CHARACTERISATION OF BEAN ROOT ROT PATHOGENS FROM SOILS IN WESTERN KENYA AND THEIR MANAGEMENT WITH BIOCHAR AND VERMICOMPOST

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UNIVERSITY OF NAIROBI

2019

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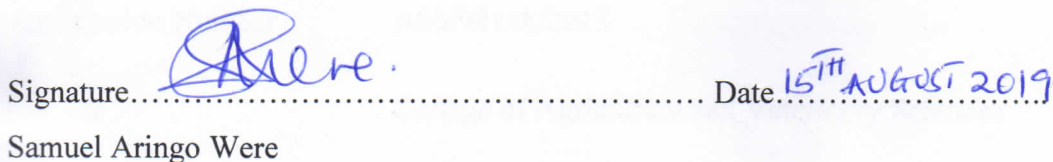

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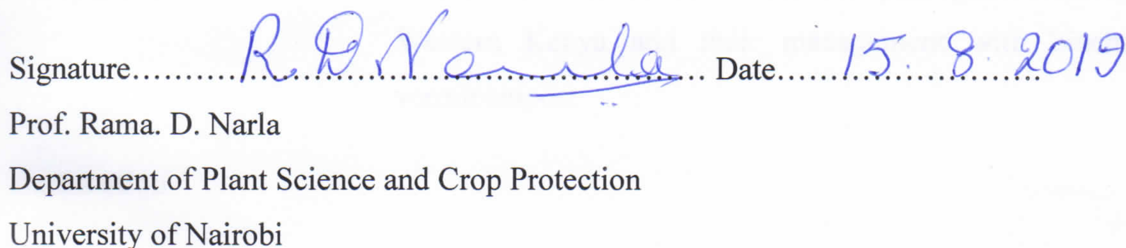

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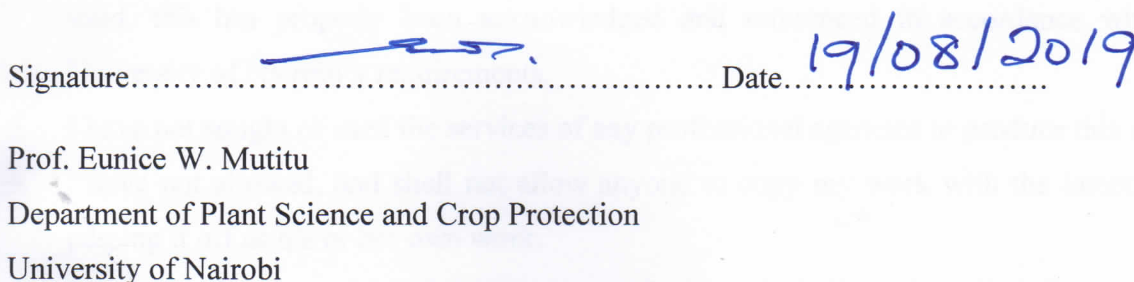

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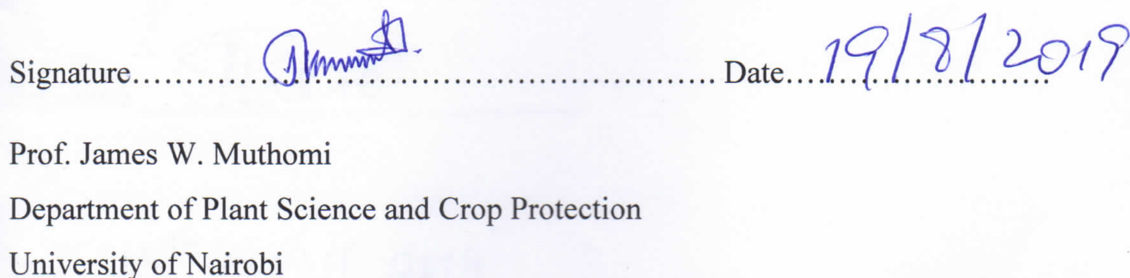

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This work is dedicated to God for giving me grace and strength to complete the research work. I also dedicate it to my dear parents, Mr. Edward Were and Mrs. Beverly Were, my wife Caroline Nyamvula Aringo, Brother Hilary N. K. Were, Sisters Rhoda E. Were and late Margaret O. Were; my son Gary E. Kondo and Nieces Louisa K. Omani; Masha K. Musau, Beverly N. Ndukwe and Gabriella Cellina Ndukwe for their love, support and encouragement.

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LIST OF ABBREVIATIONS

AEZ	Agro ecological zones
AM	Arbuscular mycorrhizal fungi
BLAST	Basic linear alignment search tool
BWES	Biochar water extractible substances
CFU	Colony forming units
CIAT	International Center for Tropical Agriculture
GIS	Geographic information system
GPS	Global Positioning System
ICIPE	International Center for Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
ISR	Induced systemic resistance
ITS	Internal Transcribed Spacer
KALRO	Kenya Agricultural and Livestock Research Organisation
KEPHIS	Kenya Plant Health Inspectorate Service
LM1	Lower midland humid
LM2	Lower midland sub humid
LR 2013	Long rains 2013
LR 2014	Long rains 2014
NAS	Non amended soils
NCBI	National Center for Biotechnology Information
NPK	Nitrogen Phosphorus and Potassium
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PGPR	Plant growth promoting rhizobacteria

qPCR	Quantitative Polymerase Chain Reaction
RH biochar	Rice husks biochar
SAR	Systemic acquired resistance
SB biochar	Sugar cane bagasse biochar
SE	Seed exudates
SM+L	Mineral Salt Media with Lecithin
SNA	Spezieller Nährstoffarmer agar
SR 2013	Short rains 2013
SR 2014	Short rains 2014
UM1	Upper midland humid
UM3	Upper midland semi-humid
UoN	University of Nairobi
WES	Water extractible substances

ABSTRACT

The importance of common bean (*Phaseolus vulgaris* L.) has continued to be emphasised in the world and as a key source of dietary protein both in the rural and urban livelihoods in Kenya. Reduction in its productivity has been attributed to variability in climatic conditions, declining soil fertility, insect pests and diseases. In Western Kenya losses due to soil borne diseases have been recorded as high as 70%. The objective of this study was to determine the prevalence of bean root rot and to contribute to improved bean productivity through management of the root rots with biochar and vermicompost soil amendments. This study evaluated the effect of biochar and vermicompost in suppression of fungal root rot of bean in farmer fields and in the greenhouse. The study also identified the mechanisms by which the disease suppression occurs.

A survey was conducted to establish the prevalence of bean root rot pathogens in four AEZ's across Kakamega, Bungoma and Busia counties of Western Kenya at the onset of long rains in 2013. Different species of *Fusarium, Pythium, Rhizoctonia* and *Macrophomina* were isolated from soils sampled from farmer fields in Western Kenya. Identification was undertaken by use of morphological and molecular means. All soils were infected with root rot pathogens including *Fusarium* species, *Pythium ultimum, Pythium irregular, Rhizoctonia solani Macrophomina phaseolina. Fusarium spp* was the most abundant across all AEZ's. *Pythium spp.* and *Rhizoctonia spp.* were most abundant in LM2 and UM3 respectively. Species of *Fusarium* were also the most abundant in different soil textures with the loamy fine sand having the highest populations of the pathogens. Quantification of root rot pathogen DNA in soils using real time PCR was recorded highest for *Rhizoctonia solani* from UM3 at 2.23pg μL^{-1}.

Treatment combinations of biochar and vermicompost had a positive impact on plant emergence in all the four seasons under field conditions. However, in the greenhouse experiments, vermicompost had phytotoxic effect on plant emergence while soils amended with sugarcane bagasse and rice husks biochar recorded 92% plant emergence. Plant growth was enhanced in soils amended with biochars and challenged with root rot pathogens than in non-challenged soils that were amended with both biochars.

Incidence and severity of bean root rot were significantly ($p<0.05$) reduced in plots amended with a combination of biochar and vermicompost as well as standalone treatments. Similar observations were made in the greenhouse experiments where the root rot severity was reduced by 27% in soils treated with sugarcane bagasse and rice husks biochar treatments. Root rot

pathogen populations were significantly ($p<0.05$) reduced in the field following application of sugarcane bagasse biochar and vermicompost. Populations of *Trichoderma spp, Paecilomyces spp, Athrobotrys spp* and *Penicillium spp* were also increased in fields where the soils were amended. Significant higher grain yields were recorded in the amended plots in the two long rain seasons and the short rain season of 2013. Plots amended with vermicompost and fertiliser had the highest yields at 565.2 kg Ha^{-1} while the lowest yields were in control plots at 311.7 kg Ha^{-1}. The highest 100 seed weight was in vermicompost treated plots which was significantly different from the control plots but not biochar treatments. Highest pH of 6.06 was recorded in biochar amended plots in LM2. Highest levels of nitrogen and phosphorus were recorded in vermicompost amended soils in LM1 and combination of biochar and vermicompost amended soils in UM3 respectively.

Sporangial and spore germination of *Pythium ultimum* s and *Fusarium solani* respectively were significantly inhibited ($p<0.05$) by biochar and vermicompost water extracts. Length of exposure period of biochar to air after pyrolysis significantly reduced its ability to suppress pathogen growth. Biochar was also found to adsorb phytochemicals from root and seed exudates in turn disrupting their ability to induce sporangial and conidial germination of root rot pathogens. In conclusion, biochar and vermicompost amendments are effective in reduction of incidence and severity of bean root rot. This study identified the mechanisms involved in control of root rot by biochar and vermicompost to include adsorption of germination trigger molecules for pathogens and inhibition of spore germination by the water extractible substances.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Common bean (*Phaseolus vulgaris* L.) is an important grain legume in Africa. It provides more than 100 million people with dietary protein in rural and urban communities (Buruchara *et al.,* 2011). The highest annual per capita for bean consumption in the world at 50-60 kg is in Eastern Africa. In Kenya, bean is the second in importance after maize as a food crop (Gicharu *et al.,* 2013).

Worldwide, common bean is grown for dry grain and green pods. It is consumed at different stages of plant development thus offering prolonged and staggered food supply. They are rich in protein (~22%) and provide iron and zinc which are key elements for mental development in addition to reducing colon cancer, breast cancer, and heart diseases (US Dry Bean Council, 2011; Aune *et al.,* 2009). Bean has also become a lucrative and steady source of income for many households in rural areas (FAO in 2011).

