EFFECT OF LABLAB (*Lablab purpureus* L.) GREEN MANURE ON POPULATION OF PATHOGENIC AND NON-PATHOGENIC SOIL MICROORGANISMS AND BEAN (*Phaseolus vulgaris* L.) CROP ESTABLISHMENT

OLIVER OTIENO OKUMU

(BSc. Agriculture, Msc, Crop Protection, University of Nairobi)

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CROP PROTECTION

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

UNIVERSITY OF NAIROBI

2018

DECLARATION

DECLARATION

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

Oliver Otieno Okumu ---------- Date: 11/12/2018

This thesis has been submitted with our approval as University supervisors.

Prof. James W. Muthomi ---------- Date: 12/12/2018

Department of Plant Science and Crop Protection
University of Nairobi

Prof. Rama D. Narla ---------- Date: 13.12.2018

Department of Plant Science and Crop Protection
University of Nairobi

Prof. John H. Nderitu ---------- Date: 12/12/2018

Department of Plant Science and Crop Protection
University of Nairobi

Dr. John Ojiem ---------- Date 12/12/2018

Kenya Agricultural and Livestock Research Organization (KALRO)
Kibos, Horticulture Research Center

DECLARATION OF ORIGINALITY

Name of Student …….

Registration Number …….

College …….

Faculty/ School/ Institute ……

Department …….

Course Name …….

Title of Work ……

Declaration

1. I understand what plagiarism is and I am aware of the University's policy.
2. I declare that this thesis is my original work and has not been submitted for examination, award of a degree or publication. Where other peoples work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not sought or used the services of any professional agencies to produce this work.
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his or her own work.
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature …….

Date …….....

DEDICATION

This thesis is dedicated to the memory of my late father, Herrington Okumu Onyuna, for his pioneering spirit, my dearest mother Julian Akoth Alolo, my beloved wife Susan Awino Wego and daughter Julie Lauren for their persistence in the face of adversity, and their belief in the value of higher education.

ACKNOWLEDGEMENTS

This research was made possible by the financial support from the McKnight Foundation under the project "Multipurpose legumes and management strategies for reinvigorating and maintaining the health and productivity of smallholder mixed farming systems" and the Technical support by the Kenya Agricultural and Livestock Research Organization (KALRO) Kibos Centre, Kisumu

I wish to express my sincere gratitude to Prof James W. Muthomi, my principal supervisor for his enormous encouragement, patience, constructive scholarly criticism and professional help throughout the project. I am also thankful to my supervisors' Prof. Rama D. Narla, Prof; I could feel your persistent struggle when you constantly encouraged me to ensure the project was done, Prof John H. Nderitu and Dr. John Ojiem for their valuable suggestions and discussions along the completion of this project. Many thanks are also extended to Dr. Julie Lauren whose advice were instrumental in understanding the concept of this project

I would also like to acknowledge the technical assistance of Simon Kamwana and James Nyongesa as well as the help of fellow PhD candidates Samuel Aringo Were and Luiza Munyua. Many thanks also to Technologists from the Department of Plant Science and Crop Protection, University of Nairobi for sharing their expertise and knowledge in the laboratory.

I would also like to acknowledge my wife Susan Awino Wego and Daughter Julie Lauren you are my pillars of support, strength and inspiration. To my mum, I would not have successfully accomplished the project without your help and encouragements. You have sacrificed everything for my better future and I really wish to live to your expectations to my very best. To each one, I am very grateful. Above all and most importantly, I thank the Almighty God for His grace and favor for the wonderful opportunity I had at the University of Nairobi

ABBREVIATIONS

AEZ	Agro-ecological zone
AG	Anastomosis group
ALS	Angular leaf spot
ANOVA	Analysis of Variance
AUDPC	Area under Disease Progress curve
BCMV	Bean Common Mosaic Virus
C	Carbon
CBB	Common bacterial blight
cfu	Colony Forming Units
DAP	Diamonium Phosphate
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GLP2	Grain Legume Program Two
Ha	Hectare
K	Potassium
KALRO	Kenya Agricultural and Livestock Research Organization
Kg	Kilogram
KK8	Kakamega eight
MGT	Mean Germination Time
ml	Milliliters
MLP	Multipurpose Legume Project
N	Nitrogen
NaCl	Sodium Chloride

NARL	National Agricultural Research Laboratories
P	Phosphorus
PGPR	Growth-promoting Rhizobacteria
Ppm	Parts per million
SPSS	Statistical Product and Service solutions
UNESCO	United Nations Education, Scientific and Cultural Organization
WHC	Water Holding Capacity

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL ABSTRACT

The use green manure as soil amendments stimulates the growth and activity of soil microorganisms, with subsequent mineralization of nutrients and increase in fertility. However, undecomposed plant residues have been reported to reduce crop emergence and establishment. The study was carried out to determine the effect of lablab green manure on population of soil microorganisms and to investigate the mechanisms contributing to poor crop establishment. A survey was conducted in Nandi South to determine the usage of green manures as soil amendment and field experiments where two bean varieties, KK8 and GLP2 were planted on plots treated with lablab green manure incorporated over the whole plots, and between rows, diamonium phosphate fertilizer and lime. The effect of time after incorporation of lablab green manure on bean root rot and establishment was determined by incorporating lablab green manure at 0, 7, 14 and 28 days before planting. Data was collected on soil nutrient content, soil microorganisms, crop emergence, plant stand, incidence and severity of root rot, biomass and grain yield. Mechanisms contributing to poor crop establishment incorporation were determined by evaluating the effect of fresh and compost extracts of lablab and also in comparison with other legumes on bean germination, mycelial growth of root rot fungal pathogens, spore germination and germ tube elongation. Potential antagonistic activity between saprophytes and the root rot fungal pathogens was also determined

Most farmers applied green manure as a soil amendment with 62% evenly distributing green manure in soil before planting and majority (48%) of the farmers planted crops two weeks after incorporation of green manure. Lablab green manure improved soil organic carbon, nitrogen, phosphorus and potassium but reduced germination percentage by 35% and increased incidences of root rot by 30% compared to the plots without green manure. Root rot pathogen population

was significantly high in green manure treated plots while the population of saprophytic fungi was low. Plots treated with lablab green manure had reduced grain and biomass yields by up to 25%. Green manure incorporated 28 days before planting resulted in improved germination by 90% with corresponding reduction in root rot incidence and severity by 8% and 36%, respectively compared to plots incorporated with lablab green manure at planting. Green manure incorporated at planting stimulated population of root rot pathogens. However, it reduced the population of saprophytic fungi such as *Trichoderma, Aspergillus,* and *Penicillium* and also significantly reduced grain yield by up to 70%.

Fresh legume extracts were found to inhibit spore germination, germ tube elongation and mycelial growth of root rot fungi while lablab extracts was stimulated. Ethanol extracts from lablab and soybean significantly inhibited mycelial growth by up to 62% but fresh aqueous extracts from lablab had minimal antifungal activity. The ethanol extracts from groundnut, lablab and soybean increased mean germination time by 120%, with corresponding decrease in final germination percentage. Aqueous extract of lablab and soybean resulted in significantly increased spore germination by over 70% and increasing germ tube length and number of germ tubes by 8.0% and 13%, respectively. The saprophytic fungi were antagonistic against the root rot pathogens with *Trichoderma* showing the highest inhibition of up to 64%. The results of the study showed that lablab green manures stimulated an increase in root rot pathogens resulting in low emergence and establishment of bean crop probably due to pre-and post-emergence damping off. However, allowing decomposition for four weeks increases nutrients release and causes decline in the population of root pathogens in the soil. Therefore, timing and method of application of green manure is important in attaining the full crop yield. A period of 28 days after incorporation of lablab green manure is recommended for planting common beans

CHAPTER ONE: INTRODUCTION

1.1 Background information

Common bean (*Phaeseolus vulgaris*) is an important legume cultivated by many small scale farmers in Eastern and Southern Africa (Leitich *et al.,* 2016). In Africa, common bean is an essential source of protein and calories (Buruchara, 2006; Langwerden, 2014) and additionally, it provides vitamins and micronutrients like Zn and Fe (Celmeli *et al*., 2018). In the East African region, beans are grown by smallholder farmers for home consumption and any excess sold to the market for income generation (Spence, 2006; Binagwa *et al*., 2016). They play an essential role in the sustainable livelihoods of smallholder farmers and their families providing both food security and income generation (Spence, 2003).

In spite of the nutritional and economic importance of beans, smallholder farmers encounter both abiotic and biotic constraints (Kajumula and Muhamba, 2012). These constraints have considerable effect on agricultural productivity and supply of beans in the market. Soilborne diseases, drought and low soil fertility are the main constraints to bean production throughout the developing world with low fertilizer applications (Lynch *et al.,* 2009; Langwerden, 2014). Root rot is a major disease complex of common beans causing substantial economic losses and is caused by several pathogenic fungi *Fusarium solani* f. sp. *phaseoli*, *R. solani, Pythium ultimum, Fusarium oxysporum* f.sp. *phaseoli, Macrophomina phaseolina and Sclerotinia sclerotiorum*. Use of green manure residues increases soil carbon and organic matter and consequently improves soil nutrient status (Talgre *et al.,* 2009), and also increase microbial activity in the soil. Green manure also enhances soil physical conditions by improving soil tilth, soil aeration, soil water holding capacity (WHC), plant root penetration, and suppression of pathogenic organisms and therefore improves plant health (Mosavi *et al.,* 2012). Lablab crop has been identified to

have the potential for improving system productivity, household food security and income. Its benefits include improving soil fertility through inputs of fixed atmospheric nitrogen (Murphy and Colucci, 1999). It can sustain, improve productivity and food security, however, the use green manure in order to improve yield of successive crops, alters the soil environment and often results in poor crop stand establishment. Furthermore, incorporation of undecomposed crop residues affects crop dynamics by decreasing or delaying germination and establishment of plant growth resulting in reduced density and vigour of the crop (Khaliq *et al*., 2011). During decomposition, phytotoxic substances are released to the soil in turn they enhance population of soilborne pathogens that inhibits crop germination (Bonanomi *et al*., 2007). There is an immediate increase in germination of propagules of pathogenic fungi such as *Pythium, Fusarium* and *Rhizoctonia* spp. after incorporation of green plant residues. This is because of the readily available nutrient resource that stimulates growth and reproduction of soil fungi which can invade the plants and cause poor crop germination and establishment

1.2 Problem statement

Smallholder farmers encounter multiple constraints during bean production, chief among them being soil borne diseases and low soil fertility. The inherent problem of low soil fertility has been attributed to soil erosion, and crop harvests. Practices such as crop rotation, cover cropping and use of green manures lead to an increase in population of soil borne pathogens. The occurrence of these diseases and their severity are connected to the intensification of land use leading to build up of soil borne pathogen inoculum. Root rot is a major disease complex of beans and is caused by several pathogenic fungi *Fusarium solani* f. sp. *phaseoli, R. solani, Pythium ultimum, Fusarium oxysporum, Macrophomina phaseolina and Sclerotinia sclerotiorum* (Abawi and Widmer, 2000). Farmers address these constraints by applying inorganic fertilizers

and fungicides that are expensive. The use of green manure in this respect has been suggested as a cheap alternative option for improving soil fertility to address both low soil fertility status and increased incidences of soil borne pathogens. However, green manure materials applied may also limit the diversity of productive lands by inhibiting crop establishment through various mechanisms such as release of phytotoxic substances (Balekari, 2013; Al-Snafi, 2017), and enhancement microbial activity that may have an effect on crop germination through provision of large amounts of nutrients.

Germination and growth inhibition ranging from 30 to 50% have been reported in barley crops (Ashrafi *et al*., 2008). The inhibition is through physical and biochemical effects that include consumption of water for green manure decomposition, and release of phytotoxins by plant residues as they decompose (Haramoto and Gallandt, 2005). However, this depends on chemical composition of different plants. Some green manure crops during decomposition release a variety of chemical substances that have negative effects on growth and development of succeeding crops reducing germination (Larkin, 2013).

Fresh green manure residues enhance the microbial activity in the soil because they are rich in cellulose (Abawi and Widmer, 2000; Manici *et al*., 2004; Bonanomi *et al*., 2013). The instant stimulation of microbial activity may result in either increase or decrease in the population of soil pathogenic fungi. Since diseases and poor soil fertility have been cited as major constraints in bean production, elucidation of the effect of lablab green manure on soil borne pathogens and crop establishment is needed for the design of soil and crop management systems.

1.3 Justification of the study

Green manuring with legumes increases soil fertility and incorporates nitrogen in the production system. Their use for bean production is economical compared to other mineral nitrogen fertilizers. Lablab is one of the most efficient legumes in terms of biomass production and N supply in soils. It has an extensive root system that makes soil more friable, improves its tilth, facilitate water infiltration and stabilize the soil. Its favourable properties have enhanced its use as a green manure crop. However, when used as a green manure, it considerably affects crop emergence and establishment thus lowering yield.

Efforts have been put to understand the mechanisms through which the green manure acts to inhibit emergence and crop establishment. Unfortunately, many studies have focused on allelopathy as the main mechanism, but its effect of these compounds can only last for a few days due to rapid breakdown of allelopathic compounds into non-toxic forms. Other inhibitory effects could be attributed to the effect of green manure on soil microorganisms that may have caused damage to root system or produced inhibitory substances.

Therefore, understanding the pattern of inhibition by lablab green manure residues is crucial in synchronizing its use for nitrogen release and uptake by plants and at the same time reduce negative effects associated with it.

1.4 Study objectives

The broad objective of the study was to improve common bean productivity through management of root rots and soil fertility by use of lablab as green manure.

The specific objectives were:

i. To determine the effect of lablab green manure incorporation on population of soil microorganisms, establishment of common bean and yield
ii. To determine the effect of time after incorporation of lablab green manure on root rot and establishment of common bean and yield
iii. To determine the effect of legume extracts on root rot pathogens and germination of common beans

1.5 Hypothesis

i. Incorporation of lablab (*Lablab purpureus* L.) green manure impacts on the level of soil microorganisms and negatively impacts on bean crop establishment and yields
ii. Timing of lablab residue (*Lablab purpureus* L.) incorporation determines the impacts on bean crop establishment, yields and root rots
iii. Legume extracts negatively impacts on common bean establishment and either stimulates or inhibits growth of root rot pathogens

CHAPTER TWO: LITERATURE REVIEW

2.1 Importance of common bean

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct consumption (Ng'ayu-Wanjau, 2013) and is produced in a wide range of cropping systems and environments. In Africa it is an important source of protein for rural households (Buruchara, 2006) contributing up to 57% of recommended protein and 23% of energy to the majority of people living in Africa (Mwanauta *et al*., 2015). Beans are mainly grown for subsistence use in Africa, Kenya having the highest per capita consumption in the world. In Kenya, the crop is important part of the diet providing up to 45% of the consumed total protein. It is also an important source of vitamins, minerals that have both curative and preventative properties to terminal diseases such as cancer, with folic acid, dietary fibre, complex carbohydrates, and iron providing up to 30% of daily recommended levels (Pachico, 1993; Romero-Arenas *et al*., 2013). Beans improve and sustain soil fertility due to their ability to fix nitrogen in the soil. They are therefore used in crop rotation, as cover crops and as green manures (Mugisha, 2008).

2.2 Production of common beans in Kenya

In Kenya, bean is ranked as the most important legume crop both in terms of utilization and production with an annual production of 461,734 metric tons. Production areas include Eastern, Central, Western, and Nyanza Provinces at altitudes varying from 1,500 to 2,500 m above sea level (Kimiti *et al*., 2009). Commonly grown varieties in Kenya include GLPX92 (Mwitemania), GLP1127 (Mwezimoja) GLP24 (Kitui), GLP2 (Rosecoco), GLP2 (Nyayo), GLP 806 (Zebra) and GLP585 (Red haricot) (Katungi *et al*., 2009). Production increased in both Eastern and Central regions in 2012 from 6,418,596 bags of 90 Kg in 2011 to 7, 358, 256 bags in 2012, representing a 14% increase, however, due to excessive rainfall leading to water logging in areas under bean cultivation, there was decline in production in the Western

and North Rift regions. Total bean production in Kenya is estimated at 35% which is mainly in Eastern regions while in Nyanza and Western regions, the production is estimated to be 22% lower than Eastern region at national output (Katungi *et al.,* 2009). Central and Western regions are the largest producers while Coast and Eastern are the least producers. Bean production varies from region to region and depends on climatic and soil conditions, seed quality level, pest management (Katungi *et al.,* 2017). Around 95% of the population in Western Kenya grow beans which is an important addition to their diets though it is faced with grave constraints particularly infection by root rot pathogens (Mugwe *et al.,* 2008).

2.3 Constraints to common bean production

Smallholder farmers encounter multiple constraints during bean production such as pests and diseases, drought, low soil fertility, weed competition, low input use, inadequate capital, poor access to improved germplasm, poor marketing infrastructure, and low labour productivity (Birachi *et al*., 2011). This has resulted in low supply of beans in the market by the farmers.

Variations in climatic conditions cause reduction in bean yield through shortened seasons accompanied by increased water stress and incidences of crop pests and diseases (Niang *et al*., 2014). The problem of drought is far the crucial constraint to bean production in the country and it continues to worsen due the effects of climate change in various parts of the world (Beebe *et al*., 2013). Widespread drought in bean growing areas of Eastern and Southern parts of Africa leads to yields of less than 400kg/ha (Rao *et al*., 2016).

Soil fertility exhaustion is a major contributing factor to low yield in common bean in Kenya (Namugwanya *et al*., 2014). There has been declining in soil fertility through nutrient mining due to cultural practices such as removal of crop residues, continuous cultivation, fallow periods that are short and application of inputs at low rates (Kinstche *et al*., 2015). Nutritional deficiencies of mineral elements like nitrogen, potassium, phosphorus, magnesium, zinc,

calcium, and aluminum and manganese toxicity greatly constrain bean production (Chekanai *et al*., 2018). Bean production requires high levels of phosphorus, potassium, sulphur, magnesium, molybdenum and in the absence of these nutrients, growth of free living rhizobacteria in the rhizosphere is limited (Argaw *et al*., 2015). Beans are produced in soil pH ranging from 5.0 and 6.0, however, many farmers grow beans on soils with pH levels less or equal to 5.0 (Wortman, 1998). Crops grown on acid soils but with high organic matter suffer more from nutrient deficiencies whose solubility decreases as pH increases (Brown and Westermann, 2000). The farmers address these constraints by applying both lime and inorganic fertilizers even though they are expensive. They also apply inorganic fertilizers minimally since they do not have access adequate quantities due to limited purchasing and transport powers

Pests and diseases are major constraints to bean production in low income countries. Bean diseases may cause losses up to 100% universally. Major bean diseases include angular leaf spot (ALS) (*Phaeiosariopsis griseola*) causing yield losses as high as 80% (Leitich *et al*., 2016), anthracnose (*Colletotrichum lindemuthianum*), root rot pathogens that are predominantly caused by *F. solani, R. solani, M. phaseolina, F. oxysporum, Pythium ultimum, and Sclerotinia sclerotiorum* are acknowledged as one of the major problems limiting bean yield with losses up to 76% (Naseri, 2008), common bacterial blight (*Xanthomonas axonopodis pv. phaseoli*), aphids, rust, and bean common mosaic virus (BCMV).

Resistant varieties have been developed and recommended to farmers for adoption but only a few farmers have been able to take up the technology (Katungi *et al*., 2009). Consequently, many continue to use the old varieties because they are unable to access the new seeds, lack of information about the newly released varieties, and lack of purchasing powers.

Conventional bean yield is very low compared to genetic potential of improved bean cultivars (Wortman, *et al*., 1998). Farmers continue to use old technologies since they lack access to seed of new varieties, information about the varieties, and/or cash to purchase inputs including seed.

2.4 Root rots of common bean

Root rots can either be caused by a single pathogen or a combination of two or more pathogens depending on the environmental conditions (Chittem *et al*., 2015). Symptoms associated with these diseases are poor seedling germination and establishment, reduced vigour, stem discoloration and rotting of tissues (Ongom *et al*., 2012). These diseases are damaging under conditions of water logging, intensive cultivation, soil acidity in poorly fertilized soils with absence of rotation and use of susceptible bean varieties (Naseri, 2014). The synergistic interaction between *Rhizoctonia solani* and *Fusarium solani* contributes to above 50% yield losses (Garcia *et al*., 2014). Root rots are caused by soil borne pathogens which attack plants through the roots and stem (Gao *et al*., 2014; Paparu *et al*., 2016). These diseases are caused by a complex of fungal pathogens that include *Pythium* spp, *F. solani* f. sp. *phaseoli*, *R. solani*, *Macrophomina* spp. and *Sclerotium rolfsii* (Mukankusi and Opala, 2012; Korayem *et al.,* 2016; Paparu *et al.,* 2016). In Kenya, *F. oxysporum*, *F. solani*, *M. phaseolina*, *R. solani* have been identified as important root rot pathogens (Mildred, 2017).

Pythium is considered the most important species causing root rot and is associated with seed rots or damping off of seedlings (Mugisha, 2008). It produces globular sporangia enclosing oospores which are the survival structure and primary inoculum (Lodhi *et al*., 2013). *Pythium* fungus survives in the soil for many years as oospores germinate to produce zoospores that infect roots and the lower stem. The virulence increases with increased exogenous nutrients and nutrients supplied by root exudates (Sutton *et al*., 2006). Entry into the host is directly

through degeneration of plant tissues using lytic enzymes such as pectinases, proteases and cellulases. The most damage is usually done to the seed and seedling roots during germination either before or after emergence. *Pythium* spp. is considered to be opportunistic pioneers that are weak competitors (Haraala, 2012). They are unable to colonize substrates already colonized and their saprophytic and pathogenic behavior is restricted to conditions of intense microbial competition.

Fusarium root rot predominantly caused by *F. solani* f. sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli*, is a major root rot disease of common beans (Mukankusi and Opala, 2012), with a wide distribution and variations in morphology, pathogenicity, microconidia and presence of sporodochia (Macedo *et al*., 2017). Increased establishment of Fusarium root rot in many regions is favored by conditions such as soil acidity in poorly fertilized soil and hot weather (Naseri, 2014). Symptoms associated with Fusarium root rots include red longitudinal lesions on the roots and complete rotting of the root system. In aboveground plant parts, symptoms such as chlorosis, stunting, and reduction in pod production are very common (Cichy *et al*., 2007). *Fusarium* spp is very pathogenic since it produces pectic enzyme that dissolve pectin cell wall thereby reducing plant turgidity (Balaali and Iranpoor, 2006). It produces macroconidia, microconidia that are septate and thick walled chlamydospores. The pathogens survive on crop debris as mycelia and when the roots disintegrate they release macroconidia, microconidia and chlamydospores that germinate on the roots of susceptible hosts (Naseri, 2008).

Rhizoctonia solani is a major soilborne root rot pathogen with a wide host range and occurring at any stage of the bean crop cycle causing complete crop failure (Valetin-Torres *et al*., 2016). The disease affects root and hypocotyl region of the host plant and usually penetrates the host through wounds or the cuticle (Mayo *et al*., 2015). Moist environments with temperature range of 15-18^{o}C favours growth of the pathogen. *Rhizoctonia* is a species

complex presently categorized into 14 anastomosis groups based on hyphal fusion, cultural morphology, pathogenicity and DNA homology (Muzhinji, 2015). Anastomisis is the hyphal fusion occurring between isolates of the same AG and this grouping method categorizes different isolates based on their ability to anastomose with established isolates (Valetin-Torres *et al*., 2016). Isolates within the same anastomosis group produce similar symptoms on hosts and may also prefer related hosts (Dorrance *et al*., 2003). Isolates of *Rhizoctonia solani* cause sudden wilting of foliage that gradually develops into chlorosis and necrosis, and root rots

Sclerotinia stem rot (*Sclerotinia sclerotiorum*) is an important bean disease causing yield losses accompanied by reduced seed quality and weight (Petlier *et al*., 2012). The symptoms include water soaked lesions that rapidly develop along the stem. Wilting, lodging, and death of the plant are observed during severe infection. The pathogen produces sclerotia which are the survival structures that allow it to colonize different environments and attacks both roots of growing and mature plants. Infection occurs either through germtube elongation of sclerotia or by ascospores produced from apothecia during carpogenic germination of sclerotia (Abdeljalil *et al*., 2016). The main source of infection is through myceliogenically germinating sclerotia on crops leading to rotting of aerial parts of the plant in contact with soil. Cool to moderate maximum daily temperatures and high relative humidity favours infection by this disease (Petlier *et al*., 2012).

Charcoal rot caused by *M. phaseolina* has been reported in many countries including Kenya, and has a diverse host range (Naseri, 2014b). On young plants, black irregular lesions form at the base of the cotyledons and extend to the hypocotyl and stem causing death. In older mature plants wilting and blockage of the vascular system occur regularly accompanied by production of black or grey microsclerotia (You *et al*., 2011). The disease is most damaging

in areas of unreliable rainfall and high temperature (30- 35^{0}C) (Reis *et al*., 2014). Infection occurs two to three weeks after seedling emergence, however, primary infection may remain latent until the conducive conditions occur. The pathogen survives in the form of sclerotium free in the soil or within diseased plant tissues.

2.5 Factors affecting population of microorganisms in the soil

The composition and abundance of microorganisms in the soils is influenced physical, chemical, and biological factors (Buyer *et al*., 2010). Soil moisture, organic and inorganic chemicals, and soil organic matter are the major factors influencing the microbial community in the soil. Many of these factors interact with each other and may have direct and indirect effect on soil microbial community (Buyer *et al*., 2010).

Soil moisture is an important factor influencing microbial population and activity in the soil (Zhang *et al*., 2012). The activity and population of microorganisms in the soil multiplies under moisture conditions ranging from 20% to 60%. In soil, water is held through hydrogen bonds on particle surfaces and within soil pores. Therefore the higher the amount of clay or organic matter the larger the volume of water can be stored. In waterlogged conditions anaerobic microorganisms become active while aerobes get suppressed (Khare and Arora, 2015). Soil temperature greatly influences the rates of biological, chemical and physical processes in the soil. Soil microorganisms are found in a wide range of temperatures, from the cold to the near-boiling environments of springs (Pettersson, 2004). These microorganisms have different relationships with the environmental conditions depending on different temperature regimes. Soil pH affects physiological, morphological, and metabolic process of soil microorganisms (Khare and Arora, 2015). Soil nutrient availability is greatly influenced by soil pH and determines the type of microorganisms that predominate in different soils (Pettersson, 2004). Although soil pH does not usually vary much over time, management practices such as liming to counteract acidification can induce rapid changes in

the soil. Liming has frequently been reported to increase numbers of bacteria measured using plate counts (Petterson, 2004).

Fertilization of soil contributes to changes in biological characteristics of the soil. The effect of chemical fertilizer and organic amendments on soil microorganisms have been studied (Li *et al*., 2017). Application of inorganic fertilizer over a long period of time had shown an increase of 15% of the microbial biomass compared to unfertilized farms (Geisseler and Scow, 2014). Minerals nitrogen and phosphorus fertilizers have been reported to have significant effect on special population of bacteria in the soil (Eco and Park, 2016). Nitrogen is an essential macronutrient required by microorganisms to support cells (Khare and Arora, 2015). Overall, organic fertilizers significantly affect the abundance of microorganisms in the soil compared with mineral fertilizers (Li *et al*., 2017). For instance, Ngosong *et al*. (2010) observed significant increase in fungal abundance of arbuscular mycorrhizal fungi (AMF) following addition of organic manure while Elfstrand *et al*. (2007) found higher populations of fungi and bacteria in soils receiving green manure

2.6 Interactions among soil microorganisms and their effect on plant health

There are many types of interactions between soil organisms. Many of which are influenced by the physical contact between the organisms themselves. The various types of possible interactions or associations among the microorganisms can be beneficial, detrimental or neutral. The associations existing between different soil microorganisms, whether symbiotic or antagonistic, influence the activities of microorganisms in the soil. Rhizosphere organisms have been studied for their beneficial effects on plants growth and health. With main focus on those that fix nitrogen, endo- and ectomycorrhizal fungi, and plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, biocontrol microorganisms, and protozoa

(Raaijmakers *et al*., 2009). The microorganisms that are deleterious to plant health include pathogenic fungi, Oomycetes, bacteria and nematodes (Raaijmakers *et al*., 2009).

Soilborne pathogens cause devastating yield losses and are difficult to manage compared to other pathogens that attack above ground plant parts (Mihajlović *et al*., 2017). These pathogens are adapted to grow and survive in the soil, and then the pathogen establishes a parasitic relationship with the plant (Raaijmakers *et al*., 2009). Complex interaction between microflora, with the pathogen may have an impact on the outcome of pathogen infection. Some bacteria genera are considered soilborne and they include *Ralstonia solanacearum*, *Agrobacterium tumefaciens* and a few species of *Streptomyces.* Oomycetes survive in the soil as chlamydospores, sclerotia, conidia, or hyphae and in conducive environments the fungi are stimulated to grow towards the plant (Raaijmakers *et al*., 2009). Soilborne pathogens clustered within the soil may stay for long and the spread of the inoculum may likely be less compare to aerial pathogens.

The soil microflora interacts with soilborne pathogens influencing the consequence of a pathogenic infection on a plant. The activity of soilborne pathogenic fungi can be inhibited by the action of beneficial microorganisms in the soil. Biocontrol agents affect the population of soilborne pathogens through competition, antagonism, and hypeparasitism (Pal and McSpadden, 2006). Some species of *Penicillium* are antagonistic against pathogen by producing antibiotics and inducing resistance in plants by activating multiple defense mechanisms. Competition for nutrients, micronutrients and rhizosphere colonization and establishment in the root zone is a prerequisite for effective biocontrol (Raaijmakers *et al*., 2009). Antagonism occurs through the production of secondary metabolites, and the toxicity and concentration of the secondary metabolites depend on the compounds and the target (Haas and Defago, 2005) and in addition to competition and antagonism, direct biocontrol effects on soilborne plant pathogens can result from hyperparasitism (Raaijmakers *et al*.,

2009). In hyperparasitism, the pathogen is attacked by the specific biological control agent that kills both the organism and the propagules (Pal and McSpadden, 2006).

2.7 Factors affecting crop emergence and crop establishment

Poor stand establishment may involve interactions of various biotic and abiotic factors. Some of factors soil physical appearances, temperature and moisture, and several cultural practices like depth of sowing, spacing, seed size (Kołodziejek, 2017), the involvement of fungal pathogens in pre and post emergent damping off is also a factor (Tabin and Shrivastava, 2014).

Success or failure of proper stand establishment also depends on soil characteristics (Grassbaugh and Bennett, 1998). Well drained soil with high water holding capacity (WHC) improves plant stand establishment while heavy soils experience crusting thus stressing seeds and young seedlings as they emerge from soil (Grassbaugh and Bennett, 1998). Reduced oxygen availability in soils due to near saturation reduce crop germination (Blake *et al*., 2003). Certain amount of oxygen must be taken up by the seed for germination to occur, similarly oxygen diffusion into the seed is crucial for certain physiological processes to occur (Budko *et al*., 2013). High amount of water within the vicinity of the seed affects seedling and early plant growth (Kołodziejek, 2017). Several days of waterlogging after germination depress plant population (Blake *et al*., 2003). Soil tilth is defined as the soil physical conditions. Soil compaction reduces seedling emergence thus contributing to poor stand. Soil compaction also increases time for emergence making seedlings vulnerable to diseases.

Temperature and soil moisture affects germination of seed besides affecting development of disease. High moisture content and temperature favours development of root and seed rots (Mundel *et al*., 1995). Seed rot at the pre- germination or germination stage or plant death

after germination are caused by damping off diseases. Soilborne diseases caused by *Pythium* rapidly colonize unprotected seeds in high moist wet soils causing seed rot since they are very virulent. These fungi gain entry to the seedlings through the roots causing rotting of young seedlings (Naseri, 2014). *Pythium* spp, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Macrophomina phaseolina* are responsible for damping off. However, root rot pathogens damage the root causing root rots and therefore restrict absorption of water and nutrients. *Pythium* spp., *F. solani, Aphanomices eutiches, Sclerotium rolfsii, Macrophomina phaseolina*, and *R. solani* are responsible for root rot of beans (Naseri, 2014a). Incidences of root rots can be reduced by enhanced seedling emergence through different sowing techniques that provide favourable microclimate for development. Use of fungicides improves seed germination thereby reducing damping off and as such improves seedling emergence.

Cultural practices like green manure application have an influence on soil properties and thereby contribute to either successful or poor establishment of common beans. Studies have shown that certain green manure crops can limit the diversity of agricultural lands through a shading, mechanical impediment and through production of chemical compounds during decomposition (Bonanomi *et al*., 2011). The chemicals released may be phytotoxic, inhibiting or delaying growth of plants. According to Bonanomi *et al*. (2006) inhibition of crop emergence and establishment is a common phenomenon and is common among nitrogen fixing plant species. However, the effect of these residues changes with decomposition, as inhibition is greatest in the early stages of decomposition and then declines. The initial decomposition phase involves tissue breakdown accompanied by release of cell contents. Phenolic compounds and other organic compounds have been shown to be phytotoxic to various crops. The extent to which these residues affects crop establishment depends on the

immediacy of seedlings and the potency of the allelochemicals released during decomposition (Blake *et al*., 2003). Growth enhancement may occur in the later stages of decomposition.

2.8 Lablab leguminous crop

Lablab purpureus, previously known as Dolichos lablab, is very popular as is demonstrated by the many names it is known with in different parts of the world (Maas *et al*., 2010). The crop is common in many African countries from Cameroon, Swaziland, Zimbabwe, and to the East African Countries Sudan, Ethiopia, Uganda, Kenya and Tanzania (Murphy and Colucci, 1999). Lablab is a summer annual perennial legume twinning, climbing or upright herbaceous plant. It is a multipurpose legume with multiple uses as it can be grown as pulse for human consumption, used as green manure and also as livestock fodder (Shivachi *et al*., 2012, Hassan *et al*., 2014). Its immature seeds, pods, and young leaves are edible and cooked as vegetables. Mature dry beans are also eaten but they require long cooking time (Robotham and Chapman, 2017).

Lablab is effective for land restoration as they can be included in crop rotations. Lablab has a potential to produce up to an average of 2600 kg ha-1 of biomass and an average of 64.1 kg of nitrogen ha-1 (Ewansiha *et al*., 2016). According to Omondi. (2011) lablab can effectively fix nitrogen of about 20 to 140kg residual N/ha in the soil. This means lablab can be utilized as green manure for soil improvement and as cover crops to protect the soil against soil erosion and dehydration. Furthermore, it provides great potential for soil conservation and stabilization of both chemical and physical soil properties (Maass *et al*. 2010). The fixed nitrogen will then be available for the succeeding crop. Lablab is greatly tolerant to drought than common bean and cowpea and it has genetic variations for tolerance among lines (Robotham and Chapman, 2017). However, despite the massive potential to serve as a multipurpose legume, lablab use and acceptability by smallholder farmers in Kenya is very

low. This is because compared with other legumes, lablab has prolonged cooking time and further have negative nutritive value (Shivachi *et al*., 2012)

2.9 Application and decomposition of green manure

Green manuring is the incorporation of green crop residues primarily as a nutrient source for subsequent crops (Larkin, 2013; Bonanomi *et al*., 2017) while green manure crop is defined as a crop derived fertilizer incorporated directly *in situ* or brought from a distant farm (Fabunmi and Balogun, 2015). Green manure sourced from legumes is a source of local nitrogen thus reduction in reliance on external inputs and the breakdown and release of nutrients from the green manure is facilitated by soil organisms (Elfstrand, 2007; Meena *et al.*, 2018). Green manure crops add organic matter, recycle nutrients in to the soil (Fanish, 2017), and improve microbial biomass activity as well as changes in the soil microbial community (Larkin, 2013).

Decomposition involves breaking down of plant residues leading to the production of colloidal complexes called humus which improves conditions of the soil (Marieh, 2012). Its initiation is facilitated by both primary and secondary colonizers (Kabuyah, 2012). Primary colonizers are a diverse community of fungi, bacteria and other microorganisms which utilize both simple and complex sugars as well as low molecular weight compounds. Secondary colonizers, are less competitive and belong to the class Basidiomycetes. Basidiomycete group of fungi are mainly responsible for white and brown rot decay.

These colonizers are responsible for the chemical changes and nutrient release in different stages. In the early stages opportunistic fungi and bacteria utilize soluble substrates like sugars and amino acids (Nazir *et al*., 2009). Complex carbohydrates, such as cellulose and non-cellulosic polysaccharides, and lignin that are not easily decayed are degraded by extracellular enzymes (Perez *et al*., 2002). The final products of decomposition include

carbon dioxide, water, energy, microbial biomass, inorganic nutrients and re-synthesized organic carbon compounds such as humus, hemicelluloses, lignin, phenolics, and celluloses, which can be utilized by plants (Shi, 2013).

The speed with which the residues breakdown, rely on factors like moisture, temperature, aeration, soil texture, lime and mineral salts, age, condition, and character of material turned under, and the presence of suitable organisms (Braun and Beckett, 2005). Soil microorganisms' function in a wide range of temperature, however, an increase in temperature speeds up the action and activity of these microbes and therefore hasten decomposition (Katterer *et al*., 1998). Quick decomposition occurs in lighter soils and fertile soils than in heavy soils. In lighter soils, there is continuous supply of air while in heavy soils, decaying proceeds slowly. Lime favours the development of microorganisms and the evolution of carbon dioxide. As the green manure plants grow older, they contain small percentages of nitrogen and larger percentages of carbon. Older crops are rich in lignin, cellulose thus it becomes woody and hard. Therefore, fresh young materials will decay more rapidly than old ones (Braun and Beckett, 2014).

2.10 Factors affecting decomposition of organic matter in the soil

Decomposition is a complex process involving physical, chemical and biological processes (Antil *et al*., 2014). The rate and process of decomposition is affected by climate, the actions of microorganisms together with other factors such as biochemical composition of green manure, temperature, rain and cultivation (Shi, 2013). Initially, the process of decomposition involves the physical breakdown of the organic residues into smaller portions by soil microorganisms (Valarades *et al*., 2016)

The rate of decomposition and nitrogen release of leguminous green manure residues are very high (Cattanio *et al*., 2008). Source of carbon, energy, or nutrient source to the organism involved in decomposition determines its quality and suitability (Shi, 2013). Concentrations of nitrogen, phosphorus, lignin, and polyphenols indicate plant residue quality and decomposition rates (Duong, 2009). Based on chemical compositions, residue can be classified as either easily decomposable sugars or amino acid and slowly decomposable like hemicellulose and recalcitrant materials such as lignin (Shi, 2013). The decomposition of plant residue with high carbon: nitrogen ratio occurs very slowly compared to residues with carbon: nitrogen ratios of below 40 (Baldock, 2007; Cattanio *et al*., 2008).

The original composition of nitrogen in the plant residue is a factor that may either accelerate or inhibit decaying process since it determines the turnover of total microbial mass mineralizing the residue. The process of decomposition is slow in plant residues with high carbon and polyphenols. The polyphenol are quite slow to decompose and they vary depending on source of plant material (Constantinides and Fownes, 1994; Valarades *et al*., 2016). Therefore decomposition rate decreases as the concentration of polyphenols, cellulose, and waxes increases in plants. The lignin content within plant residue influences decomposition rate; as it plays an important role in plant cell wall structure and makes the cell wall resistant to microbial degradation (Duong, 2009). Plant cell wall degradation is important for the process of decomposition to occur as it allows access by microorganisms. Therefore increased content of lignin reduces the residue decomposition rate and may also enhance nitrogen nutrient immobilization.

Plant physical quality like size, roughness, and surface properties also affect the rate of decomposition by either limiting or increasing surface for accessibility by soil microorganisms thus altering the colonization pattern (Shi, 2013). Residues chopped into

small portions decompose faster than those that are not chopped. Particle size increases the contact between the residues and soil particles thus exposing them to microorganisms for decomposition (Bending and Turner, 1999). Small particle influences exchange of water, nutrients and oxygen between the substrate and the soil matrix. Soil chemical properties such as soil pH, organic matter content, and nutrient status of soil may influence decomposability of plant residues. Soil pH has a direct effect on the type and population of microbes involved in decomposition. The rate of decomposition is high in neutral soils than in acidic oil.

Temperature and humidity directly affect the soil microbial activity. The enzymatic activity increases with increase in temperature or humidity (Valarades *et al*., 2016). In tropical soils with high average temperatures and humidity, decomposition rates are higher than in temperate soils (Fierer *et al*., 2005). Moisture level of about 60% of total porous content is the optimum for decomposition rate of aerobic microorganisms. Activities such as irrigation, drainage, maintenance of cover crops or mulches to aide in infiltration can be used in order to achieve high decomposition (Whallen, 2014). However, excess moisture results in anaerobic conditions that negatively influence green manure decomposition. These conditions do no favor decomposing microorganisms except for bacteria.

2.11 Effect of green manure on soil characteristics

Green manures have recently been used to enhance soil nutrition content for improving productivity. Among them, direct application of green manure improves soil quality through increase in soil organic carbon, improve microbial numbers, soil structure, and high water holding capacity (N'dayegamiye and Tran, 2001; Bonilla *et al*., 2012). However, the impacts of green manure on soil physical and chemical characteristics depend on the amount, type and size of the added organic materials (Carvallo *et al*., 2015).

