kenya**

| Family | Species | EPPO Code | Growing habit | AD | RD (%) | Rank |
|---|---|---|---|---|---|---|
| Acanthaceae | *Asystasia gangetica* (L.) T.Anderson | ASYCO | P | 0.05 | 0.03 | |
| | *Asystasia mysorensis* (Roth) T. Anderson | ASYSC | A/P | 0.27 | 0.19 | |
| | *Hygrophila auriculata* (Schumach.) Heine | HYGAU | A | 0.59 | 0.44 | |
| Amaranthaceae | *Alternanthera sessilis* (L.) R.Br. ex DC. | ALRSE | A/P | 0.78 | 0.58 | |
| | *Amaranthus cruentus* L. | AMACR | A | 0.07 | 0.05 | |
| | *Amaranthus retroflexus* L. | AMARE | A | 3.61 | 2.67 | |
| | *Amaranthus spinosus* L. | AMASP | A | 2.01 | 1.49 | |
| | *Amaranthus viridis* Hook.f | AMAVI | A | 2.02 | 1.49 | |
| | *Gomphrena celosioides* Mart. | GOMCE | A/P | 0.38 | 0.28 | |
| Apiaceae | *Centela asiatica* (L.) Urb. | CLLAS | P | 0.43 | 0.32 | |
| Asteraceae | *Aspilia mossambicensis* (Oliv.) Wild | APIMO | A/P | 0.04 | 0.03 | |
| | *Acanthospermum hispidum* DC. | ACNHI | A | 1.36 | 1.01 | |
| | *Achyranthes aspera* L. | ACYAS | P | 0.11 | 0.08 | |
| | *Ageratum conyzoides* L. | AGECO | A | 2.87 | 2.13 | |
| | *Athroisma stuhlmannii* O.Hoffm. | AJMST | A/P | 0.01 | 0.01 | |
| | *Bidens pilosa* L. | BIDPI | A | 4.07 | 3.02 | 9 |
| | *Eclipta prostata* (L.) L. | ECLAL | A | 0.07 | 0.05 | |
| | *Enydra fluctuans* Lour | ENYFL | P | 0.02 | 0.02 | |
| | *Flaveria trinervia* (Spreng.) C.Mohr | FLATN | A | 0.59 | 0.44 | |
| | *Galinsoga parviflora* Cav. | GASPA | A | 0.69 | 0.51 | |
| | *Leonotis nepetifolia* (L.) R. Br. | LEONE | A/P | 0.09 | 0.07 | |
| | *Parthenium hysterophorus* L. | PTNHY | A | 12.31 | 9.12 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Acmella radicans* (Jacq.) R.K. | SPIRA | A | 0.31 | 0.23 | |
| | *Sphaeranthus steetzii* Oliv. & Hiern | SPSST | A/P | 0.79 | 0.59 | |
| | *Sphaeranthus suaveolens* (Forssk.) DC | SPSSU | A | 0.67 | 0.50 | |
| | *Synedrella nodiflora* Gaertn. | SYDNO | A | 0.55 | 0.41 | |
| | *Tagetes minuta* L. | TAGMI | A/P | 0.08 | 0.06 | |
| | *Xanthium strumarium* L. | XANSTL | A | 7.61 | 5.63 | 5 |
| Commelinaceae | *Aneilema umbrosum* (Vahl) Kunth | ANEUM | P | 0.21 | 0.15 | |
| | *Commelina africana* L. var. *africana* | COMAF | P | 0.02 | 0.02 | |
| | *Commelina benghalensis* L. var. *benghalensis* (non Hybrid) | COMBE1 | A/P | 2.17 | 1.60 | |
| | *Commelina benghalensis* L. (Hybrid) | COMBE2 | A/P | 0.97 | 0.72 | |
| | *Commelina diffusa* Burm. f. | COMDI | A/P | 11.98 | 8.87 | 3 |
| | *Commelina erecta* L. var. *livingistonii* | COMEL | P | 0.08 | 0.06 | |
| | *Commelina forskaolii* Vahl | COMFO | A | 1.34 | 1.00 | |
| | *Commelina kotschyi* Hassk. | COMKO | A/P | 1.64 | 1.22 | |
| | *Commelina latifolia* A. Rich. var. *latifolia* | COMLF | P | 0.09 | 0.07 | |
| | *Commelina petersii* Hassk. | COMPE | P | 0.03 | 0.02 | |
| | *Commelina purpurea* C.B. Clarke | COMPU | P | 0.03 | 0.03 | |
| | *Commelina sp.* | COMSP | P | 0.07 | 0.05 | |
| Convolvulaceae | *Ipomoea aquatica* Forssk. | IPOAQ | A | 0.37 | 0.28 | |
| | *Ipomoea vagans* L. | IPOVA | P | 0.23 | 0.17 | |
| Cyperaceae | *Cyperus diffomis* L. | CYPDI | A | 1.73 | 1.28 | |
| | *Cyperus esculentus* L. | CYPES | P | 0.08 | 0.06 | |
| | *Cyperus imbricatus* Retz. | CYPIM | P | 0.04 | 0.03 | |
| | *Cyperus rotundus* L. | CYPRO | P | 10.63 | 7.88 | 4 |
| | *Fimbristylis ferruginea* (L.) Vahl | FIMFE | P | 0.14 | 0.11 | |
| | *Fimbristylis littoralis* Gaudich. | FIMLI | A | 0.04 | 0.03 | |
| | *Kyllinga bulbosa* P. Beauv. | KYLBU | P | 0.07 | 0.05 | |