Global annual production for snap and dry bean is in the excess of 43 million metric tons (FAO, 2013). This represents over half of the total production of grain legume worldwide (Miklas *et al.,* 2006). The largest producers of dry beans worldwide are India and Brazil while Kenya is ranked seventh producing 0.6 million metric tons (FAO, 2013). Its production in developing countries largely occurs under low input agricultural systems by small-scale farmers who are resource deficient. This causes common bean to be more predisposed to biotic and abiotic constraints (Miklas *et al.,* 2006). Common bean are produced under diverse cropping systems in different agro ecological zones in Eastern Africa ranging from lower midland sub humid to upper midland humid. These zones of production are mainly the low to medium altitude areas ranging from 1000-2200 m above sea level (Asrat *et al.,* 2007).

In Kenya common bean is a significant component in the farming systems where it is grown solitary or together with crops like maize (*Zea mays* L.), with low external inputs (Asrat *et al.,* 2007). Common bean is therefore a key component in intensifying production in smallholder farmer systems due to its ability to fix nitrogen. It can also encourage the much-needed improvements in soil fertility for longer periods (Buruchara *et al.,* 2011).

In recent years, common bean production trend has not been at par with the annual population growth estimated above 2 % for sub-Saharan countries. This is due to environmental stresses, notably biotic and abiotic constraints like low soil fertility and drought (Lunze *et al.,* 2011) as well as socio-economic constraints each causing significant yield reduction (Hillocks *et al.,* 2006; Katungi *et al.,* 2009). As a result low yields have been observed in the Eastern and Southern African region of about 500kg per hectare. This is in relation to potential yields of 3000 kg per hectare from improved common bean varieties when cultivated under optimal environmental conditions (Kimani *et al.,* 2005).

In order to increase production, there is need for employing management strategies to alleviate yield losses. These strategies may include but not limited to; cultural practices, biological control; application of chemical fungicides and use of resistant cultivars (Otsyula *et al.,* 2003). Since the majority are small-scale farmers, affordable and sustainable practices should be employed. The strategies like disease management and soil health improvement should put into consideration (Mwaniki*,* 2000). Use of organic amendments as a method of sustainable disease management is of great importance.

1.2 Problem Statement

Soil borne pests and diseases have increasingly become a challenge in agricultural production systems, particularly so with the same crops being continually grown in the same field (Nzungize *et al.,* 2011). Common bean production is majorly constrained by root rot pathogens which are widely spread in the soils worldwide impacting on the yield and quality of grain as has been previously reported by Buruchara *et al.,* 2015. These diseases may lead to as much as 70% reductions in grain yield of some commercial bean cultivars which are popular in Kenya. The root rot pathogens like species of *Pythium* and *Fusarium* have a wide range of host plants occupying both land-dwelling and water habitats (Paparu *et al.,* 2017). The existence and severity of disease caused by these root rot pathogens is greatly associated with intensified land use, lack of or inappropriate crop rotations and/or reduced fallowing (Nzungize *et al.,* 2011). These factors also lead to degeneration in soil fertility and pathogen inoculum build-up in the soil (Abawi *et al.,* 2006). Farmers in Kakamega and Vihiga Counties of Kenya stopped bean cultivation in 1991 through to1995 because of bean root rot problems (Otsyula *et al*., 2016). This led to serious food shortages that hiked cost of bean prices that resource poor households could not afford.

Seed coating of bean seeds has been observed to be effective for seed protection as well as young seedlings for 2 to 3 weeks after planting (El-Mougy *et al.,* 2012). However, poor farmers cannot afford chemical treatments due to their economic conditions. If used continuously, their use may also expose farmers to health hazards and complications related to poor handling of chemical pesticides (Schwartz *et al.,* 2007). Therefore, it is not sustainable to use chemicals for subsistence farming systems. Since bean root rot and a decline in soil fertility have been cited as major causes leading to bean yield losses, there is a need to establish appropriate and sustainable management strategies that combat these constraints. There is currently no sufficient information on the occurrence and variability among the root rot pathogens in different agro ecological zones of western Kenya counties of Kakamega, Bungoma and Busia. There is also lack of documentation on the effects of combining different organic amendments such as biochar and vermicompost in combating the root rot pathogens as well as the declining soil fertility.

1.3 Justification

With the ever increasing demand for production of beans and the limited arable land in Kenya, there has been an increased reliance on intensive farming with reduced periods of fallowing and no rotation. This has led to the increase in soil borne fungal diseases like root rots compounded with a decline in soil fertility which has led to suppressed yields. In Western Kenya, small holder farmers form the majority of common bean producers who are majorly resource constrained. As a result, they are unable to engage in high cost conventional root rot management strategies such as seed dressings and chemical applications. There are also increasing concerns with pesticide use which may lead to a risk of contamination to farmers and environment during application and use. Chemical use may also result in maximum residue levels being exceeded where pesticides are abused. Since there is not a single disease management strategy which is able to completely manage soil borne fungal and oomycete plant pathogens causing considerable losses in bean production every year.

In view of this, alternative management strategies that have high efficacy and low in put cost with limited environmental effects are a high priority research for modern agriculture. These limitations can thus be addressed by use of organic amendments. The use of biochar and vermicompost can provide a window of opportunity for small scale farmers to continue production of beans on their farms. Biochar has been found to have hormone and hormone-like compounds with positive physiological influences such as disease suppression and/or promoting plant growth. It also induces systemic plant defenses thereby improving plant health.

There is however little information on the performance of biochar and vermicompost in reduction of bean root rots in open fields. Consequently not much information is available on the mechanisms involved in suppression of bean root rot following biochar and vermicompost application. The findings from this study shed light on the variability of pathogens causing bean root rot in the agro ecological zones of western Kenya. It also brings to light effect of combining biochar and vermicompost on root rot pathogens, soil fertility and bean yield. Furthermore this study sheds light on the mechanisms by which biochar and vermicompost control root rot pathogens.

1.4 Objectives

Broad Objective

To contribute to improved bean productivity through management of root rots with biochar and vermicompost soil amendments.

Specific Objectives

(i) To characterise bean root rot pathogens occurring in different agro ecological zones and farming systems of Western Kenya.

(ii) To evaluate the effect of biochar and vermicompost amendments on root rot pathogens.

(iii) To test the efficacy of biochars produced from different plant materials and vermicompost in the management of bean root rot.

(iv) To elucidate the mechanism(s) exhibited by biochar and vermicompost in suppression of common bean root rot.

1.5 Hypotheses

(i) Bean root rot fungal pathogens occurring in different agro-ecological zones and farming systems of Western Kenya do not differ.

(ii) Soil organic amendments (biochar and vermicompost) have no effect on the population of common bean root rot pathogens.

(iii) Vermicompost and biochar-derived from different plant materials do not differ in their efficacy against root rot disease of common bean.

(iv) There is no specific mechanism of bean root rot disease suppression exhibited by vermicompost and biochars.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of common beans

Dry beans are grain legumes widely cultivated worldwide. They are considered an important protein and calory source both in the rural and poor urban human communities. The protein is highly competitive due to its cost in comparison with animal-based protein. This makes it important in dietary programmes for many communities in Africa (USAID, 2010). In the year 2014, it was estimated that total world production of common beans was 23 million metric tons with Kenya positioned seventh producer of dry beans worldwide (MoA, 2015). In Kenya common bean are cultivated on about one million hectares with yields averaging at 600 kg/ha (Table 2.1). The national production has been assessed at 615,000 MT in 2014 and a deficit that has been filled by imports in the last five years.

Common bean (*Phaseolus vulgaris* L. is considered the third in importance as a staple food in Kenya. In the national diet, it accounts for 5% of total food calories (Kirimi *et al.,* 2010) thus being of great importance to the food security nationally. This is due to the fact that they can be consumed at different stages of plant growth in preparation of a wide range of recipes. In Kenya it is common for beans to be consumed in various forms where fresh or dry grains can be boiled and consumed. They can also be mashed with potatoes or bananas; mixed with cereal grains such as maize and eaten as "Githeri" (Kimani *et al.,* 2006).

Table 2.1: Production and of dry beans in Kenya

Year	2010	2011	2012	2013	2014
Production area (ha)	689,377	1,036,738	1,056,046	1,083,604	1,052,408
Production (MT)	390,598	577,674	622,759	714,492	615,992
Surplus/Deficits	-21,319	-52,034	-36,685	-44,433	-87,400
Yield (MT/Ha)	0.57	0.56	0.59	0.66	0.59

Source: MoA, 2015.

2.2 Production constraints of common beans

In Kenya, bean production is mainly carried out by small holder farmers under constraining conditions (Nderitu *et al.,* 1997; Mwaniki, 2002). In turn, bean yields have declined over the years (MOA, 2011). The major constraints in bean production include, low input use, marginal lands, low soil fertility, weed competition, periodic droughts and damage due to insect pests and diseases. Of these constraints, diseases are of great importance contributing to lowering of yields averaging 600 kg/ha (Muriungi *et al.,* 2013; MOA, 2015).

Production of common bean in Kenya is affected by many biotic and abiotic stresses. Biotic stresses include insect pests and diseases which have contributed to continuous decline of production in many parts of the country. Diseases affecting common bean include angular leaf spot (*Phaeoisariopsis griseola)*, anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*, bean common mosaic virus (BCMV) and root rots (Wortmann *et al.,* 1998). The stem and root rots caused by different species of soil borne pathogens like fungi, bacteria and nematodes are considered to be diseases of great economic importance in production of beans in Africa. These diseases have been reported to cause reduction in yields of as high as 86% in fields infested with species of *Fusarium* and experiencing other constraints at the same time (Abawi and Pastor-Corrales, 1990). *Fusarium* root rot has been reported to be the most widespread disease in the bean growing areas in Kenya (Mutitu, 1988; MoA, 2011).

Under increasing population pressure, cultivation has intensified while the fallowing period or crop rotation have reduced or disappeared altogether in an effort to increase production. This has resulted in the decline of soil fertility, compaction of soil and pathogen inoculum build-up in the soil (Wortmann *et al.,* 1998).