Incorporating green manure crops in the soil improves soil nutrient status by increasing carbon and organic matter (Talgre *et al*., 2009, Zhang *et al*., 2015). Green manure influences the top soil by increasing plant nutrient availability, soil buffering capacity, stimulate root development and microbial activity, and facilitate carbon and nitrogen cycles (Abawi and Widmer, 2000). Application of green manures also influences nitrogen cycling in the soil by recovering residual mineral nitrogen, or fixing nitrogen from the atmosphere thereby contributing to subsequent crop nitrogen nutrition (Ambrosano *et al*., 2013). Green manure enhances soil organic matter thus improves soil fertility, physical and biological properties of degraded soils (Lal, 2015). Increased soil organic matter as a result of green manure applications maintain soil silt, improving water holding capacity and infiltration of air and water therefore reduces soil erosion and ensures soil aggregate stability and decreasing soil bulk density (Gregorich *et al*., 1994; Aranyos *et al*., 2016). At maturity, green manures increase the content of carbohydrate and lignin which lowers nitrogen release to subsequent crops (Ranells and Wagger, 1992; Valadares *et al*., 2016). Therefore, application of green manure must factor in timing of incorporation as this influences decomposition

The exhaustion of soil organic matter as a result of high cropping intensity is the main reason for low crop productivity (Salahin *et al*., 2013). Diminished organic matter has led to dilapidation of soil physical properties like soil water holding capacity and reduced nutrient retention capacity leading to poor nutrient release from mineralization of organic matter (Bonini and Alves, 2010).

2.12 Use of green manure in the management of soil fertility and soilborne diseases

Green manuring is defined as the incorporation into the soil fresh plant materials in order to enrich it. These materials are solely incorporated while still fresh and green and improve soil fertility through nutritional, chemical and physical changes to the soil (Larkin, 2013). The

beneficial effects of organic amendments on different aspects of plant health have been widely studied with focus on their ability to improve natural soil suppressiveness (Bonila *et al*., 2012).

Soil amendments reduce the disease incidences and have been proposed to control diseases caused by soil borne pathogens. The ability of compost to suppress crop diseases depend on microbial activities, microbial population dynamics, nutrient concentrations and chemical and physical factors. Compost manure enhances microbial activity and stimulates residential microbes in the soil (Bareja *et al*., 2010). High levels of microbial activity in the soil are the main factor in disease control. Application of organic matter including green manure and cover crops is considered good agricultural practice for soil quality restoration, maintenance of soil organic matter and supply of plant nutrients (Kumar *et al*., 2014). Plant cover creates a microclimate that reduces the speed at which organic matter decomposes and this favours its accumulation in the soil (Azlan *et al*., 2012). Residues of leguminous plants supply enough amount of nitrogen to the soil through the process of biological fixation in the nodules formed by Rhizobium bacteria. Deep rooted green manure plants recycle nitrogen and other nutrients leached to the deeper soil layers (Florentin *et al.,* 2011).

The amount of organic amendments added to the soil excites soil microbial activity (Kumar *et al.,* 2014). Increased microbial activity correlates with decrease in soil borne pathogens. However, Hoitink and Boehm (1999) reported a temporary increase in certain soil borne diseases. Green manuring are nutrients rich in organic carbon for the microbial biomass which transforms unavailable nutrients in crop residues to available ones for succeeding crops and also enhances biodiversity of soil microorganisms (Kumar *et al.,* 2014). The positive effects of green manure on population of microorganism are noted through enhanced population. Decomposition of green manure provides energy for the growth of microflora and

carbon for formation of new cell material. A series of biochemical changes occur which results in simplification of various compounds. Several nutrients released by decomposers are incorporated into the soil thereby increasing soil fertility.

Green manure provides nutrients to plants as well as reduce the disease incidence caused by various soilborne pathogens (Kumar *et al.,* 2014). Management of soil-borne pathogens through organic amendments like green manure has been reported (Larkin and Griffin, 2007). Green manure crops specifically the crucifers with high contents of glucosinolates inhibit the population of pathogens in the soil (Lazzeri and Manici, 2001). Kumar, (2014) working with *Sesbania aculeta* (L) on the potential of *R. solani, S. rolfsi* and *S. sclerotiorum* found significant reduction in mycelia growth and sclerotia production by soil-borne pathogens.

The mechanisms of action against plant diseases by green manure are varied and often not understood. They influence pathogens through breakdown of glucosinolates (Bonanomi *et al*., 2007) thereby discharging fungitoxic compounds like avenacin, saponins or allyl isothiocyanate (Wiggins and Kinkel, 2005). The efficiency of a green manure in managing plant diseases is dependent on the amount of plant biomass incorporated within a season or throughout various successive seasons. Ramirez-Villapudua and Munnecke (1988) reported a decline in *Fusarium oxysporum* populations in the soil as the amount of cabbage green manure incorporated was increased.

2.13 Mechanisms involved in management of soilborne diseases by use of green manure

Various mechanisms have been proposed in the management of plant diseases using green manure. The mechanisms include increased soil fertility, biological control, antagonisms (Mihajlovic *et al*., 2017) and natural soil fumigation (Wiggins and Kinkel, 2005).

Organic soil amendments including green manures increase organic matter, nutrients and improve soil structure (Peter *et al*, 2009). Soil organic matter and nutrient availability increases following incorporation of plant residues that creates favourable niche for soil microorganisms (Stone *et al*., 2003). Soils incorporated with green manure have higher population of fungi, bacteria, Vesicular Arbuscular Mycorrhizal and higher total microbial activity than chemically fumigated or non-amended soils (Cappaert and Powelson, 1997).

Green manures from the cruciferous family decompose sulphur containing compounds which are further broken by enzymes myrosinase producing fungicidal compounds such as allyl isothiocyanate that works as soil fumigant (Bonanomi *et al*., 2010; Larkin, 2013). In particular crops such as canola, rapeseed, broccoli, cabbage, kale, arugula, cauliflower, Brussels sprouts produce isothiocynates which are unstable compounds that acts as biofumigant once in the soil. The application of brassicas residues reduces the level of soil-borne pathogens due to the secondary metabolites, glucosinolates they contain. Brassica cover crops incorporated at maturity reduce the populations of soil-borne pathogens through the release of fungicidal isothiocyanates in the same way as some commercial fumigants (Njoroge *et al.,* 2008). The biofumigation ability of different crops vary widely based on the types and amounts of glucosinolates produced with crops like Indian mustard (Brassicae family) having highest biofumigation potential (Larkin, 2013). Other crops such as sudan grass, produce different products cyanogenic glucoside that are toxic to plant disease.

Mechanism of biological control enhanced by crop rotation, organic soil amendment or specific form of leguminous green manure involves specific interactions and host physiology (Meghvansi and Varma, 2015). Competition, antibiosis, hyperparasitism, lysis as well as the lethal effects of anaerobic conditions are probably responsible for reduction of pathogens after residue plough down (Litterick *et al*., 2004). Maturity level of organic manure

determines how effective it is in managing plant diseases. However, fresh organic matter does not usually support biological control. High concentrations of nutrients in fresh green manure residues inhibit the production of enzymes required for parasitism by biocontrol agents such as *Trichoderma* spp. (Hoitink and Boehm *et al*., 1993). Immature composts always contain toxic compounds that affect growth of plants and predispose them to attack by pests.

2.14 Potential limitations of green manure to crop production

Green manures residues have been reported to induce both positive and negative influence on the growth of plants (Bonanomi *et al*., 2006). The negative effects of green manure include increase in incidence of plant diseases, physical impediment, reduced light penetration, predation activity, and release of allelochemicals during residue decomposition (Bonanomi *et al*., 2011). Many studies have reported that the addition of undecomposed residues to the soil can inhibit plant growth (Bonanomi *et al*., 2007). These concerns and potential side effects therefore limit practical application of these materials. Green manure in certain situations may also be considered costly since one is required to purchase seeds, employ labour

The incorporation of fresh organic matter leads to an ephemeral increase in the incidence of soil-borne diseases. These materials have been associated with either increase in inoculum levels of pathogenic fungi since the organic matter provides substrate for the growth of soilborne pathogens (Bonanomi *et al.,* 2006). High amounts of readily degradable fresh organic matter results in competition for oxygen in the soil as a result of intense microbial activity. Because of this phenomenon planting immediately following incorporation of green manure results in reduced germination and crop establishment (Hoitink and Boehm, 1999). The presence of conditions such as water stress, low levels of dissolved oxygen, nutrient imbalance or presence of phytotoxic compounds intensifies the susceptibility of plant roots to

soil-borne pathogens. Phytotoxic compounds that can be released by roots and decaying organic matter, may positively affect the activity of soil-borne pathogens by reducing plant resistance.

Plant species widely used as green manures, contain metabolites that are transformed into toxic substances during their decomposition (Haramoto and Gallandt, 2005). These substances can be inhibitory to seed germination and establishment. Bonanomi *et al*., (2006) established that inhibitory effect of green manure residues may not be limited to few allelopathic plant species but is fairly prevalent among different plant functional groups. The highest phytotoxicity has been shown to be common among nitrogen fixing crops, followed by woody species, and with little with grasses and sedges (Bonanomi *et al*., 2011). Phytotoxic compounds have been identified and quantified include short chain organic compounds, tannins and phenols. The composition of these and levels of these compounds may vary overtime with changes in in environmental conditions. Decomposing plant residues exhibits severe inhibition at the early stages of decomposition, however, as decomposition continues, phytotoxicity declines and plant growth stimulation becomes evident at the later stages of decomposition (An *et al*., 2001). This means that the concentration of the compounds also changes and this is also related to phytotoxicty dynamics.

CHAPTER THREE
EFFECT OF LABLAB (*Lablab purpureus* L.) GREEN MANURE ON POPULATION OF SOIL MICROORGANISMS AND ESTABLISHMENT OF COMMON BEAN (*Phaseolus vulgaris* L.)

3.1 Abstract

Green manures improve soil health and fertility however, application of undecomposed plant residues leads to poor crop emergence and increase in incidence of soilborne diseases. The study was carried out to determine the effect of lablab green manure on microbial population and crop establishment. Survey was conducted to determine the use of green manures as soil amendments. Field experiments was conducted where two bean varieties KK8 and GLP2 were planted on plots each with lablab green manure incorporated at one ton ha^{-1}over whole plots, and in rows. Diamonium phosphate applied at 75 kg/ha while lime at 4t/ha. Data was collected on soil nutrient content, microorganisms' population, crop emergence, root rot incidence and crop yield. All farmers applied green manure with 62% evenly distributing green manure on soil before incorporation and a majority (48%) of the farmers planted crops two weeks after incorporation. Green manure incorporation improved soil organic carbon, nitrogen, phosphorus and potassium but resulted in reduced germination percentage by 35% while it increased incidences of root rot by 30% compared to plots without green manure. The population of root rot pathogens was significantly higher in plots treated with green manure two weeks after bean crop emergence while the population of saprophytic fungi was low. Plots treated with lablab green manure reduced grain and biomass yields by 25%. Green manure increase soil nutrients directly and improves crop establishment when crops are planted after decomposition. However, it increased the population of pathogenic fungi with corresponding reduction in the population of antagonistic fungi. Results suggest that low emergence and establishment of bean crop is due to pre-and post-emergence damping off caused by enhanced population.

Key words: *Lablab purpureus*, root rot complex, *Phaseolus vulgaris*, soil health

3.2 Introduction

Common bean is an important source of dietary protein and calories, iron and supplementary amino acids (Celmeli *et al*., 2018). It is widely grown in Kenya and is only second to maize in significance as staple food. Common bean is grown for its green leaves, green pods, and immature or dry seeds. In Kenya, beans are largely grown by small scale farmers with fewer than five acres and mostly intercropped with maize (Mwangi *et al*., 2008). Beans play an essential role in the sustainable livelihoods of small scale farmers by providing both food security and income generation. The crop, however, is grown under challenging conditions, including marginal lands with infertile soils prone to drought, pests, and diseases (Mildred, 2017). Soil borne diseases, drought and low soil fertility are primary constraints to crop production in most of the third world countries (Rao *et al*., 2016). Soil borne pathogens cause significant losses in common bean production from the initial stage up to harvest (Mihajlovic *et al*., 2017).

These pathogens can survive for long in the absence of the host and consequently they are difficult to predict and control. Low soil fertility has been attributed to erosion and crop harvest thus the outflow of nutrients is higher than the input in smallholder farms. This phenomenon has prompted farmers to embrace the use of inorganic fertilizers to rejuvenate the soil, however, inorganic fertilizers are expensive (Richard and Ogunjobi, 2016). The use of green manure has been suggested as a simple and cheap option to restore soil productivity by small scale farmers (Talgre *et al*., 2012a).

Green manures contain lignin, cellulose, hemicellulose, micro and macro-nutrients and their decomposition is dependent on lignin, cellulose content and carbon: nitrogen ratio, which is reliant on the type of the crop and the environmental conditions (Lemitiri *et al*., 2016). The process of decomposition is mediated by microbial activity (Schnecker *et al*., 2014) where

bacteria, fungi, molds, protozoa, actinomycetes are involved in the initial stages while millipedes, centipedes and earthworms act on the materials on the later stages (Condron *et al*., 2010) for energy use for growth and carbon for the synthesis of new cell material (Gougoulias *et al*., 2014). However, the products that are released during decomposition are toxic to plant and enhance pathogenic fungi affecting crop germination and establishment resulting in reduced density and vigour, however, the toxic effects of these products depend on their concentrations in the soil (Haramoto and Gallandt 2005). Optimum plant density allow crops to exploit resources optimally and produce high yields (Gezahegn *et al*., 2016)

The toxicity associated with green manure is common in the early stages of crop development, resulting into reduced germination, root growth and seedling weight and height (Bonanomi *et al*., 2011). Reduced nitrate because of nitrogen tie up by the soil microbes and promotion of seed and seedling pathogens due to provision of food source by the fresh residues incorporated in the soil results in poor emergence (Manici *et al*., 2004; Kumar, 2014). Therefore, if susceptible bean seeds are planted they are invaded by the high population of pathogenic fungi also causing poor germination and establishment of common beans. Proper characterization of the composting products is necessary to predict the harmful effects of green manure to the soil in order to reduce harm to crops. The study was conducted to determine effect of lablab green manure on population of soil microorganisms and establishment of common bean.

3.3 Materials and Methods

3.3.1 Description of the study site

The study was conducted in Koibem and Kapkerer locations in Nandi South Sub-County in the North of Rift valley to the north latitude 0^{o}34’N (Nyberg *et al*., 2012). These sites have been under continuous cropping which has resulted in soil degradation accompanied by

increased soilborne pathogens. In particular, root rot has been identified as a major limiting factor in bean production in Nandi South smallholder systems (Lauren *et al*., 2009). The main agro ecological zones are upper highlands (UH) forest reserves, lower highlands (LH1) zones suitable for tea and dairy production and upper midlands (UM) suitable for sorghum, millet, potatoes and coffee. The area experiences the long rain seasons occurring from March to August and the short rain seasons from September to December. The area receives an average annual precipitation of 1200mm to 2000mm with mean yearly temperature ranging from 18-25^{o}C and the soils are characterized by fine drained clay loamy soils (FAO-UNESCO, 1997).

3.3.2 Assessment of green manure use among farmers in Nandi South

A semi structured questionnaire (Appendix I) was administered to individual farmers in the form of interviews in the short rains of 2015. Farmers participating in the project were purposively selected in Kapkerer and Koibem, Nandi South Sub-County by selecting them in a transect line. The sampling frame was a list of 200 small scale farmers and the sample size was obtained using the coefficient of variation of 15% and a standard error of 0.02. The formula given by Nassiuma (2000) was used

$$n = \frac{NC2}{C2+(N-1)e2}$$

where n = Sample N = Population C = Covariance e = Standard error. A total of 41 farmers were considered for the survey. Data was collected on sources of green manure, green manure use, application time, and method of application. Additional data was collected on time of planting after green manure incorporation.

3.3.3 Determination of the effect of lablab green manure on the population of microorganism and establishment of common bean

3.3.3.1 Experimental design and layout

Lablab variety Rongai was planted during the long rains of 2015 and 2016 at a spacing of 45cm×30cm and at flowering the vegetation was harvested, cut into small portions and used as green manure in the short rains of 2015 and 2016. Treatments included even distribution of lablab, furrow application of lablab, DAP, lime and untreated plots. In each plot measuring 4m by 6m, chopped portions of lablab green manure was evenly applied at the rate of 10kg by spreading over the whole plots. About 2.5kg of chopped lablab portions was also applied in four furrows per plot. Diamonium phosphate fertilizer was applied at the rate of 75kg/ha^{-1} in furrows while lime was applied by uniformly broadcasting over the plot at the rate of 4t/ha (Kiplagat *et al*., 2014), while control plots had no treatment applied. Bean varieties KK8 and GLP2 were planted in the plots at a spacing of 50cm×10cm between and within the rows with a seed rate of 50kg/ha. Beans were planted immediately after incorporation of the soil amendments by placing two seeds per hole. The treatments were arranged in a randomized complete block design with a split plot arrangement. The bean varieties constituted the main plots while the treatments comprised the subplots. Data was collected on seedling emergence, incidence, and severity of root rot, plant stand, and yield. Soil samples were collected for isolation of soil microorganisms and for determining soil nutrient status

3.3.3.2 Sampling soil for microbial and nutrient analysis

Soil samples were collected before treatment application and at the second, fourth and sixth week after incorporation of green manure to evaluate the changes in soil's chemical and biological properties (Pfenning and de Abreu, 2008). A trowel was used to collect five samples (200g) of soil in each plot following a zigzag sampling procedure. The samples were mixed to form a composite sample of 1 kg which was kept in plastic bags and placed in the shade to prevent dehydration. The samples were divided into two sub samples for nutrient

and microbial analysis. Samples for microbial analysis were stored at 4^{o}C in the refrigerator until used. Soil samples were taken to the Kenya Agricultural and Livestock Research Institute (KALRO) Kabete for nutrient analysis

3.3.3.3 Determination of soil chemical characteristics

Soil samples collected were air dried and then pounded with pestle and mortar (Kiplagat *et al*., 2014). The samples were then subjected to chemical and physical analyses to determine the level of pH, available nitrogen, organic carbon, and available phosphorus. Soil pH and organic carbon were determined volumetric method according to Walkley and Black method as described by Okalebo *et al*. (2002). Available nitrogen was determined by Kjeldahl method, phosphorus was determined by Brays method and Potassium was determined by flame photometer (Motsara and Roy 2008).

3.3.3.4 Determination of population of microorganisms in soil

Both bacteria and fungi in the soil were isolated by taking from each soil sample one gram and dissolving in 10 ml sterile distilled water and shaking for 30 minutes. One milliliter of the soil suspension was transferred into 9 ml of sterile distilled water, shaken and the ten-fold dilution repeated up to a dilution of 10^{-3} (Saravanan *et al*., 2013). One milliliter of the third dilutions was plated in molten potato dextrose agar and nutrient agar medium, cooled to 45^{o}C. The plates with Nutrient Agar were incubated for 24 hours while those on PDA were incubated at room temperature after which the numbers of bacterial and fungal colonies were counted. The different fungal and bacterial colony types were identified based on colony color, growth type, colony reverse color and color of mycelia (Nurbaya *et al*., 2014). Population of each type of fungi and bacteria was determined following the formula cfu g^{-1} = Total number of colonies × Dilution factor.

The identification of bacterial isolates was carried out based on cultural, morphological and biochemical characteristics while the identification of fungi was based on colony characteristics as well as microscopic morphology. *Fusarium* isolates was sub-cultured on synthetic nutrient agar (Nirenberg, 1981) and on PDA media. Cultures on synthetic nutrient agar were incubated under UV light to facilitate conidial sporulation while those on PDA were incubated at room temperature for 14 days (Okumu *et al*., 2016).

3.3.3.5 Assessment of incidence of root rot

The incidence of root rot was determined by counting the number of seedlings with root rot symptoms in every plot at the second, fourth and sixth week after emergence (Muthomi *et al*., 2014). Infected plants were identified based on symptoms like yellowing of leaves, wilting, stunted growth and death (Medvecky *et al*., 2007). Area under disease progress curve for disease incidence was calculated using the formula described by (Muengula-Manyi *et al*., 2013).

AUDPC= $\sum_{i=1}^{n}(Yi + Yi + 1)/2\,(t2 - t1)$ where Yi is the incidence of disease at time I, Yi+1 is the disease incidence recorded at the time i+1, n, the number of registration on the incidence , and t days between the registration of Yi and Yi+1.

3.3.3.6 Determination of emergence, plant stand and yield

Common bean emergence was assessed by counting the number of emerged plants after one week while plant stand was determined by counting the number of surviving plants in each plot at the second, fourth and at sixth week after emergence. Yield attributes of beans were determined by taking 10 samples of bean plants randomly from two central rows in each experimental unit at physiological maturity (El-Naim *et al*., 2012). At harvest, plant biomass was determined by sampling from each plot ten plants that were completely dried in an oven

at 50^{o}C for a week and weighed then converted into kilogram per hectare. The total grain yield was calculated following the formula by Mwangi *et al*., (2008)

$$\text{Yield (t/ha)} = \frac{\text{Field weight per plot (g)} \times 10\,000\ \text{m2/hectare}}{\text{Harvest area (m2)} \times 1000\,000\ \text{g/tonne}}$$

3.3.4 Data analysis

The survey data was analyzed using the Statistical Package for Social Sciences Version 20 (Hejase and Hejase, 2013) by computing means, frequencies, and percentages. Other data was analyzed by Analysis of variance using Genstat Inc. 15th edition 9 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Means were separated by Tukey's test. Correlation analysis among soil characteristics, plant pathogenic microbes, root rot severity, disease incidence, and plant yield was performed by Genstat 15th edition

3.4 Results

3.4.1 Green manure use among farmers in Nandi South Sub County

All the respondents from both sites applied inorganic fertilizer albeit in different proportions (Fig 3.1). In addition, all the farmers in Koibem and 63% of the respondents in Kapkerer applied green manure while a few of the farmers used farm yard manure as a soil amendment. Farmers used various sources of green manure as soil amendment. Some of the sources included lablab, maize, brassicas, beans, groundnuts and soybean (Figure 3.2). Lablab was by far the most common source of green manure with close to 60% of the farmers in both sites using it. This was followed closely by maize straw. In both sites, soybean, groundnut, bean and brassicas were the least utilized sources of green manure.

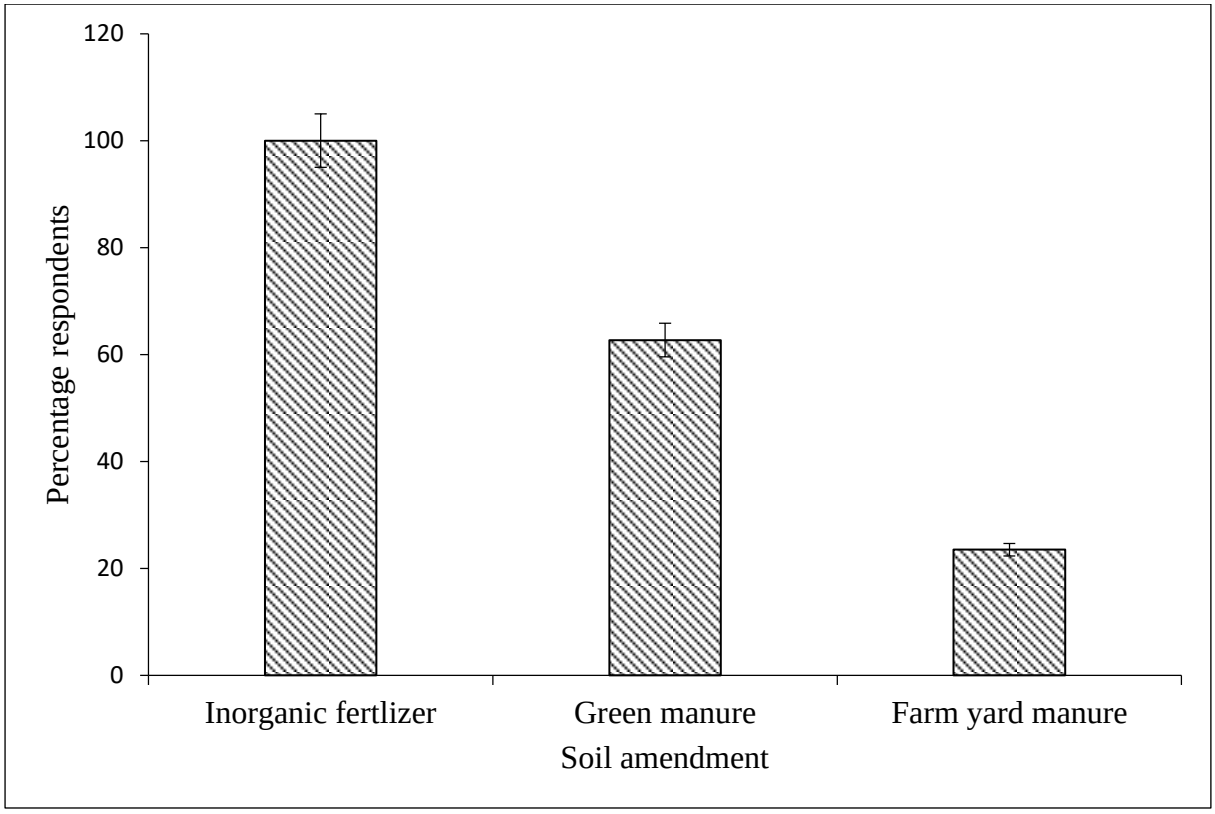

Figure 3. 1. Percentage of farmers using various soil amendments in Nandi County

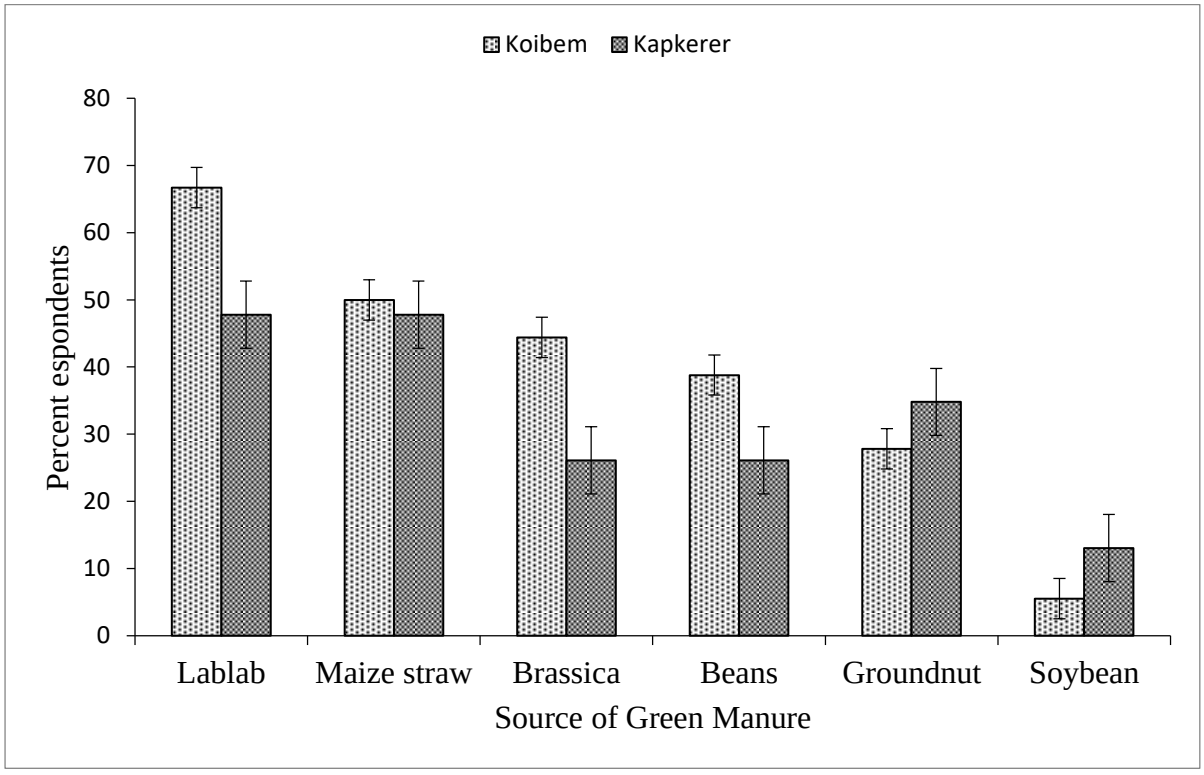

Figure 3. 2. Percentage of farmers using various crops as sources of green manure

3.4.2 Methods of applying green manure by farmers in Nandi South Sub County

Farmers used different methods of incorporating green manure in the soil (Fig. 3.3). Majority of the farmers in both sites evenly distributed green manure residues over the field while around 22% of the farmers in both sites ploughed in green manure while still standing in the field and a few others left the residues in the field as mulch.

Majority of the farmers interviewed in both sites (48%) planted two weeks after incorporation of green manure in the soil, around (34%) of the farmers planted three weeks after green manure incorporation (Fig. 3.4) while a few others planted four weeks after incorporation. However, in both sites, no farmer planted immediately after incorporation of green manure. All the farmers from Koibem reported that they planted their crop evenly over the green manure incorporated plots (Fig 3.5) while more than half of the farmers in Kapkerer evenly planted their crops after green manure application and incorporation. Other (44%) farmers from Kapkerer planted crops on top of green manure.

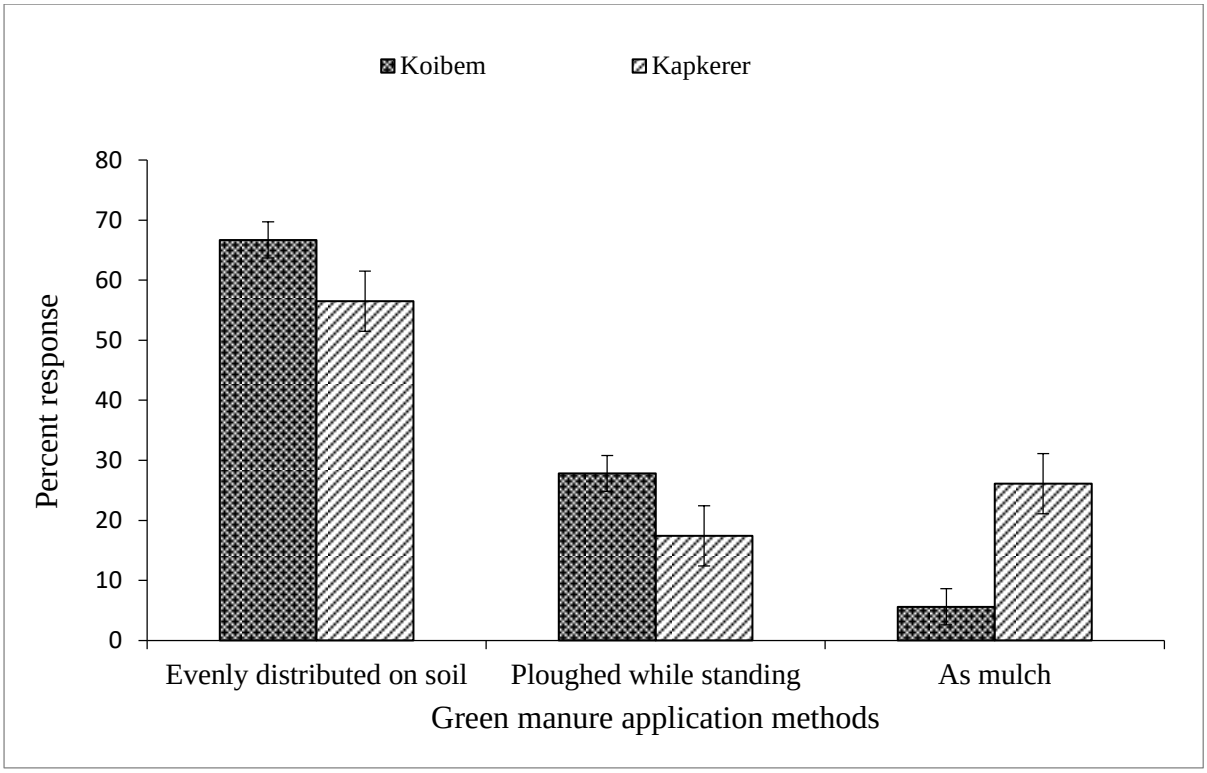

Figure 3. 3. Percentage of farmers who reported various methods of green manure application

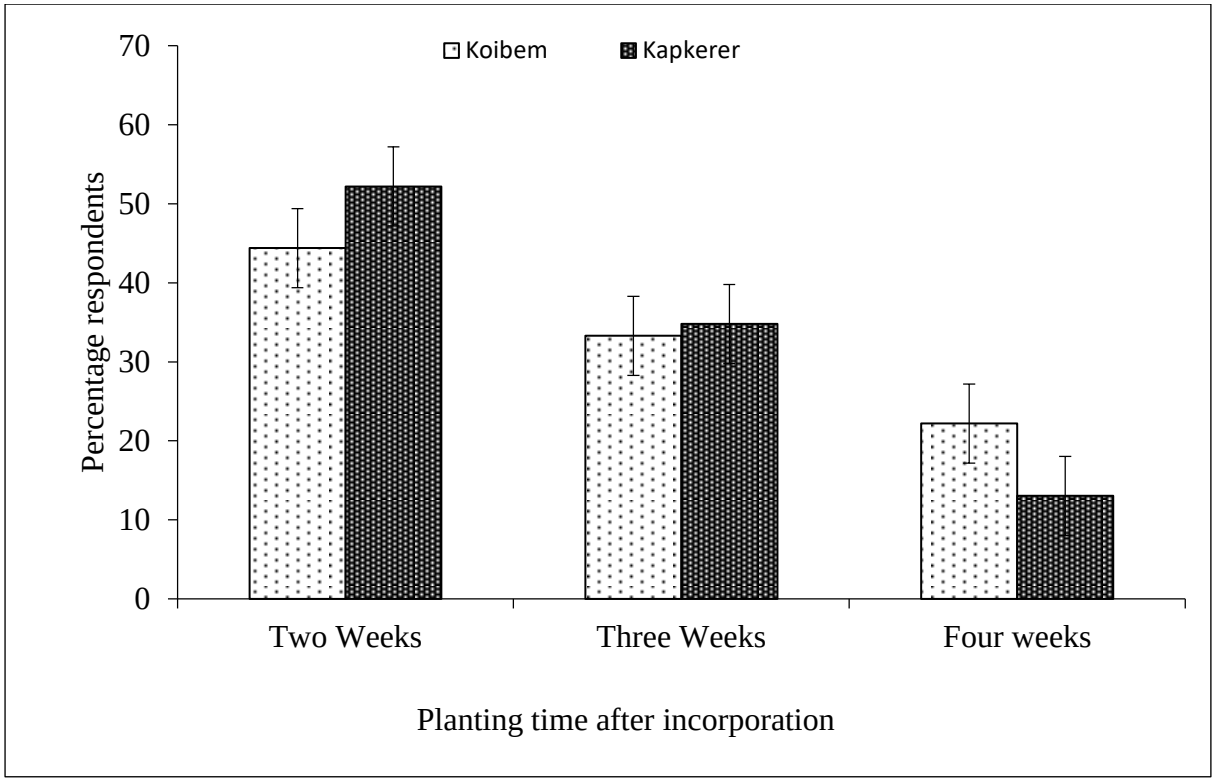

Figure 3.4. Percentage of farmers who reported the time of planting after green manure incorporation

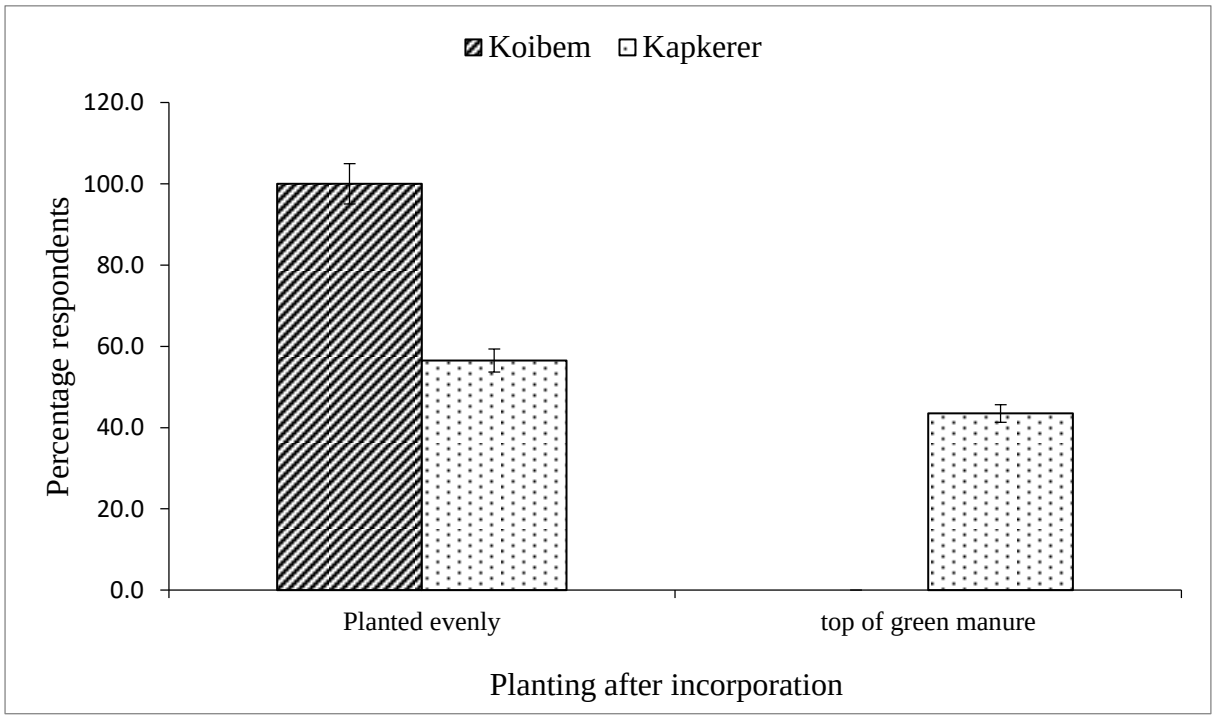

Figure 3.5. Percentage of farmers who reported different planting methods after green manure application

3.4.3: Uses and benefits of green manure as reported by farmers

Majority of the farmers in Koibem clearly understood the benefits associated with the use of green manure to crop production (Fig. 3.6). Of the farmers interviewed, 72% in Koibem specified that use of green manure improved soil fertility and therefore enhanced crop germination. Farmers in Koibem also listed high yields, reduced disease incidences and suppression of weeds as some of other benefits of incorporating green manure. However, the same cannot be reported for Kapkerer as many of the farmers did not recognize benefits accompanying green manure application as soil amendment. Only 22% of the respondents reported that green manure improved crop germination therefore enhanced crop yields. However, a few of the interviewed farmers (6%) also indicated that green manure application improved soil fertility.

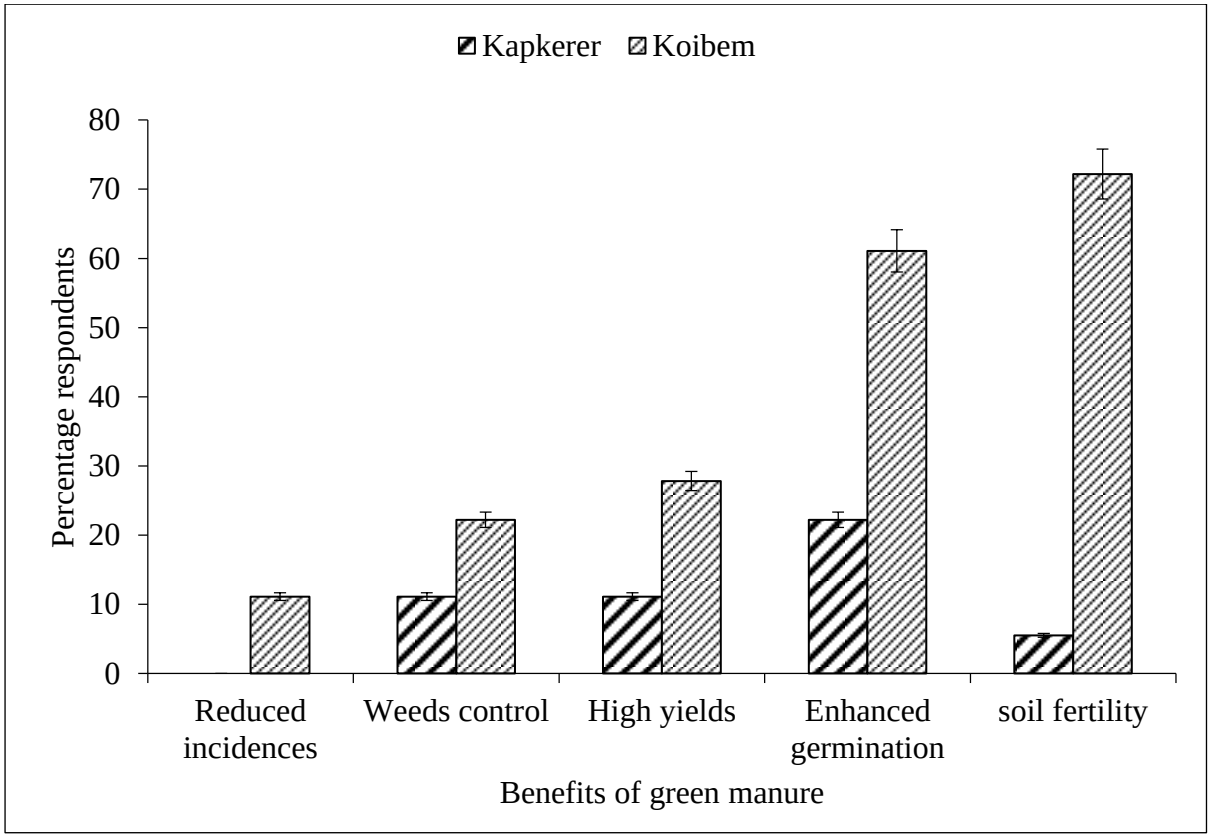

Figure 3.6. Percentage of farmers in Nandi South who reported the benefits of green manure

3.4.4 Effect of lablab green manure on chemical properties of soil

The chemical characteristics of soil were affected by incorporation of green manure in the soil (Table 3.1). The initial soil characteristics indicated strong acidity in both sites with low percent organic carbon content. However, six weeks after green manure applications, analysis showed a marginal increase in percentage organic matter in soil samples treated with green manures with values ranging from 2.2% to 2.4% in Koibem while in Kapkerer the values ranged from 0.50 to 1.00. A similar trend due to green manure treatments was be reported for soil pH, nitrogen, phosphorus in Kapkerer. The increases in soil organic carbon, and available nitrogen were highest for lablab treated plots.