| | | | | | |
|---|---|---|---|---|---|
| | *Kyllinga pulchella* Kunth | KYLPU | Unknown | 0.06 | 0.04 |
| | *Pycreus lanceolatus* (Poiret) C.B.Clarke | CYPLC | P | 0.41 | 0.30 |
| Euphorbiaceae | *Acalypha ciliata* Forssk. | ACCCI | A | 1.07 | 0.79 |
| | *Euphorbia hirta* L. | EPHHI | A | 0.51 | 0.37 |
| | *Euphorbia heterophylla* L. | EPHHL | A | 1.42 | 1.05 |
| | *Euphorbia hypericifolia* L. | EPHHY | A | 0.06 | 0.04 |
| | *Ricinus communis* L. | RIICO | P | 0.02 | 0.02 |
| Fabaceae | *Aeschynomene mimosifolia* Vatke | AESMI | A/P | 0.84 | 0.63 |
| | *Senna hirsuta (L.)* S.H.Irwin & Barneby | CASHI | P | 0.03 | 0.02 |
| | *Senna obtusifolia* (L.) Irwin & Barneby | CASOB | P | 0.48 | 0.36 |
| | *Crotalaria brevidens* Benth. | CVTBD | A/P | 0.03 | 0.02 |
| | *Crotalaria laburnifolia* L. | CVTPE | A/P | 0.02 | 0.01 |
| | *Crotalaria retusa* L. | CVTRE | A/P | 0.06 | 0.05 |
| | *Desmodium incanum* (Sw.) DC. | DEDCA | P | 0.23 | 0.17 |
| | *Desmodium tortuosum* (Sw.) DC. | DEDTO | P | 0.06 | 0.04 |
| | *Desmodium uncinatum* (Jacq.) DC. | DEDUN | P | 0.06 | 0.05 |
| | *Indigofera spicata* Forssk. | INDSP | P | 0.69 | 0.51 |
| | *Mimosa pigra* L. | MIMPI | A/P | 0.42 | 0.31 |
| | *Sesbania sesban* (L.) Merr. | SEBSE | P | 0.08 | 0.06 |
| Linderniaceae | *Crepidorhopalon hepperi* Eb. Fisch | LIDHE | A | 0.01 | 0.01 |
| Lythraceae | *Ammannia baccifera* L. | AMMBA | A | 0.23 | 0.17 |
| Malvaceae | *Abutilon mauritianum* (Jacq.) Medik. | ABUMT | P | 0.09 | 0.07 |
| | *Corchorus olitorius* L. | CRGOL | A | 1.12 | 0.83 |
| | *Malvastrum coromandelianum* (L.) Garcke | MAVCO | A | 0.89 | 0.66 |
| | *Sida acuta* Burm.f. | SIDAC | P | 0.09 | 0.07 |
| | *Sida cordifolia* L. | SIDCO | P | 0.07 | 0.05 |
| Marsileaceae | *Marsilea minuta* L. | MASMI | P | 0.14 | 0.10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Menispermaceae | *Stephania abyssinica* Oliv. | STJAB | | 0.02 | 0.02 | |
| Mollugonaceae | *Mollugo nudicaulis* Lam. | MOLNU | A | 0.01 | 0.00 | |
| Nyctaginaceae | *Boerhavia diffusa* L. | BOEDI | P | 1.98 | 1.46 | |
| Onagraceae | *Ludwigia adscendens* (L.) Hara | LUDAD | P | 1.66 | 1.23 | |
| Orobanchaceae | *Striga hermonthica* (Delile) Benth | STRHE | OP | 2.23 | 1.65 | |
| Phyllantaceae | *Phyllanthus niruri* L. | PYLNIL | P | 0.08 | 0.06 | |
| | *Phyllanthus amarus* Schum. & Thonn. | PYLAM | A | 0.24 | 0.18 | |