2.3 Root rot of common bean complex

Bean root rot is caused by a complex of soil borne pathogens such as *Pythium ultimum, Pythium irregulare*, *Fusarium solani* f. sp. *phaseoli, Fusarium oxysporum* f.sp *phaseoli, Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotium rolfsii* (Mukuma, 2016; Paparu *et al.,* 2017). These have been reported to be predominant in bean growing areas of Western, Central and Eastern regions in Kenya (Muriungi *et al.,* 2013; Mwang'ombe *et al.,* 2007; Abawi and Pastor-Corrales, 1990; Mutitu, 1988). Namayanja *et al.* (2014) and Otsyula *et al.* (2003) have reported on the importance of root rot common bean in production in Western Kenya where losses reaching to 70% have been documented. Paparu *et al.* (2017) reported these

diseases to have caused increased yield reduction of up to 100% in susceptible bean varieties. These diseases reduce quality and yield of bean (Paparu *et al.,* 2017) and in some areas they have resulted in farmers stopping cultivation of the crop altogether (Buruchara *et al.,* 2015). Root rot pathogens are difficult to control due to their complexity and mechanisms of survival in the soil as saprophytes or as resting spores in the form of oospores, chlamydospores and sclerotia over long periods of time (Rani and Sudini, 2013). The pathogens are known to worsen problems of drought or interference with acquisition of nutrients such as phosphorus by restricting root systems. The most prevalent fungal root rot pathogens found in western Kenya are *P. ultimum, F. solani, R. solani* and M. *phaseolina* (Otsyula *et al.,* 2003; Namayanja *et al.,* 2014)

Species of *Fusarium* produce three different types of spores including macroconidia, microconidia and chlamydospores (Burgess *et al.,* 1994). These spores are important in identification of different species of the genus *Fusarium*. Septate macroconidia are produced on monophialides and polyphialides in the aerial mycelium. They can also be borne in sporodochia on short monophialides (Leslie and Summerell, 2006). An important distinguishing characteristic of *Fusarium* from other genera is the presence of macroconidia. They have a crescent moon shape crest with multisepta (Alexopoulus *et al*., 1996). Leslie and Summerell, (2006) described different shapes of macroconidia with some being straight and others curving dorsal ventrally. The top and bottom cells of these macroconidia are key characteristics used to define different species of *Fusarium*. Top pointed cells can be blunt, papillate, hooked and narrowing, while the basal can be foot-shaped, elongated foot shaped, conspicuously notched and slightly notched. Chlamydospores produced by some of the *Fusarium* species are the vital characteristic for identification as well as for survival in unfavourable conditions. This is due to the presence of the thick wall that contains a lipid substance (Alexopoulus *et al*., 1996). Chlamydospores can be formed in chains, clumps, in pairs or singly. Their presence or absence together with that of microconidia and their shape, contribute to distinguishing species in *Fusarium* (Mukuma, 2016).

Pythium species are fungal-like micro-organisms with a colourless filamentous mycelium or slightly yellowish or greyish lilac in colour (Owen-Going *et al*., 2008). The hyphae are hyaline measuring 5-7 µm, occasionally reaching 10 µm in diameter. Hyphal septation is absent with exception to old hyphae or at the point where reproductive organs are delineated (Plaats-Niterink, 1981). Appresorium may be produced by pathogenic *Pythium* spp. which enables

them to attach to host cells and penetrate (Levesque and de Cock, 2004). The pathogen produces spherical sporangia containing structure and oospores which act as the survival structure and primary inoculum (Lodhi and Khanzada, 2013). The size and structure of morphological characteristics have been used as a criterion for identifying the different species within *Pythium* (Matsumoto *et al*., 1999). These characteristics include homothallic or heterothallic sexual reproductive structures, morphology of the sporangia which may be spherical or lobulated, nature of the oogonial wall which is either smooth or ornate with projections and borne intercalary or terminally.

Antheridial characteristics may be stalk-less on hyphae, interpolated, or formed at the end on an antheridial stalk (Postma *et al*., 2009) which can either be monoclinous or diclinous in nature. The type of oospores produced which may be pleurotic or apleurotic have also been used in identification of species within *Pythium.* Dick, (2001) observed that the quality of the structures used in identification often vary based on the isolate and the conditions of culture. According to Uzuhashi *et al*., (2010) these differences in character attributes have led to the continuing taxonomic system of *Pythium* spp. Kageyama *et al*., (2005) however stated the importance of combining both the morphological characteristics and molecular techniques in identification of *Pythium.*

In nature, *R. solani* reproduction is asexual and exists mainly as vegetative mycelia producing macro and micro sclerotia. The mycelia grows rapidly on PDA forming white to cream to brown colonies with the young colonies being white while the old colonies are brown in colour (Desvani *et al.,* 2014). The hyphae are septate with branches having a slight constriction with a septum at the point of branching and perpendicular. Moniliod cells are produced by *Rhizoctonia* which develop to sclerotia which are the survival and resting structures of the fungus (Strausbaugh *et al.,* 2011). Macrosclerotia are often blackish-brown measuring ≤1mm in diameter while the microsclerotia are originally white in colour appearing like thin small crusts on PDA media. The variation of colony colour, sclerotia color, size and their distribution pattern is used to distinguish the different anastomosis groups of *R. solani* (Lakshman *et al.*, 2016).

Macrophomina phaseolina is a basidiomycete fungus which is mostly known to occur in two anamorphic forms (Khaledi and Taheri, 2016). The fungus may occur as a saprophyte in the form of *Rhizoctonia baticola* where it mainly produces microsclerotia as the resting structures and primary inoculum and in its pathogenic form as *Macrophomina phaseolina* where it mainly

produces pycnidia (Fuhlbohma *et al.,* 2013). *M. phaseolina* isolates on PDA are characteristically grey to black in color. Important feature is the production of sclerotia from specialized highly compressed hyphae. They may also produce pycnidia on specific media. Morphological identification is based on the colony colour as well as the size of sclerotia and their colour (Fuhlbohma *et al.,* 2013).

Species of *Sclerotium* produce abundant white, coarse mycelium with the main branch hyphae being relatively large measuring 5-9 microns in diameter (Watanabe, 2002). The hyphae are pale brown characterized as having clamp connections (Díaz-Nájera *et al.,* 2018). The hyphae are branched, septated near the main hyphae and constricted at the base. The fungus produces compact sclerotia which are globose or sub globose with a glossy smooth surface which is well differentiated (Watanabe, 2002). These characteristics help in the identification of the fungus.

2.3.1 Symptoms of bean root rot

The expression of root rots in form of symptoms in common bean depends on the pathogen involved (Nzungize *et al.,* 2012). The common root rot symptoms may comprise one or a combination of numerous qualities such as seed rot occurring before germination resulting in poor seedling establishment. Other traits such as damping-off, jagged growth, leaf chlorosis, premature defoliation, death of severely infected plants and suppressed yield may also be observed (Abawi *et al*., 2006; Nzungize *et al.,* 2012; Namayanja *et al.,* 2014). Seedling rot on common bean develops rapidly leading to plant death within only a few weeks of sowing (Abawi and Pastor-Carrales, 1990). Infected bean seeds and/or seedlings show dark brown discoloration while the infected roots on young plants become necrotic and kill the plant. Vascular discolouration of the roots occurs on the stem when the pathogen invades the plant and produces a soft rot at or above the soil surface. This may lead to the death or wilting of larger plants resulting from water loss in leaves and stems. Affected plant parts lose their turgidity and droop. Soil temperatures as well as moisture content impact the susceptibility of common bean to *Pythium* diseases with some *Pythium* spp like *P. ultimum* and *P. irregulare* being favoured by cold temperatures (Nzungize *et al.,* 2012; Mathiesen *et al.,* 2016).

Infections of *Fusarium solani* f.sp. *phaseoli,* the first symptoms in plants develop as red to brown longitudinal streaks on hypocotyls, tap root and lateral roots of seven to ten day old bean plants with necrosis being restricted to the cortex cells (Burke and Hall, 1991). Disease severity may increase with the passing of time and the developing plant, resulting to complete rotting

of the root system. Infections by *F. oxysporum* result in plants vascular system discolouration as well as discolouration of the roots and hypocotyl tissues (Mukuma, 2016).

Typical symptoms of *Rhizoctonia* root rot include lesions that are sunken and elongated which appear reddish-brown in colour on roots and hypocotyls (Agrios, 2005). These lesions may develop to reddish-brown cankers extending longitudinally on stems of older plants as the stem becomes woody. *Macrophomina phaseolina* infected plants will often have a pale, ash-colored, dry rot on the stem (Khaledi and Taheri, 2016; Mukuma, 2016). Numerous microsclerotia and pycnidia develop on adult plant stem tissues. These appear as small black dots especially in the dead areas (Abawi and Pastor-Corrales, 1990; Mukuma, 2016). Characteristic symptoms of *Sclerotium rolfsii* will include a moldy white growth of mycelia at the base of the stem and on the ground surrounding the plant when humidity is high. The mycelium is often mixed with numerous sclerotial bodies (Le, 2011; Leoni *et al.,* 2014).

2.3.2 Conditions favouring development of root rot

Soil management practices, cropping systems and climatic conditions have been identified as factors that influence the severity of root rots (Abawi and Pastor Corrales, 1990). Soil moisture is required by these pathogens to cause infection in beans. Papura *et al.,* (2017) reported that *Fusarium* and *Pythium* root rots thrive under cool temperatures and elevated humidity whereas *Sclerotium rolfisi* is favoured by the warm temperature and moist conditions. *Rhizoctonia solani* is observed to cause seedling damping off of susceptible hosts under humid conditions, warm temperature and cool wet soil condition (Strausbaugh *et al.,* 2011; Lodhi and Khanzada, 2013). *Macrophomina phaseolina* on the other hand thrives under intermittent drought conditions producing symptoms under hot, dry weather (Almomani *et al.,* 2013; Gautam *et al.,* 2014).

Root rots also have been found to be greatly influenced by cropping history, plant spacing, and stress factors such as drought, soil compaction, or flooding (causing oxygen deprivation). Infections of parasitic and pathogenic micro-organisms affecting the roots make the disease severe (Naseri *et al.,* 2014; Leslie and Summerell, 2006). Low soil pH or acidic soils have been known to affect root rot where alkaline pH was shown to favour establishment of *Pythium* spp. Naseri, (2014) conversely observed that severity of *Fusarium* root rot was inversely correlated to pH an indication that the pathogen thrives in acidic soils. Similar observations were made in case of root rot caused by *Rhizoctonia solani* (Acharya, 2017) and

*Macrophomina phaseolina (*Sukanya *et al.,* 2016). Effective management of soil borne root rot of common bean is however difficult to achieve using a single strategy due to the involvement of multiple pathogens with diverse biology causing the disease (Ongom *et al.,* 2012; Abawi *et al.,* 2006; Abawi and Ludwig, 2000).

2.4 Management of root rots of common bean

There are various strategies available for the management of soil borne diseases which have been applied by farmers either consciously or subconsciously with an aim of improving their crop productivity (Rani and Sudini, 2013). These options include good agricultural practices such as field sanitation, crop rotation and application of organic amendments (Nzungize *et al*., 2012; Rani and Sudini, 2013). Other strategies such as use of resistant cultivars, biological control, seed dressing and chemical sprays have also been employed with the sole aim of reducing soil inoculum which in return influences both the rate and severity of root rot (Nzungize *et al.,* 2012; Spence, 2003).

The production of resting spores that are persistent in the soils for years (Abawi *et al.,* 2006) necessitates the use of different management strategies. Thus effective management of root rots depend on proper knowledge of the host, pathogens involved and the environmental conditions that favour infection and development of the disease (Rani and Sudini, 2013; Ongom *et al.,* 2012; Abawi *et al.,* 2006). A combination of compatible management strategies aiming at reducing soil inoculum which in return influences both the rate and severity of root rot should be promoted (Nzungize *et al.,* 2011; Stone *et al*., 2003; Spence, 2003).