Table 3.1. Levels of chemical characteristics before and after lablab green manure incorporation

	Koibem					Kapkerer				
	pH	OC	N	P	K	pH	OC	N	P	K
		%		Ppm	me%		%		ppm	me%
Before incorporation	4.80	2.30	0.20	15.00	0.62	4.60	0.80	0.10	17.50	0.18
Whole Incorporated	5.10	2.40	0.22	10.00	0.52	4.80	1.00	0.12	30.00	0.20
Between rows	4.60	2.20	0.19	10.00	0.50	4.80	0.50	0.07	25.00	0.16
No amendment	4.60	2.50	0.22	15.00	0.54	4.90	0.70	0.10	20.00	0.20
DAP	4.67	2.10	0.18	15.00	0.50	4.69	0.88	0.11	25.00	0.16
Lime	4.77	2.13	0.18	15.00	0.54	4.99	0.69	0.09	25.00	0.20
Mean	4.8	2.3	0.2	13.3	0.53	4.8	0.8	0.1	23.70	0.18
LSD (0.05)	0.2	0.2	NS	2.71	NS	0.1	NS	NS	4.60	NS

DAP – Diamonium phosphate, N – nitrogen, P- phosphorus, K- potassium, OC- organic carbon, Me- milliequivalent, PPM – Parts per million, NS- Not significant

3.4.5 Effect of lablab green manure on emergence and plant stand of common bean

Significant differences ($P \leq 0.05$) were observed between the green manure treatments with respect to crop emergence in both seasons (Table 3.2). Highest percentage emergence in both sites and in both seasons was recorded in lime, DAP treatments and in control plots while plots treated with lablab residues both in rows and in whole plots had the lowest percentage

emergence in both seasons. However, there was increased emergence in the second season compared to the first (85%). In both sites and in both seasons, in the second, fourth and sixth week after emergence, control and plots treated with lime and DAP treatments had the highest stand count overtime when compared with plots incorporated with lablab green manure. In addition, there was no significant (P≤0.05) difference between the two sites with respect to percentage stand count six weeks after emergence. There was high emergence in the second season of 2016 in plots treated with lime compared to the first season of 2015. There was a progressive decline in plant stand across the sites

Table 3.2. Percentage plants stand at different sampling times after green manure incorporation in Koibem and Kapkerer, Nandi South

	Weeks after emergence							
	Koibem				Kapkerer			
	0	2	4	6	0	2	4	6
2015 Short rains								
Lablab whole	55.4_{ab}	53.6_{ab}	49.3_{ab}	30.7_{a}	32.2_{c}	31.7_{c}	31.0_{c}	15.5_{b}
Lablab in rows	50.3_{b}	48.2_{b}	44.2_{ab}	25.5_{a}	39.4_{bc}	39.2_{bc}	37.4_{bc}	17.0_{ab}
No amendment	63.2_{a}	62.2_{a}	56.8_{a}	32.7_{a}	60.0_{a}	60.0_{a}	56.6_{a}	24.6_{a}
DAP	60.6_{ab}	58.8_{a}	55.2_{a}	31.3_{a}	58.0_{a}	58.0_{a}	56.1_{a}	24.2_{a}
Lime	62.4_{a}	61.6_{a}	55.6_{a}	33.0_{a}	63.2_{a}	63.2_{a}	62.2_{a}	34.0_{a}
Mean	58.4	56.9	52.3	30.6	50.6	50.4	49.2	23.1
LSD T×S ($p \leq 0.05$)	10.6	11.1	11.1	17.2				
LSD Site	4.7	4.9	4.3	7.9				
CV (%)	24.0	25.5	27.1	79.1				
2016 Short rains								
Lablab whole	46.3_{c}	41.9_{c}	31.5_{d}	19.9_{d}	41.5_{c}	39.6_{c}	37.1_{d}	27.1_{d}
Lablab in rows	47.1_{c}	43.7_{c}	40.3_{d}	26.5_{d}	48.4_{c}	44.7_{c}	43.7_{d}	31.9_{c}
No amendment	66.5_{b}	62.7_{b}	54.3_{c}	32.1_{c}	70.3_{b}	65.5_{b}	59.6_{b}	45.9_{bc}
DAP	73.9_{b}	69.9_{b}	62.1_{b}	38.9_{c}	72.0_{b}	65.4_{b}	63.7_{b}	49.5_{bc}
Lime	85.4_{a}	82.5_{a}	74.1_{a}	52.3_{b}	84.4_{a}	80.1_{a}	76.6_{a}	67.3_{a}
Mean	63.8	60.2	52.4	34.0	63.3	59.1	56.1	44.3
LSD T×S ($p \leq 0.05$)	9.7	9.5	9.9	10.8				
LSD Site	11.5	11.5	11.1	10.4				
CV (%)	18.9	19.7	22.5	34.2				

DAP – Diamonium phosphate, Means within column followed by different letters are significantly different based on Fishers Protected LSD test (P ≤0.05).

3.4.6 Effect of lablab green manure on incidence of root rot of common bean

In terms of relationship between plant stand and root rot incidence, there were significant differences (P≤0.05) between the treatments and sites in both seasons (Table 3.3). In the year 2015 in both sites, lime and untreated plots had the highest mean plant stand while plots treated with lablab had the low plant stand. High root rot incidences were recorded in plots treated with lablab green manure and the lowest incidences were recorded in plots treated with lime. In the second short rain season 2016 and in both sites, plots treated with lime had the highest mean plant stand while plots treated with lablab had the lowest plant stand. High root rot incidences were again recorded in plots treated with lablab green manure and the lowest incidences were recorded in plots treated with lime.

Table 3.3. Percentage plant stand of common bean and incidence of root rot under different treatments in Koibem and Kapkerer, Nandi South

	Koibem		Kapkerer	
	Plant stand	Incidence	Plant stand	Incidence
2015 Short rains				
Lablab whole plots	47.3_{ab}	14.4_{b}	27.6_{c}	22.8_{a}
Lablab in rows	42.1_{b}	12.3_{bc}	33.3_{bc}	18.5_{a}
No amendment	53.8_{a}	8.2_{c}	50.3_{a}	11.0_{b}
DAP	51.5_{a}	8.8_{c}	49.1_{ab}	12.2_{b}
Lime	53.2_{a}	8.2_{c}	55.6_{a}	10.6_{bc}
Mean	49.6	10.4	43.2	15.0
LSD ($p \leq 0.05$)	10.2	4.9		
CV (%)	27.2	47.4		
2016 Short rains				
Lablab whole plots	34.9_{d}	15.0_{d}	36.3_{d}	34.5_{a}
Lablab between rows	39.4_{d}	15.0_{d}	42.2_{d}	28.5_{b}
No amendment	53.9_{c}	11.0_{de}	60.3_{bc}	19.3_{c}
DAP	61.2_{bc}	11.2_{de}	62.6_{b}	23.3_{bc}
Lime	73.6_{a}	9.6_{e}	77.1_{a}	19.8_{cd}
Mean	52.6	12.4	55.7	25.1
LSD ($p \leq 0.05$)	8.9	5.8		
CV (%)	20.3	38.3		

DAP – Diamonium phosphate, means within column followed by different letters are significantly different based on Fishers Protected LSD test (P ≤0.05).

Significant differences ($P \leq 0.05$) in root rot incidences were observed among the treatments in the two sites (Table 3.4). The highest root rot incidence was recorded in Kapkerer. Overtime, there was reduction in root rot incidence in Koibem while there was an increase in root rot incidence in Kapkerer. Plots treated with lablab residues resulted in the highest root rot incidence while those treated with lime had the least root rot incidence in both sites. Four weeks after emergence, root rot incidence in Kapkerer increased in all the treatments but plots incorporated with lablab green manure both in row and in whole plots recorded the highest root rot incidences (32%).

Six weeks after emergence, significant differences were observed between the treatments and there was decline in root rot incidences in all the treatments. In the second season of 2016, there were significant differences ($P \leq 0.05$) in root rot incidences among the treatments within the two sites. Highest root rot incidence was recorded in Kapkerer than in Koibem while highest plant stand was realized in Koibem than in Kapkerer. Addition of lablab residues in whole plots resulted in the highest mean root rot incidence in Kapkerer and Koibem while application of lime resulted in least root rot incidence in Kapkerer and Koibem.

Table 3. 4. Percentage root rot incidence at different sampling times after green manure incorporation in Koibem and Kapkerer, Nandi South

	Weeks after emergence					
	Koibem			Kapkerer		
Short rains 2015	2	4	6	2	4	6
Lablab whole	18.9_{bc}	20.1_{b}	4.2_{b}	30.4_{a}	31.9_{a}	6.3_{a}
Lablab in rows	17.4_{bc}	15.3_{bc}	4.1_{b}	23.9_{a}	27.1_{a}	4.5_{ab}
No amendment	11.2_{c}	10.4_{c}	3.1_{b}	12.0_{bc}	15.5_{bc}	5.7_{ab}
DAP	11.4_{c}	11.1_{c}	4.0_{ab}	14.1_{bc}	17.7_{bc}	4.8_{ab}
Lime	10.1_{c}	11.2_{c}	3.3_{b}	11.2_{c}	15.4_{bc}	5.2_{ab}
Mean	13.8	13.6	53.7	18.3	21.5	5.3
LSD ($p \leq 0.05$)	7.4	7.6	1.9			
CV (%)	56.9	53.6	53.1			
P value	<.001	<.001	<.001			
Short rains 2016						
Lablab whole	19.6_{b}	13.7_{d}	11.8_{d}	31.2_{a}	34.4_{a}	37.7_{a}
Lablab in rows	17.4_{bc}	15.2_{d}	12.5_{d}	24.7_{ab}	29.8_{ab}	30.9_{ab}
No amendment	11.2_{c}	11.2_{d}	10.7_{d}	15.8_{bc}	19.7_{cd}	22.4_{c}
DAP	11.4_{c}	11.4_{d}	11.1_{d}	19.3_{bc}	23.4_{bc}	27.3_{bc}
Lime	10.1_{c}	10.0	8.7_{d}	16.2_{bc}	19.8_{cd}	23.3_{c}
Mean	13.9	12.3	10.9	21.4	25.4	28.4
LSD ($p \leq 0.05$)	7.3	5.7	5.8			
CV (%)	49.1	37.8	36.5			
P Value	<.001	<.001	0.005			

DAP – Diamonium phosphate, Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$).

On area under diseases progress curve, there were significant differences ($p \leq 0.05$) among the treatments in both seasons and between the two common bean varieties (Table 3.5). Largest area under diseases progress curve was observed in the 2015 season when compared with the 2016 short season. In both seasons, the plots treated with lablab green manure had the highest area under diseases progress curve while those treated with lime had the least AUDPC for root rots of common bean. The two varieties showed different levels of susceptibility with GLP2 variety being the most susceptible to root rots. Area under disease progress curve was highest for variety GLP2 in both seasons (929 and 1064 respectively) in plots treated with lablab green manure and lowest in plots treated with lime in both seasons.

Table 3. 5**.** Area under disease progress curve of common bean root rot on KK8 and GLP2 bean varieties

	2015 Short rains			2016 Short rains		
	KK8	GLP2	Mean	KK8	GLP2	Mean
Lablab whole	647.2_{a}	929.6_{a}	788.4_{a}	668.5_{a}	1064.0_{a}	866.1_{a}
Lablab in rows	497.6_{a}	793.5_{a}	645.5_{a}	614.0_{a}	909.0_{b}	761.5_{b}
No amendment	316.6_{b}	431.7_{b}	410.8_{b}	447.3_{b}	595.8_{c}	591.7_{c}
DAP	308.2_{b}	513.3_{b}	374.2_{b}	516.1_{ab}	667.3_{c}	521.6_{c}
Lime	295.1_{b}	434.5_{b}	364.8_{b}	485.9_{b}	522.7_{c}	504.3_{c}
Mean	412.9	620.5	516.7	546.4	751.7	649.0
LSD t×v ($p \leq 0.05$)	167.2			145.6		
LSD variety	59.5			75.6		
P value	<0.001			<0.001		

DAP – Diamonium phosphate, means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$).

3.4.7 Effect of lablab green manure on population of root rot pathogens in the soil

F. solani, F. oxysporum and *Pythium* were isolated form the soil samples collected from both sites. (Table 3.6). *F. solani* and *F. oxysporum* were the most dominant pathogens accounting for more than 80% of root rot pathogens isolated from the soil samples. *Pythium* species was also isolated from the soil samples in small portions. The populations of these pathogens were significantly ($P \leq 0.05$) affected by green manure treatments. In 2015 season, soil samples collected from Kapkerer had the highest population density of root rot pathogens compared to Koibem. In both seasons and sites, plots treated with green manure had the highest number of both *F. solani* and *F. oxysporum* while the plots treated with DAP had the least. In 2016 season, plots treated with green manure had the highest number of both *F. solani* and *F. oxysporum* while the plots treated with lime had the least. *Pythium* population in both sites and seasons were highest in plots treated with green manure and were least in plots treated with DAP and lime. Overall, the densities of both *F. solani* and *F. oxysporum* were significantly higher in plots treated with green manure when compared with other treatments

Table 3. 6. Population (cfu g soil $\times 10^4$) of root rot pathogens isolated from soils incorporated with lablab green manure

	Koibem			Kapkerer		
	F. solani	*F. oxysporum*	*Pythium*	*F. solani*	*F. oxysporum*	*Pythium*
2015 short rains						
Lablab whole	2.7_b	3.2_a	0.6_b	4.3_a	4.7_a	0.9_a
Lablab in rows	3.8_a	3.3_a	0.8_a	4.7_a	4.0_b	0.6_b
No amendment	2.6_b	3.1_a	0.7_a	3.4_b	3.9_{bc}	0.7_{ab}
DAP	2.7_b	2.8_b	0.5_b	3.1_b	3.3_c	0.8_a
Lime	2.6_b	2.8_b	0.7_a	4.0_{ab}	4.1_a	0.8_a
Mean	2.9	3	0.7	3.9	4	0.8
LSD path($p \leq 0.05$)	0.6	0.3	0.1	0.8	0.7	0.2
LSD S×T ($p \leq 0.05$)	0.6	2.3	0.9	0.6	2.3	0.9
2016 short rains						
Lablab whole	2.3_a	1.8_a	0.8_b	2.7_a	2.2_a	1.0_a
Lablab in rows	2.5_a	1.8_a	1.3_a	2.5_a	1.8_{ab}	1.0_a
No amendment	1.9_b	1.2_b	1.0_{ab}	1.6_b	1.4_b	0.9_{ab}
DAP	1.9_b	1.2_b	0.9_b	1.8_b	1.5_b	0.7_b
Lime	2.0_b	1.1_b	0.6_b	1.4_b	1.2_{bc}	0.7_b
Mean	2.1	1.4	0.9	2.0	1.6	0.9
LSD Path($p \leq 0.05$)	0.3	0.4	0.3	0.7	0.4	0.2
LSD S×T ($p \leq 0.05$)	0.5	1.4	0.5	0.5	1.4	0.5

DAP – Diamonium phosphate, Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$).

The population of *F. solani* in plots incorporated with lablab green manure significantly ($P \leq 0.05$) varied widely from the initial sampling to the second sampling (Figure 3.7). The population of *F. solani* responded to green manure residue by rising from the initial population to 42,500 colony forming units g^{-1} in Koibem and then reduced while in Kapkerer the population rose from 47,500 cfu g^{-1} to 67,500 cfu g^{-1} and reduced to below 10,000 cfu g^{-1} after 4 weeks. *F. oxysporum* also responded in the same way as *F. solani* increasing from the initial population to 35,833 cfu g^{-1}. *Pythium* spp. Soil populations' showed slight fluctuations, increasing between the first and the second sampling times. However, in the plots with no green manure applied, *F. solani* populations showed little fluctuation slightly increasing then reducing while *F. oxysporum* increased from the initial sampling population and continued to increase until end of the experiment in both sites. *Pythium* had a slight reduction in population in both sites

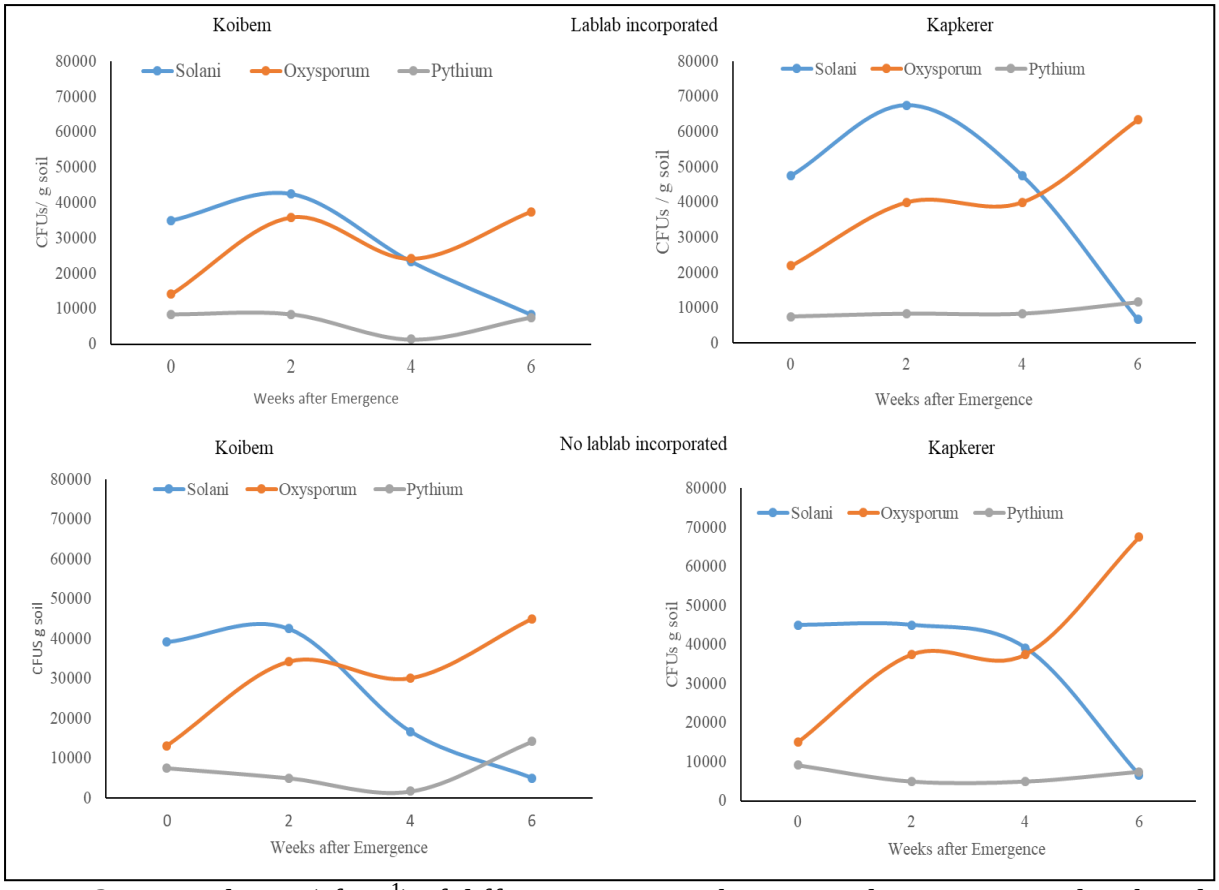

Figure 3. 7. Population (cfu g^{-1}) of different root rot pathogens in plots incorporated with and without lablab green manure in the short rains of 2015

In 2016 (Figure 3.8), the *F. solani* population responded to green manure residue by rising from the initial population to about 40,000 cfu g^{-1} in Koibem and about 30,000 cfu g^{-1} in Kapkerer then reduced. *F. oxysporum* also responded to lablab green manure in the same way as *F. solani* by significantly increasing from the initial population to 32,000 cfu g^{-1} in Koibem and 35,000 cfu g^{-1} in Kapkerer then the population decreased four weeks after crop emergence. In both sites, *Pythium* spp. populations in the soil showed slight fluctuation, increasing two weeks after emergence then reducing to below 10,000 cfu g^{-1}. However, in Koibem in plots with no green manure treatment applied, *Fusarium solani* populations did not show any fluctuation in the first four weeks after emergence but slightly increased after the fourth week then reduced while *F. oxysporum* on the other hand increased from the initial sampling population and continued to increase until end of the experiment in both sites. *Pythium* had a slight reduction in population in both sites

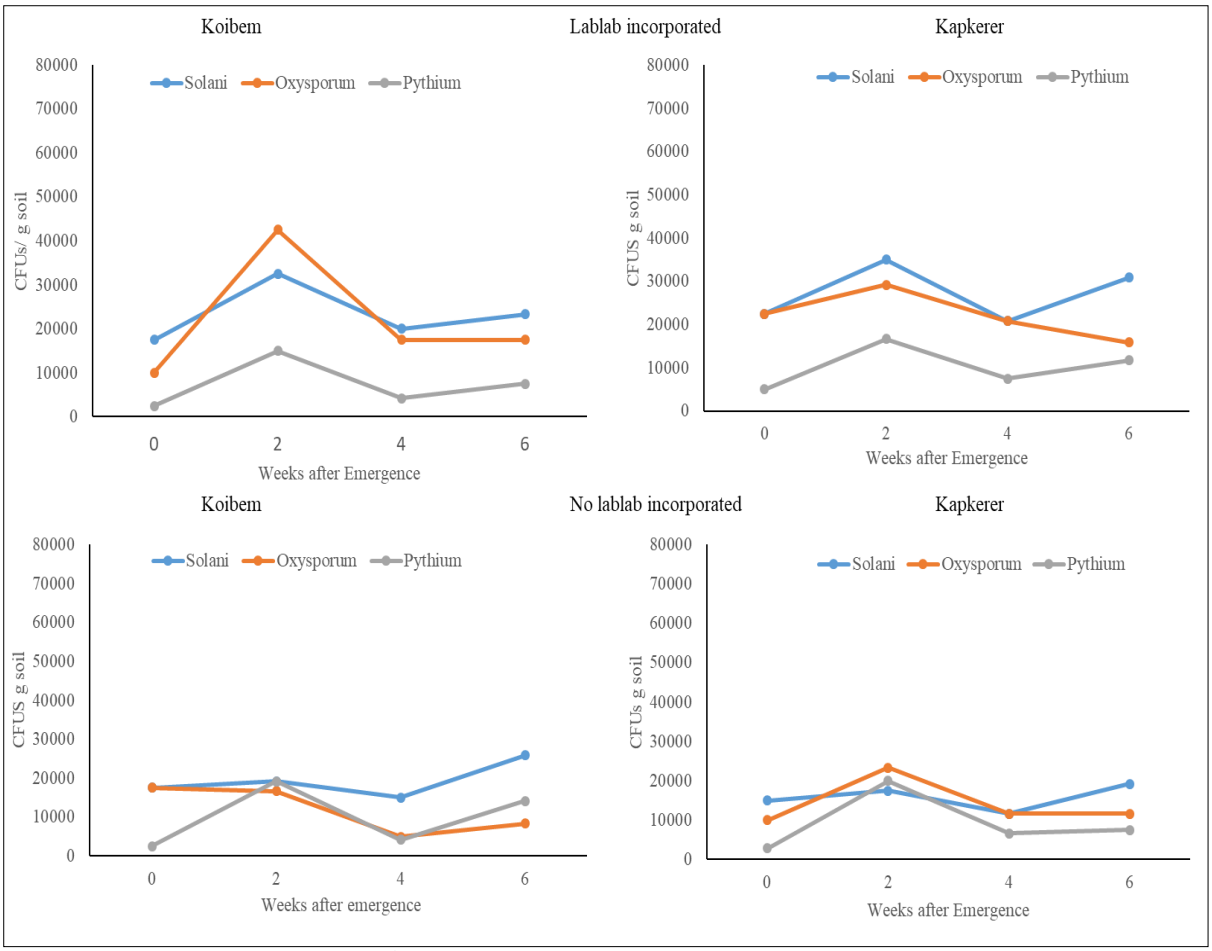

Figure 3. 8. Population (cfu g^{-1}) of different root rot pathogens in plots incorporated with and without lablab green manure in the short rains of 2016

3.4.8 Effect of lablab green manure on population of saprophytic fungi in the soil

The results obtained from the experiments indicate that application of lablab green manure had significant effect on quantitative composition of saprophytes in the soil (Table 3.7). Species including *Aspergillus*, *Trichoderma*, and *Penicillium* were dominant. In 2015 season, it was observed that soil samples from Kapkerer site had the highest population density of *Trichoderma*, and *Penicillium* than Koibem. In 2016 season, the populations of saprophytic fungi were higher in soil samples collected from Koibem than the samples from Kapkerer. In both sites and in both seasons, the densities of all the saprophytes were higher in plots treated with lablab green manure and in plots treated with diamonium phosphate fertilizer and in plot without any treatment.

Table 3. 7. Population (cfu g^{-1} soil $\times 10^4$) of saprophytic fungi isolated from plots incorporated with lablab green manure

	Koibem			Kapkerer		
	Aspergillus	*Trichoderma*	*Penicillium*	*Aspergillus*	*Trichoderma*	*Penicillium*
2015 short rains						
Lablab whole	1.9_{a}	1.1_{ab}	1.4_{a}	0.9_{bc}	0.9_{b}	1.5_{b}
Lablab in rows	1.1_{b}	1.3_{a}	0.9_{b}	1.1_{a}	1.4_{a}	1.7_{b}
No amendment	1.4_{ab}	1.4_{a}	1.1_{ab}	1.2_{a}	0.8_{b}	2.7_{a}
DAP	1.2_{bc}	0.7_{bc}	1.1_{ab}	0.8_{c}	0.5_{c}	1.4_{b}
Lime	0.9_{c}	0.5_{c}	0.7_{c}	1.0_{ab}	0.8_{b}	2.7_{a}
Mean	1.3	0.98	1.1	1.0	0.9	2
LSD ($p \leq 0.05$)	0.4	0.51	0.35	0.17	0.3	0.79
P value	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
2016 short rains						
Lablab whole	1.7_{a}	0.7_{ab}	2.1_{ab}	0.8_{b}	0.5_{b}	0.9_{b}
Lablab in rows	0.7_{d}	0.9_{a}	0.9_{b}	1.4_{a}	1.2_{a}	2.6_{a}
No amendment	0.9_{b}	0.9_{a}	2.6_{a}	1.3_{a}	0.7_{b}	2.3_{a}
DAP	0.8_{c}	0.5_{b}	1.9_{ab}	0.7_{b}	0.5_{b}	1.8_{a}
Lime	0.7_{d}	0.4_{b}	1.1_{b}	1.0_{ab}	0.7_{b}	2.4_{a}
Mean	1.0	0.7	1.7	1.1	0.7	2.1
LSD ($p \leq 0.05$)	0.5	0.2	0.8	0.3	0.3	0.8
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

DAP – Diamonium phosphate, means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$).

In plots treated with lablab green manure, the population of saprophytes *Penicillium*, *Aspergillus,* and *Trichoderma* did not show variation ($P \leq 0.05$) from the initial sampling, however, there was slight increase in the population of *Trichoderma* and *Penicilium* in Kapkerer while there was reduced population in Koibem. In plots without green manure application, there was no change in population of saprophytic fungi, either slightly increasing or reducing (Figure 3.9). There was also sharp increase in the population of *Penicillium* four weeks after incorporation

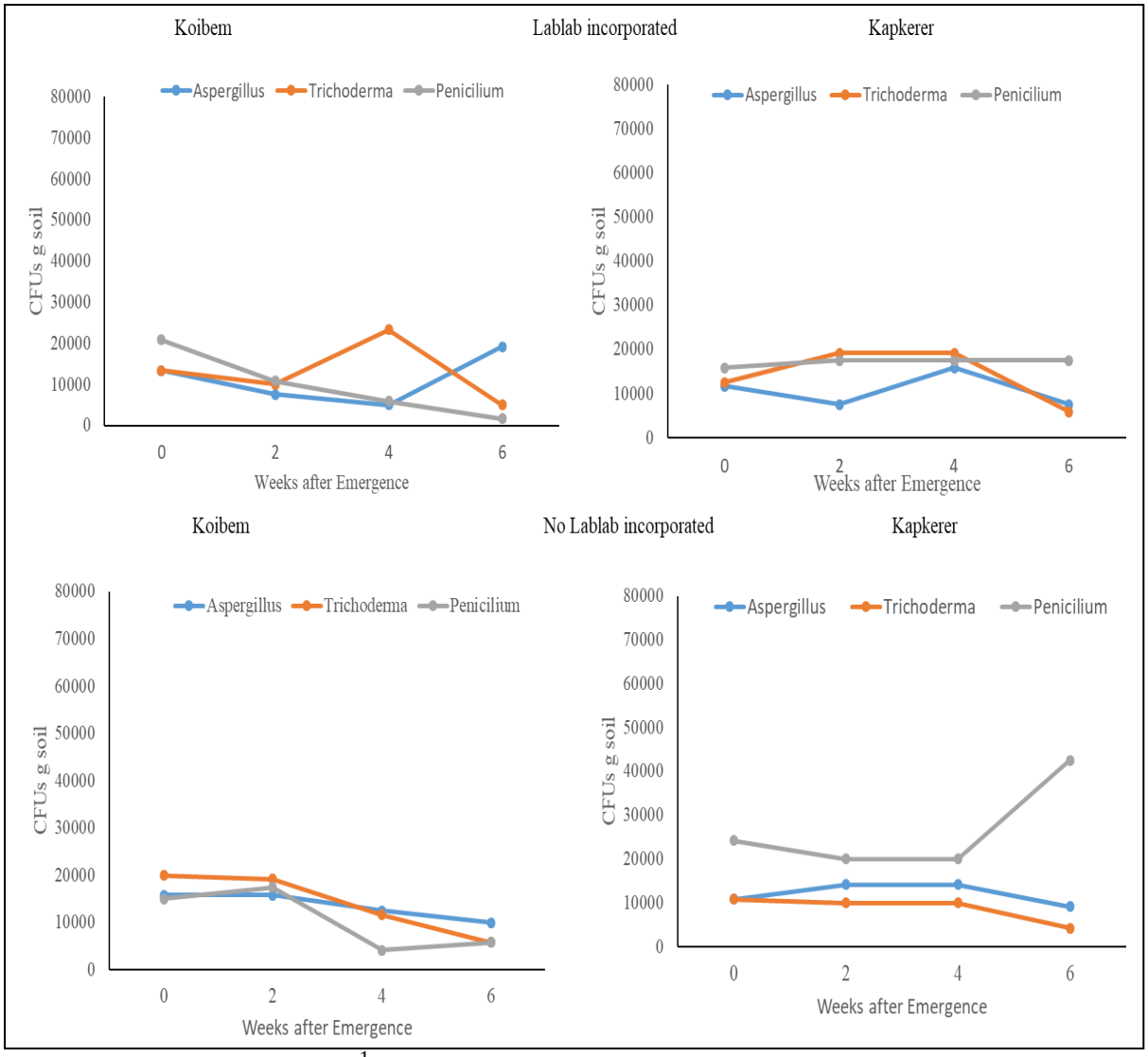

Figure 3.9. Population (cfu g^{-1}) of saprophytic fungi isolated from plots incorporated with and without lablab green manure in the short rains of 2015

In the second season (Figure 3.10), there was no variation in the population (cfu g^{-1}) of saprophytic fungi *Penicillium*, *Aspergillus*, and *Trichoderma* in plots treated with lablab green manure from the initial sampling population in Kapkerer while in Koibem, there was greater increase in the population of *Trichoderma* and *Aspergillus* from the second to the fourth week after crop emergence. In plots without green manure, there was no variation in population of saprophytic fungi, except for *Penicillium* which drastically reduced from 8.0×10^4 cfu g^{-1} to around 1×10^4 cfu g^{-1}

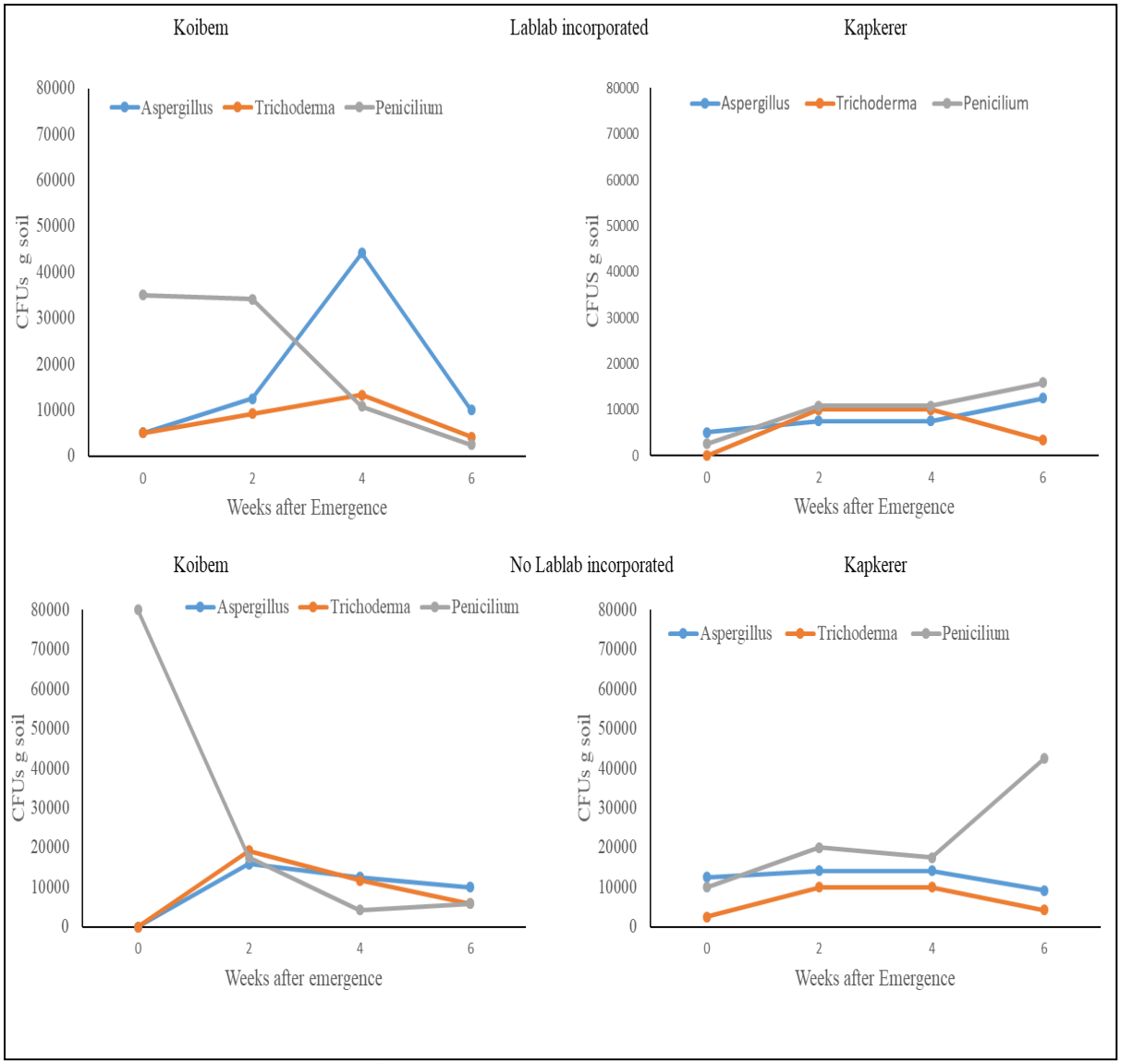

Figure 3. 10. Population (cfu g^{-1} soil) of saprophytic fungi isolated from plots incorporated with lablab green manure in 2016 short rains

3.4.9 Effect of lablab green manure on the population of bacteria in the soil

The population of bacteria did not vary between the sites and in both seasons ($p \leq 0.05$) (Table 3.8). In the first season in Koibem, control plots had the highest bacterial density while plots treated with lablab had the lowest bacterial density. In Kapkerer, there was no significant difference between the treatments in Kapkerer. In the second season, in both sites there was no significant difference ($p \leq 0.05$)

Table 3. 8. Population ($\times 10^6$) of bacteria (cfu g^{-1} soil) isolated from plots treated with lablab green manure

	Koibem			Kapkerer		
	2015 Short rain	2016 Short rain	Mean	2015 Short rain	2016 Short rain	Mean
Lablab whole	23.9_b	18.9_a	21.4_{ab}	13.4_b	15.0_a	14.2_b
Lablab in rows	27.8_b	13.2_a	20.5_{ab}	16.6_b	18.9_a	17.8_b
No amendment	41.4_a	19.2_a	30.3_a	12.3_b	18.6_a	15.4_b
DAP	25.3_b	20.0_a	22.7_{ab}	12.0_b	18.3_a	15.2_b
Lime	27.6_b	11.5_a	19.6_{ab}	15.2_b	13.6_a	14.4_b
Mean	29.2	16.6	22.9	13.9	16.9	15.9
LSD ($p \leq 0.05$)	12.3	99.3	73.2			
CV (%)	86.6	90.2	58.1			
P value	0.343	0.211	0.185			

Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$). DAP – Diamonium phosphate

3.4.10 Effect of lablab green manure on grain and biomass yield of common beans

There was significant ($p \leq 0.05$) difference in grain yield of common beans in Koibem (Table 3.9). In the first season, plots treated with lime resulted in high biomass and grain yield across the two sites, Koibem (320kg/ha^{-1}, 3t ha^{-1}) and Kapkerer (298.0kg/ha^{-1}, 2.6t ha^{-1}) while lablab incorporated plots consistently gave the lowest yield in the first season and in the second season averaging (2t ha^{-1}). Controlled plots performed better than plots incorporated with lablab green manure. In general lime and DAP plots resulted in improved common beans yield as compared to other treatments. Plots treated with lablab both in rows and in whole plots did not results in greater biomass compared to the control plots.

Table 3. 9. Biomass and grain yield of common beans harvested from plots treated with lablab green manure

	Koibem		Kapkerer	
2015 Short rains	Plant Biomass (Kg/ha)	Grain Yield (t/ha)	Plant Biomass (Kg/ha)	Grain Yield (t/ha)
Lablab whole plots	243.5_{c}	2.4_{a}	276.5_{a}	2.2_{a}
Lablab Between rows	257.2_{bc}	2.7_{a}	267.9_{a}	2.0_{a}
No amendment	345.4_{a}	2.7_{a}	265.7_{a}	2.4_{a}
DAP	343.6_{ab}	3.4_{a}	260.5_{a}	1.9_{a}
Lime	320.3_{ab}	3.2_{a}	298.0_{a}	2.6_{a}
Mean	302.0	2.9	273.7	2.2
LSD ($p \leq 0.05$)	117.4	1.4	170.8	1.7
CV (%)	25.7	27.9	34.9	33.8
P value	0.002	0.245	0.687	0.923
Short rains 2016				
Lablab whole plots	56.7_{e}	0.2_{d}	160.7_{cd}	0.5_{d}
Lablab between rows	117.2_{de}	0.4_{d}	198.9_{cd}	0.6_{cd}
No amendment	161.5_{cd}	0.6_{cd}	253.9_{bc}	1.0_{bc}
DAP	151.8_{cd}	0.7_{bc}	345.8_{b}	1.2_{b}
Lime	303.3_{b}	1.1_{bc}	484.5_{a}	1.9_{a}
Mean	158.1	0.6	288.8	1.0
LSD ($p \leq 0.05$)	108.9	0.43	108.9	0.4
CV (%)	60.2	67.2	60.2	67.2
P value	0.001	<.001	0.001	<.001

DAP – Diamonium phosphate, Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($P \leq 0.05$).