| Poaceae | *Chloris virgata* Sw. | CHRVI | A | 0.02 | 0.02 | |
| | *Cynodon dactylon* (L.) Pers. | CYNDA | P | 15.62 | 11.57 | 1 |
| | *Digitaria abyssinica* (A. Rich) Stapf. | DIGAB | A | 3.68 | 2.73 | 10 |
| | *Digitaria velutina* (Forssk.) P.Beauv. | DIGVE | A | 0.18 | 0.13 | |
| | *Dactyloctenium aegyptium* (L.) P.Beauv. | DTTAE | A | 0.76 | 0.56 | |
| | *Echinochloa colona* L. (Link) | ECHCO | A | 6.42 | 4.76 | 6 |
| | *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase | ECHPY | P | 0.43 | 0.32 | |
| | *Eleusine indica* (L.) Gaertn | ELEIN | A | 0.70 | 0.52 | |
| | *Eragrostis tenuifolia* (A.Rich.) Hochst. ex Steud. | ERATE | P | 0.11 | 0.08 | |
| | *Ischaemum rugosum* Salisb. | ISCRU | A | 1.02 | 0.76 | |
| | *Paspalum scrobiculatum* L. | PASSC | P | 0.19 | 0.14 | |
| | *Phragmites australis* (Cav.) Trin. ex Steud. | PHRCO | P | 0.17 | 0.12 | |
| | *Setaria verticillata* P.Beauv. | SETVE | A | 0.09 | 0.07 | |
| | *Sorghum arundinaceum* (Desv.) Stapf | SORVE | A/P | 0.08 | 0.06 | |
| | *Sporobolus pyramidalis* L. | SPZPY | P | 0.01 | 0.01 | |
| Polygonaceae | *Persicaria pulchra* (Blume) Soják | POLPV | P | 0.08 | 0.06 | |
| | *Persicaria setosula* (A.Rich.) K.L.Wilson | POLSM | P | 0.02 | 0.02 | |
| Pontederiaceae | *Heteranthera callifolia* Rchb. ex Kunth | HETCA | A | 0.04 | 0.03 | |
| Portulacaceae | *Portulaca oleracea* L. | POROL | A | 4.36 | 3.23 | 8 |
| | *Portulaca quadrifida* L. | PORQU | A/P | 1.86 | 1.38 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Rubiacae | *Mitracarpus hirtus* (L.) DC | MTCVI | A | 0.94 | 0.70 | |
| | *Oldenlandia corymbosa* L. | OLDCO | A | 0.05 | 0.04 | |
| Solanaceae | *Datura stramonium* L. | DATST | A | 0.08 | 0.06 | |
| | *Physalis angulata* L. | PHYAN | A | 0.56 | 0.41 | |
| | *Solanum incanum* L. | SOLIA | P | 0.12 | 0.09 | |
| | *Withania somnifera* (L.) Dunal | WITSO | A/P | 0.19 | 0.14 | |
| Sphenocleaceae | *Sphenoclea zeylanica* Gaertn. | SPHZE | A | 0.01 | 0.00 | |
| Tribulaceae | *Tribulus terrestris* L. | TRBTE | A/P | 0.98 | 0.72 | |
| Typhaceae | *Typha domingensis* Pers. | TYHDO | P | 0.15 | 0.11 | |
| Verbenaceae | *Lantana camara* | LANCA | P | 0.01 | 0.01 | |
| | *Stachytarpheta jamaicensis* (L.) Vahl | STCIN | P | 6.27 | 4.64 | 7 |
| Vitaceae | *Cyphostemma adenocaule* (A.Rich.) Wild & R.B.Drumm. | CWMAA | A | 0.01 | 0.01 | |