Severity of root rots can also be influenced by certain cultural practices which can be used as short-term measures. They can also be used as complementary strategies in integrated management of root rot diseases (Mihajlovic *et al.*, 2017; Nderitu *et al.,* 1997). Some strategies do promote conditions unfavourable for pathogen growth and survival and in turn lead to reduction in inoculum levels (Baysal-Gurel *et al.,* 2012). They may also promote plant growth and vigour leading to plant tolerance to infection in the presence of pathogens (Mehta *et al.,* 2014; Stone *et al.,* 2003). Planting at depths of 4.0 to 5.0 cm facilitates rapid emergence of the seedlings thereby reducing damage by root rot pathogens as well as disease severity on the plants. Strategies such as time, method and rate of planting that minimize competition for moisture, nutrients and light between plants do reduce the incidence and severity of root rots as reported by Schwartz, (2012). He also observed that elevated soil moisture early in the growth period increases root rot damage due to *Pythium, Rhizoctonia* and *Fusarium* spp which

can be reduced by manipulation of soil moisture. Deep ploughing and planting on raised beds or well drained soils have been observed to manage the moisture levels (Nzungize *et al.,* 2012; Schwartz, 2012; Tu*.,* 1992).

Crop rotation impacts on pathogen population in the field and on a broad range of soil characteristics thereby affecting plant disease (Baysal-Gurel *et al.,* 2012). It has been known to reduce residual populations of the root rot organisms in the soil (Schwartz *et al.,* 2001; Schwartz, 2012). Hall and Phillips, (1992) reported on population decline of *Fusarium solani* f.sp. *phaseoli* whenever a crop other than bean was grown. This is however limited in cases where the soil pathogen population is very high which is the case in many regions. On the other hand, some crops have been found to reduce populations of a specific soil pathogen but may lead to increase in population of others. Barley has been found to be successful when used for crop rotation in control of *Fusarium solani* f.sp. *phaseoli*. It is however not recommended for control of pathogens like *Pythium* spp, and *Macrophomina phaseolina*, which tend to have wide host range (Hall and Nasser, 1996). As a result, proper weed control should be undertaken when crop rotation is practiced to inhibit growth of different root rot susceptible species. In the strict sense, crop rotation is rarely practiced, which is contrary to farmer's claims of undertaking it. It is also not feasible in the small scale farmer fields due to land pressure since dominant crops in the fields shift according to seasons (Muriungi *et al.,* 2013).

In response to the root-rot crisis of late 1980's and 1990's in western Kenya , KALRO and CIAT, in collaboration with the Kenyan Department of Agriculture in 1993, introduced and evaluated several bush bean and climbing bean varieties for resistance to bean root rot (Otsyula *et al.,* 2003; Otsyula *et al.,* 2016). Out of ten bean varieties assessed, seven have been released for cultivation by farmers following successful breeding. The varieties include bush bean varieties of KK8, KK15, KK22, KK-Rose Coco 194 and climbing bean varieties of KK-Red bean16, KK-Red13 and KK-Rose Coco 33 (Ongom *et al,* 2012; Otsyula *et al.,* 2016). However, due to lack of an elaborate seed system, these varieties have not been fully accessed by the farmers in a sustainable manner (Otsyula *et al.,* 2016). As a result there has been a shortage in the market of the new varieties thereby delaying their uptake and use. Further to this, most of the varieties have not been well adapted by the farmers due to various factors related to the cultivation of these varieties. The factors hindering the successful uptake of these varieties include high demand for fertilisers, increased labour and time required at pre and post-harvest due to shattering in the field of some varieties and poor intercrop with maize thus requiring sole cropping (Otsyula *et al.,* 2016).

Graber *et al* in 2014 documented that the rhizosphere interface of root rot pathogens occur in a dynamic environment due to the high microbial populations and activity. In addition, its rapid change in pH, concentration of salts as well as water and osmotic potential makes biological control of soil-borne diseases complex (Handelsman and Stabb*,* 1996). For this reason, the ideal microorganisms for biological control are those that are resident to the rhizosphere (Bouzidi and Mederbal, 2016) since the first line of plant defence against root pathogens is provided by rhizosphere. Microbial protection of plants from fungal attack can either be through synthesis of antifungal metabolites, competition for nutrients, parasitism, niche exclusion, and by stimulating the plants resistance (Sreevidya and Gopalakrishnan, 2016; Schwartz, 2012; Whipps, 2001).

The available microbial based management options include seed dressings with *Trichoderma* spp and *Bacillus subtilis* as well as soil inoculation with these microorganisms together with *Glomus intraradices* (Khaledi and Taheri, 2016). Khaledi and Taheri, (2016) demonstrated significant reduction of *Macrophomina phaseolina* root rot disease in soy bean following seed treatment and soil application with *Trichoderma harzianum.* Their study reported that seed treatment increased suppression of root rot disease as compared to the soil application. Similarly *R. solani* root rot of beans was significantly suppressed following application of *Trichoderma harzianum* by 31% and 43% due to root colonisation by *Glomus intraradices* following soil application (Matloob and Juber, 2013). In Kenya Muriungi *et al.,* (2013) demonstrated effective suppression of *F. oxysporum* root rot of beans by different species of *Trichoderma.* In other experiments, *T. harzianum* strain T22 trading as Trianum and *Bacillus subtilis* trading as Nemix suppressed *Fusarium* wilt in tomatoes (Wanjohi *et al.,* 2018). Other commercial root rot biological control agents available include *T. asperellum* trading as Trichotech.

When root rot pathogens infect soils, they can survive for prolonged periods in their survival structures such as chlamydospores, oospores and sporangia (Gossen *et al.,* 2016). Available options for management of root rot disease after planting in such soils are limited and their effectiveness is questionable. Here, chemical application can be an efficient strategy when targeting to kill the pathogen (Abawi and Pastor-Corrales 1990). Many broad spectrum and highly specific soil fumigants' that are effective in control of root rot pathogens are available in form of fumigants and seed dressers. Their use is however limited due to their high costs and toxicity to man and environment (Nolling, 1991; UNEP, 2008).

Seed and young seedlings can be protected from root rot infection for two to three weeks of sowing following the application of soil and seed treatments (Abawi *et al.,* 2006; Schwartz *et al.* 2007). Chemicals such as Ridomil (Metalaxyl-M) have been used in the management of *Pythium* spp. whereas *Rhizoctonia solani* has been successfully managed by application of Chloroneb (1,4-Dichloro-2,5-dimethoxybenzene), Topsin (dimethyl 4,4'-0-phenylenebis [3-thioallophanate] and Terraclor or Pentachloronitrobenzene (Schwartz, 2012). Initial infection by *Fusarium* has been observed to be delayed, but not prevented, by soil fumigation or treatment with Terracoat, Topsin M or Terraclor Super X. However, their effectiveness can be lost quickly when plant growth and development is constrained by environmental or cultural conditions (Schwartz, 2012).

Other chemicals employed in the management strategies and used as seed treatments include Thiram (Thiram 70 S), Monceren (difenoconazole) and Apron star (Thiamethoxam + metalaxyl-M + difenoconazole). They were however found to be partially effective since damage occurred on fibrous roots at some distance from seed placement (Abawi and Pastor-Corrales, 1990). However, these localized treatments that control root rots and seedling damping off help ensure optimal plant populations, which in turn help offset yield depression by root rots (Burke and Miller, 1983). Development of resistance to different seed dressing fungicides in the market makes the use of chemical strategy unsustainable. This is as a result of the multiple genera and species of pathogens involved as well as degradation following continued use (Abawi and Pastor Corrales, 1990 and Nolling, 1991). Seed dressing chemicals available in Kenya include but not limited to Monceren® 125 DS -Imidacloprid 233g/l, Pencycuron 50g/l, Thiram107g/l.), Seed plus® (10% Imidacloprid, 10% Metalaxyl, 10% Carbendazim) and Murtano super® (20% Lindane, 26% Thiram)

2.5 Use of organic amendments

Organic amendments are any material of plant or animal origin added to the soil for purposes of improving the soils physical properties, soil fertility recovery (Diacono and Montemurro, 2010) and increasing microbial activity (Melero *et al.,* 2006). There are different types of organic amendments such as farmyard manure, green manure (Tejada *et al.,* 2009; Himmelstein *et al.,* 2014), organic wastes (Torres *et al.,* 2015), composts (Noble and Coventry, 2005; Bastida *et al.,* 2015), and biochar (Jones *et al.,* 2012; Lehmann *et al.,* 2011).

Organic soil amendments are known to suppress soil-borne pathogens and have been used in the management of root rot disease in common beans (Mehta *et al*., 2014; Bonanomi *et al*., 2017). It has been reported that oomycetes such as *Pythium* spp. have been suppressed by various soil organic amendments like vermicompost (Mehta *et al*., 2014). Organic amendments affect root rot diseases in several ways including the release of compounds that affect pathogen survival and expression of disease in a host (Bonanomi *et al*., 2017). Accumulation of ammonia has been reported to be responsible for the death of soil pathogens where green manure is applied (Mason and Gillespie, 2013). According to findings by Tenuta and Lazarovits, (2002), nitrous acid (HNO_2) was responsible for the death of soil-borne pathogens in soils that were amended with nitrogenous organic substances. The level of toxicity and killing capacity of nitrogenous organic substances was however found to vary from soil to soil (Mason and Gillespie, 2013).

There are reports that some decomposed products from plant residues may be injurious to bean roots and predispose them to increased root rots (Baysal-Gurel *et al.,* 2012). It therefore requires one to screen for their suitability in the control of bean root rots. Soil organic amendments have been perceived to enhance microbial activities that are antagonistic to soil-borne pathogens (Agrios, 2005). *Trichoderma*, a biological control agent, was found to increase in soils treated with swine manure and was found to be responsible for the reduction of *Verticillium dahliae* that causes *Verticillium* wilt in potato (Shafique *et al.,* 2015). There are other microorganisms whose activities are enhanced by addition of soil amendments and are thought to be antagonistic to soil pathogens. These include; Pseudomonads, *Penicillium*, *Bacillus* and *Streptomyces* (Agrios, 2005).

Additionally, several soil amendments improve soil physical properties and nutrient levels. These in turn improve root penetration; water and nutrient absorption capacity resulting to increased plant vigour and resistance to root rot pathogens (Benedict *et al.,* 1988; Hall and Nasser, 1996). Organic amendments increase or reduce the carbon to nitrogen ratio of soil. When soil has a high C: N ratio, soil microbes immobilize the available nitrogen, thus depriving the plants of nitrogen. This increases stress and predisposes plants to damage from root rot pathogens. Therefore the choice of organic amendments should be done carefully especially when these residues are from the graminae family as these have a high C: N ratio.