3.4.11 Correlation among root rot pathogens, saprophytes, root rot incidence and bean yield

Significant negative correlation was exhibited between *F. oxysporum* and *Pythium* (-0.567, -0.661, $P \leq 0.05$) to crop emergence and plant stand respectively (Table 3.10). Incidence of root rot pathogens was negatively correlated to beneficial saprophytic fungi isolated from the soil. There was strong negative correlation between *F. solani*, *F. oxysporum* and *Pythium* (-0.578, -0.626, -0.606 $P \leq 0.05$) respectively to yield, however, the saprophytic fungi had significant positive relation with yield. The crop emergence was positively correlated to yield (+0.776, $P \leq 0.05$) but was negatively correlated to root rot incidence (-0.547, $P \leq 0.05$). Root rot incidence correlated negatively with grain yield and beneficial saprophytic (-0.687, $P \leq 0.05$) but was positively associated with the population of *F. solani*, *F. oxysporum* and *Pythium*

Table 3.10. Correlation coefficients among root rot pathogens, saprophytes, emergence, disease incidence, and yield

	F. o	*F. s*	*Pyth*	*Trich*	*Pen*	*Asper*	Emerg	P.S	Incide	Yield (t/ha)
F. oxysporum	-									
F. solani	0.398	-								
Pythium	0.186	-0.009	-							
Trichoderma	0.056	-0.157	0.08	-						
Penicillium	0.099	-0.138	0.139	0.4	-					
Aspergillus	-0.081	-0.316	0.002	0.141	0.009	-				
Emergence	-0.567*	0.137	-0.661*	-0.202	-0.24	-0.05	-			
Plant stand	-0.294	0.016	-0.163	-0.182	-0.193	-0.07	0.493	-		
Incidence	0.524*	0.701*	0.403	0.205	-0.014	0.069	-0.547	-0.28	-	
Yield (t/ha)	-0.578*	-0.626*	-0.606*	0.824	0.842	0.275	0.776*	0.557	-0.687*	-

***** Significantly correlated

3.5 Discussion

3.5.1 Types and utilization of organic manure by farmers

The information sources were evaluated by asking the individual farmers where they obtained information on green manure use. All the farmers accessed information on green manure use through the KALRO staff in the Multipurpose Legume Project (MLP), however, not all the farmers adopted the technology. Sources of information on green manure use are the channels through which farmers obtain information for farming and utilization of green manure as a soil amendment. Most used type of soil amendment was inorganic fertilizer, and this is because application of inorganic fertilizer is one of the easiest ways of increasing productivity per unit area.

The level of adoption of green manure usage amongst the interviewed farmers was average contradicting findings by Usman *et al*. (2016) that the extent of adoption is expected to be high for educated farmers and less for small scale farmers and those practicing traditional farming systems. Some of the factors influencing decisions by farmers to adopt organic

manure as a soil amendment are farming experience, high cost of inorganic fertilizers, level of education and estimated yield from organic manure in the short term (Odendo *et al*., 2009). Farming experience brings specialization and observance to the use of a new technology (Usman *et al*., 2016). Some farmers did not adopt this technology since using green manuring requires the purchase of seed, acquiring labour for seedbed preparation and residue incorporation (Fabunmi, and Balogun, 2015). Therefore for easy adoption, a green manuring technology should be simple and concise. Organic manure increases yield by enhancing soil organic matter, soil pH, nutrient availability and exchange and water holding capacity (Williams 1999). Many crops respond well to the application of organic manure than inorganic fertilizers. However, despite the beneficial effects of organic manure in crop production and soil fertility management, it is not frequently applied by most farmers (Oyesola and Obabire, 2011).

Majority of the respondents planted crops two to three weeks after incorporation. This was done to avoid the deleterious effects associated with undecomposed plant residues. Some of the deleterious effects associated with undecomposed plant residues include release of phytotoxic compound products, enhancement of pathogenic organisms and increased competition (Bonanomi *et al*., 2011). Moreover, legume residues are beneficial to the subsequent crops when nutrients release pattern from the decaying residue and the plant needs occur simultaneously (Odhiambo, 2010). Again after decomposition, organic P and K bound in green manure provide easily available form of P and K to succeeding crops (Talgre *et al.*, 2012b). The effect of plant residues on seedling emergence can be influenced by maturity of the tissues and their carbon: nitrogen ratios and the time elapsed between incorporation and planting. Immediately after incorporation, there is an unusual explosion of microbial activity that includes germination of propagules of pathogenic fungi that may invade susceptible hosts and this may result in poor crop establishment.

The farmers should be encouraged to consider application of green manures as an alternative to the expensive inorganic fertilizers. This, however, calls for training of the farmers on the benefits of green manure technology. When applying green manures, a fallow period of several weeks between incorporation time and planting may allow for the drop in pathogen populations and reduction of other deleterious effects associated with decomposing green manure. It is therefore important that farmers allow for time between incorporation and planting to ensure adequate decomposition. To sustain this technology, access to information and extension services are to be improved to increase adoption and use of green manures.

3.5.2 Effect of green-manure and other soil amendments on soil physical and chemical characteristics

Organic Matter, pH, available nitrogen, available phosphorus and exchangeable potassium of the plots treated with organic amendment marginally increased. The changes in soil properties are associated with the type of soil amendment applied (Brown and Cotton, 2011). This is probably due to the addition of organic amendments as they are the source of organic carbon and nitrogen to the soils. The results herein concur with those reported by Mahmood *et al*. (2017) who found that organic materials applied alone or in combination with inorganic fertilizers increase soil fertility in terms of organic carbon and the available P, N and K. The soils where green manure was incorporated had higher N concentrations than the untreated plots. This is because of higher N levels in lablab (Sitienei *et al*., 2017) and intercropping with lablab may result in increased amounts of N fixed by legume. Leguminous green manures provide large amount of nitrogen available in soils by the decomposition of biomass (Benjawan *et al*., 2015). Soil organic carbon is a source for plant nutrients in soils and maintains soil silt thereby aiding infiltration of air and water (Gregorich *et al*., 1994). Organic amendments supplement liming sources permitted to accrue in soil. The result of the soil

chemical parameters thus indicated that the treatments applied had effect on the chemical parameters of the soil albeit in small quantities

3.5.3 Effect of lablab green manure on germination and establishment of common beans

Common bean emergence was suppressed by incorporation of lablab green manure when compared with other treatments in both seasons. The highest germination percentage was observed in plots treated with lime and DAP. Results show that lablab treated plots consistently had the lowest germination percentage. Bonanomi *et al*. (2017) reported inhibition of 48%, however, the response varied according to litter type. Green manure can either stimulate or inhibit microbial growth in the soil and also make plant roots vulnerable to root infection. The present study indicated specificity of action of the lablab residues and the concentrations effects of the inhibitors on common beans emergence. According to Islam and Kato-Noguchi, (2014), the decrease in crop emergence could be as a result of phytotoxins since there was increased germination after washing the soil with water. Green crop residues during decomposition may result in production of phytotoxic products that may limit seedling germination and establishment

The initial stage of decomposition involves breakdown of plant tissue by microorganisms and subsequent release of contents of the cell with oxidized functional group while at late stages there is an increase of phenolic functional groups indicating degradation of lignin in the maturing compost (Bonanomi *et al*., 2017). Chemical nature and toxicity of inhibitors derived from undecomposed plant residues are different and the residues contain water soluble phytotoxins which are responsible for depressed germination and seedling establishment (Bonanomi *et al*., 2017). The growth inhibitors from decomposing plant residues are most concentrated in the soil close to particles of decomposing plant materials. Roots of common beans are sensitive to these chemicals and showed symptoms of damage which resulted in

stunted growth of seedlings. Phytotoxicity was severe in the initial stages of decomposition since toxicity of green manure develops relatively early in the decomposition process. When lablab green manure was applied in rows, relative uniform damage was observed in beans planted. This may be due to the inhibitory substances being produced during decomposition. Soils modified by addition of green manure preconditions plant roots to attacks by root rot pathogens and the phytotoxins produced during decomposition conditions roots for pathogen attack. This may explain the role of lablab green manure as a physical impediment to emerging seedlings and the etiology of pathogen attack of young seedlings.

Population of Fusarium root rot pathogens in lablab treated plots was higher in the second week after treatment application. Interactions of green manure with fungal pathogens like *Fusarium* may be involved in suppression of germination of common beans. Bonanomi *et al*. (2017) showed that undecomposed plant residues promote fungal growth, although the effect on microbial response can vary depending on the residue type applied in the soil. However, as decomposition proceeds, plant residues become more suitable for plant growth while bacteria and fungi are inhibited. Addition of green manure improves speedy multiplication of microorganisms in the soil (Talgre *et al*., 2014) resulting in increased metabolism which causes high consumption of oxygen in soil resulting in increased carbon dioxide production which may retard common bean germination (Jeon *et al*., 2008). Large number of organisms in soil may also result in possible accumulation of toxic substances to germination. These waste products are less decomposable than the original plant material.

Pathogenic *F. oxysporum, F. solani* and *Pythium* spp. were found to dominate plots treated with organic manure. This finding is consistent with Abawi and Widmer (2000) who reported an increase in pathogenic fungi with organic fertilizer application. Pathogenic *Fusarium* and *Pythium* species cause reduction in seed germination potential (Berg *et al*., 2017). Bonanomi

et al. (2007) reported increased symptoms such as stunting, yellowing and wilting. Symptoms associated with undecomposed plant residue were evident immediately after planting but overtime the plant partially recovered from wilting. Decomposition of lablab residue shows their negative effects as soon as they come into contact with plant roots because they interfere with water uptake (Blum *et al*., 1999).

The addition of green manure provides significant inputs of organic carbon, which increases fungal population (Yang *et al*., 2016). Antagonistic fungi isolated in small proportions were *Trichoderma, Penicilium* and *Aspergillus*. High concentrations of nutrients in fresh green manure residues inhibit the production of enzymes required for parasitism by biocontrol agents such as *Trichoderma* spp. (Hoitink *et al*., 1999). As a consequence, the weakened biological barrier due to low population of saprophytic fungi could be the cause of intensive symptoms of root rot disease. The environment in which green manures is applied may contain high concentrations of salt, ammonium salt and low oxygen which is unsuitable for growth and multiplication of antagonistic microorganisms (Aryantha *et al*., 2000).

Higher levels of soil infestation with root rot pathogens result in increased disease potential as shown in plots with lablab green manure incorporated either in rows or fully on the plot. This result conforms to findings by Marzano. (2012) who reported higher levels of soil infestation following plant residue incorporation and therefore increase in disease pressure. The fungal response to available plant materials in the soil suggests the need for great care in managing green manure and plant debris incorporation (Manici *et al*., 2004). In order to exploit the richness in moisture, nutrient and physical soil characteristics, there should be great care in organic farms where crops are planted immediately after green manure incorporation. Net immobilization of nitrogen has also been explained as a phenomenon that reduces emergence (Ambrosano *et al*., 2013).

3.5.4 Effect of green manure and other soil amendments on soil microbial population

The results obtained from both seasons indicate that incorporation of lablab green manure diversified the numerical and qualitative composition of soil fungi. The highest total number of colony forming units (cfu) of root rot pathogens was observed in plots with fresh lablab tissue incorporated and the lowest cfu in the other plots. Fresh green manure results in rapid increase in soil microbial biomass, of which fungi are commonly the largest component (Manici *et al*., 2003). Among the selected colonies, pathogenic fungi were represented by *F. solani, F. oxysporum*, and *Pythium*. Their numbers increased two weeks after treatment application and at the same time the lowest number of saprophytic fungi was obtained. Among the saprophytic fungi *Penicillium*, *Trichoderma*, and *Aspergillus* were identified. The saprophytic fungi had a low share of fungal community, similar results were reported by Cwalina-Ambroziak and Bowszys, (2009). The results demonstrate that total microbial population increased in organically amended plots compared to inorganically treated plots and control plots.

Application of organic manure stimulates the growth of total soil microbial populations through supply of nutrients which have different effects on individual microorganisms. Fresh green manure and the amount returned to the soil improve microbial activity (Nakhro and Dkhar, 2010) and provide significant inputs of organic carbon, which increases both fungal and bacterial populations (Yang *et al*., 2016). Organic manure when applied to the soil provides readily available substrate to the decomposing fungi (Swer *et al*., 2011). Nonetheless, *F. oxysporum* and *F. solani* that are destructive to common bean were very dominating in soils treated with lablab green manure two weeks after incorporation. This result is supported by findings by Abawi and Widmer (2000) who reported an increase in pathogenic fungi when organic fertilizer was applied in the soil. This is shown by higher population of root rot pathogens in organically amended plots. The fungi from the control

plots help in estimating and identifying the indigenous fungal population and diversity of the study sites. The population of the pathogenic fungi resulted in high root rot incidences in organically treated plots when compared with control

The addition of soil amendments resulted in either reduced population or maintained the initial population of antagonists but appeared to support the population of pathogenic fungi. These changes were observed in the initial stages of decomposition in all the farms regardless of previous history of the farm in which the experiment was conducted. Green manure stimulates biological and microbial activities thus speed up the breakdown of organic substances (Zhang et a., 2008; Zhang *et al*., 2015). Results show that the soil amendments used had no suppressive effects on pathogenic microbes like *Fusarium* and *Pythium* species. The negative effect of green manure has been verified with the use of red clover green manure where there was increased incidence of disease in wild mustard seeds (Conklin *et al*., 2002). However, other studies by Wigins and Kinkel, (2005) suggest that green manure treatments may help in disease control by activating pathogen, by releasing phytotoxins produced during product storage or by subsequent microbial decomposition

In intensively cultivated soils like the ones where the experiment was set up, where saprophytic pathogens have been increased by earlier soil management practices, the ploughing in of organic debris like green manures enhances the population of the pathogens and in so doing increases root rot incidence and severity in subsequent crops. Exhaustive cropping system leads to poor soil fertility with low organic matter in the soil accompanied by increased pathogenic population in the soil (Manici *et al*., 2004). The response of the pathogens and the lack of suppression of the same pathogens reveal that the pathogens may pose potential problems during the first two weeks after green manuring in the soil thus result in poor crop germination and establishment.

3.5.5 Effect of lablab green manure on the yield of common bean

Results observed shows that beans planted in plots in which lime and DAP and even in control plots outperformed plots treated with green manure. The differences observed between the treatments may be as a result of soil nutrient content and type and nature of the microorganism present in the soil (Nnabude *et al*., 2015). There was positive correlation detected between severity and incidence of root rot disease, this relationship suggests that root rot pathogens could be interacting to cause the intense root rot. Severity and incidence had negative correlation with both plant stand and total yield suggesting that damage or presence of these pathogens translates into lower yields. Yield is reported to increase in organic production systems (Roos *et al*., 2018). However, this was not the case in this study. To record high yields in organically amended fields, organic productions systems require 3-5 years of manure application (Altieri, 1995) to be more productive due to beneficial effects on soil properties of long term soil organic amendment applications (Bulluck *et al*., 2002). This explains the reason the results of the current study do not support the high yield theory when it comes to green manure application since it was not long term and plants were planted immediately after treatment applications.

3.6 Conclusion

In conclusion, the participation of farmers through surveys gave valuable insights on green manure sources, use, and application methods. Farmers preferred to use green manure from different sources as soil amendments. Results of this study demonstrate that green manure enhanced both chemical and physical attributes of soil and thus may have positive impact on soil microbial population and activity. Green manure had significant impacts on diversity, composition and structure of soil microbial communities. Given the complex nature of the interactions among residues in soil, microbes, plants as well as soil conditions, it becomes difficult to demonstrate that incorporated residues reduce crop emergence and vigour.

Understanding the relative importance of these effects is important to manage green manure hence, focusing on the balance between negative effects like phytotoxicity and N immobilization and positive effects like nutrient release, water retention, and disease suppression. The research underlines the need to apply green manure at the right time without compromising crop establishment thus accruing economic benefits

CHAPTER FOUR

EFFECT OF TIME AFTER INCORPORATION OF LABLAB GREEN MANURE ON ROOT ROT AND ESTABLISHMENT OF COMMON BEANS (*Phaseolus vulgaris* L.)

4.1 Abstract

Green manure incorporation is an important practice for restoring soil quality by through maintaining organic matter and supplying nutrients to plants. However, green manure residues may limit the productivity of soils by inhibiting establishment and plant stand through different mechanisms. Therefore, there is need to determine the suitable time for green manure incorporation before planting. The effect of time after incorporation of lablab green manure on soilborne pathogens and bean crop establishment was evaluated in the field by incorporating 12t/ha of lablab green manure at 0, 7, 14, and 28 days before planting. Soil samples were collected before and after incorporation of green manure during planting, and later at two, four and six weeks after planting. Data was collected on crop emergence, plant stand, yield, incidence and severity of root rot and population of root rot pathogens in the soil. Incorporation of lablab residues 28 days before planting resulted in improved germination by 21% and with corresponding reduction in root rot incidence and severity by 8% and 36% compared to plots incorporated with green manure at planting. Incorporation of green manure at planting was found to enhance the population of root rot pathogens while it reduced the population of saprophytic microorganisms such as *Aspergillus, Trichoderma,* and *Penicillium* and also reduced grain yield by up to 71% compared to plots where lablab residue was incorporated 28 days before planting. The results of the study showed that a period of 28 days between green manure incorporation and planting is necessary to allow for proper decomposition resulting in reduction in root rot incidence, severity and increased grain yield.

Keywords*:* Green manures, *Lablab purpureus*, Soil health soil borne pathogens

4.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is known as staple legume (Petry *et al*., 2015) and low cost protein source consumed in many developing countries where protein malnutrition is widely prevalent (Rezende *et al*., 2017). However, bean productivity is usually very low in most countries in Latin America and Africa (Araujo and Texeira, 2008). The decline in soil fertility is a major constraint to bean production and farmers usually apply below recommended rates of fertilizer along with agricultural practices that lead to nutrient depletion (Mugwe *et al.,* 2009). Application of green manure from leguminous crops is considered a sustainable option for the supply of nitrogen for organic production systems (Ferreira *et al*., 2013). The release of nutrients from these manure types are slower than from inorganic fertilizers, contributing to better sustainability of production system (Chabi-Olaye *et al*., 2005).

Plant residues are important component of the soil and they can either be living, dying, or dead with enormous chemical diversity, and are eventually decomposed through the action of biotic and abiotic agents (Miller, 2016). The residue decomposition is influenced by various factors including environment, decomposer community and residue quality (Rath and Rousk, 2015). Green manure residues have been associated with higher seedling mortality resulting in low yields (Schroeder *et al*. (1998). These materials on decomposition release phytotoxic substances that enhance the population pathogenic fungi and at the same time interfere with crop emergence (Wall, 1984). The injury to roots of seedlings is due to seedlings' root coming into contact with the immediate vicinity of the decomposing residues. Thus the effects on the seedling establishment through injury by the substances produced during decomposition or indirect through stimulation of pathogenic fungi (Wall, 1984). However, there effects on seedling growth and disease incidence depend on maturity of the tissues, carbon: nitrogen ratio and time elapsed between incorporation and planting (Lemitiri *et al*.,

2016). The inhibitory effects of decomposing plant residues regularly change, with inhibition being greatest within the first days of decomposition (Bonanomi *et al*., 2011). Stimulation and enhancement of crop growth may therefore occur in the later stages of decomposition since the compounds are degraded by soil microorganisms into substances less harmful to pants (Bonanomi *et al*., 2006). Therefore, even with the importance of green manure, there is little information how soon crops can be safely planted after application to achieve good establishment, especially common beans. The objective of this study was to investigate effect of time after incorporation of lablab green manure on establishment of common beans and population of microorganisms

4.3 Materials and Methods

4.3.1 Description of the study site

The study was conducted in Koibem and Kapkerer located in Nandi South Sub-County in the North of Rift valley to the north latitude 0^{o}34'N (Nyberg *et al*., 2012). The main agro ecological zones are upper highlands (UH) forest reserves, lower highlands (LH1) zones suitable for tea and dairy production and upper midlands (UM) suitable for sorghum, millet, potatoes and coffee. The area experiences two diverse climatic seasons, the long rain seasons occurring from March to August and the short rain seasons from September to December. The area receives an average annual precipitation of 1200mm to 2000mm with mean yearly temperature ranging from 18-25^{o}C and the soils are characterized by fine drained clay loamy soils (FAO-UNESCO, 1997).

4.3.2 Experimental design and layout

Lablab variety Rongai was planted in plots measuring 4m by 6m at a spacing of 45cm by 30cm and at flowering stage the vegetation was harvested, chopped into tiny portions. To assess the dynamics of phytotoxicity during decomposition chopped lablab residues were

incorporated at the rate of 12 tons/ha at planting, 7, 14, and 28 days before planting. Common bean varieties KK8 and with GLP2 were planted at a spacing of 50 by 10cm in each plot. The plots were separated by 1m paths and the treatments were arranged in a randomized complete block design with a split plot arrangement where bean varieties comprised the main plots while incorporation times were the subplots. The study was carried out over two short seasons of 2016 and 2017. Data on crop emergence was collected one week after planting while, incidence and severity of root rot, plant stand, were collected after every two weeks until flowering and yield was collected after maturity. Soil samples were collected for isolation of soil microorganisms at planting, two, fourth and sixth week after emergence.

4.3.3 Isolation of root rot pathogens from bean stem bases

Roots showing root rot symptoms were washed in running tap water, surface sterilized in 2% sodium hypochlorite for 2 minutes, then rinsed in three changes of sterile distilled water. 1cm long portions of bean stems were cut from infected bean roots were plated on potato dextrose agar (PDA) amended with antibiotics. The cultures were incubated at room temperature for 7 to 10 days and the root rot fungi isolated were identified and the frequency of isolation was recorded. The incidence of root rot pathogens was expressed as the total number of isolations from root sections (Naseri, 2008). Morphological and cultural characteristics were used to identify the fungi following general identification keys.

4.3.4 Isolation of microorganisms from the soil

To determine fungi counts dilution plate technique was used where one gram of each soil sample was suspended in 10 ml sterile distilled water (Bi *et al*., 2011). Tenfold dilution series from soil suspensions was performed and aliquots of 1ml from 10^{-3} of the soil suspension was plated on molten potato dextrose agar (PDA) after which the plates were incubated for four to seven days at room temperature until colonies appeared which were then counted. The different fungal colony types were identified following evaluation of colony colour, growth

type, colony reverse colour of mycelia and as well as microscopic morphology (Nurbaya *et al*., 2014). Fungal population was determined by multiplying the number of colonies by the dilution factor (cfu g^{-1} = Total number of colonies × Dilution factor)

4.3.5 Determination of emergence, plant stand and yield of common beans

Emergence was evaluated by counting the number of emerged plants after one week while plant stand was determined by counting the number of surviving plants in each plot at the second, fourth and at sixth week after emergence (Muthomi *et al*., 2014). Yield attributes of beans were determined by taking 10 samples of bean plants randomly from two central rows in each experimental unit at physiological maturity (El-Naim *et al*., 2012). Plant biomass was determined by sampling from each plot ten plants that were completely dried in an oven at 50^{o}C for a week and weighed then converted into kilogram per hectare. The total grain yield was calculated following the formula by Mwangi *et al*., (2008)

$$\text{Yield (t/ha)} = \frac{\text{Field weight per plot (g)} \times 10\ 000\ \text{m2/hectare}}{\text{Harvest area (m2)} \times 1000\ 000\ \text{g/tonne}}$$

4.3.6 Determination of incidence and severity of root rots

Incidence of root rot on bean seedlings was determined by counting the number of bean seedlings showing root rot symptoms per plot at the second, fourth and sixth week after crop emergence, while root rot severity was determined four weeks after emergence based on a scale of 0-5 (Naseri, 2008) where 0-no root discoloration, 1- 1~25% root discoloration, 2- 26~50% root discoloration, 3- 51~75% root discoloration, 4-up to 76% root discoloration, 5- completely dead plants. Area under disease progress curve for disease incidence was calculated using the formula described by (Muengula-Manyi *et al*., 2013).

AUDPC= $\sum_{i=1}^{n}(Yi + Yi + 1)/2\,(t2 - t1)$ where Yi is the incidence of disease at time I, Yi+1 is the disease incidence recorded at the time i+1, n, the number of registration on the incidence and t, days between the registration of Yi and Yi+1.

4.3.7 Data analysis

Data on emergence, plant stand, incidence, and severity and soil microorganisms was subjected to analysis of variance using Genstat (Version 15) computer software (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Mean separation was done by Fisher's Least-significant difference test (LSD) at $p \leq 0.05$.

4.4 Results

4.4.1 Effect of time after green manure incorporation on common bean establishment

Significant ($P \leq 0.05$) differences were observed between the different incorporation times with respect to emergence. The interaction between treatment and site was not significant. In the first short rain season of 2016 and in both sites, plots incorporated at planting had the lowest percentage emergence (84%) while the other incorporation times recorded the higher percentage emergence (Table 4.1). The plant stand continued to decline overtime. In the second short rain season of 2017, plots incorporated at planting and those planted seven days after incorporation recorded considerably low percentage germination (57% and 59%) respectively while other incorporation times had high percentage emergence. In 2017, greatest decline in plant population was observed across the two sites. Plant stand declined markedly to low levels in Kapkerer and Koibem over the entire sampling period.

Table 4.1. Percentage plant emergence and stand of common bean at different sampling times after incorporation of lablab green manure

Days after incorporation	Weeks after emergence							
	Koibem				Kapkerer			
	Emerg	2	4	6	Emerg	2	4	6
2016 Short rains								
28 days	99.8_a	96.5_a	94.9_a	93.8_a	99.8_a	81.5_a	51.4_a	50.7_a
14 days	99.6_a	94.5_a	92.7_a	91.8_a	100.0_a	69.5_b	35.0_{ab}	34.4_b
7 days	96.4_a	92.8_a	90.7_a	89.8_a	100.0_a	69.7_b	50.4_a	49.7_a
0 days	82.1_b	62.6_b	59.5_b	57.9_b	87.2_b	70.3_b	39.6_{ab}	38.8_b
Mean	94.5	86.6	84.4	83.3	96.9	72.7	44.1	43.4
LSD ($p \leq 0.05$)	5.9	10.4	16.9	16.8				
LSD site ($p \leq 0.05$)	3.4	6.2	2.4	7.6				
P value	0.021	0.002	0.065	0.06				
2017 Short rains								
28 days	84.1_a	80.6_a	61.8_a	30.4_a	78.7_a	74.0_a	52.8_a	32.9_a
14 days	84.2_a	78.3_a	47.8_b	24.3_a	75.7_a	68.5_{ab}	55.3_a	26.4_a
7 days	57.4_b	48.7_b	44.7_b	21.5_a	59.0_b	55.6_b	50.3_a	25.3_a
0 days	59.3_b	54.7_b	39.3_{bc}	28.4_a	46.3_b	43.2_b	40.8_{ab}	24.0_a
Mean	71.3	65.6	48.4	26.2	64.9	60.3	49.8	27.2
LSD ($p \leq 0.05$)	17.4	17.3	15.4	18.2				
LSD site ($p \leq 0.05$)	2.6	2.3	4.2	6.8				
P value	0.001	<.001	<.001	0.211				

Emerg – Emergence. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

4.4.2 Effect of time after incorporation of lablab green manure on incidence and severity of root rot

In the first season, there were significant ($p \leq 0.05$) differences among incorporation times, higher root rot incidences were observed in plots incorporated with green manure at planting in both sites (Table 4.2). Two weeks after emergence, root incidence was low in both sites but immensely increased in plots incorporated at planting (Koibem 35% and Kapkerer 27%).

The incidence of root rot in Kapkerer continued to increase while in Koibem the incidence reduced six weeks after emergence. In both sites higher root rot incidences were observed in the second season which was characterized by long and heavy rainfall and plots incorporated at planting had the highest root rot incidence except at two weeks after emergence

Table 4.2. Percentage root rot incidence at different sampling times after green manure incorporation in Koibem and Kapkerer, Nandi South

Days after incorporation	Weeks after emergence					
	Koibem			Kapkerer		
	2	4	6	2	4	6
2016 Short rains						
28 days	0.9_{b}	6.8_{b}	10.6_{b}	0.5_{a}	12.6_{b}	28.4_{b}
14 days	0.9_{b}	5.2_{b}	4.3_{b}	0.4_{a}	12.8_{b}	40.1_{ab}
7 days	1.4_{b}	4.9_{b}	7.6_{b}	0.9_{a}	21.5_{ab}	31.6_{b}
0 days	4.9_{a}	35.6_{a}	29.2_{a}	1.3_{a}	27.1_{a}	77.4_{a}
Mean	2.0	13.1	12.9	0.8	18.5	44.4
LSD ($p \leq 0.05$)	3.2	9.6	7.8	1.1	12.8	35.9
P value	<0.001	<0.001	<0.001	0.46	<0.001	0.001
2017 Short rains						
28 days	36.1_{a}	57.6_{a}	32.1_{a}	37.8_{a}	47.6_{a}	49.8_{a}
14 days	36.2_{a}	57.5_{a}	36.0_{a}	45.8_{a}	54.0_{a}	56.2_{a}
7 days	35.1_{a}	51.0_{a}	32.9_{a}	36.7_{a}	39.5_{a}	45.4_{a}
0 days	34.7_{a}	72.6_{a}	46.2_{a}	48.9_{a}	77.1_{a}	56.9_{a}
Mean	35.5	57.5	36.8	42.3	54.6	52.1
LSD ($p \leq 0.05$)	16.5	27.5	39.1	16.5	27.5	39.1
P value	0.35	0.117	0.716	0.35	0.117	0.716

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

There were no significant ($P \leq 0.05$) differences in disease severity observed among the treatments and seasons (Table 4.3). In both sites and seasons, root rot severity was highest in plots incorporated at planting when compared to other incorporation times. However, the least severity rating was observed in plots incorporated with green manure at 14 to 28 days before planting. In both site and seasons, severity rating of up to 70% were observed in plots incorporated at planting in bean variety GLP2 while in variety KK8 severity ratings of about 50% was recorded

Table 4.3. Percentage severity of root rot after incorporation of lablab green manure in Koibem and Kapkerer

Days after incorporation	Koibem		Kapkerer	
	KK8	GLP2	KK8	GLP2
Short rain 2016				
28 days	28.0_{a}	30.0_{b}	40.0_{ab}	46.0_{b}
14 days	32.0_{a}	42.0_{ab}	30.0_{b}	22.0_{c}
7 days	32.0_{a}	50.0_{ab}	50.0_{a}	54.0_{ab}
0 days	52.0_{a}	70.0a	50.0_{a}	76.0_{a}
Mean	36.0	48.0	42.5	49.5
LSD ($p \leq 0.05$)	37.5	38.9	18.6	23.7
CV (%)	32.7	25.5	13.7	15.0
P value	0.624	0.002	0.001	<.001
Short rain 2017				
28 days	50.0_{ab}	62.0_{b}	44.0_{b}	70.0_{ab}
14 days	46.0_{b}	68.0_{ab}	50.0_{ab}	74.0_{a}
7 days	50.0_{ab}	70.0_{a}	56.0_{a}	66.0_{b}
0 days	56.0_{a}	72.0_{a}	52.0_{a}	78.0_{a}
Mean	50.5	68.0	50.5	72.0
LSD ($p \leq 0.05$)	8.45	8.4	8.45	8.4
CV (%)	12.2	7.2	12.2	7.2
P Value	<.001	0.001	0.001	0.001

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

There were significant differences ($p \leq 0.05$) among the treatments in both seasons for area under disease progress curve (Table 4.4). There were also significant differences ($p \leq 0.05$) between the two varieties of common bean. Largest area under diseases progress curve was recorded in the second season 2017 compared with the first season 2016. In both seasons and in both sites, the plots incorporated at planting had the highest area under disease progress curve while those incorporated 28 days before planting had the least area under disease progress curve. The two varieties showed different levels of susceptibility with GLP2 variety being the most susceptible to root rots. GLP2 variety had the largest area under disease progress curve in both seasons in plots incorporated at planting while variety KK8 had the least area under disease progress curve.

Table 4.4. Area under disease progress curve on KK8 and GLP2 bean varieties after incorporation of lablab green manure

Day after incorporation	2016 Short rains			2017 Short rains		
	KK8	GLP2	Mean	KK8	GLP2	Mean
28 days	211.7_{b}	367.8_{ab}	289.8_{b}	646.0_{b}	726.9_{ab}	686.5_{b}
14 days	226.7_{b}	387.4_{ab}	307.1_{b}	732.3_{ab}	831.8_{ab}	782.1_{ab}
7 days	239.4_{b}	433.0_{ab}	336.2_{b}	661.4_{b}	637.6_{b}	649.5_{b}
0 days	620.3_{a}	846.0_{a}	733.2_{a}	957.8_{a}	969.6_{a}	963.7_{a}
Mean	324.5	508.6	416.6	749.4	791.5	770.5
LSD ($p \leq 0.05$)	359.4	359.4	241.4	273.1	273.1	241.4
P value	0.045	0.045	0.065	0.012	0.012	0.065

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

4.4.3 Effect of time after green manure incorporation of lablab green manure on root rot pathogens

Fusarium solani and *F. oxysporum* were the most dominant root rot pathogens associated with common beans isolated from bean roots collected from both sites (Table 4.5). However, other root rot pathogens *M. phaseolina, P. ultimum,* and *Rhizoctonia solani* were also isolated in both seasons and site in small proportions were *M. phaseolina, P. ultimum,* and *R. solani*. There was significant difference ($P \leq 0.05$) in the incidence of *Pythium, F. oxysporum* and *Macrophomina* and also between the difference incorporation times across the study sites. In the first season, bean stems collected from Kapkerer had significantly higher (32%) incidence of root rot pathogens compared to stems from Koibem. In both sites, plots incorporated at planting had the highest number of *Fusarium, Macrophomina,* and *Pythium* while plots treated 28 days before planting had the lowest population of root rot pathogens. A significant ($P \leq 0.05$) variation in the incidence of root rot pathogen population was found between the sites.

In the second season, there was significant ($P \leq 0.05$) variation in the incidence of root rot pathogens isolated from different plots with different incorporation times in both sites. *F. solani* and *F. oxysporum* were the most dominant isolated from roots collected from different farms from both sites. However, stems collected from Koibem had the highest number of root rot pathogens compared to those from Kapkerer. Other root rot pathogens isolated in small incidences included *M. phaseolina, P. ultimum* and *R. solani*. In both sites, bean stems collected from plots incorporated with lablab green manure at planting had the highest incidence of root rot pathogens *Fusarium, Macrophomina,* and *Pythium* while plots incorporated 28 days before planting had the lowest incidence of root rot pathogens. There was significant ($p \leq 0.05$) variation in the incidence of root rot pathogen population found between the sites and between the treatments.

Table 4.5. Percentage incidence of root rot pathogens contaminating bean stems after lablab green manure incorporation

Root rot pathogens	Weeks after incorporation									
	2016 Short rains					2017 Short rains				
	0	7	14	28	Mean	0	7	14	28	Mean
Koibem										
F. solani	60.0_{a}	60.0_{a}	66.7_{a}	50.0_{a}	59.2_{a}	81.1_{a}	64.4_{a}	63.3_{a}	58.9_{a}	69.9_{a}
F. oxysporum	48.3_{a}	20.0_{b}	23.3_{a}	33.3_{ab}	31.2_{b}	85.6_{a}	67.8_{a}	60.0_{a}	61.1_{a}	68.6_{a}
M. phaseolina	45.0_{ab}	20.0_{b}	25.0_{a}	11.7_{bc}	25.4_{b}	15.6_{b}	12.2_{b}	7.8_{b}	15.6_{b}	12.8_{b}
P. ultimum	41.7_{b}	10.0_{b}	6.7_{b}	5.0_{c}	15.9_{b}	14.4_{b}	3.3_{b}	11.1_{b}	11.1_{b}	9.9_{b}
R. solani	25.0_{c}	18.3_{b}	0.0_{b}	10.0_{bc}	13.3_{b}	25.6_{b}	12.2_{b}	10.0_{a}	13.3_{b}	12.5_{b}
Mean	44.0	25.6	24.3	22.0	29.0	44.5	31.9	30.4	32.0	34.7
LSD ($p \leq 0.05$)	15.7	24.4	32.3	23.6	22.8	27.4	20.9	26.0	28.1	39.1
P value	0.001	0.01	<0.001	<0.001	0.01	<0.001	<0.001	0.01	0.032	0.01
Kapkerer										
F. solani	73.3_{a}	55.0_{a}	71.7_{a}	66.7_{a}	66.7_{a}	57.8_{ab}	60.0_{a}	62.2_{a}	72.2_{a}	63.1_{a}
F. oxysporum	50.0_{ab}	21.7_{b}	30.0_{b}	31.7_{b}	33.4_{b}	71.1_{a}	67.8_{a}	64.4_{a}	64.4_{a}	66.9_{a}
M. phaseolina	33.3_{bc}	25.0_{b}	40.0_{b}	6.7_{c}	26.3_{b}	32.2_{c}	6.7_{b}	11.1_{b}	7.8_{b}	14.5_{b}
P. ultimum	13.3_{c}	13.3_{bc}	18.3_{b}	18.3_{bc}	15.8_{b}	45.6_{bc}	8.9_{b}	18.9_{b}	18.9_{b}	23.1_{b}
R. solani	41.7_{b}	15.0_{bc}	25.0_{b}	25.0_{bc}	26.7_{b}	26.7_{c}	8.9_{b}	22.2_{b}	5.6_{b}	15.9_{b}
Mean	42.3	26.0	37.0	29.7	33.8	42.3	30.5	35.8	33.8	36.7
LSD ($p \leq 0.05$)	27.4	20.9	26.1	23.6	24.2	22.6	38.1	31.6	39.8	32.4
P value	<0.001	<0.001	0.01	<0.001	0.01	0.001	<0.001	<0.001	<0.001	<0.001

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

4.4.4 Effect of different application times of green manure on the population of microorganisms isolated from the soil

A significant ($p \leq 0.05$) variation in fungal population was found between treated plots (Table 4.6). In all the treated plots, the highest cfu of root rot pathogens was detected for *F. solani*, followed by *F. oxysporum*, and *Pythium*. In the two seasons, the population of *F. solani*, and *F. oxysporum* was highest in Kapkerer than in Koibem. In the two seasons, plots treated with lablab green manure at planting in both sites had the highest population of both *F. solani* and *F. oxysporum*, those treated with green manure seven days before planting had the least population while the population of *Pythium* remained low

Table 4.6. Population (cfu g^{-1} soil $\times 10^4$) of root rot pathogens recovered from soil after incorporation of green manure at different times

Days after incorporation	Koibem			Kapkerer		
	F. solani	*F. oxysporum*	*Pythium*	*F. solani*	*F. oxysporum*	*Pythium*
2016 Short rains						
0 days	1.9ab	1.6a	0.5ab	3.1a	1.5ab	0.3a
7 days	1.6b	0.9b	0.4b	2.0b	1.7a	0.2a
14 days	2.0a	1.6a	0.4b	2.4b	1.6a	0.3a
28 days	1.9ab	1.7a	0.8a	2.9ab	0.9b	0.3a
Mean	1.9	1.5	0.5	2.8	1.4	0.3
LSD ($p \leq 0.05$)	0.3	0.6	0.3	0.8	0.6	0.13
P value	<.001	<.001	<.001	<.001	0.054	0.557
2017 Short rains						
0 days	2.6a	0.9b	0.3a	3.5a	2.1a	0.5b
7 days	2.2ab	1.6a	0.2a	2.1b	1.3b	0.5b
14 days	2.2ab	1.6a	0.3a	1.8b	1.3b	0.8a
28 days	1.8b	1.0ab	0.3a	3.0ab	1.6ab	0.6ab
Mean	2.2	1.3	0.3	2.6	1.6	0.6
LSD ($p \leq 0.05$)	0.56	0.63	0.04	1.2	0.64	0.22
P value	0.014	0.070	0.125	0.05	0.01	0.470

Values followed by the same letter within the same column are not significantly different between root rot pathogens using Fishers Protected LSD test ($P \leq 0.05$).

Root rot fungal population varied under different incorporation times (Figure 4.1). The highest population was observed 14 days after incorporation of lablab green manure. The population of both *F. oxysporum* and *F. solani* was significantly higher in plots incorporated at planting and showed an increasing trend at the second week after crop emergence then drastically reduced four weeks later. Two weeks after emergence, in plots with lablab incorporated at planting the population of *F. solani* increased from 2.4 to 3.6×10^4 cfu g^{-1} while that of *F. oxysporum* increased to 1.8×10^4 cfu g^{-1} then drastically reduced four weeks after emergence reduced. The population of *Pythium* remained low and slightly reduced from the initial population then slightly increased six weeks after emergence.

In plots incorporated with lablab green manure seven days before planting, *F. solani* population varied from 2.8 to 1.4×10^4 cfu g^{-1} while that of *F. oxysporum* varied from 1.8 to 0.4×10^4 cfu g^{-1} soil under different sampling times. *Pythium* spp. populations in soil showed slight fluctuations, increasing between the first and the second sampling times. The population of *F. solani* slightly increased from 1.9 to 2.6×10^4 cfu g^{-1} then reduced while that of *F. oxysporum* reduced from the initial population to 1.0×10^4 cfu g^{-1} soil six weeks after crop emergence in plots incorporated with lablab 14 days before planting. The population of *Pythium* was low however, it slightly increased from the initial population then reduced to near zero six weeks after emergence. Plots incorporated with lablab 28 days before planting had population of *F. solani* increased from 2.4 to 2.5×10^4 cfu g^{-1} then decreased while that of *F. oxysporum* rose up to 1.8×10^4 cfu g^{-1}. The population of *Pythium* remained low over the entire sampling period however, it slightly increased six weeks after emergence.