Note: LC: Life Cycle (A: Annual, P: Perennial, A/P: Short-lived Perennial, OP: Obligate hemi-parasite and Unknown) — AD: absolute density, RD (%): Relative density expressed in percentage. Background shading indicates the rank of the 10 predominant weed species based on relative densities. Five weed species namely, *Aeschynomene mimosifolia* Vatke, *Aspilia mossambicensis* (Oliv.) Wild, *Commelina petersii* Hassk, *Commelina latifolia* A. Rich. var. *latifolia* and *Commelina purpurea* C.B. Clarke were not recognized in the European and Mediterranean Plant Protection system (EPPO), and hence were coded as AESMI, APIMO, COMPE, COMLA, COMPU, respectively.

**Appendix 3. Summary of Canonical Correspondence Analysis (CCA) of counting data of 11 species of *Commelina* sampled in agroecological zones in Western Kenya, showing results of the corresponding Monte Carlo permutation tests**

| **Total Inertia (sum of eigenvalue): 7.564** | | | | |
|---|---|---|---|---|
| Axes | 1 | 2 | 3 | 4 |
| Eigenvalues | 0.458 | 0.294 | 0.193 | 0.148 |
| Species-environment correlations | 0.744 | 0.658 | 0.547 | 0.498 |
| Cumulative percentage variance of species data | 6.1 | 9.9 | 12.5 | 14.4 |
| Cumulative percentage variance of species-environment relation | 37.5 | 61.5 | 77.3 | 89.5 |
| Monte Carlo test (999 permutations) | *F-ratio* | *p-value* | | |
| Significance of first canonical axis | 10.501 | 0.002 | | |
| Significance of all canonical axes | 2.091 | 0.001 | | |