2.5.1 Use of biochar as a soil amendment

Biochar is the solid co-product of pyrolysis or the thermal degradation of biomass in the absence of oxygen and is used as a soil amendment for improving soil quality and crop productivity (van Zwieten *et al.,* 2010). It is differentiated from charcoal by its deliberate addition to the soil (Lehmann and Joseph, 2009). It has been promoted as a potential way of improving fertility in soil and other ecosystem services including sequestration of carbon (Lehmann *et al.,* 2006; Laird, 2008; Sohi *et al.,* 2010).

There are four reasons as to the promotion of biochar application as a soil amendment which includes; Generation of usable energy products such as fixed carbon (charcoal) also referred to as biochar or agrichar when used for agricultural purposes by means of pyrolysis. This method can also be used to treat many organic wastes and convert them into useable energy and biochar. Secondly when biochar is used as a soil amendment, fixed carbon is observed to greatly improve soil properties such as tilth, nutrient retention, and availability to plants which translates to improved crop productivity. The water holding capacity and the soil aggregate stability are also greatly improved (Glaser *et al.,* 2002). Depending on the feed stock and pyrolysis conditions, biochar half-life has been estimated to hundreds to tens of thousands of years (Lehmann, 2007). Emissions of greenhouse gases such as N_2O from cultivated soils have been reduced greatly by addition of biochar. This has been documented to be a reduction of up to 80% (Yanai *et al.,* 2007).

Alkaline biochars increase pH in acidic soils which explains the observable effects of biochar on soil fertility and /or improved nutrient retention (Liang *et al.,* 2006; Van Zwieten *et al.,* 2010). Soil biological community abundance and composition has also been changed with the use of biochar (O'Neill *et al.,* 2009; Jin, 2010). The changes in the soil community may affect nutrient cycles (Steiner *et al.,* 2008) and soil structure. These may in turn indirectly affect plant growth (Warnock *et al.,* 2007).

2.5.2 Effect of biochar on plant growth

Many observations have been made demonstrating the positive effects of biochar amendments on field crops. Major *et al.,* (2010) demonstrated a 28 to 140% increase in maize yield two to four years after biochar application. This was in comparison to non-amended control plots. Vaccari *et al.,* (2011) also reported a 30% increase in wheat biomass and yield following an

application of biochar in the Mediterranean basin. They observed sustained effect of the biochar for two consecutive seasons.

Crop response to biochar application can be ascribed to the direct effects of biochar-supplied nutrients which was earlier suggested by Silber *et al.,* (2010). There are also other indirect effects such as soil pH improvements, increased soil CEC, increased retention of nutrients (Yamato *et al.,* 2006). Other effects such as improved soil physical properties which affect water retention and soil microbial populations and functions have also been reported (Wamock *et al.,* 2007; Graber *et al.,* 2010; Kolton *et al.,* 2011).

Graber *et al*. (2010) reported an increase in a number of growth parameters for plants under different biochar treatments in the absence of nutritional and physical aspects of soil. This indicated that plant growth stimulation induced by application of biochar surpasses their influences to plant nutrition and improvement of soil properties. From their findings they hypothesised the effects to be a shift in populations of plant growth promoting Rhizobacteria (PGPR) resulting from either chemical or physical attributes of biochar; and stimulation of growth as a result of biochar borne chemicals in low doses which may otherwise be phytotoxic or biocidal at high concentrations Graber *et al.,* (2014a).

2.5.3 Effect of biochar on plant diseases

Biochar additions to soil have been shown to reduce root lesions caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* (Elmer and Pignatello, 2011). Improved arbuscular mychorrizal fungal (AMF) colonization of asparagus roots in the biochar treated field soils contributed to the suppression of the diseases. Biochar influences microbial communities and populations leading to increased populations of useful microorganisms (Jaiswal *et al*., 2017). These can protect plants from soil pathogens in a number of ways such as out competing pathogens; production of antibiotics and /or grazing on the pathogens. In addition, direct toxic effects on soil pathogens may occur by addition of chemical compounds in to the soil such as tars with the addition of biochar (Graber *et al*., 2014b).

Several compounds known to negatively affect survival of harmful microorganisms have been identified on biochar (Graber *et al*., 2014b; Graber *et al.,* 2010). These compounds include but are not limited to ethylene, propylene, benzoic acid and *O*-cresol. Sensitive soil microorganisms could be suppressed by low levels of these toxic compounds, resulting in proliferation of the resistant microorganisms such as *Nocardioides nitrophenolicus* and

Pseudomonas aeruginosa. This mechanism can be supported by the presence of *Norcardioides nitrophelonicus* and *P. mendocina* in biochar amended soils (Graber *et al.,* 2010). In the same study, they also demonstrated that microorganisms which are successful in degrading toxic organic compounds are generally resistant to a range of toxic organic compounds.

Induction of plant systemic resistance response (ISR) against disease causing microorganisms following application of biochar has been studied in different systems with foliar pathogens. Elad *et al.,* (2010) and Bonanomi *et al*., (2017) observed significant reduction in disease severity of *Botrytis cinerea* and *Oidiopsis sicula* in pepper and tomato grown in biochar amended soils. Molecular evidence for induction of systemic plant defences in the ISR and systemic acquired resistance (SAR) pathways was presented by Meller Harel *et al.,* (2012). Biochar amendments significantly increased expression of defense related genes in leaves by one to three percent.

2.6 Use of vermicompost as a biological soil amendment

2.6.1 Effect of vermicompost on plant growth

Vermicompost is a humic substance produced through an accelerated composting process by the feeding of earthworms thereby producing vermicast (Chan and Griffiths, 1988). Nutrients such as nitrogen, phosphorus, and potassium in feed material are converted by microbial action into forms that are more soluble and available to the plants (Ndegwa and Thompson, 2001). The large surface area of vermicompost provides numerous microsites for microbial activity. This is as a result of the high porosity, aeration and water holding capacity exhibited by the vermicast (Edwards and Burrows, 1988). The nutrient status of vermicompost varies greatly depending on the organic wastes used to feed the worms. The composition ranges between; organic carbon 9.15–17.98%, total nitrogen 0.5–1.5%, available phosphorus 0.1–0.3%, available potassium 0.15, calcium and magnesium 22.7–70 mg per 100 g, copper 2–9.3 ppm, Zinc 5.7–11.5 ppm, and available sulfur 128–548 ppm (Kale, 1995).

This makes vermicompost rich in macro and micronutrients and is ideal in improving yield of many crops (Hidalgo, 1999; Pashanasi *et al.,* 1996). Fresh vermicasts often contain high ammonium levels which are stabilized following rapid nitrification and protection of organic matter in dry casts (Decaens *et al.,* 1999). Vermicompost has increased availability of C, P, K, Ca, and Mg than in the starting feed stock. This is in addition to increased N availability (Orozco *et al.,* 1996). Subler *et al.,* in 1998 also reported on how vermicomposts differ in

nitrogen levels from other composts. Edwards and Burrows, (1988) reported that vermicomposts' resulted to crop improvement at early plant growth as compared to commercial growing medium. Seeds also germinated faster in vermicompost. In subsequent experiments, Edwards *et al.,* (1995) demonstrated that soil amendments of vermicompost increased plant dry weight while Agapit *et al.,* (2018) and Tomati *et al.,* (1994) found an increase in plants uptake of nitrogen. The quality of soil is often affected by its aggregates which usually determine water retention and its movement, diffusion of gasses as well as the development of roots in the soil. Vermicomposts beneficial effect to plant growth can therefore be broadly put as their contribution towards production of plant growth hormones as a result of increased microbial populations produced during formation and breakdown of organic substrates (Edwards and Arancon, 2004a).

2.6.2 Effect of vermicompost on plant diseases

Plant growth promoting hormones produced by microorganisms in vermicompost are believed to be adsorbed on to the humates produced during the vermicomposting process (Edwards and Arancon, 2004 b). It was observed that the benefits of these hormones were not only confined to plant growth but were apparently responsible for reduction of disease incidence. Chaoui *et al.,* (2002) have shown in their study a reduction in attack by *Pythium* spp on cucumbers following relatively small applications of commercially produced vermicomposts. Attack by other pathogens such as *Rhizoctonia* spp, *Verticillium* spp and *Sphaerotheca fulginae* on crops was greatly reduced following application of vermicompost (Ersahin, 2010). Suppression of pathogens was almost eliminated when vermicompost was sterilised before it was applied. Edwards and Arancon, (2004b) consider that the effect of vermicompost on plant diseases most likely arises through antagonism by microbial communities stimulated by the amendment. Other studies by Jack, (2012) reported on masking of the pathogen germination signaling molecules resulting to reduced germination of the pathogen propagules.

CHAPTER THREE

PREVALENCE AND CHARACTERISATION OF BEAN ROOT ROT PATHOGENS IN DIFFERENT AGRO ECOLOGICAL ZONES OF WESTERN KENYA

Abstract

The root rot disease complex has continued to be a major constraint in the production of common beans (*Phaseolus vulgaris*) resulting in losses of up to 70% in Kenya. The aim of this study was to establish (i) the occurrence and quantification of root rot fungal pathogens of common bean in Western Kenya and (ii) the effect of farming practices on the populations of the pathogens. A survey was conducted in Western Kenya's LM1, LM2, UM1 and UM3 AEZ's to obtain data on different farming practices and soil characteristics. Pathogens were isolated and identified using morphological and molecular techniques. Soil pH ranged from 4.59 to 6.01, Percent carbon and nitrogen ranged from 9.8 g/Kg to 19 g/Kg and 0.8 g/Kg to 1.5 g/Kg. All farms were infected with root rot fungi, including *Fusarium solani, Pythium ultimum, Rhizoctonia solani* and *Macrophomina phaseolina*. *Fusarium spp.* was the most abundant with the highest populations of 62 X 10^3 cfu/g soil recorded in lower midland zone 2. Isolation frequency of *Fusarium spp., Pythium spp.* and *Rhizoctonia spp.* was high in upper midland zone 1. Quantification of genomic DNA from soil by qPCR was highest for *Rhizoctonia solani* (2.23 pg μL^{-1}). Sand had a positive correlation with *Pythium ultimum* DNA and *Rhizoctonia solani* DNA while clay had a negative correlation with *Fusarium spp.* and *Rhizoctonia solani* DNA. In conclusion, soil properties, management practices and elevation affected root rot pathogen populations and should be considered when developing management strategies.