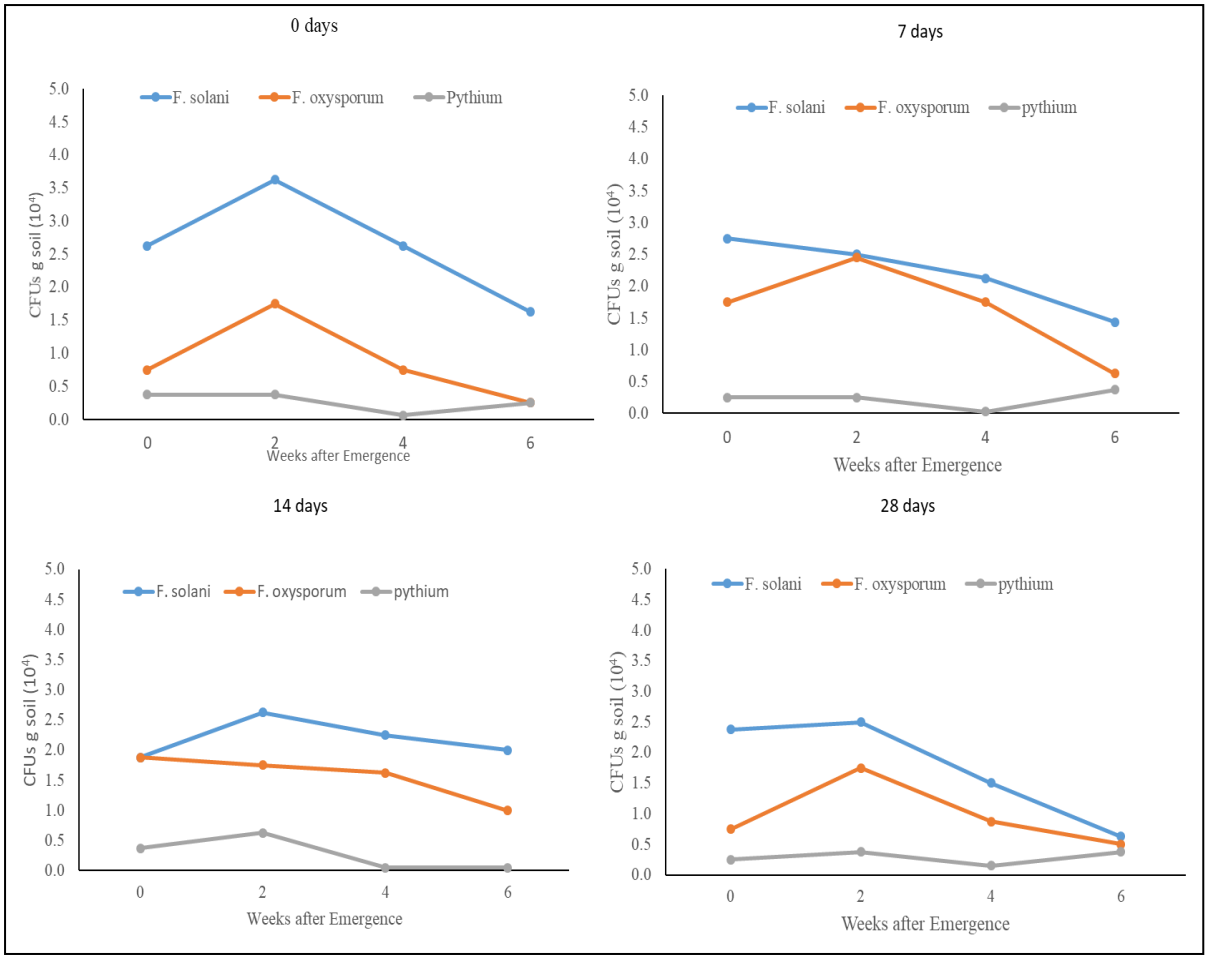

Figure 4.1. Population (cfu g^{-1}) ($\times 10^4$) of root rot pathogens in plots incorporated with lablab green manure at different times during the short rains of 2016

In 2017 season, the populations of root rot pathogens in plots incorporated with lablab green on the day of planting significantly ($P \leq 0.05$) increased rapidly two weeks after emergence while there was little variation in population in other plots (Figure 4.2). It was observed that the lablab green manure increased the population of *F. oxysporum* and *F. solani* 14 days after the treatment application from 3.0 to 4.5×10^4 cfu g^{-1} soil and 2.0×10^4 cfu g^{-1} to 2.5×10^4 cfu g^{-1} respectively. *Pythium* spp. showed little fluctuation under treated conditions, increasing slightly from 0.8×10^4 cfu g^{-1} soil to 1.3×10^4 cfu g^{-1} soil then reduced to near zero 28 days after treatment applications. In plots incorporated with lablab green manure seven days before planting, the population of *F. solani* slightly increased from 2.5 to 3.0×10^4 cfu g^{-1} while the population of *F. oxysporum* increased from 1.5 to 2.0×10^4 cfu g^{-1} soil two weeks

after crop emergence. However, the population then reduced drastically to low levels as the initial populations. *Pythium* spp. populations showed insignificant (P≤0.05) variations, increasing between the first and the second sampling times then reducing to very low levels. In plots with lablab incorporated 14 days before planting, the population of both *F. solani* and *F. oxysporum* slightly increased from 1.5 to 2.2×10^4 cfu g^{-1} then reduced from the initial population soil six weeks after crop emergence. The population of *Pythium* remained low but slightly increased from the initial population then reduced to near zero six weeks after emergence. In plots with lablab incorporated 28 days before planting, the population of *F solani* reduced from 3.1 to 2.3×10^4 cfu g^{-1} then increased to 3.6×10^4 cfu g^{-1} while that of *F. oxysporum* increased to 1.8×10^4 cfu g^{-1} then reduced. The population of *Pythium* remained low over the entire sampling period then slightly increasing two weeks after crop emergence.

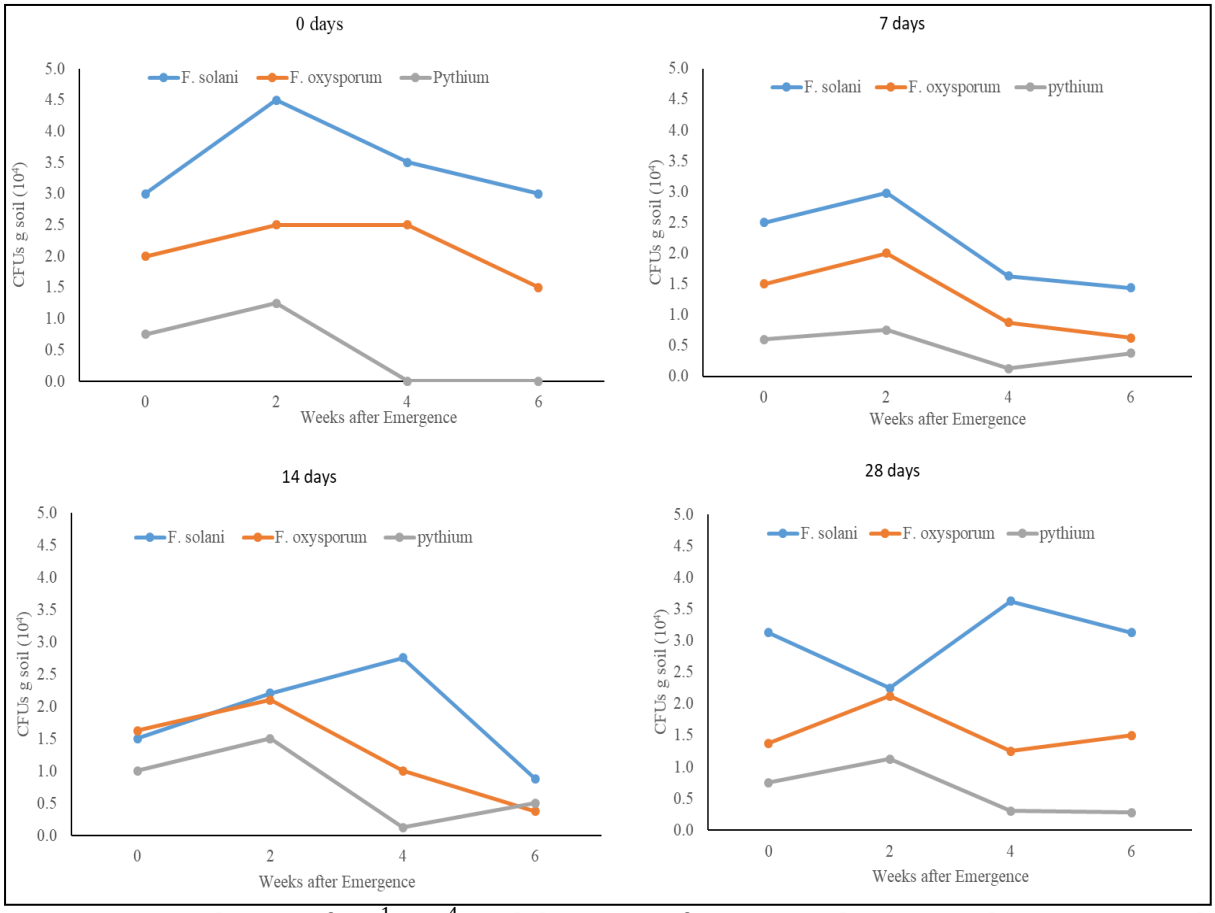

Figure 4.2. Population (cfu g^{-1} $\times10^4$) soil dynamics of root rot pathogens in plots incorporated with lablab green manure at different times in 2017 short rains

4.4.5: Effect of different application times of green manure on the population of saprophytic fungi in the soil

Aspergillus, Penicillium and *Trichoderma* were isolated from the samples (Table 4.7). In both seasons the population of *Aspergillus, Penicillium* and *Trichoderma* was high in the plots incorporated 14 days before planting and minimum in plots incorporated with green manure at planting in both sites. A significant ($p \leq 0.05$) variation in saprophytes population was found between treated plots in the second season. In Koibem, the lowest number of total lowest population of *Aspergillus, Penicillium* and *Trichoderma* was observed in plots incorporated with lablab green manure seven days before planting and the highest fungal colonies was found in the soil treated 28 days before planting. However, in Kapkerer, the plots treated with lablab green manure seven and 14 days before planting had the highest population while those treated lablab at planting had the least

Table 4.7. Population (cfu $g^{-1} \times 10^4$) of saprophytes recovered from soil treated with lablab green manure at different times

Days after incorporation	Koibem				Kapkerer			
	Asper	*Pen*	*Tricho*	Mean	*Asper*	*Pen*	*Tricho*	Mean
2016 Short rains								
0 days	0.4_a	1.3_{ab}	0.9_b	0.8_b	0.8_a	0.9_b	1.3_a	1.0_a
7 days	1.0_a	2.1_a	1.1_{ab}	1.4_{ab}	0.4_a	1.1_b	1.4_a	0.9_a
14 days	1.8_a	1.8_a	1.7_a	1.8_a	0.9_a	1.1_b	1.2_a	1.1_a
28 days	0.4_a	1.1_b	0.9_b	0.8_b	0.9_a	1.2_a	1.2_a	1.1_a
Mean	0.9	1.6	1.1	1.2	0.7	1.3	1.3	1.0
LSD ($p \leq 0.05$)	1.02	0.8	0.6	0.8	0.4	0.4	0.2	0.15
P value	0.249	0.063	0.019	0.001	0.302	0.333	0.658	0.269
2017 Short rains								
0 days	0.9_a	1.3_a	1.1_a	1.1_a	0.3_b	1.2_b	0.9_b	0.7_b
7 days	0.5_b	0.9_b	0.9_b	0.8_b	1.4_a	1.5_a	1.4_a	1.4_a
14 days	0.4_b	1.3_a	1.2_a	0.9_{ab}	1.3_a	1.6_a	1.1_a	1.3_a
28 days	0.8_{ab}	1.4_a	1.3_a	1.1_a	0.6_b	1.2_b	0.9_b	0.8_b
Mean	0.65	1.22	1.13	1.00	0.9	1.35	1.09	1.1
LSD ($p \leq 0.05$)	0.36	0.34	0.22	0.25	0.83	0.36	0.35	0.6
P Value	0.083	0.128	0.012	0.056	0.07	0.001	0.09	0.045

Values followed by the same letter within the same column are not significantly different between the saprophytes using Fishers Protected LSD test ($P \leq 0.05$). Asper – *Aspergillus*, Pen- *Penicillium*, Tricho- *Trichoderma*

After incorporation of fresh lablab tissues the mean population of *Aspergillus*, *Trichoderma*, *Penicillium* had different responses, however, there was no significant differences ($p \leq 0.05$) between the treatments in both seasons. The population of saprophytic fungi in plots incorporated with green manure at planting was the lowest when compared with other incorporation times, however, there was increase in the population from the fourth week after crop emergence. In Koibem, the population of *Aspergillus* (7500 cfu g^{-1}), *Trichoderma* (20000 cfu g^{-1}) and *Penicillium* (15000 cfu g^{-1}) were lower in plots incorporated with lablab green manure at planting, compared to other incorporation times. In Kapkerer, the population of *Aspergillus*, *Trichoderma* and *Penicillium* increased from the original population at planting and the highest was recorded in plots incorporated 14 days before planting. In both sites the population of *Aspergillus*, *Trichoderma* and *Penicillium* slightly increased when compared with the initial saprophytic population level at planting.

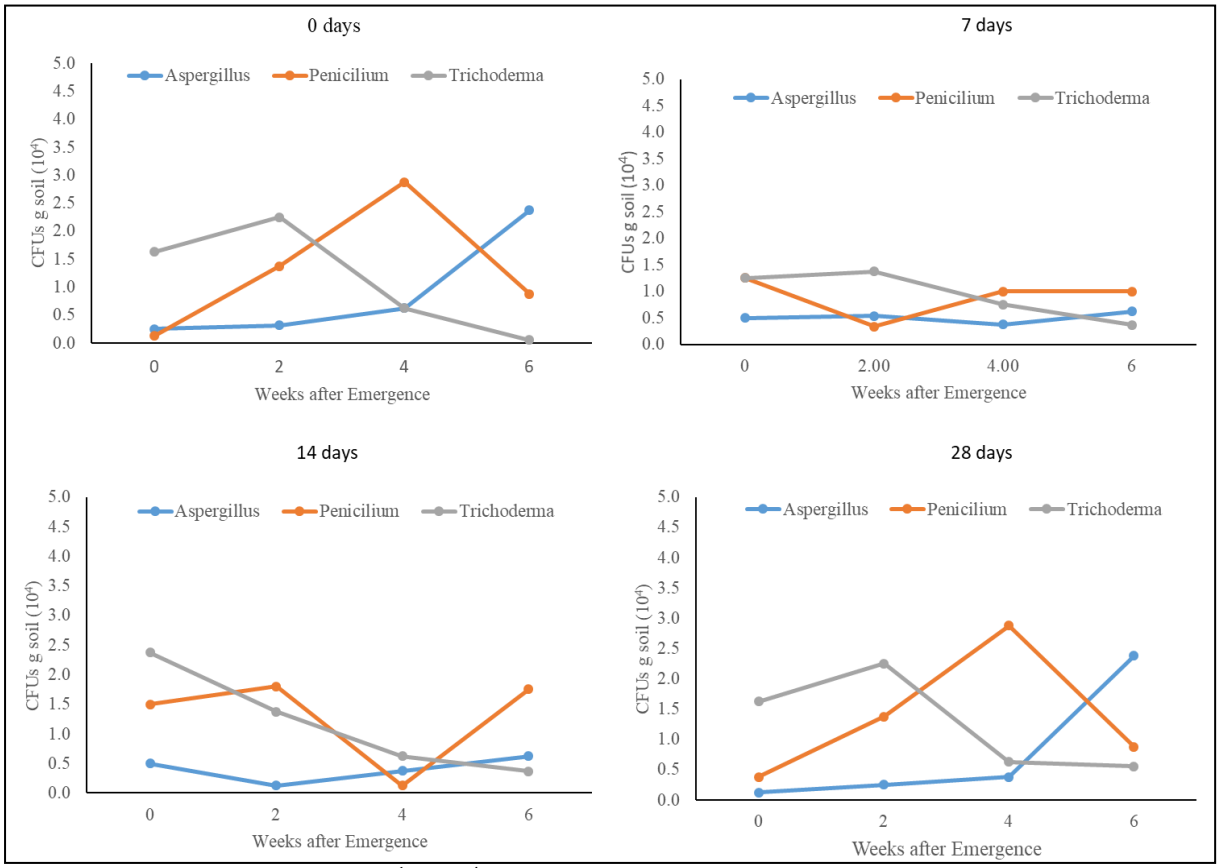

Figure 4.3. Population (cfu g^{-1} $\times 10^4$) of saprophytic fungi isolated from plots incorporated with lablab green manure at different times in the short rains of 2016

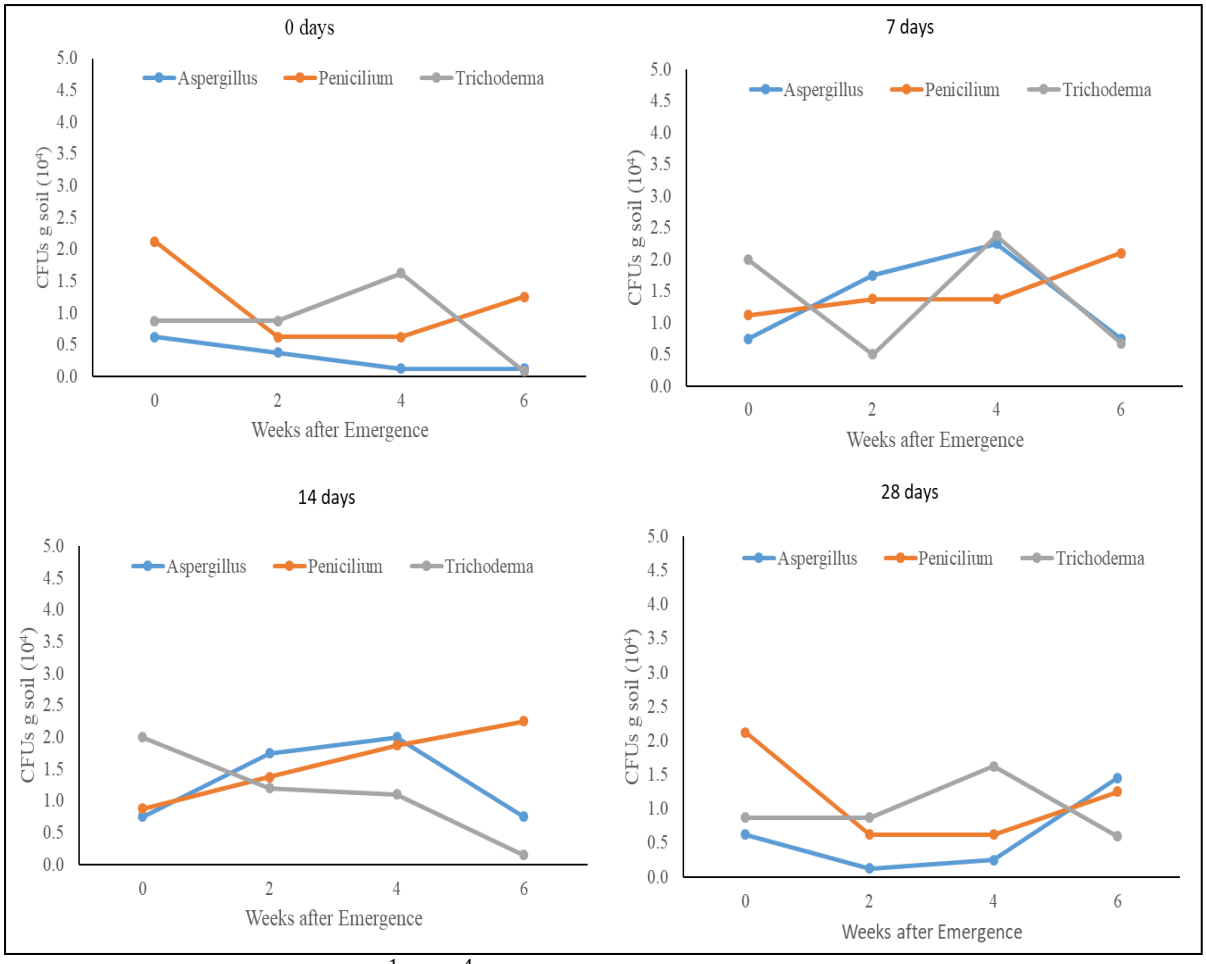

Figure 4.4. Population (cfu g^{-1} $\times 10^4$) of saprophytic fungi isolated from plots incorporated with lablab green manure at different times in 2017 short rains season

4.4.6 Effect of different application times of lablab green manure on the population of bacteria in the soil

Bacterial densities did not vary significantly among the different incorporation times (Table 4.8). However, plots incorporated with lablab green manure 28 days before planting in both seasons had high bacterial densities than soils from other plots. In particular, plots incorporated with lablab green manure seven days before planting and on the planting day had lower densities of *Bacillus* spp. There were no significant differences in both sites and in both seasons among the treatments ($p \leq 0.05$). The bacterial population varied from 13 to 28 $\times 10^4$cfu g^{-1} soil in Koibem and 7.0 to 15×10^4 cfu g^{-1} soil in Kapkerer under different treatment application times.

Table 4.8. Population (cfu g^{-1} $\times 10^5$) of bacteria isolated from soils incorporated with lablab at different times

Days after incorporation	Koibem			Kapkerer		
	2016 Short rain	2017 Short rain	Mean	2016 Short rain	2017 Short rain	Mean
0 days	17.3_{ab}	10.5_{a}	13.9_{ab}	0.0_{b}	13.5_{a}	6.8_{b}
7 days	8.3_{ab}	17.8_{a}	13.0_{ab}	2.0_{b}	15.8_{a}	8.9_{b}
14 days	25.3_{ab}	4.8_{a}	15.0_{ab}	7.0_{ab}	14.8_{a}	10.9_{ab}
28 days	38.3_{a}	18.3_{a}	28.3_{a}	15.5_{ab}	14.5_{a}	15.0_{ab}
Mean	22.3	12.8	17.5	6.1	14.6	10.4
LSD ($p \leq 0.05$)	20.1	11.2	11.4			
CV (%)	121.3	69.9	70.3			

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

4.4.7: Effect of different green manure incorporation times on yield of common beans

Yield attributes of common beans were significantly ($P \leq 0.05$) affected by different times of green manure application (Table 4.9). The grain yield was significantly different ($P \leq 0.05$) in both cropping seasons. In 2016 season, incorporation of green manure 28 days before planting resulted in high grain yields in both sites, Koibem yielded above 5tonnes /ha^{-1} while Kapkerer recorded less than 1ton/ha^{-1}. Plots incorporated at planting recorded the lowest means for biomass and grain yield in both the sites. Nonetheless, plots incorporated with lablab green manure seven and fourteen days before planting improved yield than plots treated with green manure at planting. In Koibem, the highest biomass was observed in plots treated with green manure 14 and 7 days earlier while in Kapkerer in plots incorporated 28 days before planting recorded the highest biomass weight. During the second season, plots incorporated 28 days before planting recorded the highest grain yield of 4.5tons/ha^{-1} in Kapkerer and 3.8tons/ha^{-1} in Koibem while those that were incorporated at planting recorded the least. However, there were no significant variations ($P \leq 0.05$) in terms of grain yields in

the second season. Plots planted immediately after incorporation gave consistently the lowest yield (average 1.0 t/ha^{-1}) across all seasons. The highest biomass was found in plots treated with green manure 28 days before planting in Kapkerer while in Koibem highest biomass per Kg was recorded in plots that had been incorporated with green manure 7 days earlier. Plots planted immediately after incorporation had the lowest biomass yield in both sites in both seasons.

Table 4.9. Grain and biomass yield of common beans affected by different incorporation times of lablab green manure

Days after incorporation	Koibem		Kapkerer	
	Plant Biomass (kg/ha)	Grain Yield (t/ha)	Plant Biomass (kg/ha)	Grain Yield (t/ha)
2016 Short rains				
28 Days	1176.0a	5.1a	613.0a	0.4ab
14 Days	1470.0a	5.2a	329.0a	0.5a
7 Days	1578.0a	5.1a	541.0a	0.3b
0 Days	251.0b	0.4b	181.0a	0.1c
Mean	1119.0	3.9	416.0	0.3
LSD ($p \leq 0.05$)	727.7	2.9	562.0	0.2
CV (%)	25.0	21.3	42.5	25.6
P value	<.001	<.001	0.454	<.001
2017 Short rains				
28 Days	310.5a	3.7ab	251.8a	4.3ab
14 Days	236.8a	2.3ab	179.2a	3.2a
7 Days	123.8b	1.3ab	124.2ab	2.3a
0 Days	116.8b	1.1b	113.0b	1.1b
Mean	196.9	3.9	167.1	2.9
LSD ($p \leq 0.05$)	102.6	3.2	102.6	3.2
CV (%)	29.7	54.2	29.7	54.2
P value	<.001	<.001	<.001	<.001

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

4.4.8 Relationships among root rot pathogens, severity and yield

For bean root rot, the isolation frequency of *F. oxysporum* and *Pythium ultimum* was positively correlated with severity (r = 0.3239, r= 0.209, $p \leq 0.05$) but was negatively correlated with emergence (r = -0.1532, r= -0.3042, $p \leq 0.05$). Seedling emergence showed negative relation with root rot pathogens and positive correlation with grain yield. However, root rot severity showed a positive insignificant correlation with yield (r = 0.0049, $p \leq 0.05$). However, the correlation coefficient revealed that grain yield was positively correlated with emergence (r = 0.2016, $p \leq 0.05$) but yield (r = - 0.1296, $p \leq 0.05$) showed negative and significant association with root rot pathogens (Table 4.10)

Table 4.10: Correlation coefficients among roots rot pathogens, severity of root rot, biomass and grain yield

	F. oxysporum	*F. solani*	*Pythium*	Emergence	Severity	Biomass (Kg/ha)	Yield (t/ha)
F. oxysporum	-						
F. solani	0.2256	-					
Pythium	0.3149	-0.147	-				
Emergence	-0.1532	-0.304	-0.2551	-			
Severity	0.3239	-0.053	0.2069	-0.4505	-		
Biomass (Kg/ha)	0.0435	-0.048	0.0444	-0.4794	0.3514	-	
Yield (t/ha)	-0.1296	-0.348	-0.2278	0.2091	0.0049	0.3468	-

4.5 Discussion

4.5.1 Effect of different green manure application times on common bean emergence and establishment

Although seedling emergence significantly varied amongst the different lablab green manure application times, incorporation at planting in both sites and in both seasons had considerable negative effect on emergence and early establishment of beans in the field. In contrast, plots incorporated seven days earlier had none or only slight effects on common beans emergence. It has been demonstrated that increasing crop residues on top of the seed can reduce emergence, plant establishment, growth and, in some instances, yield (Hiel *et al*., 2016). The high germination percent in other plots incorporated earlier with green manure can be attributed to high porosity, aeration, water holding capacity, and presence of humic like substances produced during decomposition of green manures (Sarma and Gogai 2015). The enhancement of seed germination may be attributed to organic manure allowed to decompose enhancing availability of macro and micronutrients in soil for germination (Vaithiyanathan and Sundaramoorthy, 2016). Green manure residues allowed to decompose at field capacity become lethal during the initial stages of decomposition therefore, the inhibition in germination varied with decomposition period. Similar results were reported by Al-Harun *et al*. (2015). Crop residues when incorporated in the soil reduce germination and thus results in poor seedling establishment (Conklin *et al*., 2002; Yerima *et al*., 2015). The effect of green manure on emergence of crops is associated with the stress experienced by seeds during green manure breakdown. Plant residues should be placed away from the sowing rows to lower the problems associated with poor crop growth but still maintain benefits of plant residues.

Organic materials are made of large quantities of easily decomposable organic matter that have harmful effect on crops. Within four to seven days, the carbon dioxide production

reaches maximum when easily decomposable green manure is incorporated (Shiga, 1997). Heavy application of these types of organic matter may result in oxygen deficiency in the plant roots and production of substances that are harmful to plants thus reducing conditions necessary for crop growth. Composting materials before being incorporated improves the physical and chemical conditions of the soil (Shiga, 1997).

The effect of green manure on crops depend on factors such as environment, nature and quantity of the green manure, levels of moisture during decomposition and timing of sowing following green manure incorporation (Conklin *et al*., 2002). Green manure from cowpea incorporated before planting broccoli crop resulted in poor germination (Schroeder *et al*., 1998). Our results confirm the need to synchronize the date of green manure biomass incorporation to the soil and the crops needs. Green manure incorporation from 14 up to 28 days before planting allows the reduction of green manure dose and thus improves crop emergence and establishment. Haramoto and Gallandt. (2005) reported a reduction in crop emergence by about 23 to 33% following crimson clover green manure application. Reduction in emergence due to crimson clover green manure has been noted and was attributed to either nitrogen immobilization or phytotoxins release.

Nitrate levels are always depressed following legume green manure legume incorporation compared with synthetic nitrogen fertilizer. Decomposition of incorporated legume green manure may discharge large flush of ammonia that is toxic to newly germinating seedlings. Slow decomposition of organic matter under drier conditions may induce inhibition of germination and initial growth of a consecutive crop. However, nutrient immobilization hypothesis presupposes that incorporation of undecomposed green manure reduces the available nutrients, which therefore result in reduced shoot: root ratio because of more energy being allocated to the root system for the limiting resources (Bonanomi *et al*., 2017). We

observed the opposite response, with a sharp increase in the shoot: root ratio after the addition of undecomposed lablab green manure. This conforms to the work by Haramoto and Gallandt. (2005) who suggested that crimson clover effects on emergence could be as a result of released phytotoxins in addition to possible nitrogen immobilization or ammonia phytotoxicity during the breakdown. Each material should be utilized and processed on the basis of its characteristics, and the objective of its utilization.

4.5.2 Effects of different application times of green manure on root rot incidence and severity

High incidences and severity with large area under disease progress curves were observed in Kapkerer than in Koibem where soil fertility is regarded as low in both seasons. The lower incidence of root rot in plots that had earlier been incorporated is due to the greater decomposability of lablab green manure. In these areas, there has been decline in soil fertility due to intensive farming practices as well as high population of root rot pathogens. Root rot pathogens are more devastating in soils of low fertility (Ahanger *et al*., 2013). From our results, low population of root rot pathogens were observed in plots that had earlier been incorporated with lablab green manure confirming earlier findings that root rot pathogens are low in high fertility soils (Muthomi *et al*., 2014).

Root rot pathogens isolated from stem bases of beans included *F. oxysporum*, *F. solani*, *Pythium* spp, *Macrophomina* and *Rhizoctonia* spp with Kapkerer having the highest infection rate compared to Koibem because of the low fertility status of farms. Kapkerer site has been under cultivation for over 80 years compared to Koibem which has been under cultivation for a period of between 5 to 30 years (Odundo *et al*., 2010). Continuous cropping changes soil physiochemical parameters and microorganisms thereby affecting negatively soil fertility leading to decline in crop productivity (Li *et al*., 2016). Susceptible crops cultivated overtime

increases the population density of pathogen in soil and this is directly proportional to the disease intensity in the crop (Bareja *et al*., 2010).

4.5.3 Effect of different lablab green manure application times on soil microbial population

Different application times of lablab green manure had significant impacts on soil microbial community composition and structure. Among microbial populations that are reported to be related to bean health, *Fusarium* was the most abundant isolated from all the soil samples. Fourteen days after application of lablab residues, high densities of *F. oxysporum*, *F. Solani* and *Pythium* spp. were observed. Gomez *et al*. (2016) reported similar fungal species in their study. They established that these pathogens predominated over other fungal populations because they are capable of using different substrates in the soil. There were low incidences of other fungal genera since the saprophytic fungi were relatively high.

The populations of both pathogenic and non-pathogenic microbes are influenced by factors such as the nature of green manure, degree of decomposition, carbon: nitrogen ratio, time of application and amount of fresh organic residues applied (Van Bniggen and Termorshuizen, 2003). The quality of the organic matter determines whether beneficial or facultative pathogens multiply fast (Litterick *et al*., 2004). Fresh or barely decomposed organic residues may lead to short-term upsurge in populations of soilborne pathogens such as *Rhizoctonia* and *Pythium* spp. because they reproduce easily in such materials. Van Bniggen and Termorshuizen, (2003) reported an increase in severity of Pythium damping-off between 7 – 10 days after incorporation of cover crops. In view of the risks associated with green manure incorporation, although temporary, the timing of incorporation in relation to planting is very critical.

There is much difference between fresh green manure and those that are partially decomposed. Increased disease pressure following green manure application is because organic manures provide environments that are not conducive for growth and explosion of antagonistic microorganisms which is characterized by high salt and ammonium concentrations, and lack of oxygen (Litterick *et al*., 2004). Use of composts that vary in salinity, nitrogen availability, and degree of decomposition may lead to increase in disease incidence and severity (Walters, 2009)

Direct incorporation of lablab amendments increased bacterial and fungal population initially, indicating that the fresh lablab promotes long-term stimulation of the microbial population. According to Elfstrand *et al*. (2007) the increase in population could be due to high availability of carbon compared with the processed forms fertilizer, resulting in faster growth of microorganisms after direct incorporation. They further noted that, timing of incorporation is the cause for the observed differences, since direct incorporation of the red clover ley was carried out two weeks before the incorporation of slurry and compost

4.6 Conclusion

Common bean plant emergence decreased linearly with delay in green manure application. The highest emergence was attained in plots incorporated 28 days earlier. Incorporation of green manure at planting results in reduced plant growth, underlying the importance of allowing green manure to decompose. Green manure had significant impacts on soil microbial community composition and structure. Among microbial populations that are reported to be related to bean health, *Fusarium* was the most abundant. The efficiency of green manure depends on the time of biomass application. Residues must be allowed to decompose sufficiently and be colonized to allow microbiostasis. Immature residues frequently contain toxic compounds that affect growth of plants and predispose plant root to

attack by diseases. The results confirm the need to synchronize the date of green manure application to the soil and the crop needs. Incorporation of green manure incorporation earlier 28 days before planting allows for the reduction of the dosage of green manure that is lethal to plant germination and establishment, resulting in increased yield.

CHAPTER FIVE
EFFECT OF LEGUME EXTRACTS ON ROOT ROT PATHOGENS AND GERMINATION OF COMMON BEANS

5.1 Abstract

Application of some undecomposed green manure causes poor germination and crop establishment in the field. Therefore, mechanisms' contributing to poor establishment after incorporation were determined by evaluating the effect of fresh and decomposed extracts of lablab in comparison with other legumes on bean germination, mycelial and bacterial growth, spore germination and germtube elongation. Antagonistic activity amongst saprophytes and pathogenic microorganisms was also evaluated in dual cultures. Data was collected on seed germination, mycelial growth, spore germination, and elongation. Fresh lablab extracts prepared with ethanol showed inhibitory effects on germination accompanied by increased mean germination time, and decrease in germination index while ethanol extracts of groundnut and beans caused highest inhibition in beans shoot length and reduced biomass. Fresh ethanol extracts significantly inhibited mycelial growth of the tested fungi, aqueous extracts from beans, groundnuts and soybean had significant level of antifungal activity while aqueous lablab extracts stimulated mycelial. Aqueous extract of lablab and soybean enhanced spore germination by over 70% with more pronounced effect on germtube length and number of germtubes by 8.0% and 13% respectively. The antagonists *Penicillium, Paecelomyces* and *Trichoderma* effectively checked the growth of root rot pathogens with *Trichoderma* inhibiting radial growth by 64%. This study comparatively reveals that the extract of lablab was inhibitorier to common bean germination compared to other legume extracts and also stimulated the growth of root rot pathogens thus poor establishment of beans.

Key words: Antagonists, legume extracts, microbial decomposition, root rot pathogens

5.2 Introduction

Common bean is an important food crop as well as soil improvers (Masangwa *et al*., 2012). Regardless of their economic importance, farmers have been successful to realize potential yields because of several limiting factors chief among them low soil fertility. Green manures have been introduced as way of improving soil nutrient fertility. However, upon decomposition these crops introduce other problems as they release secondary metabolites that can be phytotoxic to succeeding crops (Kaur *et al*., 2012). However, there phytotoxicity depends on the amount of plant residues, the environment of decomposition, duration of decomposition, residue placement and weathering (Jilani *et al*., 2008; Kaur 2012). These phytotoxins have specific communication in terms of growth inhibition and stimulation and they are either inhibitory or stimulatory to crop growth and microorganisms in the soil (Farooq *et al*., 2013; Cheng and Cheng, 2015).

The toxic chemicals released into the soil during breakdown of the residues may cause severe inhibition to germination, however, the concentrations phytotoxins decline as decomposition proceeds (Jilani *et al*., 2008, Bonanomi *et al*., 2017). The chemicals released alter the plant environment which may result in either poor crop germination or reduced growth (Herro and Callaway, 2003) and the seeds allowed to germinate in such environments require more time for germination (Ayub *et al*., 2012). Lertmongkol *et al*. (2011) reported that continuous cropping of mung bean led to plant growth inhibition by between 10 to 25% of successive crop growth.

Substances capable of inhibiting germination and growth of seedlings arise under some conditions of decomposition. Production of phytotoxic substances depends on residue maturity, water content, pH and length of decomposition (Al-Mughraby, 2003). Aqueous extracts of crop residues contain toxic substances that can greatly delay germination and

reduce shoot and root length of crops (Dhole *et al*., 2013; Chukwuka *et al*., 2014; Afzal *et al*., 2015). However, the chemical contents of the residues differ based on the nature of the solvents used in the extraction process as higher quantities of phenolics have been consistently isolated in alcohol extracts (Jules *et al*., 2011). Therefore, seeds treated with extracts results in lower germination percentage and the inhibition or stimulation is as a result of phytotoxins released by a crop during the growth or when decaying.

Compost and compost extracts improve soil quality by changing chemical and physical properties of soil, increasing organic matter content, water holding capacity, general diversity of microbes, providing macro- and micro-nutrients crucial for plant growth and suppressing diseases thereby improving plant health. The effect of the residues raises the question about the use of green manure in the field. While this practice has practical value in enhancing soil nutrients, the residues contain substances that affect germinations and growth of beans. The objective of this study was to investigate the effect of lablab extracts in comparison with other legume extracts on establishment of common beans and on the growth of soil microorganism.

5.3 Materials and methods

5.3.1 Preparation of various legume extracts

Compost preparation was done following the method described by Inckel *et al*., (2002) with minor modification. Chopped portions of green manure were piled in compost bin then a thin layer of soil was added to obtain the microorganisms needed for composting and covered. Water was occasionally added to maintain 60% moisture content, and turned once per week to maintain porosity and facilitate homogenous decomposition and maintain equilibrium between microbial activities. To determine compost maturity, periodic temperature readings using a soil thermometer were taken until a constant temperature of about 30.5^{o}C was

obtained. Once decomposed, the compost was macerated using a blender in sterile distilled water and in 80% ethanol separately in the ratio of 1:10 as legume based compost extracts (Cieniak *et al*., 2015). Separately, fresh chopped legume residues were macerated following the procedure stated above. The mixture was left standing for 2 hours then the macerated mass of fresh and compost materials was squeezed through three layers of sterile cheese cloth and separated by filtration using Whatman no.1 filter paper (Whatman plc, Maidstone, Kent, UK) and stored at 4^{o}C until further use. Solvent was evaporated at lower temperature under reduced pressure in rotary flash evaporator to get the crude extracts (Hadi *et al.*, 2013). Two filtrates obtained served as 100% aqueous and ethanol based extracts of the legume plants

5.3.2 Determination of the effect of legume extracts on bean seed germination

Four legume extract of common bean, lablab, soybean and groundnut at 100% concentration were used for seed bioassay. Common bean seed variety GLP2 were washed in tap water and surface sterilized in 5% sodium hypochlorite for two minutes. The seeds were rinsed in four changes of sterile distilled water after which fifty uniform bean seeds were soaked in 200ml of different legume extracts overnight then sown in moist chamber lined with sterile paper towel. In each moist chamber, 20 ml of each aqueous extract was used to wet the seeds while sterile distilled water was used for the control (Chukwuka *et al*., 2014). The treatments were replicated four times in a completely randomized design and repeated twice. The moist chamber boxes were incubated and seeds allowed to germinate. After incubation, the shoot lengths of 10 randomly selected seedlings were measured with a ruler and digital slide calipers. Dry weights of seedling were measured by electric digital balance at fourteen days of age. The germination percentage determined using the formula:

$$\text{Percent germination (\%)} = \frac{\text{number of seeds germinated}}{\text{number of seeds used in bioassay}} \times 100.$$

Mean germination time was calculated using the equation by Dezfuli *et al*. 2008. MGT = ΣDn/ Σn where N: Number of seeds which were germinated on day D, D: Number of days counted from the beginning of germination

5.3.3 Determination of the effect of legume extracts on fungal mycelial growth

Stimulatory or inhibitory effects of the legume extracts on mycelial growth were tested using poison food technique. Two milliliters of each legume extract was dispensed per petri dish and 15ml of molten potato dextrose agar (PDA) added. Two milliliters of sterile distilled water was dispensed in control plates. The plates were gently rotated to ensure even spread of the extracts and allowed to solidify. Petri dishes containing PDA amended with 2ml of legume extracts and those with sterile water were inoculated with 5mm of mycelial discs cut from actively growing 8 day old cultures of *Pythium, Fusarium, Aspergillus* and *Trichoderma* at the centre of each plate. Radial growth was measured each day from the second day after incubation at 25°C, until the 6th day. The percentage growth inhibition of each extract was calculated by the formula Percent inhibition = $\frac{\text{growth in control} - \text{growth in sample}}{\text{growth in control}} \times 100$ (Hadi *et al*., 2013)

5.3.4 Determination of the effect of legume compost extracts on spore germination

Spore germination assay was done according to Nollet and Rathore (2010). *Fusarium* was grown on PDA medium and spores were harvested after 10 days of incubation when cultures were fully sporulated. The spores were collected by adding 5ml of sterile water with tween 80 0.1% (v/v) to each petri dish and scrapping the surface using sterile glass slide. The suspension collected was centrifuged at 25^{o}C at 2000r/min for five minutes and the supernatant was discarded and pellet re-centrifuged until a highly concentrated spore solution remained. A haemocytometer slide was used to count the spore production to have

approximately 10^2 spores/ ml. Using sterile pipette, a drop 50µl of spore suspension was mixed with 50µl of the extract in a cavity of sterile slides. The slides were kept in moist chamber lined with moist paper towel. Spores showing elongation of germtube were recorded after incubation of 24 hours, number of germtubes per spore and germtube length was also recorded in each of the four replicates. The treatments were arranged in a completely randomized design. The experiment was done in duplicate and the slides were examined under the microscope for conidial germination and the numbers of germtubes were counted. The germtube length was measured using ocular micrometer. Percentage spores that germinated were calculated according to Amadi *et al*., (2014) thus:

$$\text{percent germination} = \frac{\text{no.of germinated spores}}{\text{total number of spores}} \times 100$$

While Spore germination inhibition was determined using the formula:

$$\text{Spore germination inhibition} = \frac{\text{spores germinated in control} - \text{spores germinated in treatments}}{\text{spore germinated in control}} \times 100$$

5.3.5 Determination of the effects of the legume extracts on bacterial growth

Pure colonies of *Bacillus* spp. were transferred to 5ml nutrient broth and incubated overnight at 37^{o}C. A loop-full each of the bacteria was introduced separately by spreading evenly on Petri dishes containing Nutrient agar with a loop (Masoud and Gouda 2012). The plates were cultured at 37°C for 10mins after which a sterile standard of 8mm cork borer was used to cut two uniform wells on the surface of inoculated agar. The growth inhibition was determined by the agar well diffusion method. About 100µl legume extracts was poured in the wells in the agar and allowed to diffuse at room temperature for 20 minutes. Sterile distilled water was poured in the wells treated as control. The plates were incubated at 37°C for 24 h and inhibition zone diameters were measured (mm) using a ruler. The experiment was done in duplicate and the growth inhibitory effect of plant extracts was recorded.