**Appendix 4. Picture of the 11 species of *Commelina* recorded in agroecological zones of Western Kenya**

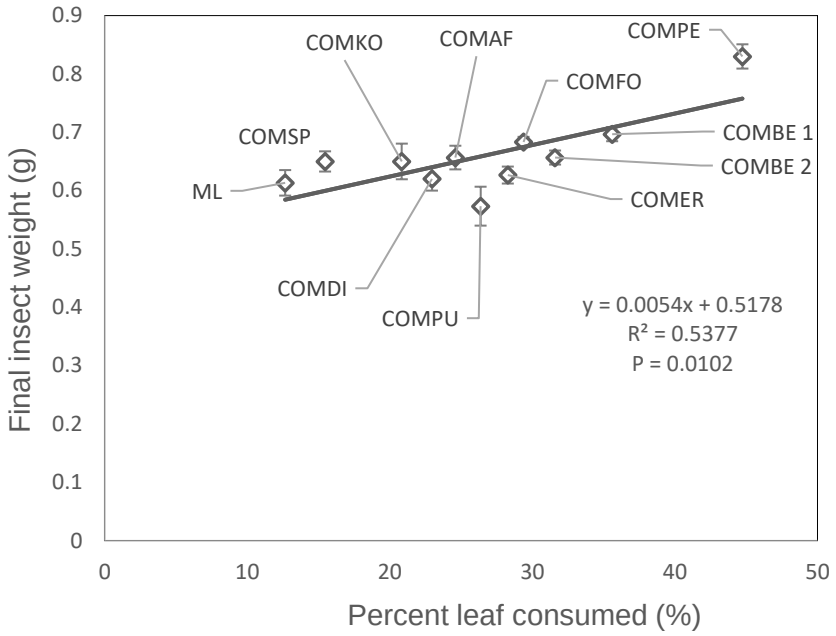
A: COMDI B: COMPU C: COMEL D: COMAF

E: COMPE F: COMLA G: COMBE 1 H: COMBE 2

I: COMSP K: COMFO J: COMKO

Note*: COMDI: Commelina diffusa; COMPU: Commelina purpurea; COMEL: Commelina erecta* subsp. *livingstonii; COMAF: Commelina africana; COMPE: Commelina petersii; COMLA: Commelina latifolia* var. *latifolia; COMBE1: Commelina benghalensis* var. *benghanlensis* (non-hybrid variant), *COMBE2: Commelina bengalensis* (hybrid variant); *COMSP: Commelina sp., COMFO: Commelina forskaolii; COMKO: Commelina kotschyi*

**Appendix 5. Relationship between final insect gained weight (g) and percentage of leaf consumed (%) recorded in five days**



**Appendix 6. ANOVA's of 10 nutrients from proximate composition and Van soest fibers analysis system. Data are reported as Mean (± SE) and coefficient of variation (CV) and analyzed after a logarithmic transformation [log(x+1)].**

| Species | %DM | %ASH | %EE | %CP | %NFE | %NDF | %ADF | %ADL | % Hemicellulose | % Cellulose |
|---|---|---|---|---|---|---|---|---|---|---|
| COMPU | 90.53±0.03$^{a}$ | 24.14±0.005 | 3.25±0.01 | 21.67±0.005$^{a}$ | 25.6±2.03$^{f}$ | 73.36±0.03 | 28.95±0.01 | 3.25±0.03$^{cd}$ | 44.41±0.00 | 29.64±0.02 |
| COMLA | 89.24±0.07 | 14.83±0.06 | 2.07±0.06$^{a}$ | 27.53±0.025 | 34.89±0.15$^{ad}$ | 58.18±0.03 | 18.54±0.01 | 2.43±0.02$^{a}$ | 39.64±0.02$^{b}$ | 18.83±0.01 |
| COMDI | 90.08±0.01$^{b}$ | 12.41±0.01 | 2.59±0.005$^{cd}$ | 20.81±0.005 | 45.55±0.13$^{cd}$ | 59.22±0.02 | 17.85±0.04 | 2.73±0.02$^{b}$ | 41.375±0.01 | 18.65±0.04 |
| COMFO | 91.25±0.03$^{d}$ | 19.60±0.02 | 1.70±0.03 | 21.91±0.05$^{b}$ | 31.23±0.02$^{ab}$ | 58.545±0.03 | 19.31±0.04 | 3.31±0.01$^{d}$ | 39.235±0.07 | 19.065±0.02 |
| COMPE | 90.08±0.03$^{b}$ | 16.76±0.02 | 3.85±0.03$^{f}$ | 25.10±0.04 | 37.74±0.06$^{be}$ | 48.715±0.07 | 15.37±0.10$^{a}$ | 1.84±0.02 | 33.34±0.03$^{a}$ | 14.465±0.02 |
| COMEL | 90.42±0.03$^{ac}$ | 13.10±0.03 | 2.09±002$^{a}$ | 22.44±0.02 | 39.90±0.01a$^{cd}$ | 57.53±0.03 | 24.30±0.01 | 2.03±0.01 | 33.23±0.02$^{a}$ | 22.725±0.03 |
| COMKO | 90.30±0.05$^{bc}$ | 15.91±0.01 | 2.90±0.02$^{e}$ | 23.93±0.02 | 33.50±0.04$^{f}$ | 62.56±0.02 | 16.35±0.04 | 3.14±0.02$^{c}$ | 47.205±0.02 | 23.46±0.01 |
| COMBE1 | 90.47±0.02$^{ac}$ | 32.89±0.02 | 2.43±0.02$^{bc}$ | 23.20±0.01 | 23.06±0.02$^{be}$ | 57.13±0.03 | 19.81±0.01 | 2.37±0.01$^{a}$ | 36.32±0.05 | 17.83±0.03 |
| COMBE2 | 91.20±0.05$^{d}$ | 20.80±0.01 | 2.76±0.01$^{de}$ | 21.77±0.01$^{ab}$ | 40.07±0.09$^{ab}$ | 54.87±0.03 | 15.19±0.02$^{a}$ | 3.86±0.01 | 39.68±0.01$^{b}$ | 15.45±0.02 |
| COMSP | 90.59±0.02$^{a}$ | 22.55±0.01 | 2.37±0.02$^{b}$ | 20.54±0.02 | 38.00±0.01$^{c}$ | 61.36±0.03 | 20.86±0.02$^{b}$ | 2.66±0.02$^{b}$ | 42.5±0.01 | 16.54±0.01 |
| COMAF | 91.28±0.03$^{d}$ | 21.30±0.04 | 3.82±0.06$^{f}$ | 23.70±0.03 | 29.36±0.07 | 68.525±0.005 | 20.91±0.03$^{b}$ | 4.37±0.08 | 47.61±0.03 | 23.21±0.04 |
| % CV | 0.61 | 2.15 | 16.56 | 1.52 | 13.66 | 0.86 | 2.91 | 12.34 | 1.04 | 2.11 |
| P-value | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