3.1 Introduction

Bean root rot caused by a complex of soil borne pathogens is a major constraint to production of common bean in Kenya (Buruchara *et al.,* 2015). The complex of pathogens reported to occur in Kenya are species of *Fusarium*, *Pythium*, *Rhizoctonia, Macrophomina* and *Sclerotium* (Buruchara *at al.,* 2015; Okoth and Siameto, 2010). These pathogens are known to cause high losses in susceptible bean varieties resulting in total crop failure under high moisture and nutrient depleted soils (Paparu *et al.,* 2017). These pathogens are widely distributed in the bean growing regions of Kenya (Otsyula *et al.,* 2013; Mwangombe *et al.,* 2007). Crops get infected at the seedling stage and the disease progresses through vegetative and reproductive growth stages of the bean plants (Hagerty, 2013) causing losses of up to 100% when they occur with other pathogens such as nematodes and bean stem maggot. Root rot is favoured by long rainfall, intermittent droughts and fluctuation in soil moisture condition (Gautam *et al.,* 2014). Due to the threat of climate change and uncertain environmental conditions, Farrow *et al.,* (2011) predicted that the incidence of root rots would rise in East Africa.

Management of soil borne diseases of common bean has been hindered by the persistence of these pathogens in soils over long periods of time in form of mycelia, conidia, oospores, sclerotia or chlamydospores. Continuous cultivation of the same crop in the same field for many years also leads to build up of soil borne pathogen inoculum leading to increased infections (Marzano, 2012). However, Meenu *et al.,* (2010) reported that employing different agronomic practices such as crop rotation, deep tillage, fallowing and application of organic amendments reduces disease inoculum in the soil. They also deprive the pathogen its host and create conditions that favour the growth and development of microorganisms that are antagonists to plant pathogens. These practices have also been shown to have positive changes in the soil structure and root rot disease dynamics leading to increased yields (Bailey and Lazarovits, 2003). This study therefore sought to determine the fungal spectrum associated with common bean farming systems in different agro ecological zones of Western Kenya as well as characterize the fungal root rot pathogens in the area of study.

3.2 Materials and methods

3.2.1 Description of the study site

This study was carried out in three regions of Western Kenya including North Teso situated at latitude 0° 38' 7.0008" N; longitude: 34° 16' 31.0008" E, Bungoma situated at latitude: 0° 34' 10.29" N, longitude: 34° 33' 30.1536" E and Kakamega county at latitude: 0° 17' 1.1796" N, longitude: 34° 45' 5.2668" E. The regions covered four different agro ecological zones which included lower midland humid (LM1), lower midland sub humid (LM2), upper midland humid (UM1) and upper midland semi humid (UM3). The climate in the agro ecological zones varied greatly in relation to rainfall and elevation (Table 3.1).

Table 3.1: Climatic conditions and elevation within each agro ecological zone

AEZ	Annual mean temperature (°C)	Altitude (m)	Annual average rainfall (mm)	Long rainy season (mm)	Short rainy season (mm)
LM1	22.2 – 21.0	1300-1500	1650-1850	750-850	550-730
LM2	22.3 - 21.4	1200-1350	1450-1650	650-700	550-580
UM1	21.0 – 18.5	1500-2000	1600->2000	700- 1000	650-800
UM3	21.0-18.8	1450-1910	1200-1500	550-650	450-580

Source: Jaetzold *et al.*, (2005)

3.2.2 Identification of farmer field sites using the Global Positioning System

At the beginning of this study, collection of soil samples was undertaken from sixty famer fields. These farms were selected from a sampling frame of 280 small holder bean growers in the three counties of western Kenya that had previously undertaken common bean cultivation the previous season under Tropical Soil Biology and Fertility project. The sample size was calculated following Nassiuma, (2000) formula where an 18% coefficient of variation and a standard error of 0.02 was used to calculate the sample size following the formular below.

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

where:

n = Sample, N = Population, C = Covariance, e = Standard error

The Geographical positioning System (GPS) coordinates were taken and recorded for each respective site. These were combined with other site information like administrative locality

and the allocated farm code to uniquely identify the spatial location of each farm on a map. Geographic information system (GIS) maps were developed using the ArcGIS software and converted to shape files that contained data of the spatial location of the farms over the earth surface and GPS coordinates from the farms. The data in spatial format was then overlaid on administrative boundaries as well as agro-ecological zones (AEZ) and soil typology to produce maps indicating the location of farms in the different regions.

3.2.3 Assessment of farming practices in Western Kenya

A survey was conducted in March 2013 using a semi structured questionnaire (Appendix I) to establish the farming systems in the area of study. The sampling frame was a list of 280 small holder bean growers in the three counties of western Kenya. This was done as described in section 3.2.2 above. Farmer selection was based on history of bean production, gender and household income. The total population assessed consisted of sixty households in the different agro ecological zones of the study area.

3.2.4 Collection of soil samples from farmer fields

To determine the occurrence of mycoflora in the farmer's fields, soil samples were collected from sixty bean fields in four AEZ's. Each farmer field was demarcated to an equivalent of 0.0475 ha which was used for the whole period of the study. Two sets of circles measuring 6 meters in diameter were drawn in each selected field. Smaller circles measuring 3 meters were then drawn within the larger circles to have a pair of two concentric rings in each field. Thirteen sampling points were randomly selected around each pair of two concentric circles and at the center in each field as described by Huising *et al.*, (2008).

A core was made at each sampling point to a depth of 20cm from where two hundred and fifty to three hundred grams of soil was collected. The cored soil samples were then mixed in a bucket from which a 1 kg composite sample was taken per field, put in a plastic bag and kept in a shade to prevent dehydration of soils (Cares and Huang, 2008). The soil samples were then placed in a cool box containing ice and later transported to the CIAT Maseno Laboratory and stored at 4°C to stabilize the soil (Cares and Huang, 2008; Pfenning and de Abreu, 2008). The soils were then transported to the University of Nairobi's (UoN) plant pathology laboratory. All sampling tools were surface sterilized with 70% ethanol before sampling of every field to avoid cross contamination across the fields.

3.2.5 Determination of the soil texture, pH and chemical composition

3.2.5.1 Soil texture determination

The particle size distribution of sand, silt and clay for the soils sampled from each farmer field was determined by the hydrometer method (Bouyoucos, 1962). One hundred and fifty grams of each soil sampled was air dried for a period of 48 hours then pulverised and sieved using 2 mm sieve to remove any organic matter from the soils. Fifty (50) grams of air dried soil was mixed with 50 ml of Calgon dispersing agent and 300 ml distilled water in a 500 ml plastic shaking bottle. The mixture was then shaken for 24 hours on a mechanical shaker after which the soil water mixture was plunged into a 1000 ml sedimentation cylinder and 10 seconds later hydrometer readings were recorded. The mixture was then let to sit undisturbed for 2 hours and then the second reading was taken. The hydrometer readings were used to calculate the percentage of sand, clay and silt particles for each soil sample using the equations below.

Calculation of percentage sand particles: $\textbf{Percentage Sand} = \mathbf{100} - ((\mathbf{R1} \div \mathbf{50}) \times \mathbf{100})$
Calculation of percent Clay particles: $\textbf{Percent Clay} = (\mathbf{R2} \div \mathbf{50}) \times \mathbf{100}$
Calculation of percent silt particles: $\textbf{Percent Silt} = \mathbf{100} - (\%\,\textbf{sand} + \%\,\textbf{clay})$
Where R_1 = first hydrometer reading and R_2 = second hydrometer reading

Soil textural class was then determined using United States Department of Agriculture textural triangle (USDA, 1951).

3.2.5.2 Determination of soil pH

A method by Rhoades, (1982) was used to determine the soil pH by weighing 25 grams of air dried soil sample. This was placed into a 100 ml plastic beaker to which 50 mL distilled water was added. It was followed by shaking for 10 minutes on a mechanical shaker and left to stand for 30 minutes. The soil water mixture was stirred again for 2 minutes and the pH of the sample taken using a PL-600 Lab pH meter (MRC Ltd. Tel-Aviv, Israel).

3.2.5.3 Determination of soil chemical composition

Two hundred cubic centimeters of soil was subjected to chemical and physical analyses to measure total nitrogen (% N), available nitrogen (NO_3^- and NH_4^+), organic carbon (% OC), available phosphorus (P_2O_5), exchangeable potassium (K_2O_5), calcium (Ca_2^+) and magnesium (Mg^{++}) ions and soil texture. Soil pH in water was determined with a pH meter. Soil pH for each soil sample was determined in a 1: 2.5 soil water suspension using a calibrated field scout pH meter (Spectrum Technologies, Inc. Paxinos, Pennsylvania, USA). The soil electrical

conductivity (EC) for the same soil sample was subsequently determined using a saturated soil paste using the same meter. Percentage organic carbon was determined according to Walkley and Black (1934) as described by Nelson and Sommers (1996). Total N was determined by the micro-Kjeldhal distillation method as described by Bremner (1996). NO_3^- and NH_4^+ were determined using a colorimetric assay of the soil extract as described by Bremner *et al*. (1965). Exchangeable Ca^{++} and Mg^{++} in an ammonium lactate solution was determined by Atomic Adsorption Spectrophotometry (AAS) while K^+ was determined by flame photometry as per Osborne (1973). Phosphorus was determined as described by Olsen *et al*. (1954).

3.2.6 Isolation, identification and quantification of fungal flora from selected farmer fields in Western Kenya

Soil inhabiting fungi were isolated from soils collected in the sixty sites in western Kenya. Three sub samples each weighing 1g were taken from each 1 kilogram of soil, dissolved in 10ml sterile distilled water in three different universal bottles, mixed by shaking for 1 minute followed by a 10-fold serial dilution series for each sample to achieve a 10^{-4} dilution. One milliliter of 10^{-4} dilution was plated on potato dextrose agar (PDA-HIMEDIA®) medium using pour plate method. The PDA was amended with 50ppm streptomycin sulphate antibiotic to suppress bacterial growth and allow only the mycoflora to thrive.