5.3.6 Determination of the effect of saprophytic fungi on the growth of root rot pathogens

The method by Rahman *et al*. (2009) was used in this experiment. A agar disc (5-mm) of the antagonists *Trichoderma*, *Penicilium*, and *Paecelomyces* was placed 2cm away from the edge of the Petri dish. A same sized agar disc of the test fungus, *Pythium ultimum, Fusarium solani*, *Fusarium oxysporum*, and *Macrophomina phaseolina*, was in the same way placed 2 cm away from the periphery of the Petri dish on the opposite end of the antagonist. For the control, the tested fungi were placed in the same position on fresh PDA plate without the antagonists. All pairings were done in quadruplicate and incubated at 28^{o}C. The activity of the antagonists was determined by measuring the radius of the fungi colony in the direction of the antagonist colony and the radius of the different fungi colony in the control plate four days after incubation. Percentage inhibition of radial growth (PIRG) was done using the formula PIRG = $\frac{R1-R2}{R1} \times 100$

where R2 antagonist colony, R1 radius of fungi colony in control plates. The number of days required for the antagonist to overgrow the whole colony was also recorded

5.3.7 Data analysis

The data collected was analyzed statistically using the Fisher's analysis of variance technique by Genstat statistical computer package version 15 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 1991) and least significant differences (LSD) tested at 5% probability to compare the treatments' means

5.4 Results

5.4.1 Effects of extracts on germination, mean emergence time, shoot length and dry weight of common beans

The results of analysis of variance revealed that all the tested extracts had different effect on seed germination (Table 5.1 and 5.2). Furthermore, analysis showed the different effects between the legume extracts. In both experiments there was significantly ($P \leq 0.05$) high germination percentage in aqueous legume extracts when compared with ethanol extracts. However, seeds treated with aqueous lablab extracts constantly had the lowest germination percentage 42% and 46% respectively while the maximum seed germination was recorded in seeds treated with aqueous groundnut extracts (85%) and bean compost extracts (91%). Seeds treated with ethanol extracts had significantly ($P \leq 0.05$) low germination percentages, with lablab compost extracts recording the least at 38% followed by fresh lablab extracts prepared with ethanol at 38% compared with other extracts. On the other hand, in both experiments, regular recording of germination percentage showed a delay phase in germination of treated beans, however, inhibition of germination depended on the type of the extract used.

Concerning mean germination time (MGT), in both experiments, aqueous extracts except for lablab significantly shortened the mean germination time. The maximum mean germination times of 7.8 days was recorded in seeds treated with soybean compost ethanol extracts and beans treated with fresh beans ethanol extracts while minimum mean germination time was recorded in seeds treated with sterile distilled water followed by seeds treated with aqueous bean compost extracts. Similar to germination, legume ethanol extracts had significant high effect negative ($P\leq 0.05$) on the seedling growth of common beans. The ethanol extracts showed inhibitory activities on the shoot and dry weight of beans.

The sensitivity of seedling growth to the extracts was higher than the germination rate beans. In addition, shoot growth was more sensitive and the inhibitory effect was plant extract dependent. Concerning the shoot length and dry weight, maximum shoot length 12.6 cm was recorded in control treatments and minimum value (0.2 cm) was recorded in seeds treated with lablab fresh ethanol extracts while the greatest weight was recorded in seeds treated with sterile distilled water.

Table 5. 1. Percentage germination, mean germination time, shoot length and dry weight of bean seeds treated with different legume extracts in 2017

Legume extracts	Fresh				Compost			
	GP	MGT (Days)	S.L	DW	GP	MGT (Days)	S.L	DW
Water								
Lablab	57.5_{d}	4.4_{cd}	5.9_{bc}	16.9_{b}	60.0_{c}	3.9_{cd}	4.8_{c}	18.6_{a}
Bean	72.5_{c}	3.9_{cd}	4.6_{c}	13.4_{bc}	78.1_{b}	3.5_{cd}	8.2_{b}	13.3_{bc}
Soybean	76.9_{b}	4.9_{bc}	13.2_{a}	14.7_{bc}	61.9_{c}	4.1_{cd}	6.2_{bc}	12.5_{cd}
Groundnut	85.0_{b}	3.8_{cd}	4.7_{c}	12.6_{cd}	36.8_{e}	3.7_{cd}	8.2_{b}	15.6_{bc}
Ethanol								
Lablab	41.3_{e}	7.5_{a}	0.2_{e}	10.5_{d}	37.5_{e}	7.6_{a}	0.5_{e}	14.2_{bc}
Bean	65.0_{c}	4.6_{cd}	1.5_{de}	12.5_{cd}	68.8_{c}	6.4_{ab}	1.0_{e}	12.3_{cd}
Soybean	76.3_{bc}	7.1_{a}	1.3_{de}	13.9_{bc}	38.1_{e}	7.8_{a}	2.0_{de}	12.1_{cd}
Groundnut	48.1_{de}	6.9_{a}	2.1_{de}	16.2_{ab}	61.3_{cd}	7.4_{a}	1.2_{ef}	12.9_{cd}
Control	99.4_{a}	3.0_{d}	12.6_{a}	18.6_{a}				
Mean	62.6	5.34	4.6	14.2				
LSD ($p \leq 0.05$)	12.4	0.96	2.4	2.4				
CV (%)	13.9	12.8	36.7	11.8				
P value	<.001	<.001	<.001	<.001				

G.P- Germination percentage, G.I- Germination index, MGT- Mean germination time, S.L- shoots length, DW- Dry weight. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

Table 5.2. Percentage germination, mean germination time, shoot length and dry weight of bean seeds treated with different legume extracts 2018

Legume Extracts	Fresh				Compost			
	GP	MGT (Days)	S.L	DW	GP	MGT (Days)	S.L	DW
Water								
Lablab	77.0_{b}	3.2_{e}	6.4_{bc}	15.4_{b}	80.5_{b}	4.3_{d}	3.3_{d}	16.1_{b}
Bean	88.0_{ab}	3.7_{e}	4.7_{c}	17.3_{a}	90.5_{ab}	3.7_{e}	8.2_{b}	15.7_{b}
Soybean	88.5_{ab}	4.7_{d}	5.2_{c}	16.8_{ab}	90.0_{ab}	3.9_{e}	12.6_{a}	15.8_{b}
Groundnut	84.5_{b}	4.5_{d}	4.5_{cd}	16.3_{a}	81.0_{b}	3.7_{e}	12.6_{a}	18.6_{a}
Ethanol								
Lablab	46.5_{d}	6.8_{b}	0.2_{e}	17.3_{a}	84.0_{b}	5.5_{c}	2.9_{de}	15.7_{b}
Bean	62.0_{c}	7.8_{a}	1.6_{e}	16.6_{a}	64.5_{c}	7.1_{ab}	1.0_{e}	16.3_{b}
Soybean	61.5_{c}	6.8_{b}	1.3_{e}	17.8_{a}	79.5_{b}	6.4_{bc}	2.0_{e}	16.1_{b}
Groundnut	63.0_{c}	7.3_{ab}	2.1_{e}	18.9_{a}	65.5_{c}	7.9_{a}	1.2_{e}	15.8_{b}
Control	99.5_{a}	3.2_{e}	12.6_{a}	15.2_{b}				
Mean	76.8	5.3	4.9	16.6				
LSD ($p \leq 0.05$)	13.8	0.7	1.9	2.1				
CV (%)	12.6	9.4	27.7	9.0				
P value	<.001	<.001	<.001	<.001				

G.P- Germination percentage, G.I- Germination index, MGT- Mean germination time, S.L- shoots length, DW- Dry weight. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

5.4.2 Relationship among mean germination time and various common bean germination indices and seedling characteristics

The mean germination time (MGT) of all seed treatments was related to shoot length, seedling vigour index and hypocotyl length in the laboratory germination tests after 14 days. Seeds germinating earlier over a shorter period of time with lower MGT produced long shoots, larger seedlings, and higher seedling vigour index (Figure 5.1) that were less variable. The relative mean germination time values were closely related (R2 = 0.54, 0.66, 0.71, $p<0.05$)

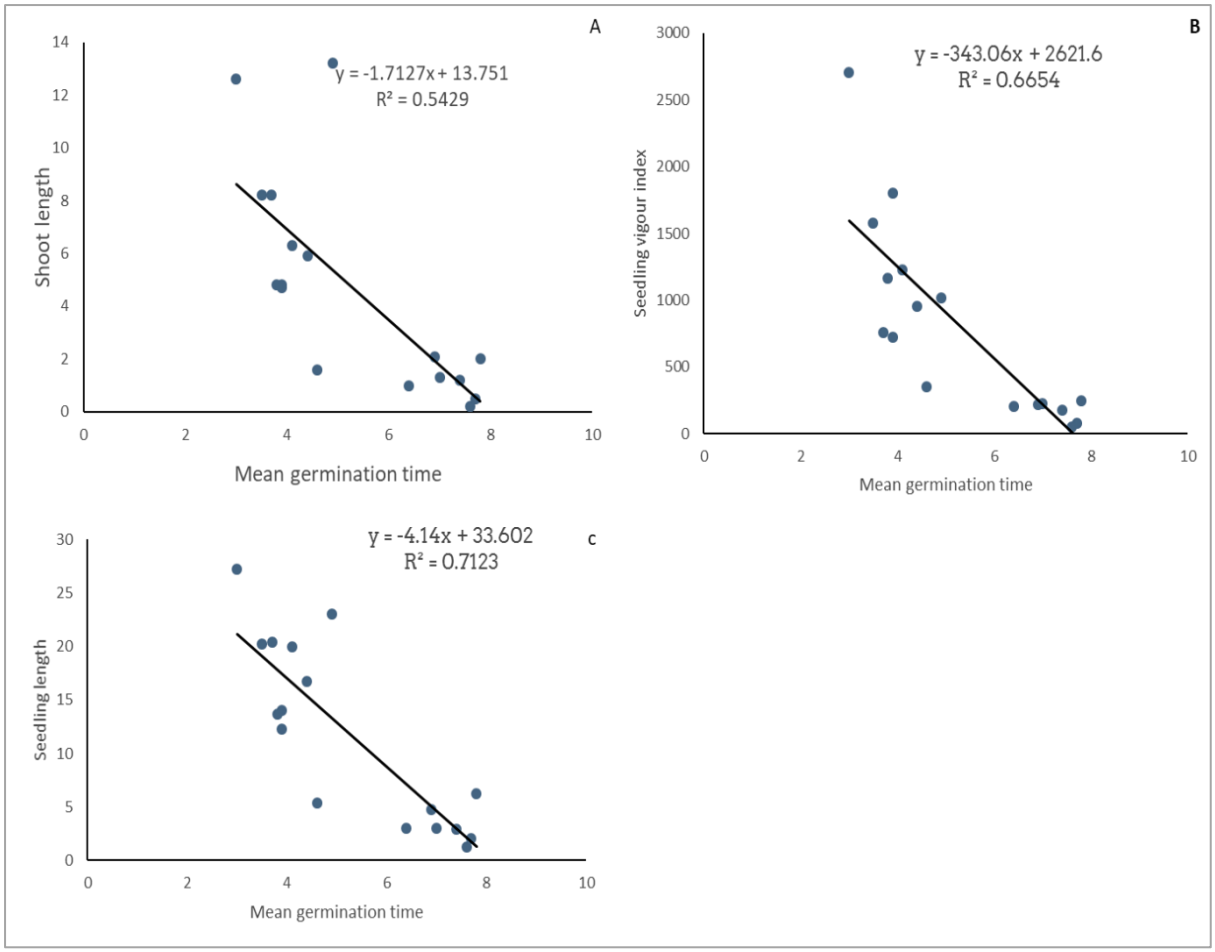

Figure 5. 1. Relationship among mean germination time, and shoot length, seedling vigour index, and seedling length

5.4.3 Effects of fresh and compost extract of legumes on mycelial growth of different fungi

Analysis of the effect of the legume extracts indicated that all tested extracts showed varied degree of inhibition compared to control (Table 5.3 and 5.4). Even though some legume extracts exhibited certain levels of antagonism against mycelia of the tested fungi, the fresh aqueous and ethanol extract of lablab and soybean had the highest inhibition of mycelial growth of *Pythium* and *Fusarium*. Fresh aqueous extracts from beans and groundnut extracts were found to have low antifungal activities on *Pythium* but had strong antifungal effect on *Fusarium* mycelial growth. In both experiments the highest percentage inhibition (70%) in *Pythium* was observed in fresh soybean ethanol extracts while the least inhibition percentage was observed in fresh aqueous groundnut extracts. The highest percentage inhibition (53.3%)

in *Fusarium* was observed in fresh soybean ethanol extracts while the least was observed in bean compost extracts.

The extracts showed varied degree of inhibition with both aqueous fresh and compost extracts were found to stimulate the growth of *Trichoderma* except for fresh lablab and soybean while all the ethanol extracts inhibited mycelial growth of *Trichoderma*. However, the same extracts inhibited the growth *Aspergillus* by percentages ranging from 4% - 46%. Increased mycelial expansions were observed with groundnut, soybean and bean fresh extracts while absolute inhibition was observed with ethanol based extracts from groundnut, soybean, beans and lablab respectively while in the second season, near complete inhibition were observed with beans, lablab and soybean extracts.

Table 5.3. Percentage inhibition or stimulation by extracts of different legumes on mycelial growth of different fungi

Legume Extracts **Experiment one**	Fresh Extracts				Compost Extracts			
	Pyth	*Fus*	*Tricho*	*Asperg*	*Pyth*	*Fus*	*Tricho*	*Asperg*
Water								
Lablab	11.2_{c}	12.2_{b}	0.8_{d}	28.8_{b}	-2.3_{d}	8.9_{bc}	-19.2_{e}	22.6_{b}
Bean	3.5_{c}	32.2_{a}	-8.9_{d}	17.6_{bc}	5.8_{c}	8.9_{bc}	-12.6_{e}	32.6_{ab}
Soybean	-2.7_{d}	44.4_{a}	1.4_{d}	23.8_{b}	-1.4_{d}	-1.1_{c}	-0.1_{d}	25.1_{b}
Groundnut	-11.7_{d}	46.7_{a}	-28.0_{e}	16.4_{b}	-4.1_{d}	3.3_{c}	-3.0_{d}	28.8_{b}
Ethanol								
Lablab	13.4_{c}	33.3_{a}	37.5_{c}	43.8_{a}	-2.7_{d}	0.0_{c}	-8.9_{d}	3.9_{c}
Bean	54.2_{a}	38.9_{a}	53.6_{b}	46.3_{a}	69.9_{a}	8.9_{bc}	4.3_{d}	31.3_{ab}
Soybean	69.9_{a}	20.0_{ab}	56.6_{b}	43.8_{a}	48.0_{b}	1.1_{c}	-13.3_{e}	20.1_{b}
Groundnut	69.0_{a}	36.7_{a}	75.0_{a}	41.3_{a}	39.0_{b}	12.2_{c}	3.6_{d}	33.8_{ab}
Mean	22.4	20.4	8.6	28.8				
LSD ($p \leq 0.05$)	10.7	15.2	10.6	17.6				
P Value	<.001	<.001	<.001	<.001				

Means followed by different letter(s) within each column are significantly different at $p \leq 0.05$, Pyth- *Pythium*, Fus- *Fusarium*, Tricho- *Trichoderma*, Asperg – *Aspergillus.* – denotes stimulation

Table 5. 4. Percentage inhibition or stimulation by extracts of different legumes on mycelial growth of different fungi

Legume Extracts **Experiment Two**	Fresh Extracts				Compost Extracts			
	Pyth	*Fus*	*Tricho*	*Asperg*	*Pyth*	*Fus*	*Tricho*	*Asperg*
Lablab	2.7_{c}	8.4_{a}	8.2_{ab}	11.5_{ab}	-50.6_{e}	-1.6_{b}	-4.7_{b}	-3.1_{b}
Bean	5.6_{c}	14.2_{a}	-2.8_{b}	3.1_{b}	-25.9_{d}	-11.6_{b}	-0.1_{b}	2.1_{a}
Soybean	6.7_{c}	17.0_{a}	-0.1_{b}	1.0_{b}	3.8_{c}	-14.4_{b}	33.9_{a}	-4.2_{b}
Groundnut	-7.4_{c}	22.7_{a}	-12.0_{c}	4.2_{b}	-4.9_{c}	-7.3_{b}	41.3_{a}	-8.3_{b}
Ethanol								
Lablab	26.4_{b}	4.1_{a}	21.0_{ab}	13.5_{a}	-6.5_{c}	7.0_{a}	-2.8_{b}	-3.1_{ab}
Bean	39.3_{b}	11.3_{a}	26.5_{ab}	15.6_{a}	62.9_{a}	-5.9_{b}	6.3_{b}	5.2_{b}
Soybean	53.3_{a}	19.9_{a}	-9.3_{b}	13.5_{a}	34.8_{b}	-8.7_{b}	-4.7_{b}	-7.3_{ab}
Groundnut	48.3_{a}	19.9_{a}	3.6_{b}	15.6_{a}	32.5_{b}	4.1_{a}	16.4_{ab}	2.1_{a}
Mean	13.8	5	7.5	3.8				
LSD ($p \leq 0.05$)	14.4	12.4	10.4	17.4				
P value	<.001	<.001	<.001	<.001				

Means followed by different letter(s) within each column are significantly different at $p \leq 0.05$, Pyth- *Pythium*, Fus- *Fusarium*, Tricho- *Trichoderma*, Asperg – *Aspergillus.* – denotes stimulation

5.4.4 Effect of different legume extracts on spore germination of *Fusarium oxysporum*

The extracts had variable effects on spore germination of *F. oxysporum* (Table 5.5). In 2017, aqueous extract of lablab resulted in the highest spore germination percentage (84.0%), followed by aqueous fresh soybean extract (71.8%). In 2018, spores treated with aqueous extracts from beans had the highest (80%) spore germination followed by spores treated with aqueous lablab extracts (71%) (Table 5.6). This was significantly ($P \leq 0.05$) higher than for the other extracts. The least spore percentage germination was observed with fresh ethanol lablab and soybean extracts in both experiments. The germtube formation was significantly affected by the different extracts treatments after 24 hours of incubation. The longest germ tube formation (1.8µm) was recorded in samples treated with fresh aqueous lablab extracts followed by the lablab compost aqueous extracts (1.4 µm) in 2017. In 2018, samples treated

with fresh aqueous bean extracts had the longest germtube length (1.9 µm) followed by samples treated with lablab aqueous extracts. The least germ tube length ranging from 0.20 µm to 0.33 µm was recorded in samples treated with bean compost ethanol extracts, soybean compost ethanol extracts, and lablab ethanol based fresh extracts.

In the first experiment 2017, the maximum number of germ tubes per spore (3.4) was recorded with spores treated with soybean compost ethanol extracts followed by spores treated with lablab and soybean aqueous fresh extracts. In the second experiment, the maximum number of germtubes was recorded in the control followed by those treated with fresh aqueous extracts from beans, and ethanol based compost extracts from soybean and groundnuts. In both experiments, the minimum number of germ tube per spore was recorded in treatments with ethanol lablab fresh extract, followed by bean fresh extract, and soybean aqueous extracts. Spores treated with ethanol lablab extracts recorded the highest inhibition (98%) in spore germination. This was significantly ($P \leq 0.05$) higher than other extracts. In both experiments, ethanol extracts of soybean, groundnut and beans had similar inhibitory effects on spore germination, with percentage inhibitions of above 90%. In both experiments increased spore elongation were with water extracts of lablab (-13%) and (-41%). Ethanol extracts were relatively inhibitive on germinating spores than water extracts of the legume plants. Longer germtube lengths were observed under the aqueous lablab extracts while shorter germ tubes were observed under the ethanol extracts. This was noted particularly with lablab and soybean ethanol extracts

Table 5.5. Percentage spore germination, germtube length, number of germtubes per spore of *F. oxysporum* treated with different legume extracts and incubated for 24 hours

Legume Extracts	Fresh Extracts				Compost Extracts			
	Experiment one							
	GP	GL	Germtube	PI	GP	GL	Germtube	PI
Water								
Lablab	84.0_{a}	1.84_{a}	2.8_{a}	13.9_{c}	55.6_{a}	1.4_{abc}	2.1_{ab}	48.6_{b}
Bean	54.6_{a}	0.67_{b}	1.9_{ab}	77.8_{ab}	61.1_{a}	0.9_{abcd}	1.9_{ab}	62.5_{b}
Soybean	71.8_{a}	0.82_{b}	2.8_{a}	48.6_{b}	31.5_{ab}	0.8_{abcd}	0.5_{b}	73.6_{ab}
Groundnut	50.4_{a}	0.62_{b}	2.7_{a}	63.9_{b}	45.3_{a}	0.4_{cd}	1.3_{ab}	68.1_{ab}
Ethanol								
Lablab	2.2_{b}	0.27_{bc}	0.22_{c}	98.6_{a}	22.1_{ab}	0.5_{cd}	1.1_{ab}	90.3_{a}
Bean	5.9_{b}	0.30_{bc}	0.44_{bc}	95.8_{a}	13.3_{b}	0.2_{d}	0.7_{b}	91.7_{a}
Soybean	8.7_{b}	0.27_{bc}	0.77_{bc}	94.4_{a}	59.5_{a}	0.3_{cd}	3.4_{a}	48.6_{ab}
Groundnut	10.0_{b}	0.22_{c}	0.44_{bc}	94.4_{a}	16.7_{ab}	0.3_{cd}	1.1_{ab}	93.1_{a}
Control	73.3_{a}	1.7_{a}	2.5_{a}	0.0_{c}				
Mean	39.2	0.67	1.57	68.5				
LSD ($p \leq 0.05$)	47.1	0.61	1.41	31.0				
CV (%)	72.3	54.6	54.2	27.3				

G.P- Germination Percentage, G.L- Germtube length, P.I- Percent inhibition. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

Table 5.6: Percentage spore germination, germtube length, number of germtubes per spore of *F. oxysporum* treated with different legume extracts and incubated for 24 hours

Legume Extracts	Fresh Extracts				Compost Extracts			
	Experiment two							
	GP	GL	Germtube	PI	GP	GL	Germtube	PI
Water								
Lablab	71.1_{ab}	1.6_{a}	1.7_{a}	41.8_{b}	51.4_{bc}	0.7_{c}	1.6_{a}	78.6_{b}
Bean	80.4_{a}	1.9_{a}	2.7_{a}	71.4_{ab}	39.4_{c}	0.8_{c}	1.0_{ab}	87.8_{a}
Soybean	43.2_{bc}	0.9_{b}	1.9_{a}	78.6_{ab}	60.0_{b}	0.4_{d}	1.2_{b}	85.7_{a}
Groundnut	81.9_{a}	1.8_{a}	2.1_{a}	63.3_{b}	34.5_{c}	0.4_{d}	1.1_{b}	90.8_{a}
Ethanol								
Lablab	3.0_{d}	0.1_{d}	0.2_{c}	97.9_{a}	53.6_{bc}	0.9_{b}	1.9_{a}	70.4_{b}
Bean	25.5_{cd}	0.5_{c}	1.0_{b}	82.6_{a}	50.1_{bc}	0.9_{b}	1.9_{a}	69.4_{b}
Soybean	1.4_{d}	0.1_{d}	0.3_{c}	97.9_{a}	57.8_{bc}	1.4_{ab}	2.2_{a}	76.5_{b}
Groundnut	0.0_{d}	0.0_{d}	0.0_{c}	100.0a	46.5_{bc}	1.0_{b}	2.2_{a}	50.0_{c}
Control	93.7_{a}	1.6_{a}	2.78_{a}	0.0_{c}				
Mean	46.7	0.8	1.5	73.1				
LSD ($p \leq 0.05$)	28.9	0.5	0.9	19.6				
CV (%)	37.3	35.7	39.4	16.4				
P value	<.001	<.001	<.001	<.001				

G.P- Germination Percentage, G.L- Germtube length, P.I- Percent inhibition. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

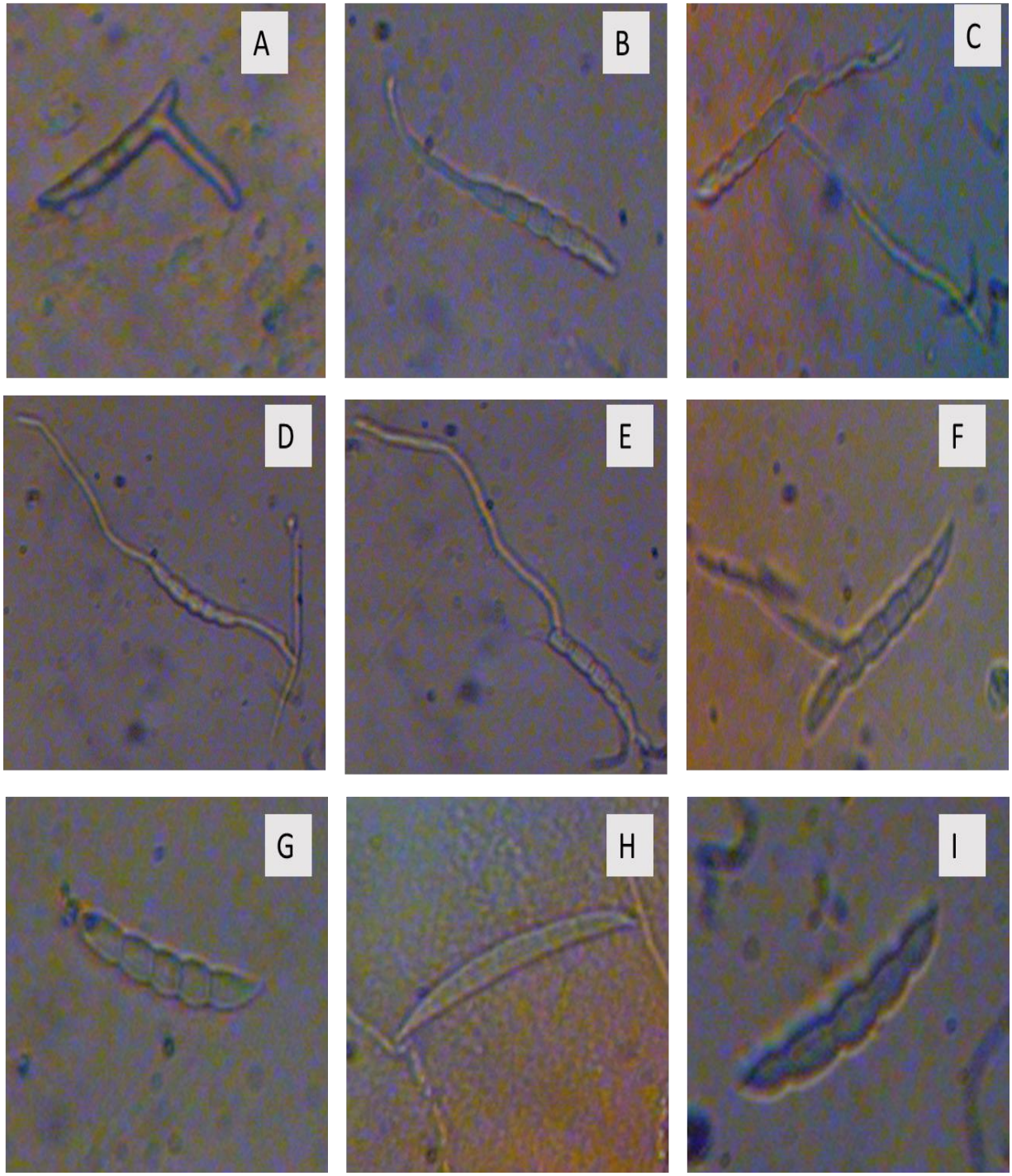

Figure 5.2. Germinated spores of *Fusarium oxysporum* after 24 hours of incubation on different legume extracts.

A – soybean fresh extracts, B- lablab compost extracts, C- Bean fresh extracts, D- lablab fresh extract, E- bean compost extract, F- groundnut compost extracts, G- lablab ethanol fresh extracts, H- soybean ethanol fresh extracts, I- groundnut ethanol fresh extracts

5.4.5 Effects of different legume extracts on bacterial growth

All the extracts of the legumes showed varying degrees of antibacterial activities against *Bacillus* spp. The results of the inhibition zone diameter and percentage growth inhibition in table 5.6 shows the antibacterial activity of legumes aqueous and ethanol extracts against bacteria. Results indicate that, the effect of the tested extract showed variable inhibition zones ranging from 0.1mm to 14.5 mm (Table 5.6). Soybean (14.6 mm), and groundnut (14.5 mm) ethanol extracts showed the highest zone of inhibition on *Bacillus* spp. lablab and beans extracts had the lowest antibacterial activity against *Bacillus* spp. In general, fresh ethanol based extracts had the greatest growth inhibition on *Bacillus* spp growth when compared with other extracts

Table 5.7. Percentage inhibition by fresh and compost legume extracts on bacteria growth

Legume Extracts	Experiment one			Experiment two		
	Fresh	Compost	Mean	Fresh	Compost	Mean
Water						
Lablab	7.2_{d}	0.0_{f}	3.6_{bc}	2.0_{c}	0.2_{f}	1.1_{c}
Beans	5.8_{d}	0.0_{f}	2.9_{c}	6.6_{b}	0.6_{ef}	3.6_{b}
Soybean	5.9_{d}	1.6_{ef}	3.8_{bc}	4.9_{b}	1.1_{ef}	3.0_{bc}
Groundnut	5.1_{d}	2.3_{ef}	3.7_{bc}	4.9_{b}	1.9_{ef}	3.4_{b}
Ethanol						
Lablab	16.5_{ab}	3.7_{e}	10.1_{a}	11.5_{a}	2.9_{e}	7.2_{a}
Beans	10.6_{c}	1.8_{e}	6.2_{b}	12.5_{a}	2.8_{e}	7.7_{a}
Soybean	17.8_{a}	3.9_{e}	10.9_{a}	11.5_{a}	2.4_{e}	6.9_{a}
Groundnut	14.8_{b}	5.9_{d}	10.4_{a}	14.3_{a}	4.1_{b}	9.2_{a}
Mean	5.1		6.5	4.2		5.3
LSD ($p \leq 0.05$)	2.9		2.9	1.7		2.4
CV (%)	40.5		12	30.1		8.1

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

5.4.6 Effect of antagonists on the growth of root rot pathogens of common bean

Data in Table 5.8 reveal that all the tested antagonists significantly (P≤0.05) reduced the linear growth of all the tested fungi compared to the control, albeit, with varying efficiencies for different pathogens. In general, the radial growth values ranged from 13mm to 27mm for *Fusarium* spp, 36mm to 56mm and 14mm to 29mm for *M. phaseolina*. *Trichoderma* was the most effective antagonist with the highest percentage checks in radial growth. *Paecelomyces* and *Penicilium* also had significant effect on the radial growth of various pathogens compared to the control. Trichoderma completely overgrew the colony of the three root rot pathogens within seven days while both *Penicilium* and *Paecelomyces* took over 12 days (Table 5.9). No distinct inhibition zone towards *Fusarium* spp, and *Pythium* spp. was observed with *Trichoderma*. Colony overgrowth times varied from 5-14 days. For all the antagonists, the minimum colony overgrowth time recorded was for *Trichoderma* and the maximum recorded was for *Penicilium*

Table 5. 8. Radial Growth (mm) of various root rot pathogens in dual culture method

Isolates	Experiment one				Experiment two			
	F.s	*F. o*	*Pythium*	*M. p*	*F.s*	*F. o*	*Pythium*	*M. p*
Trichoderma	13.9_{b}	14.5_{b}	28.3_{c}	15.6_{b}	23.7_{a}	22.0_{b}	38.3_{bc}	24.7_{b}
Penicillium	15.4_{b}	13.1_{b}	39.5_{b}	14.8_{b}	24.7_{a}	24.7_{ab}	49.5_{ab}	29.7_{a}
Paecilomyces	15.3_{b}	13.8_{b}	36.3_{c}	17.7_{b}	23.4_{a}	23.3_{b}	43.3_{b}	24.0_{b}
Control	20.5_{a}	22.5_{a}	56.6_{a}	23.0_{a}	27.7_{a}	27.7_{a}	56.6_{a}	29.7_{a}
Mean	16.3	15.9	45.2	17.8	24.8	24.4	45.2	27.0
LSD ($p \leq 0.05$)	2.14	5.4	8.9	3.4	3.7	2.9	8.9	1.96
CV (%)	8.3	21.2	12.4	11.8	9.2	7.4	12.4	4.5

Means followed by different letter(s) within each column are significantly different at $p \leq 0.05$. *F.o Fusarium oxysporum, F.s –Fusarium solani, M.p- Macrophomina phaseolina*

Table 5. 9. Colony overgrowth time (days) of various antagonists against root rot pathogens

Isolates	Experiment one				Experiment two			
	F.s	*F.o*	*Pythium*	*M.p*	*F.s*	*F.o*	*Pythium*	*M.p*
Trichoderma	9.1_{b}	9.3_{c}	4.3_{c}	4.8_{b}	10.3_{a}	9.0_{b}	6.3_{b}	6.3_{b}
Penicillium	16.8_{a}	16.5_{a}	7.0_{b}	9.7_{a}	14.3_{a}	14.3_{a}	6.5_{b}	10.3_{a}
Paecilomyces	14.0_{ab}	13.5_{b}	9.8_{a}	9.5_{a}	13.5_{a}	15.5_{a}	10.5_{a}	10.5_{a}
Mean	13.3	13.1	7.0	8.0	12.7	12.9	7.8	9.0
LSD ($p \leq 0.05$)	3.9	2.1	1.4	1.7	5.1	3.7	3.9	3.4
CV (%)	17.1	9.4	11.9	12.3	23.6	16.6	29.7	21.8

Means followed by different letter(s) within each column are significantly different at $p \leq 0.05$. *F.o Fusarium oxysporum, F.s –Fusarium solani, M.p- Macrophomina phaseolina*

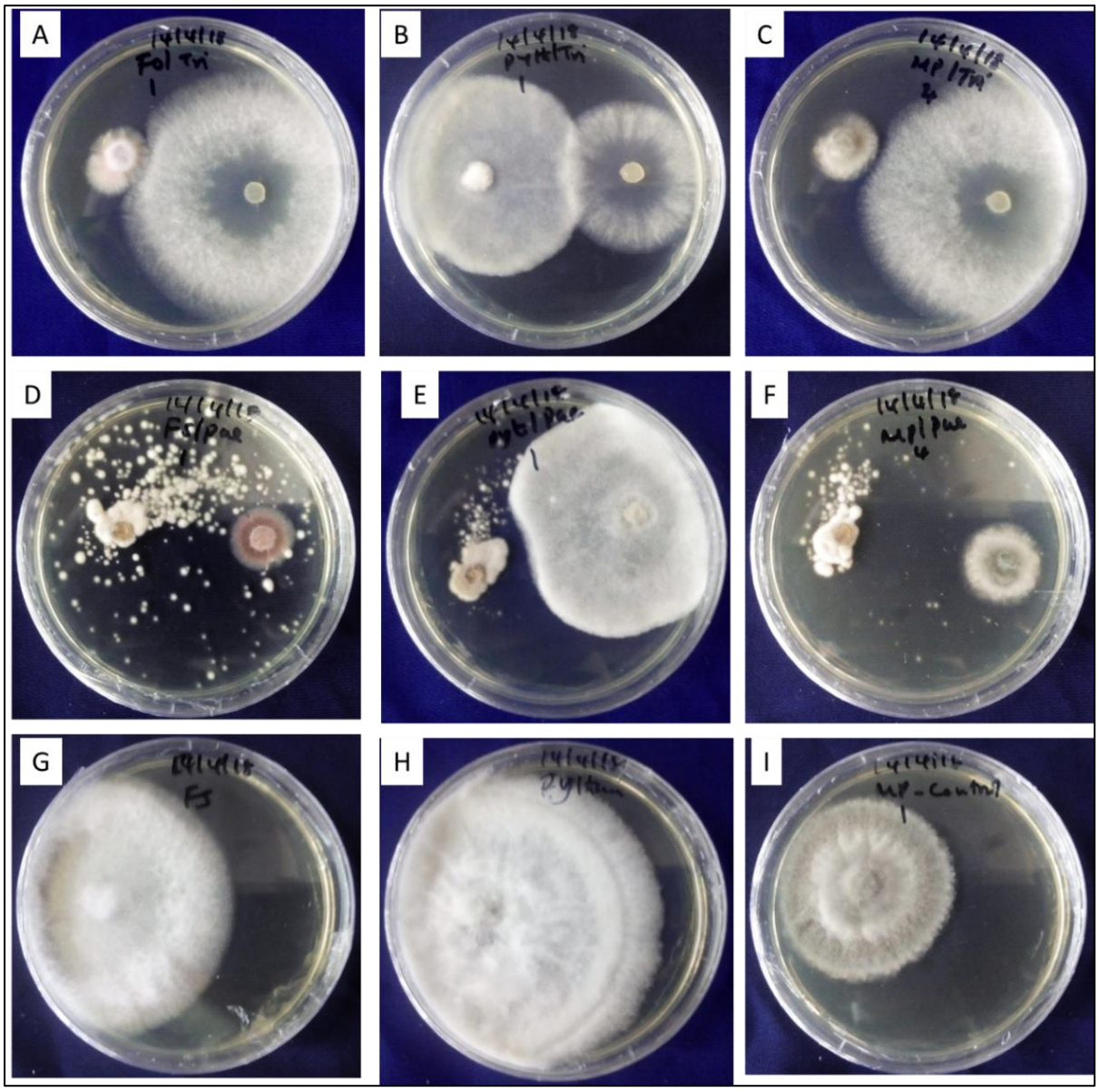

Figure 5.3. Interactions of soilborne pathogens and antagonists isolated from the soils.
A- *F. solani* and *Trichoderma* spp. B-*Pythium* and *Trichoderma* spp. C- *M. phaseolina* and *Trichoderma* spp. D- *F. solani* and *Paecilomyces* spp. E-*Pythium* and *Paecilomyces* spp. F- *M. phaseolina* and *Paecilomyces* spp. G- *F.solani,* H- *Pythium,* I- *M. phaseolina*. Control plates

5.5 Discussion

5.5.1 Effect of various legume extracts on bean germination and seedling establishment

The results indicate that ethanol legume extracts displayed adverse effects on seed emergence and germination. This suggests that ethanol is effective in extracting substances due to high polarity and good solubility (Onivogui *et al*., 2016). The presence of extracts potentially reduced seed germination and the seeds that germinated with extracts required more time for mean germination times (Ayub *et al*., 2012). However, germination was significantly improved by aqueous extracts except for lablab aqueous extracts. Germination percent is a commonly used index to measure the effects of phytotoxic substances on germination and mainly depends on final measurements (Kato-Noguchi *et al*.,, 2014). However, germination index cannot explain the delay in germination caused by legume extracts.

Seed germination is a process of growth and development of the embryo that initiates growth of plant (Hillary and Nuringtyas, 2016). The difference in seed germination between compost and fresh extracts may suggest that chemical in compost extracts may have degenerated and enhanced germination and emergence. Germination index, seedling vigour index, and mean germination time together with germination percentage were considered in order to understand inhibition in germination. The delay in germination was more pronounced in seeds treated with ethanol extracts, and in lablab aqueous extracts and less pronounced in seeds treated with aqueous extracts of soybean, groundnuts and beans. These results show the inhibitory potential of the legume extracts and type but this is dependent on the extract medium used since ethanol extracts were more efficient in extracting bioactive compounds in the legume plants. The delay in germination and inhibition has been reported by Hussain *et al*. (2008) since early seedling growth is very sensitive to phytotoxins (Soltys *et al*., 2012). Results show that the chemicals from legume extracts had severe effect on seedling growth, significantly reducing shoot, secondary root formation and dry weight of bean seedlings.