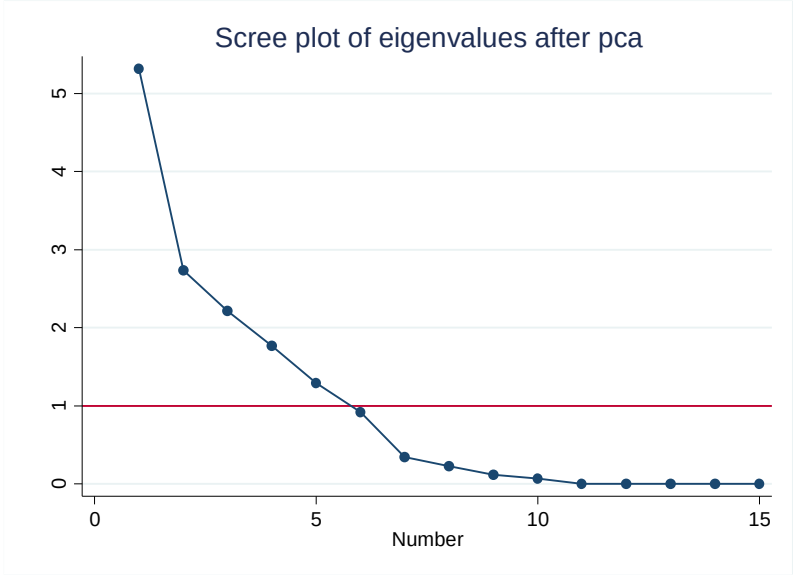
Note: Means sharing the same superscript letter in species are not significantly different at $P < 0.05$.