Each dilution was replicated three times and incubated for 7 days at room temperature. Different fungal colonies were counted and quantified per gram of soil. These were then sub cultured on fresh PDA medium and upon identification, different genera of fungi were sub-cultured on different media. *Fusarium* spp. was sub-cultured on Low nutrient agar (KH2PO4 - 1g, KNO3- 1g, MgSO4* 7H20- 0.5g, KCl- 0.5 g, Glucose- 0.2 g, Sucrose - 0.2 g and Agar 20 g in 1 L) (Nirenberg, 1981) and PDA media (39 g commercial PDA powder/ L water). Sporulation of cultures on SNA was facilitated by incubation of cultures under UV light for 14 to 21 days at 25 ºC. Cultures on PDA were incubated under normal light at 25ºC for 14- 21 days to study the cultural characteristics of the colonies. Identification of *Fusarium* isolates was done based on morphological characteristics which included size and septation of the macro conidia, micro conidia, spore-bearing phialid, presence/absence of chlamydospores and their formation. Identification was done to species level following identification keys by Nelson *et al*., (1983) and the *Fusarium* laboratory manual (Leslie and Summerell, 2006).
Pythium sp. were sub cultured on corn meal agar (corn meal, infusion from 50g, Agar 15g/L water) and observed for production of sporangia, oogonia and antheridia used in identification

based on keys by Plaats-Niterink (1981) and Dick (1990). Identification of other fungi was based on morphological and cultural features such as colour of the colony, growth type, colour of mycelia and spore types (Zhou *et al.,* 2010). The total colony forming units for each genus per gram of soil was determined by obtaining the product of the colonies and the dilution factor. Relative isolation frequency of each genus was determined using Gonzalez *et al.,* (1999) formula with the following equation;

$$\text{Frequency (\%)} = \frac{\text{number of isolates of a genus}}{\text{total number of all isolates}} \text{x } 100$$

All the fungal isolates were maintained on PDA slants at 4 ºC at the University of Nairobi's Plant Pathology laboratory for further identification by gene sequencing.

3.2.7 Molecular characterisation of fungi isolated from the soils of selected farmer fields in Western Kenya

Molecular identification was undertaken by gene sequencing of the isolated fungal DNA. The key fungi from which DNA was extracted from included *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina, Trichoderma* spp*, Paecilomyces lillacinus* and the oomycetes *Pythium ultimum* and *Pythium irregulare.*

3.2.7.1 Extraction of DNA from soils and root rot pathogens

DNA extraction was conducted from the sixty soil samples collected during the survey period. Twenty grams from each of the sixty samples were stored at -20°C until they were processed. Total microbial DNA was extracted from 0.25 g (fresh weight) of each soil sample. The Power Soil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) was used to extract the DNA from the soil. Manufacturer's instructions were followed with modifications which included the use of a bead beater (BioSpec 1001 Mini-Beadbeater-96 Cell Disruptor, Bartlesville, OK, USA) in place of vortexing. This helps to dislodge DNA from the substrate when run at high speed for 10 minutes. The DNA was then lyophilized and stored at −20 °C until it was used for further downstream processes (Fillion *et al.,* 2003).

Fungal cultures of root rot isolates obtained from the soil samples were grown for seven days on PDA (HIMEDIA®) in 9 cm diameter petri dishes incubated at 25°C. Mycelia were gently scrubbed and collected from the surface of the medium with a sterile glass slide after addition of 0.05% (v/v) Tween 80 in sterile distilled water. The suspensions were then transferred to a 1.5 mL micro tube. The tubes were then centrifuged at 3000 g for 5 min with the temperature

being maintained at 4°C. The resulting supernatant was discarded and the pellet used for DNA extraction. The phenol and chloroform protocol was used to extract DNA followed by isopropanol precipitation as per the procedure by González-Mendoza *et al.,* (2010). The extracted DNA samples were then lyophilised and stored at -20 °C at IITA-ICIPE, Nairobi Kenya waiting further downstream processing.

The fungal DNA and soil DNA was rehydrated with 50 µL and 100 µL of nuclease free water respectively before further processing. They were then quantified using the Qubit® 2.0 Flourometer at the Biotechnology Research Center of Cornell University Ithaca, NY. USA.

3.2.7.2 Amplification of DNA extracted from fungi isolated from selected farmer fields in Western Kenya

Conventional polymerase chain reaction (PCR) was used in amplification of the internal transcribed spacer (ITS) region of the pathogens using ITS1 and ITS4 universal primers. Preparation of a 50 µL reaction volume containing 10 µL nuclease free water (IDT), 25.0 µL of IQ SYBR Green Super Mix 2X (Bio Rad 170-8880), 5 µl of each 2µM primer [ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3')] (White *et al.,* 1990) and 5 µL of DNA template was used for all the pathogens. Aliquots of forty five (45µL) PCR reaction mix were pipetted into PCR tubes. To the mix, 5 µL of the pathogen DNA was added and mixed using a pipette. All the workings were done on ice.

PCR amplifications were done as previously described by White *et al.,* (1990) in a T100 thermal cycler (Bio-Rad Laboratories, Inc.). The PCR program used for *Fusarium* spp*, Rhizoctonia* spp*, Macrophomina* spp. *and Paecilomyces* spp were an initial denaturation at 95°C for 3 min, followed by 25 cycles denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min with a final step of extension held at 72°C for 10 min at the end of the amplification reaction. An annealing temperature of 58 °C was used for *Pythium* isolates with all the other temperatures and cycles being the same as above.

Electrophoresis of the PCR amplicons was run on 1% agarose gels dissolved in 1× TAE (Tris-Acetate EDTA) concentration buffer solution after staining with sybr green (0.5 µl/4.5 µl sample). Electrophoresis was carried out for 45 min at 80 V and thereafter visualized under ultraviolet (UV) light. To estimate the sizes of the PCR amplicons, a 100 bp 1Kb plus molecular ladder (Bio-Rad Laboratories, Inc. CA, USA) was used (Fillion *et al.,* 2003).

3.2.7.3 Gene sequencing of soil borne fungi isolated from selected farmer fields in Western Kenya

Thirty two (32) PCR amplicons were purified with the Wizard PCR Clean Up System (Promenga, USA) as per the manufacturer's instructions. Twelve and a half microlitres (12.5 µL) of each amplicon was then mixed with 2.5 µL of the forward primer (ITS 1) and then submitted to the Biotechnology resource center (BRC Genomics facility, Institute of Biotechnology Cornell University Ithaca, NY USA) for sequencing. A comparison of ITS sequences of the isolates was done with those of known species available in the GenBank database. This was done by performing a nucleotide search using the basic linear alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) website http://blast.ncbi.nlm.nih.gov/Genbank/ (Geiser *et al*., 2004).

3.2.8 Molecular quantification of root rot fungal DNA in soils from selected farmer fields in Western Kenya

Quantification of DNA of major root rot pathogens (*F. solani, R. solani, M. phaseolina* and *P. ultimum*) associated with common beans in western Kenya was conducted from total soil DNA. This was undertaken by quantitative PCR amplifications using ABI ViiA7 Real-Time PCR system (Life Technologies, USA) in a total volume of 20µL on a 96 well plate. The 20 µL reaction mixtures contained a final concentration of (2X) IQ SYBR Green Supermix (BioRad), 2µM each of forward and reverse primers for respective fungi, 1 µL of soil DNA template and sterile Nuclease free water. Primers used were; *F. solani*- AFP346 (5' GTATGTTCACAGGGTTGATG 3') Lievens *et al.,* (2006) and ITS1f (5' CTTGGTCATTTAGAGGAAGTAA 3') Gardes and Bruns, (1993); *P. ultimum* - AFP276 (5' TGTATGGAGACGCTGCATT 3') (Lievens *et al.,* (2005) and ITS4 (5' TCCTCCGCTTATTGATATGC 3') White *et al.,* (1990); *R. solani* - ST-RS1 (5' AGTGTTATGCTTGGTTCCACT 3') Lievens *et al.,* (2005) and ITS4 (White *et al.,* 1990); *Macrophomina phaseolina* primers were designed based on the available *Macrophomina phaseolina* sequences' at NCBI database to give a product length of 218 base pairs. The sequences of the primers used was Upper Primer (5' TCCCGATCCTCCCACCCTTTGTAT 3'), and Lower Primer (5' CATTTCGCTGCGTTCTTCATC 3'). Different thermal cyclic conditions were used for amplification of each target fungus. Conditions used were; *Fusarium solani* denaturation at 95°C for 3 min, then 40 cycles each comprising of denaturation at 95°C for 15 s, annealing at 58°C for 30 s and elongation at 72°C for 30s; *Pythium ultimum* and *Rhizoctonia solani*, the thermal-cycling conditions were an initial denaturation of 95°C for 3

min, followed by 40 cycles each consisting, denaturation at 95°C for 15 s, annealing at 60°C for 30 s and a final step at 72°C for 30s. *Macrophomina phaseolina* thermal-cycling conditions were an initial denaturation of 95°C for 15 min, followed by 40 cycles each consisting, denaturation at 94°C for 15 s, annealing at 60°C for 30 s and a final step at 72°C for 30s. All samples were run in triplicate. The amplification results were analysed with ABI ViiA7 Real-time PCR Software v1.2 (Life Technologies, USA).

3.2.9 Standard curve and qPCR efficiency

Deoxyribonucleic acid (DNA) used for preparation of the standard curves were the same as the ones used in the identification of *F. solani, P. ultimum, R. solani* and *M. phaseolina* as previously described. The isolates were amplified using AFP346 and ITS1f; AFP276 and ITS4; ST-RS1 and ITS4; and upper and lower primers respectively. The standard curves were generated by seven fold dilutions of each of the fungal DNA. Cycle threshold (Ct) values were calculated by the ABI ViiA7 Real-time PCR software v1.2 (Life Technologies, USA). The cycle thresholds indicate fluorescent signals rising above background during the early cycles of the exponential phase of the PCR amplification process. Cycle threshold (Ct) values were used to obtain standard curves. They were plotted against the logarithm of the concentration of each 10-fold dilution series of fungal genomic DNA.

In every qPCR run, seven of the respective DNA dilutions (10; 1; 0.1; 0.01; 0.001; 0.0001; 0.00001 ng) with three replicates of each were included in the 96-well plate. This was done to interpolate the amplification results to the absolute quantity of the target in each sample since Ct values may slightly vary between experiments (Fillion *et al.,* 2003).

3.2.10 Data collection and analysis

Survey data on farming practices was collected with the help of a semi-structured questionnaire (Appendix I). This was then analyzed using IBM Statistical package for social science (SPSS) version 20 by computing means, frequencies and percentages. Fungal counts were done following isolation from the soil, while other data such as soil particle size percentages, soil pH measurements and soil nutrient content were collected following laboratory analysis. These data was subjected to analysis of variance (ANOVA) by GENSTAT version 14 and the Tukey test Least Significant difference (LSD) was used for mean separation at 5% level of significance.

The DNA quantified from the soils was subjected to a correlation analysis with soil properties and the fungal populations obtained from the laboratory. This was done using IBM Statistical package for social science (SPSS) version 20.

3.3 Results

3.3.1 Agro-ecological zones and soil typology of the study area

The study was carried out in four agro ecological zones (AEZ) which included Lower midland zone 1 (LM1) 9 farms, Lower midland zone 2 (LM2) 10 farms, Upper midland zone 1 (UM1) 11 farms and Upper midland zone 3 (UM3) 30 farms cutting across the different counties of Kakamega, Bungoma and Busia in Western Kenya (Fig. 3.1).