These results are comparable to those reported by Haramoto and Gallandt, (2005); Bonanomi *et al*. (2011); Kato-Noguchi *et al*. (2014) where extracts were phytotoxic to seedlings and decreased radicle elongation. There was also great inhibition in the seedling length because after germination the sustenance of the seedlings was done with the extracts even. Phytochemicals from green manure are not only inhibitory to germination but also retard seedling growth after germination (Shankar *et al*., 2014). Similar result was reported by Terzi, (2008) with walnut juice where high inhibition was recorded with juglone.

There was close relationship between mean germination time and the shoot length, seedling length and seedling vigour index, with regression analysis ranging from 0.54 to 0.71. Thus, the laboratory assessment of mean germination time was highly predictive of all the growth parameters. This implies that later germinating seeds resulted in smaller seedlings and with greater spread of germination. Similar findings were reported in comparisons of seed lots of maize (Matthews and Khajeh-Hosseini, 2007). The shorter shoot length produced by the seeds having high MGT, may have resulted from the spread in time of germination of seeds (Demir *et al*., 2005). Seed germination involves both biochemical and physiological changes and any interruption to the two processes by chemical substance may result into germination failure and the germination bioassays done explains the effect of exogenously applied material (Tanveer *et al*., 2014). The implication of these substances is the injurious effect they impart on the crops that result in reduced and delayed germination ultimately leading to decline in plant stand and yield. In the present study, the vigour index ranged from 2.1 to 5.7 with a mean of 3.6, and since the percentage germination inhibition was high for beans with lablab green manure, legible vigour index was not observed. The results show that the fresh ethanol extracts influenced more vigour loss. Similar results in loss of vigour were reported by Pawar and Chavan. (2007) where the allelopathic effects of Eucalyptus, Melia, Moringa, and Parthenium were observed on wheat, rice, millet, and sorghum

5.5.2 Effects of aqueous and alcoholic extract of legume plants against fungal and bacterial growth and spore germination

The effect of four plant extracts resulted in different levels of antifungal activity against various fungi. Results showed that the aqueous extracts from soybean, groundnuts and bean showed more inhibitory effect against mycelial germination of the tested fungi when compared with the lablab aqueous extracts and control. However, results of the mycelial growth assay suggested that crude ethanol extracts from Lablab and soybean were the most active against fungi *Aspergillus, Pythium* and *Fusarium*. Aqueous crude extracts from lablab stimulated germination of spores and enhanced germtube elongation. Several reports have shown that plant extracts have inhibitory effects against pathogenic fungi (Mohammadi and Atik, 2013). The toxicity observed against these fungi may be due to alteration of cell wall permeability, interference with electron transport, the nutrient absorption, and other metabolic processes of the cell. The result shows that the tested compost extracts had different effects on the fungi tested. Composts prepared from lablab and soybean, groundnut and beans had no relative effects on the growth of these fungi when compared with the control. This may be due to the absence of biologically active antifungal compounds.

The current study shows that fresh ethanol extracts prepared from various legumes had high inhibitory effects against *Fusarium solani, Fusarium oxysporum*. The presence and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms (Mohammadi and Atik, 2013). Phytochemical analysis by Torres and Manalo. (1990) and Balekeri. (2013) showed that the fresh leaf extracts of lablab (*Lablab purpureus* L.) contains sugar, alcohols, phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids pigments, glycosides and anthnanoids. However, the total phenolic contents (TPC) were lower compared with other plants.

The bioactive compounds in extracts may have applied two inhibitory actions on mycelia and spores. They act simultaneously and differently on various targets (Okigbo and Odurukwe, 2009). Arif *et al*. (2009) suggested enzyme inhibition by oxidized compounds through reaction with sulfhydryl groups, or through non-specific interactions with proteins as main mechanisms responsible for phenol toxicity. The notable fungitoxic ability of the legume extracts suggests that the contents of the plant material are highly soluble in the extracting solvents used (Nweke, 2015). Legume extracts were effective in reducing the radial growth of the pathogens after two days in culture, which decreased as incubation period increased indicating that the efficacy of the active compounds is not persistent in the culture medium or they degenerated in toxicity levels after two days of culture.

Different extracts had different effects on spore germination. However, most of the extracts macerated with sterile distilled water, were only moderately or slightly inhibitory to spore germination and elongation. The maximum inhibition in spore germination was found in ethanol extracts of all legumes in both experiments. However, fresh lablab aqueous extract was found to stimulate the germination of *F. oxysporum* conidia with increased number of germ tubes per spore. The other plants extracts had intermediary effect. The results show that the legumes have antifungal properties against *F. oxysporum* and the amount of the fungitoxic substances extracted may be significantly increased when different extraction methods are used. Water, acetone, ether, and chloroform were reported not to be very effective in extracting the inhibitory substances (Ho *et al*., 2007).

5.5.3 Effects of legume extracts on bacterial growth

In the present study, ethanol extracts of lablab and soybean showed inhibitory activity against *Bacillus* spp. The assessment of the antibacterial activity of several leguminous extracts showed that the highest growth inhibitory effect relative to the diameter of inhibition zones

was as a result of lablab ethanol extracts while the least diameter was shown by compost extracts. When inhibition zone size is considered as indicators of antibacterial effectiveness soybean and lablab crude extracts emerged as the most potent of all plant extracts tested. As lablab crude extract produced the largest inhibition zones compared to the rest. Chemical contents differ depending on the type and nature of solvent used in the extraction technique (Masoud and Gouda, 2012) while the sensitivity of the extracts depends on the concentrations and the effectiveness of extracts constituents (Masoud and Gouda, 2012). Plant extracts possess antibacterial characteristics against pathogenic bacteria since they are hydrophobic and can bond both lipidic layer of the cellular membrane and mitochondria of the bacteria resulting into rupture and the important molecules and ions exit from the cell, leading to the eventual death of the bacteria (Shojaee *et al*., 2017).

5.5.4 Effect of saprophytic fungi against root rot pathogens of common beans

All the tested fungi exhibited antagonism against the tested root rot pathogens by inhibiting the mycelial growth but had various levels of antagonism. Differences between the fungal isolates were observed with regards to hyphal interaction and mycelial growth inhibition. Of the tested antagonists, *Trichoderma* demonstrated stronger antagonistic activity, inhibiting radial growth of root rot pathogens using dual culture technique. Mycelial interaction is a basic method of assessing antagonistic properties of microorganisms (Rahman *et al*., 2009). *Trichoderma* parasitizes through coiling around the mycelia and producing antibiotic metabolites that penetrates and dissolves the target pathogens (Raut *et al*., 2014; Vinale *et al*., 2014). Volatile and non-volatile antibiotics produced by *Trichoderma* and *Penicillium* are responsible for the inhibitory action against root rot pathogens. *Trichoderma* spp. are producers of enzymes that degrade cell wall (Bech *et al*., 2015). Spiegel and Chet. (1998) reported that *Trichoderma* spp. are known as biological mycoparasites which are used

commercially as biocontrol agents of a range of plant pathogenic fungi such as *Fusarium, Pythium,* and *Rhizoctonia* strains.

Results showed that the test antagonists *Trichoderma* grew faster than the pathogen in dual culture. The time for colony to over growth the pathogen is needed to assess antagonistic ability to compete in an environment with limited resources (Begum, *et al*., 2008). Of the antagonists tested, *Trichoderma* was able to fully overgrow the tested pathogens within seven days in the dual culture test. Fast growth is an important factor for use as a biocontrol study, the effectiveness of biocontrol agents depends on their ability to thrive during favourable environmental conditions before they come across any plant pathogen. *Paecilomyces* spp. showed strong antagonism and produced zones of inhibition at the point of contact with the pathogen. They produce antifungal metabolites that may be responsible for the inhibition (Pusztahelyi *et al*., 2015). The effect of the culture filtrates on the pathogen might be due to toxin production into the culture medium which accumulate overtime accounting for the observed increased toxicity. Complete inhibition of pathogens growth was not observed in this study since biocontrol agents do not completely inhibit the pathogens they antagonize (Heydari and Pessarakli, 2010). Going by the results of this study, *Paecilomyces, Trichoderma,* and *Penicilium* spp. may probably function as good biocontrol agents against root rot pathogens of common beans

5.6 Conclusion

Experiments were conducted to determine the mechanisms responsible for poor emergence of bean after green manure applications. The addition of the extracts to the germinating seed also affected seedling length. Aqueous lablab extracts stimulated growth and germination of mycelial spores of *F. oxysporum*. The results revealed that ethanol extracts were more efficient in inhibiting spore and mycelial germination of fungi than aqueous extracts. The low

inhibition effects of aqueous extract were probably as a result of antimicrobial compounds and fungicidal materials being not lipophilic. The present study shows that poor emergence and establishment of common bean in the field following lablab green manure application is due to a combination of various factors. The factors include stimulation of mycelial and spore growth while inhibiting the population of saprophytic fungi, phytotoxicity and presence of inhibitory substances in lablab green manure during decomposition that affect the root elongation of beans. Further investigation on the identity of the inhibitory substances released during decomposition is needed and to determine their significance under field conditions on other crops

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMDENATIONS

6.1 General discussion

In the first study lablab green manure was incorporated in the soil but was compared with other soil amendments. The results showed that incorporation of green manure in the soil prior to planting improved soil pH, and organic matter. Similarly, no significant differences were reported for pH. Results of this study are comparable to those of Ibrahim *et al*. (2017), who reported an increase in soil organic matter, pH, phosphorus and potassium following green manure application. Results from the study show that there was significant reduction in emergence and bean crop establishment in the plots treated with fresh lablab green manure. These results are comparable to findings by Muthomi *et al.* (2014) who reported that common bean emergence and establishment are sensitive to green manure from lablab and therefore results in poor emergence and establishment.

Following green manure incorporation, growth inhibitors are mostly concentrated in the soil close around the decomposing plant materials. The bean roots which are sensitive show symptoms of damage that result in stunted growth. Similar were described by Bonanomi *et al*. (2017) who reported germination inhibition of up to 48% when residues were planted one day after incorporation. Similarly, high root rot incidence and severity was observed in the plots treated with green manure at planting. Root rot pathogens have previously been reported to increase following green manure incorporation (Wall, 1984; Muthomi *et al*., 2014). Furthermore root rot pathogens like *Fusarium,* and *Pythium* were isolated in high incidences from symptomatic bean stems. These findings suggest that these pathogens may play critical role in common bean emergence and establishment.

From the soil samples collected from different sites, the amount of *Fusarium* and *Pythium* counts increased in the second week after treatment applications. Green manure provided nutrient rich organic carbon which stimulated the population and growth of root rot pathogens, however, the population of saprophytic fungi *Penicillium, Aspergillus,* and *Trichoderma* reduced at the same period. In intensively cultivated farms with high population of pathogens, such as *Pythium* and *Rhizoctonia* due to previous soil management practices, application of fresh organic debris into the soil can stimulate pathogen population thereby increasing root rot severity in subsequent crops (Manici *et al*., 2004). High levels of soil infestations may lead to increased disease potential and thus reduce germination, establishment and crop seedling stand.

Planting beans in plots incorporated with green manure at planting had negative effects on emergence and early establishment in the field. In contrast, incorporation seven days before planting had positive, none or only minor effects on establishment of common beans. Sarma and Gogai (2015) attributed the high germination rate in Okra to high porosity, aeration, high water holding capacity, and presence of humic like substances due to organic manure decomposition. Green manure residues allowed to decompose at field capacity become more toxic during the early stages of decomposition. The inhibition in germination varies with decomposition period (Al-Harun *et al*., 2015). During decomposition, chemical substances such as phenols, tannins that inhibit germination and establishment may arise under some conditions. Maturity of plant residue, length of decomposition are the important factors that may influence production of phytochemicals. The effect was greatly observed in the plots with 2 weeks' incubation after incorporation. Seeds in the immediate vicinity of the decomposing plant residues had the greatest germination inhibition

In the present study, it is clear that several legume extracts were active against mycelial growth and spore germination of fungi isolated from beans. The results revealed that ethanol extracts were more efficient in inhibiting both mycelial growth and spore germination than aqueous extracts. The lower inhibition effect of aqueous extracts could be due to low active antimicrobial compounds since fungicidal substances are lipophilic (Cohen *et al*., 2002).

Fresh aqueous extracts from lablab stimulated mycelial and spore germination of root rot pathogens. The highest spore germination was recorded in lablab and soybean aqueous extracts. The extracts had little impact on germination of spore and promoted rapid germination, probably because they contain little amount of phenols therefore reduced antifungal properties. Radial growth was high in the presence of lablab fresh extracts that also induced an abundant production of profuse mycelium. This can be due to the ability of the pathogens to utilize the energy and nutrients released during decomposition of residues. *Fusarium* species are aggressive saprophytes (Gordon and Okamoto, 1990), and pathogenic species maintain the ability to colonize crop residues (Bonanomi *et al*. 2007). High population of *F. solani* and *F. oxysporum* was isolated from plots that were incorporated with lablab green manure. Bonanomi *et al*. (2006) showed that *Fusarium* is able to use decomposed olive mill residue for its saprophytic growth. Beans planted in plots amended with undecomposed lablab green residues symptoms such as yellowing and wilting were observed, similar to those caused by root rot pathogens two weeks after emergence. Generally, phytochemicals released during decomposition show their negative effects on plants as soon as they come into contact with roots probably because they reduce water uptake by the roots (Blum *et al*., 1999).

Mechanisms have been proposed to be reasons behind the harmful effects of green manure residues. In this study, allelochemicals and other compounds that might be present in lablab

were not isolated. When the significance of these harmful effects are understood, one is be able to devise ways of reducing deleterious impacts of green manure on crops which contributes to poor crop establishment. In many studies, the poor crop establishment has been attributed to phytotoxicity, however, effect on soilborne pathogens may have been ignored. The pathogen activity may have been confounded by the chemicals released during decomposition. The laboratory studies provide clear evidence that the proximity of the seed to green manure was closely related to level of seedling establishment.

6.2 Conclusion

There has been interest in green manuring because of need to develop sustainable agricultural systems at the same time it is important to establish salient benefits and shortcomings of the green manuring. The present study indicated specificity of action of the lablab residue and the concentrations effects of the inhibitors on common beans emergence and establishment. Phytotoxicty was severe in the initial stages of decomposition and toxicity of green manure develops rather early in the decomposition process. When lablab green manure residue was applied in rows, inhibitory substances produced during decomposition were also unevenly distributed and relatively uniform damage was observed on beans especially along the rows. The successful adoption of green manure for soil nutrients enhancement depends on identifying green manures that do not suppress establishment of crops. Soilborne fungal pathogens associated with beans increased thus increase in disease incidence and severity however, the population of beneficial microorganisms did not proliferate. Therefore, the population of antagonists was not able to ameliorate the effects of root rot pathogens inciting high root rot incidences

A combination of toxins from microorganisms whose growth was stimulated by substances in the residues contributed to high populations of root rot causing pathogens especially

Fusarium spp which accounted for the high plant stand decline. The role of lablab green manure as physical impediment to emerging seedlings and in the etiology of root rot pathogen attack of young seedlings could be mechanisms responsible for reduced plant densities. The inhibitory effect of lablab green manure on germination is a phenomenon caused mainly by phytotoxicity accompanied by the effect of root rot pathogens. The extracts experiment supported the hypothesis that toxicity could be one of the mechanisms inhibiting crop establishment. The presence of decomposing residue had significant effect on common bean germination and establishment and in turn enhanced the population of root rot pathogens. Aqueous extracts of lablab revealed toxicity activity limiting seed germination and seedling establishment.

Lablab green manure can improves soil nutrient status and also increase yield. However, negative effects of planting the very day of green manure can be avoided by adopting a relay planting of at least 14 days. Earlier residue incorporation allows more time for residue decomposition and release of nutrients in the soil. The reduction in crop stand in the first experiment must be taken into account for putative green manure applications. Consequently, a second experiment was designed to establish a relay-planting of beans after incorporation of lablab green manure. Seedlings grown in soil incorporated with lablab are affected in ways comparable to those exposed to residue extracts. Despite the laboratory and field results obtained, the issue of whether lablab residues are allelopathic remains contentious. The residue mediated effects may result from physical, biological or chemical changes to the soil environment. By employing several experimental approaches, the results of the various experiments conducted in this study points to a common conclusion that enhanced pathogenic population and competitive occurrence of chemicals during decomposition are the dominant mechanism inhibiting crop emergence and establishment. Therefore, these results are

important for practical applications in agriculture in general in order to consider the potential harmful effects of organic matter as crop residues, cover crop and compost amendments

6.3 Recommendations

i. Application of lablab green manure done earlier 2 to 4 weeks to ensure adequate decomposition so as to enhance emergence, stand establishment and reduce the population of soil borne pathogens

ii. Farmers should be encouraged to adopt lablab as green manure since it improves nutrient regime, structure, physical and biological properties of the soil.

iii. Further studies should be done on isolation, identification and characterization of the composting products of lablab that results in poor emergence and establishment and their effect also tested on other crops

iv. Further studies should be done on the role of microbially produced and transformed chemicals and their effect on crop emergence

v. Further studies should be done on root development where green manure has been applied to determine the processes within the root that are disrupted by the process of decomposition

REFERENCES

Abawi, G, S., Widmer, T, L. 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology, 15: 37-47.

Abdeljalil, N., Vallance, J., Gerbore, J., Rey, P., Daami-Remadi, M. 2016. Bio-suppression of Sclerotinia stem rot of tomato and biostimulation of plant growth using tomato-associated rhizobacteria. Journal of Plant Pathology and Microbiology, 7: 2-11.

Afzal, M. I., Iqbal, M. A., Cheema, Z. A. 2015. Triggering growth and boosting economic yield of late-sown wheat (*Triticum aestivum* L.) with foliar application of allelopathic water extracts. World Journal of Agricultural Sciences, 11: 94-100.

Ahanger, R. A., Bhat, H. A., Shah, S. A. G. A. H. 2013. Root disease management in organic agriculture. Journal of Genetic and Environmental Resources Conservation, 1:158-169.

Al-Harun, M. A. Y., Johnson, J., Uddin, M. N., Robinson, R. W. 2015. The effects of temperature on decomposition and allelopathic phytotoxicity of bone seed litter. Journal of Environmental Sciences, 33: 1-11.

Al-Mughrabi, K. 2003. Antimicrobial activity of extracts from leaves, stems and flowers of *Euphorbia macroclada* against plant pathogenic fungi. Phytopathologia Mediterranea, 42(3): 245-250. doi:10.14601/Phytopathol_Mediterr-1712.

Al-Snafi. 2017. The pharmacology and medical importance of dolichos lablab (*Lablab purpureus*) – A review. IOSR Journal of Pharmacy, 7: 22-30.

Altieri, M. A. 1995. Agro-ecology: The science of sustainable agriculture (No. Ed 2. Intermediate Technology Publications Limited. Westview Press, Boulder, CO, p. 433.

Amadi, J. E., Adeleke, E. E., Olahan, G., Garuba, T., Adebola, M. O. 2014. Effect of plant extracts on sporulation and spore germination of stored melon seed fungi. International Journal of Research-Granthaalayah, 1(1): 21-29.

Ambrosano, E. J., Cantarella, H., Ambrosano, G. M. B., Dias, F. L. F., Rossi, F., Trivelin, P. C. O., Muraoka, T. 2013. The role of green manure nitrogen uses by corn and sugarcane crops in Brazil. Agricultural Sciences, 4: 89-92.

An, M., Pratley, J. E., Haig, T. 2001. Phytotoxicity of vulpia residues: IV. Dynamics of allelochemicals during decomposition of vulpia residues and their corresponding phytotoxicity. Journal of Chemical Ecology, 27(2): 395-409.

Antil, R., Raj D., Abdalla N., Inubushi K. 2014. Physical, chemical and biological parameters for compost maturity assessment: A Review. In: Maheshwari D. (eds) Composting for Sustainable Agriculture. Sustainable Development and Biodiversity, 3. Springer, Cham.

Aranyos, J. T., Tomócsik, A., Makádi, M., Mészáros, J., Blaskó, L. 2016. Changes in physical properties of sandy soil after long-term compost treatment. International Agrophysics, 30(3): 269-274.

Araujo, A. P., Teixeira, M. G. 2008. Relationships between grain yield and accumulation of biomass, nitrogen and phosphorus in common bean cultivars. Revista Brasileira de Ciência do Solo, 32: 1977-1986.

Argaw, A., Mekonnen, E., Muleta, D. 2015. Agronomic efficiency of N of common bean (*Phaseolus vulgaris* L.) in some representative soils of Eastern Ethiopia. Cogent Food and Agriculture, 1(1): 1-15.

Arif, T., Bhosale J. D., Kumar, N., Mandala ,T. K., Bendre R.S., Lavekar G. S., Dabura, R. 2009. Natural products – antifungal agents derived from plants. Journal of Asian Natural Products Research, 11: 621-638.

Aryantha, I. P., Cross, R., Guest, D. I. 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. Phytopathology, 90(7): 775–782.

Ashrafi, Z. Y., Sadeghi, S., Mashhadi, H. R., Hassan, M. A. 2008. Allelopathic effects of sunflower (*Helianthus annuus*) on germination and growth of wild barley (*Hordeum spontaneum*). Journal of Agricultural Technology, 4(1): 219-229.

Ayub, M., Ijaz, M. K., Tariq, M., Tahir, M., Nadeem, M. A. 2012. Allelopathic effects of winter legumes on germination and seedling indicators of various summer cereals. Agricultura Tropica et Tubtropica, 45: 179-183.

Azlan, A., Aweng, E. R., Ibrahim, C. O., Noorhaidah, A. 2012. Correlation between soil organic matter, total organic matter and water content with climate and depths of soil at different land use in Kelantan, Malaysia. Journal of Applied Sciences and Environmental Management, 16(4): 353-358.

Balaali, G. R., Iranpoor, M. 2006. Identification band genetic variation of *Fusarium* species in Isfahan, Iran, using pectic zymogram technique. Iranian Journal of Science and Technology, 30(1): 92-100.

Baldock, J. A. 2007. Composition and cycling of organic carbon in soil. in nutrient cycling in terrestrial ecosystems (pp. 1-35). Springer, Berlin, Heidelberg.

Balekari U. 2013. Antihyperglycemic and antihyperlipidaemic activities of Dolichos Lablab (*Lablab purpureus*) seed extract on streptozotocin - nicotinamide induced diabetic rats. International Conference and Exhibition on Pharmacognosy, Phytochemistry and Natural Products, Hyderabad- India, 21-23.

Bareja M, Kumar, P., Lodha, S. 2010. Effect of composts on microbial dynamics and activity, dry root rot severity and seed yield of cowpea in the Indian arid region, Phytopathologia Mediterranea, 49: 381-392.

Bech, L., Busk, P. K., Lange, L. 2015. Cell wall degrading enzymes in *Trichoderma asperellum* grown on wheat bran. Fungal Genetics and Biology, 4: 116- 128.

Beebe, S., Rao, I., Blair, M., Acosta, J. 2013. Phenotyping common beans for adaptation to drought. Frontiers in Physiology, 4(35): 1-19.

Begum M, M., Sariah M., Zainal M. A., Puteh A. B., Rahman, M. A. 2008. Ultrastructural studies of soybean seed-borne infection by *Diaporthe phaseolorum* var. *sojae* and screening of antagonistic potentiality by selected biocontrol agents *in vitro*. Pertanika Journal of Tropical Agricultural Science, 31(2): 247–256.

Bending, G. D., M. K. Turner. 1999. Interaction of biochemical quality and particle size of crop residues and its effect on the microbial biomass and nitrogen dynamics following incorporation into soil. Biology and Fertility of Soils, 29: 319-327.

Benjawan, L., Sihawong, S., Chayaprasert, W., Liamlaem, W. 2015. Composting of biodegradable organic waste from Thai household in a semi-continuous composter. Compost Science and Utilization, 23(1): 11-17.

Berg, L. E., Miller, S. S., Dornbusch, M. R., Samac, D. A. 2017. Seed rot and damping-off of alfalfa in Minnesota caused by *Pythium* and *Fusarium* species. Plant Disease, 101: 1860-1867.

Bi, R., Spadiut, O., Brumer, H., Henriksson, G. 2012. Isolation and identification of microorganisms from soil able to live on lignin as a carbon source and to produce enzymes which cleave the β-o-4 bond in a lignin model compound. Cellulose Chemistry and Technology, 46(3-4): 227-242.

Binagwa, P. H., Bonsi, C. K., Msolla, S. N., Ritte, I. I. 2016. Morphological and molecular identification of *Pythium* spp. isolated from common beans (*Phaseolus vulgaris* L.) infected with root rot disease. African Journal of Plant Science, 10(1): 1-9.

Birachi, E. A., Ochieng, J., Wozemba, D., Ruraduma, C., Niyuhire, M. C. 2011. Factors influencing smallholder farmers' bean production and supply to market in Burundi. African Crop Science Journal, 19: 335-342.

Blake, J. J., Spink, J. H., Dyer, C. 2003. Factors affecting cereal establishment and its prediction. HGCA Research Review. Retrieved from https://cereals.ahdb.org.uk/media/306036/rr51_complete_final_report.pdf. Accessed on 15 June 2017.

Blum U., Shafer S.R., Lehman M.E., 1999. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. an experimental model. Critical Review of Plant Science, 18: 673-693.

Bonanomi, G., Sicurezza, M.G., Caporaso, S., Esposito, A., Mazzoleni, S., 2006. Phytotoxicity dynamics of decaying plant materials. New Phytologist, 169: 571-578

Bonanomi, G., Antignani, V., Pane, C., Scala, F. 2007. Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology, 89: 311-324.

Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology and Biochemistry, 4: 136-144.

Bonanomi, G., Incerti, G., Barile, E., Capodilupo, M., Antignani, V., Mingo, A., Mazzoleni, S. 2011. Phytotoxicity, not nitrogen immobilization, explains plant litter inhibitory effects: evidence from solid-state 13C NMR spectroscopy. New Phytologist, 191: 1018-1030.

Bonanomi, G., Incerti, G., Giannino, F., Mingo, A., Lanzotti, V., Mazzoleni, S. 2013. Litter quality assessed by solid state 13C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. Soil Biology and Biochemistry, 56: 40-48.

Bonanomi, G., Cesarano, G., Lombardi, N., Motti, R., Scala, F., Mazzoleni, S., Incerti, G. 2017. Litter chemistry explains contrasting feeding preferences of bacteria, fungi, and higher plants. Scientific reports, 7(1): 1-13.

Bonilla, N., Gutiérrez-Barranquero, J., Vicente, A., Cazorla, F. 2012. Enhancing soil quality and plant health through suppressive organic amendments. Diversity, 4(4): 475-491.

Bonini, C. S. B., Alves, M. C. 2010. Relation between soil organic matter and physical properties of a degraded oxisol in recovery with green manure, lime and pasture. Paper presented at the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, 1-6.

Braun, J., Beckett, P. 2005. Factors affecting decomposition properties of paper mill sludge spread on mine tailings. (unpublished report). 7-14

Brown, B., Westermann, D. 2000. Soil fertility and bean production. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Soil+Fertility+and+Be

an+Production&btnG=#d=gs_cit&p=&u=%2Fscholar%3Fq%3Dinfo%3An-H97-GX0EUJ%3Ascholar.google.com%2F%26output%3Dcite%26scirp%3D0%26hl%3Den (Accessed on 21st June 2016).

Brown, S., Cotton, M. 2011. Changes in soil properties and carbon content following compost application: results of on-farm sampling. Compost Science and Utilization, 19: 87-96.

Budko, N., Corbetta, A., van Duijn, B., Hille, S., Rottschäfer, V., Wiegman, L., Zhelyazov, D. 2013. Oxygen transport and consumption in germinating seeds. SWI Proceedings, 5: 1-30.

Bulluck , L. R., Brosiusb, M, Evanylob, G. K., Ristainoa, J. B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology, 19: 147-160.

Buruchara, R. A. 2006. Background information on common beans. Biotechnology, breeding and seed systems for African crops. [Online]. Available by Rockefeller foundation, Nairobi, Kenya. http://www.africancrops.net/rockefeller/crops/beans/index.htm Accessed on 21st June 2016.

Buyer, J. S., Teasdale, J. R., Roberts, D. P., Zasada, I. A., Maul, J. E. 2010. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biology and Biochemistry, 42(5): 831-841.

Cappaert, M. R., Powelson, M. L. 1997. Potential of a green manure to suppress early dying of potato in the Columbia Basin. American Potato Journal, 74: 421-426.

Carvalho, N. S., Oliveira, A. B. B., Pessoa, M. M. C., Neto, V. P. C., de Sousa, R. S., da Cunha, J. R., de Araujo, A. S. F. 2015. Short-term effect of different green manure on soil chemical and biological properties. African Journal of Agricultural Research, 10(43): 4076-4081.

Cattanio, J. H., Kuehne, R., Vlek, P. L. 2008. Organic material decomposition and nutrient dynamics in a mulch system enriched with leguminous trees in the Amazon. Revista Brasileira de Ciencia do Solo, 32: 1073-1086.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., Toker, C. 2018. The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. Agronomy, 8(9): 166-175.

Chabi-Olaye, A., C. Nolte, F. Schulthess, Borgemeister, C. 2005. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller)

(Lepidoptera: Noctuidae) in the humid forest of Southern Cameroon. Agriculture, Ecosystem and Environment Journal, 108: 17–28.

Chekanai, V., Chikowo, R., Vanlauwe, B. 2018. Response of common bean (*Phaseolus vulgaris* L.) to nitrogen, phosphorus and rhizobia inoculation across variable soils in Zimbabwe. Agriculture, Ecosystems and Environment, 266: 167-173.

Cheng, F., Cheng, Z. 2015. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Frontiers in Plant Science, 6: 1020-1018.

Chittem, K., Mathew, F. M., Gregoire, M., Lamppa, R. S., Chang, Y. W., Markell, S. G., Goswami, R. S. 2015. Identification and characterization of *Fusarium* spp. associated with root rots of field pea in North Dakota. European Journal of Plant Pathology, 143(4): 641-649.

Chukwuka, K. S., Obiakara, M. C., Ogunsumi, I. A. 2014. Effects of aqueous plant extracts and inorganic fertilizer on the germination, growth and development of maize (*Zea mays* L.). Journal of Agricultural Sciences, Belgrade, 59(3): 243-254.

Cichy, K. A., Snapp, S. S., Kirk, W. W. 2007. Fusarium root rot incidence and root system architecture in grafted common bean lines. Plant and soil, 300: 233-244.

Cieniak, C., Walshe-Roussel, B., Liu, R., Muhammad, A., Saleem, A., Haddad, P.S., Cuerrier, A., Foster, B.C. and Arnason, J. T., 2015. Phytochemical comparison of the water and ethanol leaf extracts of the cree medicinal plant, *Sarracenia purpurea* L. (Sarraceniaceae). Journal of Pharmacy and Pharmaceutical Sciences, 18: 484-493.

Cohen, Y., Baider, A., Ben-Daniel, B., Ben-DanieI, Y. 2002. Fungicidal preparations from Inula viscose. [Extrakte aus Inula viscose zur Bekämpfung von Pilzkrankheiten.] In: Boos, Markus (Ed.) 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture. Proceedings to the Conference from 4th to 7th Feb. 2002 at Weinsberg/Germany, pp. 152-156.

Condron, L., Stark, C., O'Callaghan, M., Clinton, P., Huang, Z. 2010. The role of microbial communities in the formation and decomposition of soil organic matter: In Soil microbiology and sustainable crop production (pp. 81-118). Springer, Dordrecht. Retrieved from https://pdfs.semanticscholar.org/217b/4fb4320bc26125cc81000ba3f3c64a001d1c.pdf. Accessed on 21st June 2016.

Conklin, A. E., Erich, M. S., Liebman, M., Lambert, D., Gallandt, E. R., Halteman, W. A. 2002. Effects of red clover (*Trifolium pratense*) green manure and compost soil

amendments on wild mustard (*Brassica kaber*) growth and incidence of disease. Plant and Soil, 238: 245-256.

Constantinides, M., Fownes, J. H. 1994. Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin, and soluble polyphenol concentrations. Soil Biology and Biochemistry, 26: 49-55.

Cwalina-Ambroziak, B., Bowszys, T. 2009. Changes in fungal communities in organically fertilized soil. Plant, Soil and Environment, 55(1): 25-32.

Demir, I., Ermis, S. Okcu, G., Matthews, S. 2005. Vigour tests for predicting seedling emergence of aubergine (*Solanum melongena* L.) seed lots. Seed Science and Technology, 33: 481-484.

Dezfuli, P. M., Sharif-Zadeh, F., Janmohammadi, M. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). Journal of Agriculture and Biological Science, 3(3): 22-25.

Dhole, J. A., Bodke, S., Dhole, N. 2013. Allelopathic effect of aqueous extract of five selected weed species on seed mycoflora, seed germination and seedling growth of *Sorghum vulgare* Pers. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(3): 142-148.

Dorrance, A. E., Kleinhenz, M. D., Mclure, S. A., Tuttle,N. T. 2003. Temperature, moisture and seed treatments effects on *Rhizotonia solani* root rot of soybean. Plant Disease, 87: 533-538.

Duong, T. T. T. 2009. Dynamics of plant residue decomposition and nutrient release. Pp. 1-109. Master's Dissertation. University of Adelaide, Australia.

Eco, J., Park, K. C. 2016. Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. Agricultural Ecosystem and Environment, 231: 176–182.

Elfstrand, S., Hedlund, K, Martensson, A. 2007. Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. Applied Soil Ecology, 35: 610–621.

El-Naim, A. M., Jabereldar, A. A., Ahmed, S. E., Ismaeil, F. M., Ibrahim, E. A. 2012. Determination of suitable variety and plants per stand of cowpea (*Vigna unguiculata* L. Walp) in the sandy soil, Sudan. Advances in Life Sciences, 2: 1-5.

Ewansiha, S. U., Ogedegbe, S. A., Falodun, E. J. 2016. Utilization potentials of lablab (*Lablab purpureus* (L.) Sweet) and the constraints of field pests and diseases in Nigeria. Agro-Science, 15(1): 11-16.

Fabunmi, T. O., Balogun, R. O. 2015. Response of maize (*Zea mays* L.) to green manure from varying populations of cowpea in a derived savannah of Nigeria. African Journal of Food, Agriculture, Nutrition and Development, 15(3): 10138-10152.

Fanish, S. A. 2017. Impact of green manure incorporation on soil properties and crop growth environment: A Review World Journal of Agricultural Sciences, 13: 122-132.

FAO-UNESCO. 1997. Soil map of the world. Revised Legend, edited by ISRIC, Wageningen.

Farooq M., Bajwa A. A., Cheema S. A., Cheema Z. A. 2013. Application of allelopathy in crop production. International Journal of Agriculture and Biology, 15: 1367–1378.

Ferreira, E. P., Stone, L. F., Didonet, A. D. 2013. Green manure species and sowing time effects on the agronomic performance of common bean. Agronomy Journal, 105: 1721-1727.

Fierer N., Craine J.M., McLauchlan K., Schimel J.P. 2005. Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326.

Florentin, M. A., Penalva, M., Calegari, A., Derpsch, R. 2011. Green manure/cover crops and crop rotation in conservation agriculture on small farms (pp.97). Rome: Food and Agriculture Organization.

Gao, X., Wu, M., Xu, R., Wang, X, and Pan, R. 2014. Root interactions in a maize/soybean intercropping system control soybean soilborne disease. Public Library Science. 95: 1-8.

Garcia, R.A., Miranda, A.M., Junior, M.L., Araujo, F.G. and Cunha, M. 2014. Effect of organic compounds on root rots of common beans. Bioscience Journal. 30: 25-32.

Gatiboni, L. C., Coimbra, J. L. M., Denardin, R. B. N., Prado Wildner, L. D. 2011. Microbial biomass and soil fauna during the decomposition of cover crops in no-tillage system. Revista Brasileira de Ciência do Solo, 35: 1051-1057.

Geisseler D, Scow KM. 2014. Long-term effects of mineral fertilizers on soil microorganisms-A review. Soil Biology and Biochemistry, 75: 54–63.

Gezahegn, A. M., Tesfaye, K., Sharma, J. J., Belel, M. D. 2016. Determination of optimum plant density for faba bean (*Vicia faba* L.) on vertisols at Haramaya, Eastern Ethiopia. Cogent Food and Agriculture, 2(1): 1-10 doi.org/10.1080/23311932.2016.1224485.

Gomez, R, P., Aulicino, M, B., Monaco, C., Kriplelz, N., Cordo, C. A. 2016. Composition and dynamics of the fungal population in a typical Phaeozem luvico in Argentina. Cogent Food and Agriculture, 2: 1-15 doi.org/10.1080/23311932.2016.1180965.

Gordon, T. R., Okamoto, D. 1990. Colonization of crop residue by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium*. Phytopathology, 80(4): 381-386.

Gougoulias, C., Clark, J. M., Shaw, L. J. 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. Journal of the Science of Food and Agriculture, 94: 2362-2371.

Grassbaugh, E. M., Bennett, M. A. 1998. Factors affecting vegetable stand establishment. Scientia Agricola, 55: 116-120.

Gregorich, E. G., Carter M, R., Angers, D. A, Monreal, C. M., Ellert B. H. 1994. Towards a minimum data set to assess soil organic matter quality in agricultural soils. Canadian. Journal of Soil Science, 74: 367–385.

Haraala, C. 2012. Compost and its effects on soilborne plant pathogens. Swedish University of Agricultural Sciences, Uppsala. Retrieved from https://stud.epsilon.slu.se/4261/12/haarala_c_a_120531.pdf. Accessed on 12th June 2017

Haas, D., and Défago, G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology, 3(4): 307- 314.

Hadi, M., Kashefi, B., Sobhanipur, A., Rezaarabsorkhi, M. 2013. Study on effect of some medicinal plant extracts on growth and spore germination of *Fusarium oxysporum* schlecht. *in vitro*. American-Eurasian Journal of Agricultural and Environmental Science, 13(4): 581-588.

Hassan, M. R., Amodu, J. T., Muhammad, I. R., Jokthan, G. E., Abdu, S. B., Abdullahi, B., Akpensuen, T. T. 2014. Forage yield and quality of Lablab (*Lablab purpureus* L. Sweet) intercropped with maize (*Zea mays* L.) with flooded irrigation system in the semi-arid zone of Nigeria. Journal of Agricultural Science, 6(11): 196-201.

Haramoto, E. R., Gallandt, E. R. 2005. Brassica cover cropping: II. Effects on growth and interference of green bean (*Phaseolus vulgaris* L.) and redroot pigweed (*Amaranthus retroflexus*). Weed Science, 53: 702-708.

Hejase A. J, Hejase H. J. 2013. Research methods a practical approach for business students. (2nd edn.), Philadelphia, PA, USA: Masadir Inc.

Herro, J.L, Callaway RM 2003. Allelopathy and exotic plant invasion. Plant and Soil, 256: 29-39.

Heydari, A., Pessarakli, M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences, 10(4): 273-290.

Hiel, M. P., Chélin, M., Parvin, N., Barbieux, S., Degrune, F., Lemtiri, A., Garre, S. 2016. Crop residue management in arable cropping systems under a temperate climate. Part 2: Soil physical properties and crop production. A review. BASE, 20: 245-256.

Hillary, J., Nuringtyas, T. R. 2016. Inhibitory effect of wild mango (*Mangifera foetida* L.) extract on seed germination of *Cynodons dactylon* (L.) Pers. In AIP Conference Proceedings (Vol. 1744, No. 1, p. 020029). AIP Publishing.

Ho, W. C., Wu, T. Y., Su, H. J., Ko, W. H. 2007. Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from speedweed. Plant Disease, 91: 1621-1624.

Hoitink, H. A. J., Boehm, M. J., Hadar, Y. 1993. Mechanisms of suppression of soilborne plant pathogens in compost amended substrates. In: Science and Engineering of composting: Design, Environmental, Microbiological and Utilization Aspects. (Eds H. A. J. Hoitink, H. M. Keener). Ohio state University, Ohio, 601 to 621.

Hoitink, H. A .J., Boehm, M. J. 1999. Biocontrol within the context of soil microbial communities: a substrate- dependent phenomenon. Annual Reviews of Phytopathology, 27: 427- 446.

Hussain, M. I. Gonzalez-Rodriguez, L., Reigosa, M. J. 2008. Germination and growth response of four plant species to different allelochemicals and herbicides, Allelopathy Journal, 22:101–110.

Ibrahim, A. K., Ibrahim, S. A., Voncir, N., Hassan, A. M. .2017. Effects of green manuring and nitrogen levels on the yield and yield attributes of maize (*Zea mays* L.). Asian Journal of Soil Science and Plant Nutrition, 2(4): 1-11.

Inckel, M., Smet, P. D., Tersmette, T., Veldkamp, T. 2002. The preparation and use of compost. Agromisa Foundation, Wageningen. Retrieved from http://www.journeytoforever.org/farm_library/AD8.pdf. Accessed on 26th November 2017.

Islam, A. K. M., Kato-Noguchi, H. 2014. Phytotoxic Activity of Ocimum tenuiflorum Extracts on Germination and Seedling Growth of Different Plant Species," The Scientific World Journal, vol. 2014, Article ID 676242, 8 pages. https://doi.org/10.1155/2014/676242.