**Appendix 7. Kruskal-Wallis test of the eight mineral nutrients. Data are reported as Mean (± SE) and coefficient of variation (CV).**

| Species | Na% | K% | Ca% | Mg% | Zn% | Mn% | Fe% | Cu% |
|---|---|---|---|---|---|---|---|---|
| | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean |
| COMPU | 0.0130 ± 0.002 | 0.1700 ± 0.000 | 0.4700 ± 0.010 | 0.5000 ± 0.020 | 0.0036 ± 2.000e-004 | 5.5110 ± 0.001 | 0.2450 ± 0.005 | 0.0019 ± 3.000e-004 |
| COMLA | 0.0039 ± 1.000e-004 | 0.1700 ± 0.010 | 1.4600 ± 0.020 | 0.4300 ± 0.000 | 0.0034 ± 3.000e-004 | 3.2300 ± 0.010 | 0.0048 ± 2.000e-004 | 0.0014 ± 3.000e-004 |
| COMDI | 0.0073 ± 1.000e-005 | 0.1200 ± 0.010 | 0.4800 ± 0.000 | 0.3800 ± 0.020 | 0.0030 ± 0.001 | 5.5770 ± 0.000 | 0.1100 ± 0.000 | 0.0018 ± 0.000 |
| COMFO | 0.0036 ± 0.000 | 0.1700 ± 0.000 | 1.4300 ± 0.000 | 0.4900 ± 0.020 | 0.0030 ± 0.001 | 4.1280 ± 0.002 | 0.0560 ± 0.047 | 0.0033 ± 0.001 |
| COMPE | 0.0100 ± 0.000 | 0.0970 ± 0.001 | 6.2000 ± 0.100 | 0.4300 ± 0.020 | 0.0050 ± 0.001 | 2.9800 ± 0.000 | 0.0430 ± 0.001 | 0.0021 ± 3.000e-004 |
| COMEL | 0.0013 ± 1.000e-004 | 0.1700 ± 0.000 | 1.5100 ± 0.020 | 0.4400 ± 0.010 | 0.0040 ± 0.001 | 6.3040 ± 0.004 | 0.1400 ± 0.030 | 0.0028 ± 2.000e-004 |
| COMKO | 0.0009 ± 1.000e-006 | 0.1400 ± 0.000 | 1.1500 ± 0.020 | 0.5000 ± 0.100 | 0.0039 ± 1.000e-004 | 4.1650 ± 0.000 | 0.0810 ± 0.003 | 0.0026 ± 1.000e-004 |
| COMBE 1 | 0.0050 ± 0.001 | 0.1700 ± 0.000 | 1.9500 ± 0.020 | 0.3600 ± 0.020 | 0.0043 ± 2.000e-004 | 4.2030 ± 0.001 | 0.1500 ± 0.000 | 0.0037 ± 2.000e-004 |
| COMBE 2 | 0.0130 ± 0.002 | 0.1700 ± 0.000 | 1.7100 ± 0.030 | 0.4400 ± 0.000 | 0.0040 ± 0.001 | 6.3040 ± 0.002 | 0.1400 ± 0.001 | 0.0028 ± 3.000e-004 |
| COMSP | 0.0522 ± 3.000e-004 | 0.1850 ± 0.005 | 0.3200 ± 0.010 | 0.3200 ± 0.000 | 0.3800 ± 0.000 | 7.0150 ± 0.001 | 0.2460 ± 0.000 | 0.0018 ± 1.000e-004 |
| COMAF | 0.0018 ± 0.000 | 0.1000 ± 0.000 | 2.8220 ± 0.001 | 0.4900 ± 0.010 | 0.0045 ± 0.001 | 6.7220 ± 0.002 | 0.1960 ± 0.001 | 0.0023 ± 3.000e-004 |
| %CV | 87.47 | 25.38 | 19.96 | 67.69 | 239.53 | 0.76 | 177.13 | 158.91 |
| Kruskal Wallis statatistic | 20.80 | 19.21 | 20.64 | 16.91 | 11.79 | 20.81 | 18.23 | 20.07 |
| P-value | 0.0225* | 0.0377* | 0.0237* | 0.0765ns | 0.2994ns | 0.0225* | 0.0286* | 0.0512ns |

Note: Significant difference assessed at different levels of P- value, *: $P < 0.05$ and ns: not significant at $P > 0.05$.

**Appendix 8. Scree plot eigenvalues of the first five important principal components**



**Appendix 9. Detail of a plot with dots indicating *Commelina* hills and grey area indicating sampling area at central harvest**