Jeon, W. T., Kim, M. T., Seong K. Y., Oh I. S. 2008. Changes of soil properties and temperature by green manure under rice-based cropping system. Korean Journal of Crop Science, 53: 413-416.

Jilani, G., Mahmood, S., Chaudhry, A. N., Hassan, I., Akram, M. 2008. Allelochemicals: sources, toxicity and microbial transformation in soil - a review. Annals of Microbiology, 58(3): 351-357.

Jules, C. N., Assob, Henri L. F., Dickson S. Nsagha, A. L. Njunda, P. F N., Emmanuel A., Asongalem, A., Njouendou, J., Bertrand, S., Veronique, B. P. 2011. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. BMC Complementary and Alternative Medicine. 11:70. doi:10.1186/1472-6882-11-70.

Kabuyah, R. T. N. M. 2012. The decomposition of organic matter in soils by fungi. Doctoral Dissertation, University of Manchester.

Khare, E., Arora, N. K. 2015. Effects of soil environment on field efficacy of microbial inoculants. In Plant Microbes Symbiosis: Applied Facets (pp. 353-381). Springer, New Delhi.

Kajumula M., S., G. Muhamba T. 2012. Evaluation of Common Bean (Phaseolus vulgaris L.) Genotypes for Adaptation to Low Phosphorus," ISRN Agronomy, vol. 2012, Article ID 309614, 9 pages. Https://doi.org/10.5402/2012/309614.

Kato-Noguchi, H., Mominul, A. K. M. Islam. 2014. Phytotoxic Activity of Ocimum tenuiflorum Extracts on Germination and Seedling Growth of Different Plant Species," The Scientific World Journal, vol. 2014, Article ID 676242, 8 pages. https://doi.org/10.1155/2014/676242.

Katterer, T., Reichstein, M., Andrén, O., Lomander, A. 1998. Temperature dependence of organic matter decomposition: a critical review using literature data analyzed with different models. Biology and Fertility of Soils, 27(3): 258-262.

Katungi, E., Farrow, A., Chianu, J., Sperling, L., Beebe, S. 2009. Common bean in Eastern and Southern Africa: a situation and outlook analysis. International Center for Tropical Agriculture (CIAT), 6:1–61.

Katungi, E., Magreta, R., Letaa, E., Chirwa, R., Dambuleni, K., Nyamwaro, S. 2017. Adoption and impact of improved bean varieties on food security in Malawi. Technical Report. CIAT- PABRA.

Kaur, S., Singh, H. P., Batish, D. R., Kohli, R. K. 2012. Phytotoxicity of decomposing below-ground residues of *Ageratum conyzoides*: nature and dynamics of release of phytotoxins. Acta Physiologiae Plantarum, 34(3): 1075-1081.

Khaliq, A., Matloob, A., Farooq, M., Mushtaq, M. N., Khan, M. B. 2011. Effect of crop residues applied isolated or in combination on the germination and seedling growth of horse purslane (*Trianthema portulacastrum*). Planta Daninha, 29(1): 121-128.

Kimiti, J. M., Odee, D. W., Vanlauwe, B. 2009. Area under grain legumes cultivation and problems faced by smallholder farmers in legume production in the semi-arid eastern Kenya. Journal of Sustainable Development in Africa, 11: 305-315.

Kiplagat, J. K., Okalebo, J. R., Serrem, C. K., Mbakaya, D. S., Jama. B. 2014. Determination of appropriate rate and mode of lime application on acid soils of Western Kenya: targeting small scale farmers. in challenges and opportunities for agricultural intensification of the humid highland systems of Sub-Saharan Africa, doi.org/ 10.1007/978-3-319-07662-1_15.

Kinstche, K., Guibert, H., Bonfoh, B., Tittonell, P. 2015. Long-term decline in soil fertility and responsiveness to fertilizer as mitigated by short fallow periods in Sub-Sahelian area of Togo. Nutrient Cycling in Agroecosystems, 101: 333-350.

Kołodziejek, J. 2017. Effect of seed position and soil nutrients on seed mass, germination and seedling growth in *Peucedanum oreoselinum* (*Apiaceae*). Scientific Reports, 7(1): 1959 doi.org/ 10.1038/s41598-017-02035-1.

Korayem, A.M., Dieb, M. M. M. Mohamed, M. M. M. Saied, N.M. 2016. Interrelationships of root-knot nematodes with root-rot fungi and their effect on common bean grown in natural infestation. International Journal of PharmTech Research. 9(10): 25-32.

Kumar, R., Mahajan, G., Srivastava, S., Sinha, A. 2014. Green manuring: A boon for sustainable agriculture and pest management–A review. Agricultural Reviews, 35: 196-206.

Lal, R. 2015. Restoring soil quality to mitigate soil degradation. Sustainability, 7(5), 5875-5895

Langwerden, J. E. 2014. Soil fertility constraints for the production of common bean (*Phaseolus vulgaris* L.) in the Usambara Mountains of Northern Tanzania. MSc. Thesis. Wageningen University.

Larkin, R. P. 2013. Green manures and plant disease management. CAB Reviews, 8(37): 1-10.

Larkin, R. P., and Griffin, T. S. 2007. Control of soilborne potato diseases using Brassica green manures. Crop protection, 26: 1067-1077.

Lauren, J., Ojiem, J., Medvecky, B., Odendo, M., Pell, A., Mwonga, S.,Duxbury, J., Okalebo, J., Hobbs, P., Nderitu, J., Stoltzfus, R., Muthomi, J., Barrett,C., Buruchara,

R. 2009.Using Improved Pulse Crop Productivity to Reinvigorate Smallholder Mixed Farming Systems in Western Kenya. Technical Progress Reports. Dry Grain Pulses Collaborative Research Program (CRSP) pp 1-23.

Lawes Agricultural Trust, Harpenden, 1991. Guide to the classical experiments. Rothamsted Experimental Station. UK.

Lazzeri, L., Manici L. M. 2001. Allelopathic effect of glucosinolate containing plant green manure on *Pythium* spp. and total fungal population in soil. Horticulutral Science 36**:**1283–1289.

Leitich, R. K., Omayio, D. O., Mukoye, B., Mangeni, B. C., Wosula, D. W., Arinaitwe, W., Abang, M. M. 2016. Pathogenic variability of angular leaf spot disease of common bean in Western Kenya. International Journal of Applied Agricultural Sciences, 2(6): 92-98.

Lemtiri, A., Degrune, F., Barbieux, S., Hiel, M.P., Chélin, M., Parvin, N., Vandenbol, M., Francis, F., Colinet, G., 2016. Crop residue management in arable cropping systems under temperate climate. Part 1: Soil biological and chemical (phosphorus and nitrogen) properties. A review. Biotechnology, Agronomy, Society, and Environment, 20: 236-244.

Lertmongkol., Sarobol, E. D., Premasthira. C. 2011. Allelopathic effects of mung bean (*Vigna radiata* L.) on subsequent crops. Kasetsart Journal of Natural Sciences, 45:773-779.

Li, X., Lewis, E. E., Liu, Q., Li, H., Bai, C., Wang, Y. 2017. Effects of long-term continuous cropping on soil nematode community and soil condition associated with replant problem in strawberry habitat. Scientific reports, 6: 30466- 30471.

Litterick, A. M., Harrier, L., Wallace, P., Watson, C. A., Wood, M. 2004. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—A review. Critical Reviews in Plant Sciences, 23:453-479.

Lodhi, A. M., Khanzada, M. A., Shahzad, S., Ghaffar, A., Lévesque, C. A. 2013. Prevalence of *Pythium aphanidermatum* in agro-ecosystem of Sindh Province of Pakistan. Pakistan Journal of Botany 45(2): 635-642.

Lynch, J., Rosas, J. C., Magalhaes Miguel, I. I. A. M., Brown, M. K., Findeis, J., Celestina Jochua, I. I. A. M., Xerinda, M. S. A. 2009. Improving bean production in drought-

prone, low fertility soils of Africa and Latin America-an integrated approach. Phase I-Pennsylvania State University as Lead University–Project Google Scholar.

Maass B, Knox M, Venkatesha S, Angessa T, Ramme S, Pengelly, B. 2010. *Lablab purpureus* - a crop lost for Africa? Tropical Plant Biology 3:123–135.

Macedo, R., Sales, L. P., Yoshida, F., Silva-Abud, L. L., Junior, M. L. 2017. Potential worldwide distribution of Fusarium dry root rot in common beans based on the optimal environment for disease occurrence. PloS one, 12(11), e0187770. https://doi.org/10.1371/journal.pone.0187770

Mahmood, F., Khan, I., Ashraf, U., Shahzad, T., Hussain, S., Shahid, M., Ullah, S. 2017. Effects of organic and inorganic manures on maize and their residual impact on soil physico-chemical properties. Journal of Soil Science and Plant Nutrition, 17(1): 22-32.

Manici, L. M., Caputo, F., Babini, V. 2004. Effect of green manure on *Pythium* spp. population and microbial communities in intensive cropping systems. Plant and Soil, 263: 133-142.

Manici, L. M., Ciavatta, C., Kelderer, M., Erschbaumer, G. 2003. Replant problems in South Tyrol: role of fungal pathogens and microbial population in conventional and organic apple orchards. Plant and Soil, 256 (2): 315-324.

Marieh, N. 2012. Characterization of soil humic substances in Hungarian and Iranian soils. Unpublished Ph.D. Thesis, Szentistvan University.

Marzano, S. Y. 2012. Assessment of disease suppression in organic transitional cropping systems. Doctoral Dissertation, University of Illinois at Urbana-Champaign.

Masangwa, J. I. G., Kritzinger, Q., Aveling, T. A. S. 1. 2017. Germination and seedling emergence responses of common bean and cowpea to plant extract seed treatments. Journal of Agricultural Science, 155: 18–31.

Masoud, E. A. and Gouda, H. A. 2012. Effect of some natural plant extracts against gram negative bacteria in Njran Area, Saudi Arabia. Egyptian Academic Journal of Biological Sciences, 4: 85-92.

Matthews, S. and Khajeh Hosseini, M. 2007. Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize (*Zea mays*). Seed Science and Technology, 35: 200-212.

Mayo, S., Gutierrez, S., Malmierca, M.G., Lorenzana,A., Campelo, M.P. Hermosa, R. and Casquero, P.A. 2015. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth

of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. Frontiers in Plant Science, 6: 685-692.

Medvecky, B., Ketterings, Q., Nelson, E. 2007. Relationships among soil borne bean seedling diseases, *Lablab purpureus* L. and maize stover residue management, bean insect pests and soil characteristics in Trans Nzoia district, Kenya. Applied Soil Ecology, 35: 107-119.

Meena, B. L., Fagodiya, R. K., Prajapat, K., Dotaniya, M. L., Kaledhonkar, M. J., Sharma, P. C., Kumar, S. 2018. Legume green manuring: an option for soil sustainability. *In legumes for soil health and sustainable management* (pp. 387-408). Springer, Singapore. Retrieved from https://link.springer.com/chapter/10.1007%2F978-981-13-0253-4_12. Accessed on 3rd October 2018.

Meghvansi, M. K., Varma, A. 2015. Organic amendments and soil suppressiveness in plant disease management. Switzerland: Springer International Publishing. Retrieved from https://www.springer.com/gp/book/9783319230740. Accessed on 3rd October 2018.

Mihajlovic, M., Rekanović, E., Hrustić, J., Grahovac, M., Tanović, B. 2017. Methods for management of soilborne plant pathogens. Pesticidi i Fitomedicina, 32(1): 9-24.

Mildred, M. M. 2017. Effect of soil fertility and intercropping on the incidence and severity of root rot diseases of common bean *Phaseolus vulgaris* L. pp 1-124. MSc. Thesis, University of Nairobi.

Miller, J. O., 2016. Organic matter is an essential component of soils. University of Maryland Extension. Retrieved from https://extension.umd.edu/sites/extension.umd.edu/files/_docs/articles/FS-1045%20Soil%20Organic%20Matter.pdf. Accessed on 08th July 2018.

Mohammadi, Z., Atik, F. 2013. Fungitoxic effect of natural extracts on mycelial growth, spore germination and aflatoxin B1 production of *Aspergillus flavus*. Australian Journal of Crop Science, 7(3): 293- 298.

Mosavi, S. B., Jafarzadeh, A. A., Nishabouri, M. R., Ostan, S., Feiziasl, V., Karimi, E. 2012. The effect of different green manure application in dry land condition on some soil physical properties. International Journal of Agricultural Crop Science, 4: 1233-1239.

Motsara, M. R., Roy, R. N. 2008. Guide to laboratory establishment for plant nutrient analysis. Rome: Food and Agriculture Organization of the United Nations, 19: 45-64.

Muengula-Manyi, M., Mukwa, L., Nkongolo, K. K., Tshilenge-Djim, P., Winter, S., Bragard, C., Kalonji-Mbuyi, A., 2013. Assessing reactions of genetically improved and local

cassava varieties to Cassava Mosaic Disease (CMD) infection in a savannah region of the DR-Congo. Annals of Agricultural Science, 62: 99–104.

Mugisha, C. 2008. Improving resistance to *Fusarium* root rot (*Fusarium solani* (mart) Sacc. f. sp. *phaseoli* in common beans (*Phaseolus vulgaris* L.). Doctoral Dissertation, University of Kwa Zulu-Natal, Pietermaritzburg.

Mugwe, J., Mugendi, D., Mucheru-Muna, M., Odee, D., Mairura, F. 2009. Effect of selected organic materials and inorganic fertilizer on the soil fertility of a humic nitisol in the central highlands of Kenya. Soil Use and Management, 25(4): 434-440.

Mugwe, J., Mugendi, D., Mucheru-Muna.. M, Merckx, R., Chianu, J. and Vanlauwe B. 2008. Determinants of the decision to adopt integrated soil fertility management practices by smallholder farmers in the central highlands of Kenya. Experimental Agriculture, 45: 61-75.

Mukankusi, C., Opala, J. 2012. Development of Fusarium root rot resistant ideotypes in common bean. Third RUFORUM Biennial Meeting 24 - 28 September. Entebbe, Uganda.

Mundel, H. H., Huang, H. C., Kozub, G. C., Barr, D. J. S. 1995. Effect of soil moisture and temperature on seedling emergence and incidence of Pythium damping-off in safflower (*Carthamus tinctorius* L.). Canadian Journal of Plant Science, 75(2): 505-509.

Muthomi, J. W., Mugambi, I. K., Ojiem, J., Chemining'wa, G. N. and Nderitu, J. H. 2014. Effect of incorporating lablab biomass in soils on root rot disease complex and yield of beans intercropped with maize. International Journal of AgriScience, 4: 515-524.

Murphy, A. M., Colucci, P. E. 1999. A tropical forage solution to poor quality ruminant diets: A review of *Lablab purpureus*. Livestock Research for Rural Development, 11(2): 25-34.

Muzhinji, N., Truter, M., Woodhall, J. W., and van der Waals, J. E. 2015. Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* from potato in South Africa. Plant Disease. 99:1790-1802.

Mwanauta, R. W., Mtei, K. M., Ndakidemi, P. A. 2015. Potential of controlling common bean insect pests (bean stem maggot (*Ophiomyia phaseoli*), Ootheca (*Ootheca bennigseni*) and Aphids (*Aphis fabae*)) using agronomic, biological and botanical practices in field. Agricultural Sciences, 6: 489 – 497.

Mwangi, S.N., Deng, A.L. and Kamau, A.W. 2008. Response of Kenyan varieties of common bean, *Phaseolus vulgaris* L., to infestation by *Aphis fabae* Scopoli. African Entomology, 16:196-202

N'Dayegamiye, A., Tran, T. S. 2001. Effects of green manures on soil organic matter and wheat yields and N nutrition. Canadian Journal of Soil Science, 81(4): 371-382

Nakhro, N., Dkhar, M. S. 2010. Populations and biomass carbon in paddy field soil. Journal of Agrobiology, 9: 102-110.

Namugwanya, M., Tenywa, J. S., Otabbong, E., Mubiru, D. N., Masamba, T. A. 2014. Development of common bean (*Phaseolus vulgaris* L.) production under low soil phosphorus and drought in Sub-Saharan Africa: a review. Journal of Sustainable Development, 7(5): 128-135.

Naseri, B. 2008. Root rot of common bean in Zanjan, Iran: major pathogens and yield loss estimates. Australasian Plant Pathology, 37: 546-551.

Naseri, B. 2014b. Bean production and Fusarium root rot in diverse soil environments in Iran. Journal of Soil Science and Plant Nutrition. 141:177-188.

Naseri, B. 2014a. Charcoal rot of bean in diverse cropping systems and soil environments. Journal of Plant Diseases and Protection, 121(1): 20-25.

Nassiuma D. K. 2000. Survey sampling: Theory and methods. Njoro, Kenya: Egerton University Press.

Nazir, R., Warmink, J. A., Boersma, H., Van Elsas, J. D. 2009. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. FEMS Microbiology Ecology, 71(2): 169-185.

Ng'ayu-Wanjau B. N. 2013. Breeding for durable resistance to angular leaf spot (*Pseudocercospora griseola*) in common bean (*Phaseolus vulgaris*) in Kenya. Doctoral Thesis. University of Kwa Zulu Natal. Pp 1-156.

Ngosong, C., Jarosch, M., Raupp J, Neumann E, Ruess L. 2010. The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. Applied Soil Ecology, 46: 134–142.

Niang, I., O. C. Ruppel, M. A. Abdrabo, A. Essel, C. Lennard, J. Padgham. 2014. Africa. Pp. 1199–1265 in V. R. Barros, C. B. Field, D. J. Dokken, M. D. Mastrandrea, K. J. Mach, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, L. L. White, eds. Climate change 2014: impacts, adaptation, and vulnerability. Part B: regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the

Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY.

Nirenberg, H. I. 1981. A simplified method of identifying *Fusarium* spp occurring on wheat. Canadian Journal of Botany, 9: 1599-1606.

Njoroge, S. M. C., Riley, M. B., Keinath, A. P. 2008. Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on damping-off and *Fusarium* wilt of watermelon. Plant Disease, 92:287-294.

Nnabude, P. C., Nweke, I. A., Nsoanya, L. N. 2015. Response of three varieties of tomatoes (*Lycopersicon* esculentus) to liquid organic fertilizer (alfa life) and inorganic fertilizer (NPK 20: 10: 10) and for soil improvements. European Journal of Physics and Agricultural Sciences, 3: 28-37.

Nollet, L. M., Rathore, H. S. 2010. Handbook of pesticides: Methods of pesticides residue analysis. USA: Taylor and Francis; p. 608. Retrieved from https://www.crcpress.com/Handbook-of-Pesticides-Methods-of-Pesticide-Residues-Analysis/Nollet-Rathore/p/book/9781420082456. Accessed on 13th August 2017.

Nurbaya. K, Tutik Kuswinanti., Ade Rosmana, Baharuddin., Syamsuddin Millang. 2014. Growth rate and identification of *Fusarium* spp. associated with *Aquillaria* spp. from Nunukan regency, North Kalimantan. International Journal of Current Research and Academic Review, 2(11): 7 – 17.

Nweke, F. U. 2015. Effect of some plant leaf extracts on mycelia growth and spore germination of *Botryodiplodia theobromae* causal organism of yam tuber rot. Journal of Biology, 5: 67-71.

Nyberg, G., Tobella, B., Kinyangi, J., Ilstedt, U. 2012. Soil property changes over a 120-yr chronosequence from forest to agriculture in Western Kenya. Hydrology and Earth System Sciences, 16: 2085-2094.

Odendo, M., Obare, G., Salasya, B. 2009. Factors responsible for differences in uptake of integrated soil fertility management practices amongst smallholders in western Kenya. African Journal of Agricultural Research, 4: 1303-1311.

Odhiambo, J. J. 2010. Decomposition and nitrogen release by green manure legume residues in different soil types. African Journal of Agricultural Research, 5(1): 090-096.

Odundo, S., Ojiem, O., Okalebo, J., Othieno, C., Lauren, J., Medvecky, B., 2010. Effect of phosphorus on survival, nodulation and yield of cowpea (*Vigna unguiculata* L.) varieties across a soil fertility degradation gradient in Western Kenya. World cowpea 2010 conference. 27th September to 1st October 2010, Saly, Senegal.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments - a review. Applied Soil Ecology, 44(2): 101-115.

Okalebo, J. R., Gathua, K. W., Woomer, P. L. 2002. Laboratory methods of plant and soil analysis: a working manual. TSBF-UNESCO, Nairobi, Kenya.

Okigbo, R.N., Odurukwe, C. N. 2009. Occurrence and control of fungal rot pathogens of yams (*Dioscorea* spp) with leaf extracts of *Chromolena odorata*, *Carica papaya* and *Aspilia africana*. Nigerian Journal of Mycology, 2(1):154-165.

Okumu, O. O., Muthomi, J. W., Mutegi, C. K., Wagacha, J. W. 2016. Resistance of Kenyan wheat germplasm to Fusarium head blight and deoxynivalenol contamination. International Journal of Agronomy and Agricultural Research, 9: 22-35.

Omondi, O. S. 2011. The potential for njahi (*lablab purpureus* l.) in improving consumption adequacy for protein, iron and zinc in households: A Case for Nandi South District, Kenya. MSc. Dissertation, University of Nairobi.

Ongom, P. O., Nkalubo, S. T., Gibson, P. T., Mukankusi, C. M., Rubaihayo, P. R. 2012. Evaluating genetic association between *Fusarium* and *Pythium* root rots resistances in the bean genotype RWR 719. African Crop Science Journal, 20(1): 31-39.

Onivogui, G., Letsididi, R., Diaby, M., Wang, L., Song, Y. 2016. Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of *Anisophyllea laurina* R. Br. ex Sabine fruits. Asian Pacific Journal of Tropical Biomedicine, 6(1): 20-25.

Oyesola, O.B. Obabire, I. E. 2011. Farmers' perceptions of organic farming in selected local government areas of Ekiti State, Nigeria. Journal of Organic Systems, 6:20-26.

Pachico D. 1993. The demand for bean technology. In*:* Henry G., ed. Trends in CIAT's Commodities. CIAT working document No. 128. Cali, Colombia: CIAT, 60-74

Pal, K. K., B. McSpadden G, 2006. Biological control of plant pathogens. The Plant Health Instructor doi.org/10.1094/PHI-A-2006-1117-02.

Paparu, P., Acut, A., Kato, F., Acam, C., Nakibuule, J., Musoke, S., Nkalubo, S. and Mukankusi, C. 2016. Prevalence and Incidence of Four Common bean root rots in Uganda. Experimental Agriculture.1-13. doi:10.1017/S0014479717000461.

Parks, R. 1998. Influence of a Sudan grass green manure on microorganisms and earl dying of potatoes in two soils. M.Sc. Thesis, Oregon State University, Corvallis.

Pawar, K. B., Chavan, P. D. 2007. Influence of leaf leachates of soybean, Moringa, Parthenium and Eucalyptus on carbohydrate metabolism in germinating seeds of *Sorghum bicolor* (L.) Moench. Allelopathy Journal, 19: 543–548.

Petlier, A. J., Bradley, C. A., Chilvers, M. I., Malvick, D. K., Mueller, D. S., Wise, K. A., Esker, P. D. 2012. Biology, yield loss and control of Sclerotinia stem rot of soybean. Journal of Integrated Pest Management, 3(2): 1-7.

Perez, J., Munoz-Dorado, J., de la Rubia, T. D. L. R., Martinez, J. 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. International microbiology, 5(2): 53-63.

Peter, K. H., Swella, G. B., Mushobozy, D. M. 2009. Effect of plant populations on the incidence of bean stem maggot (*Ophiomyia* spp.) in common bean intercropped with maize. Plant Protection Science-UZEI (Czech Republic). Retrieved from http://agris.fao.org/agris-search/search.do?recordID=CZ2010000064. Accessed on 16th March 2017

Petry, N., Boy, E., Wirth, J. P., Hurrell, R. F. 2015. Review: the potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. Nutrients 7, 1144 – 1173. doi: 10.3390/nu7021144.

Pettersson, M. 2004. Factors affecting rates of change in soil bacterial communities. Doctoral Thesis, Lund University, Sweden.

Pfenning, L. H., De Abreu, L. M. 2008. Saprophytic and plant pathogenic soil fungi. Chapter eight in A hand Book of Tropical Soil Biology, E. Jeroen Husing and David E. Bignel, Fatima, M. S, 153. London: Earth Scan.

Pusztahelyi, T., Holb, I. J., Pócsi, I. 2015. Secondary metabolites in fungus-plant interactions. Frontiers in Plant Science, 6: 573-584. https://doi.org/10.3389/fpls.2015.00573.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil, 321: 341-361.

Rahman, M. A., Begum, M. F., Alam, M. F. 2009. Screening of Trichoderma isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. Mycobiology, 37(4): 277-285.

Ramirez-Villapudua, J., Munnecke, D.E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. Phytopathology, 78:289-295.

Ranells, N. N., Wagger, M. G. 1992. Nitrogen release from crimson clover in relation to plant growth stage and composition. Agronomy Journal, 84: 424- 430.

Rao, I. M., Miles, J.W., Beebe, S.E., Horst, W. J. 2016. Root adaptations to soils with low fertility and aluminium toxicity. Annals of Botany, 118: 593-605.

Rath, K. M., Rousk, J. 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Biology and Biochemistry, 81: 108-123.

Raut, I., Badea-Doni, M., Calin, M.., Oancea, F., Vasilescu, G., Sesan, T. E., Jecu, L. 2014. Effect of volatile and non-volatile metabolites from *Trichoderma* spp. against important phytopathogens. Revista de Chimie, 65(11): 1285-1288.

Reis, E. M., Boaretto, C., Danelli, A. L. D. 2014. *Macrophomina phaseolina*: density and longevity of microsclerotia in soybean root tissues and free on the soil, and competitive saprophytic ability. Summa Phytopathologica, 40(2): 128-133.

Rezende, A. A., Pacheco, M. T. B., Silva, V. S. N D., Ferreira, T. A. P. D. C. 2017. Nutritional and protein quality of dry Brazilian beans (*Phaseolus vulgaris* L.). Food Science and Technology, 10.1590/1678-457x.05917.

Richard, P. O., Ogunjobi, A. A. 2016. Effect of organic and inorganic fertilizer applications on phosphate solubilizing bacteria in the rhizosphere of maize (*Zea mays* L.). African Journal of Microbiology Research, 10: 2021- 2028.

Robotham, O., Chapman, M. 2017. Population genetic analysis of hyacinth bean (*Lablab purpureus* (L.) Sweet, Leguminosae) indicates an East African origin and variation in drought tolerance. Genetic Resources and Crop Evolution, 64(1): 139-148.

Romero-Arenas, O., Damián-Huato, M. A., Rivera-Tapia, J. A., Báez-Simón, A., Huerta-Lara, M., Cabrera-Huerta, E. 2013. The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding of rural communities in Puebla, Mexico. International Research Journal of Biological Sciences, 2(8): 59-65.

Roos, E., Mie, A., Wivstad, M., Salomon, E., Johansson, B., Gunnarsson, S., Watson, C. A. 2018. Risks and opportunities of increasing yields in organic farming. A review. Agronomy for sustainable development, 38(2): 14-35. https://doi.org/10.1007/s13593-018-0489-3.

Salahin, N., Alam, M. K., Islam, M. M., Naher, L., Majid, N. M. 2013. Effects of green manure crops and tillage practice on maize and rice yields and soil properties. Australian Journal of Crop Science, 7(12): 1901- 1908.

Saravanan, S., Sivakumar, T., Thamizhmani, R., Senthilkumaran, R. 2013. Studies on Microbial Diversity in Marine ecosystem of Parangipettai, Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences, 2: 20-32.

Sarma, B., Gogoi, N. 2015. Germination and seedling growth of Okra (*Abelmoschus esculentus* L.) as influenced by organic amendments. Cogent Food and Agriculture, 1(1): 1-6 https://doi.org/10.1080/23311932.2015.1030906

Schnecker, J., Wild, B., Hofhansl, F., Alves, R. J. E., Bárta, J., Čapek, P., Hofer, A. 2014. Effects of soil organic matter properties and microbial community composition on enzyme activities in cryoturbated arctic soils. PLoS One, *9*(4), e94076. https://doi.org/10.1371/journal.pone.0094076

Schroeder, J. L., Kahn, B. A., Lynd, J. Q. 1998. Utilization of cowpea crop residues to reduce fertilizer nitrogen inputs with fall broccoli. Crop Science, 38: 741-749

Shankar, M, R., Veeralakshmi, S., Sirajunnisa, A. R., Rajendran, R. 2014. Effect of allelochemicals from leaf leachates of *Gmelina arborea* on inhibition of some essential seed germination enzymes in green gram, red gram, black gram, and chickpea. International scholarly research notices, Article ID 108682, http://dx.doi.org/10.1155/2014/108682.

Shi, J. R. 2013. Decomposition and Nutrient release of different cover crops in organic farm systems. Master Thesis. University of Nebraska, Lincoln, NE.

Shiga, H. 1997. The decomposition of fresh and composted organic materials in soil. Food and Fertilizer Technology Centre. Retrieved from http://www.fftc.agnet.org/library.php?func=view&id=20110804181904. Accessed on 29th January 2018.

Shivachi A, Kiplagat K, Kinyua G 2012. Microsatellite analysis of selected Lablab purpureus genotypes in Kenya. Rwanda Journal 28:39–52.

Shojaee, F., M., Sadeghi Mahoonak, A. R., Khomeiri, M., Ghorbani, M. 2017. Antibacterial effects of stevia *Rebaudiana bertoni* extract on pathogenic bacteria and food spoilage. Journal of Food Biosciences and Technology, 7(1): 57-64.

Sitienei, R. C., Onwonga, R. N., Lelei, J. J., Kamoni, P. 2017. Use of Dolichos (*Lablab Purpureus* L.) and combined fertilizers enhance soil nutrient availability, and maize (*Zea Mays* L.) yield in farming systems of Kabete Sub County, Kenya. Agricultural Science Research Journal, 7: 47 – 61.

Soltys., D., Bogatek, H., Gniazdowskas, A. 2012. Phytotoxic effects of cyanamide on seed germination and seedling growth of weed and crop species, Acta Biologica Cracoviensia, 54: 87–92.

Spence, N. 2003. Characterization and epidemiology of root rot diseases caused by *Fusarium* and *Pythium* spp. Beans in Uganda R7568. Final Technical Report. CIAT Bean Program, Cali, Colombia.

Spence, N. 2006. Bean root rot disease management in Uganda (DFID project ref, R8478, NR Int Code ZA0689). Final technical report, International Development Team, Central Science Laboratory (DEFRA).

Spiegel, Y., Chet, I. 1998. Evaluation of *Trichoderma* spp. as a biocontrol agent against soilborne fungi and plant-parasitic nematodes in Israel. Integrated Pest Management Reviews, 3: 169-175.

Stone, A. G., Vallad, G. E., Cooperband, L. R., Rotenberg, D., Darby, H. M., James, R. V., Stevenson, W. R., and Goodman, R. M. 2003. Effect of organic amendments on soilborne and foliar diseases in field-grown snap bean and cucumber. Plant Disease, 87:1037-1042.

Sutton, J. C., Sopher, C. R., Owen-Going, T. N., Liu, W., Grodzinski, B., Hall, J. C., Benchimol, R. L. 2006. Etiology and epidemiology of Pythium root rot in hydroponic crops: current knowledge and perspectives. Summa Phytopathologica, 32(4): 307-321.

Swer, H., Dkhar, M. S., Kayang, H. 2011. Fungal population and diversity in organically amended agricultural soils of Meghalaya, India. Journal of Organic Systems, 6(2): 3-12.

Tabin, T., and Shrivastava, K. 2014. Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (*Thymelaeaceae*). Asian Journal of Plant Science Research, 4(6): 41-6.

Talgre L., Lauringson E., Roostalu H., Astover A., Eremeev V., Selge A. 2009. The effects of pure and undersowing green manures on yields of succeeding spring cereals // Acta Agriculturae Scandinavica. Section B: Soil and Plant Science. 59: 70–76.

Talgre, L., Lauringson, E., Makke, A., Lauk, R. 2012a. Biomass production and nutrient binding of catch crops. Žemdirbystė Agriculture Journal, 98: 251-258.

Talgre, L., Lauringson, E., Roostalu, H., Astover, A., Makke, A. 2012b. Green manure as a nutrient source for succeeding crops. Plant Soil Environment, 58(6): 275-281.

Talgre, L., Lauringson, E., Roostalu, H., Makke, A. 2014. Phosphorus and potassium release during decomposition of roots and shoots of green manure crops. Biological Agriculture Horticulture, 30(4): 264-271.

Tanveer, A., Safdar, M. E., Tariq, M. A., Yasin, M., Noorka, I. R. 2014. Allelopathic inhibition of germination and seedling vigor of some selected crops by *Achyranthes aspera* l. Herbologia, 14: 35-46.

Terzi, I. 2008. Allelopathic effects of Juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. African Journal of Biotechnology, 7: 1870–1874.

Torres, R. C, Manalo, J. B. 1990. Phyto-chemical investigation of Dolichos lablab L. by thin layer chromatography. Philippine Technology Journal, 15(1): 41-50.

Usman, I. S., Aliyu, A., Qasimu, A. I., Adamu, T. 2016. Farmers' perception on organic manure usage among arable crop farmers in Jalingo local government area of Taraba state, Nigeria. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 16: 353 – 359.

Vaithiyanathan, T and Sundaramoorthy, P .2016. Impact of organic manure and inorganic fertilizers on seed germination of green gram (*Vigna radiata* L.). World Scientific News, 35: 111-122.

Valadares, R. V., de Ávila-Silva, L., da Silva Teixeira, R., de Sousa, R. N., Vergütz, L. 2016. Green Manures and crop residues as source of nutrients in tropical environment. In Organic Fertilizers-From Basic Concepts to Applied Outcomes. IntechOpen., 7:52-67.

Valetin Torres, S., Vargas, M. M., Godoy-Lutz, G., Porch, T. G., Beaver, L. 2016. Isolates of *Rhizoctonia* can produce both web blight and root rot symptoms in common bean (*Phaseolus vulgaris*, L.). Plant Disease 100: 1351-1357.

Van Bniggen, A. H., Termorskuizen, A. J. 2003. Integrated approaches to root disease management in organic farming systems. Australasian Plant Pathology 32: 141-156.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nigro, M., Marra, R., Manganiello, G. 2014. *Trichoderma* secondary metabolites active on plants and fungal pathogens. The Open Mycology Journal, 8: 127- 139.

Vicente, M. F., Basilio, A., Cabello, A., Peláez, F. 2003. Microbial natural products as a source of antifungals. Clinical microbiology and infection, 9(1), 15-32.

Wall, R. E. 1984. Effects of recently incorporated organic amendments on damping-off of conifer seedlings. Plant disease, 68: 59-60.

Walters, D. 2009. Disease control in crops: biological and environmentally-friendly approaches. John Wiley and Sons. Retrieved from https://www.wiley.com/en-us/Disease+Control+in+Crops%3A+Biological+and+Environmentally+Friendly+Approaches-p-9781405169479. Accessed on 15th May 2016

Whalen J. K. 2014. Managing soil biota-mediated decomposition and nutrient mineralization in sustainable agroecosystems. Advances in Agriculture 384–604.

Wiggins, B. E., Kinkel, L. L. 2005. Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous *Streptomycetes*, Phytopathology, 95: 178-185.

Williams, T. O. 1999. Factors Influencing manure application by farmers in semi-arid West Africa. Nutrient Cycling in Agroecosystems, 55: 15-22.

Wortman, C.S., Kirkby, R.A., Elude,C.A., Allen. D.J. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali, Colombia.

Wuest, S., Skirvin, K. 1999. Crop residue and plant health: research overview and implications for no-till. Columbia Basin Agricultural Research Annual Report. Special Repeat, 999: 81-84.

Yang, H., Niu, J., Tao, J., Gu, Y., Zhang, C., She, S., Yin, H. 2016. The impacts of different green manure on soil microbial communities and crop health. 10.20944/preprints201609.0056.v1.

Yerima, B. P. K., Tiamgne, Y. A., Tziemi, T. C. M. A., Van Ranst, E. 2015. Effect of substrates on germination and seedling emergence of sunflower (*Helianthus annuus* L.) at the Yongka Western Highlands Research/Garden Park, Bamenda-Cameroon. Tropicultura, 33(2): 91-100.

You, M. P., Colmer, T. D., Barbetti, M. J. 2011. Salinity drives host reaction in Phaseolus vulgaris (common bean) to *Macrophomina phaseolina*. Functional Plant Biology, 38(12): 984-992.

Zhang, D., Hui, D., Luo, Y., Zhou, G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology, 1(2): 85-93.

Zhang, Q., Zhou, W., Liang, G., Wang, X., Sun, J., He, P., Li, L. 2015. Effects of different organic manures on the biochemical and microbial characteristics of albic paddy soil in a short-term experiment. PloS one, 10(4), e0124096. doi:10.1371/journal.pone.0124096

Zhang, X. F., Zhao, L., Xu Jr, S. J., Liu, Y. Z., Liu, H. Y., Cheng, G. D. 2012. Soil moisture effect on bacterial and fungal community in Beilu River (Tibetan Plateau) permafrost soils with different vegetation types. Journal of Applied Microbiology, 114(4): 1054-1065.

APPENDIX 1 : QUESTIONNAIRE

BEAN PRODUCTION SURVEY QUESTIONNAIRE

A. Background information

Farmer ID: ……. Name of the farmer: ……. Date: …….…….

Age: ……...……. Sex: ……. Village: ……..

Agro-ecological zone: ……

Latitude: …….. Longitude: …….. Elevation: ……

Head of household (M/F): ……Highest level of education: ……..

B. Bean production practices

1. Acreage/ Size of Farm: (Acres/ Ha)

……..

2. Duration of land use [Years]: [1] <5 [2] 5-10 [3] 11-15 [4] 16-20 [5] 21-25 [6] 26-30 [7] 31>

3. What is your type of land ownership? [1] Own [2] Family Owned [3] Communal [4] Rented/ Hired [5] Borrowed

   Others Specify

   …….

1. Crop Farming History (Beginning with the most recent in the experimental site):

Last crop grown	**Period of production**

2. Which season do you cultivate beans?

   (1) Long rain season ☐ (2) short rain season ☐

3. What is the area under bean production (acres):

……?

4. What varieties of bean do you grow in your farm? Please state the reason for preferring the stated variety?

.....

8. From which sources do you obtain bean seeds?

a). Own ☐ b). Neighbor c). Market ☐ d) Agro-shops ☐

9. What other crops do you grow in your farm?

……

10. Methods of field preparation …….

(1) Hand tillage ☐ (2) Oxen plough ☐ (3) Machine tillage ☐

(4) Other (specify) ☐

11. Do you practice crop rotation? (Yes/No)

If yes, how often do you rotate your crop and with which crops?

……

If no, why don't you rotate your crops?

(1) Size of farm ☐ (2) Limited crop diversity ☐ (3) Lack of information ☐

(4) Others (Specify) ☐

12. How do you cultivate beans? (1) Pure sta ☐ (2) intercrop with mai ☐

Reason for how the crop is cultivated

……

……

13. Do you apply green manure? (Yes/No)

14. Do you apply Dolichos lablab green manure as a soil amendment or any other amendment?

Type of amendment	Quantity applied (Kg)	How often
Code A	Code B	Code C
1= DAP 2= NPK 3=CAN 4= Urea 5=Foliar feed 6=Lime 7= green manure 8=chicken manure 9= cow/ sheep/ goat manure 10= other	1=6 kg (Debe) 2=30 kg (wheel barrow) 3=150kg (oxcart) 4=450kgs (pick up) 5= Other (Specify)	1= seasonal 2= annually

15. If applying green manure, from which source?

(1) Brassicas (Cabbages, kales) ☐ (2) Bean, ☐ (3) Dolichos lablab ☐ (4) Groundnut ☐ (5) Soybean ☐ (6) cowpea ☐ (7) Maize straw ☐ (8) Others (Specify)

16. How much land do you set aside for Dolichos lablab/ green manure plant establishment for use as green manure?

……

……

17. From where did you get the information about green manure especially the use of Dolichos lablab as green manure?

……

Of the above mentioned green manure types, which one do you prefer and why?

……

18. Which variety of Dolichos lablab do you plant for use as green manure?

……

……

19. At what stage of the plant (green manure plant) do you use them as green manure?

(1) At flowering ☐ (2) At maturity ☐

20. What method do you use in application of these green manures in the soil?

(1)Left as mulch on soil surface ☐ (2) incorporated after evenly distributed on the soil (3) ☐ incorporated in furrows then covered with soil ☐

21. After incorporation of green manure in the soil, how long does it take you to plant your crop?

(1) Immediately after incorporation ☐ (2) Two weeks after incorporation ☐

(3) One month after incorporation ☐ (4) others (Specify) ☐

22. After application of the green manure, how do you plant maize or beans in your farm?

(1) Planted evenly across the field ☐ (2) On top of the covered green manure furrows ☐

(3) On the side of green manure covered furrows ☐

23. Have you noticed benefits/ difficulties with bean crop germination and establishment when you apply Dolichos lablab green manure or any other green manure?

……

24. What are the other uses of Dolichos lablab apart from being used as green manure?

……

25. What amount of bean yield do you expect in your farm (Kg/ unit area)?

……

Any other observation

……

Vote of thanks

Thank the farmer and promise to share the findings once the research is complete