The farms situated in the different agro ecological zones had varying soil types which include 32 farms with acrisols, 16 farms having gleysols and 12 farms with ferrasols spread out across the three different counties of western Kenya (Fig 3.2). The farms have been identified with dots and numbers on the maps.

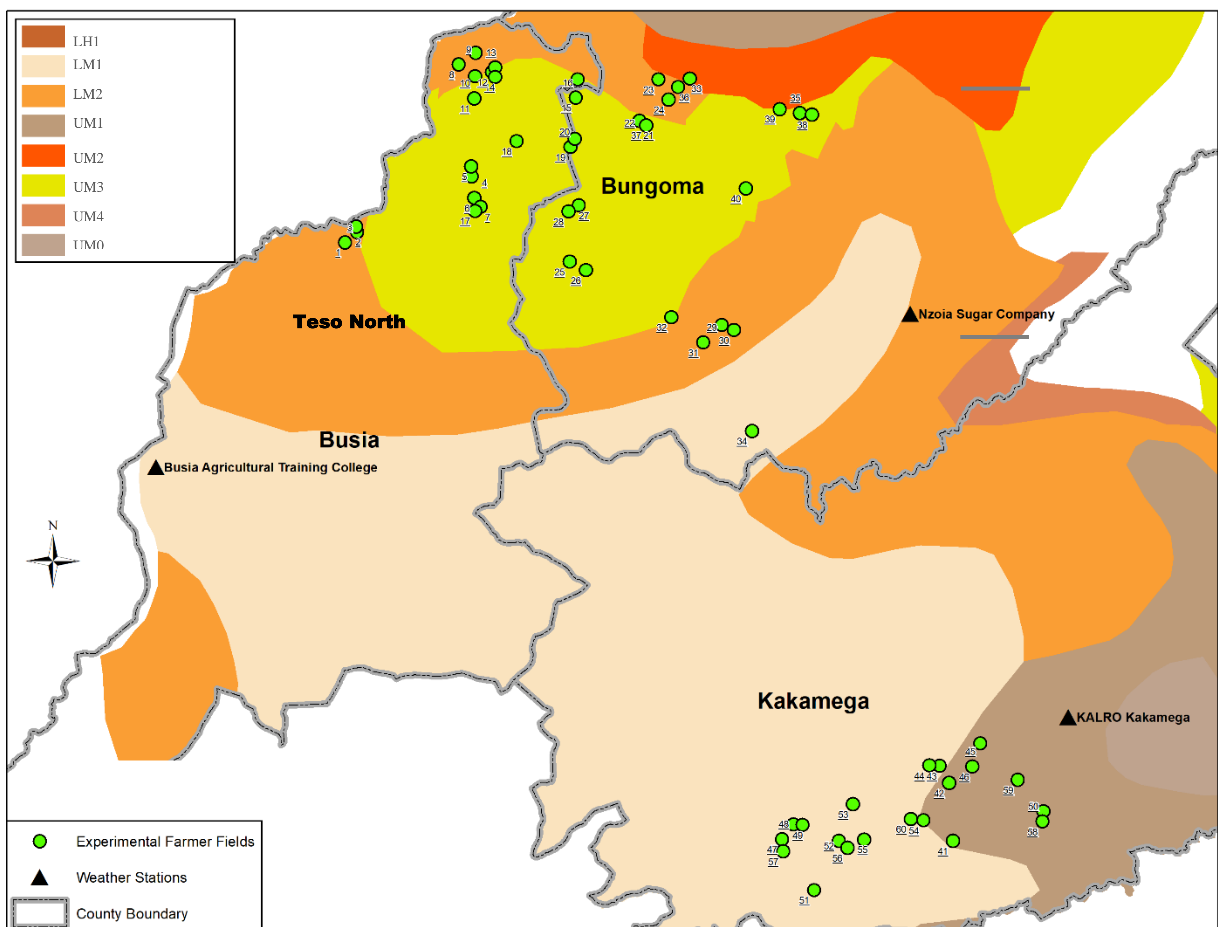


Figure 3.1: Agro ecological zones of study sites in Western Kenya.

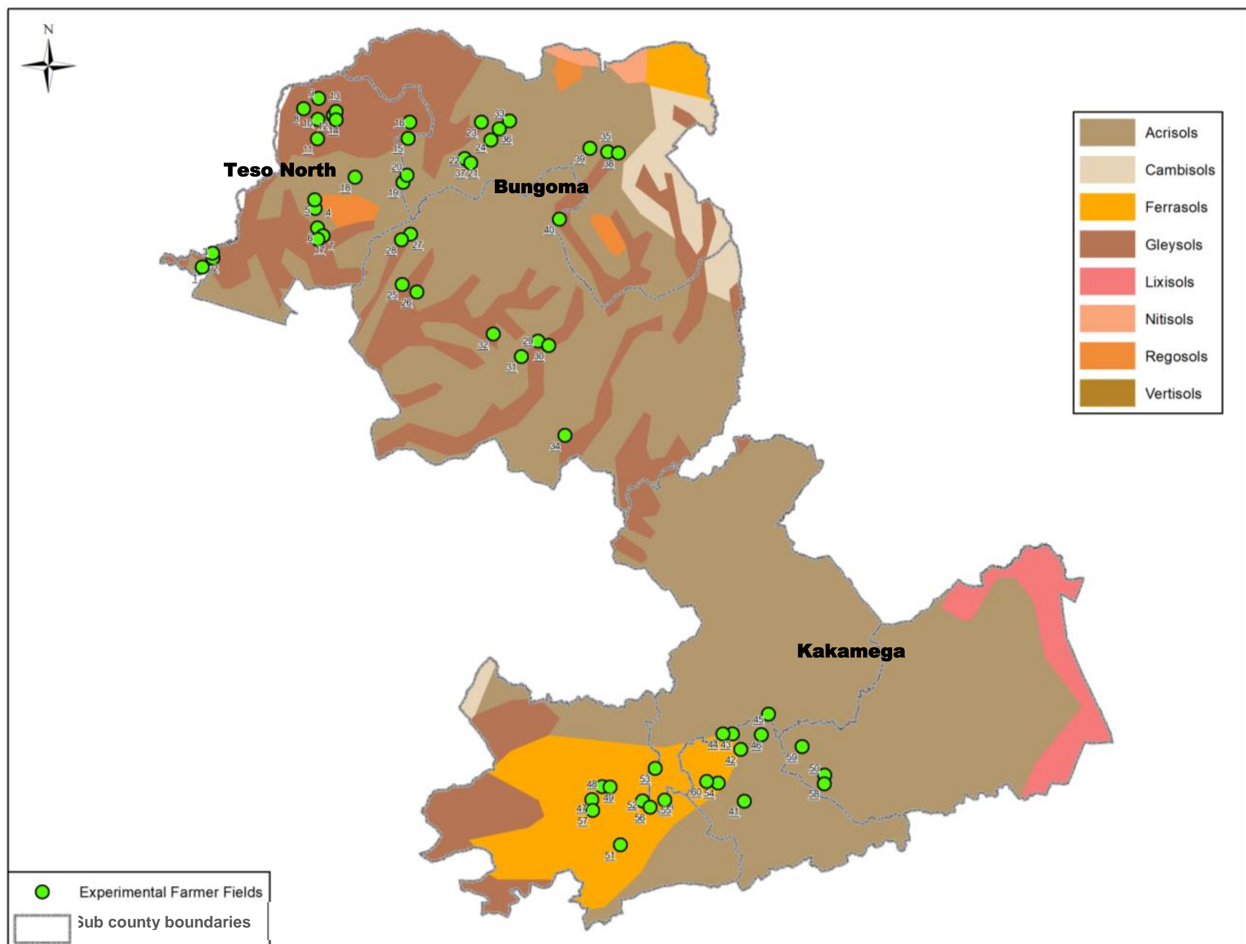


Figure 3.2: Soil typology of study sites in Western Kenya.

3.3.2 Farming practices in western Kenya

Information collected with the help of a semi structured questionnaire indicated that the total farm size and the respective acreage under bean production varied in the four different AEZs. Fifty eight percent (58.2%) of the farmers across the AEZ's owned farms ≥ 2.1 to 5 acres while 26.5% owned farms < 2 acres. Most of the farmers in all the AEZ's had less than 2 acres under bean production and 8.1% of the farmers produced beans on more than 5 acres of land across the four AEZ's. Lower Midlands zone 2 (LM2) had the lowest percentage of farmers with less than 2 acre farm sizes whereas UM1 had the highest percentage (45.5%) of farmers with less than 2 acres of land size. LM1 had the highest percentage of farmers (100%) with less than 2 acres under bean production. The proportion of farmers producing beans on more than 5 acres was in LM2 at 10% (Table 3.2).

The duration of land use varied across the farmers and the different regions. Majority (47.3%) of the farmers had used their farms for cultivation for over 20 years. Lower midland zone 2 had the highest percentage (60%) of the farmers with over 20 years of land use while UM1 had

the lowest (36.4%). The percentage of the farmers who had used their farms for less than 5 years was 7.1%. The highest proportion of these farmers was from UM1 at 18.2% (Table 3.3).

Forty seven percent (47.9%) of the sampled farmers undertook crop rotation on their farms. Lower midland zone 2 had the majority (70%) of farmers who undertook crop rotation whereas UM1 had the least proportion (27.3%) of farmers who undertook crop rotation across the four AEZ's. The percentage of farmers who did not undertake crop rotation was 52.1% (Table 3.4). Tillage practices also varied across the AEZ's where 73.9% of farmers used oxen plough for land preparation while 25.4% and 0.8% of farmers undertook land preparation by hand using a hoe and by tractor respectively. Upper midland zone 1 had the highest percentage (45.5%) of farmers who undertook tillage by hand while 93.8% of the farmers in UM3 used oxen plough to till their land which was the highest across all AEZ's (Table 3.4).

Table 3.2: Percentage (%) of farmers, farm size and acreage under beans in different AEZs of Western Kenya.

AEZ	Total farm Size (acres)				Area under beans (acres)		
	0.5-2	2.1-5	5.1-10	>10	0.5-2	2.1-5	5.1-10
LM1 (n=7)	28.6	71.4	-	-	100	-	-
LM2 (n=10)	10	60	30	-	60	30	10
UM1 (n=11)	45.5	54.5	-	-	90	9.1	-
UM3 (n=32)	21.9	46.9	21.9	9.4	75	18.8	6.2
Mean	26.5	58.2	25.95	9.4	81.25	19.3	8.1

AEZ –Agro-ecological zone

Table 3.3: Percent duration of land under cultivation in different regions of Western Kenya

AEZ	≤5 years	>5 to 10 years	>10 to 20 years	>20 years
LM1 (n=7)	0	0	57.1	42.9
LM2 (n=10)	10	0	30	60
UM1 (n=11)	18.2	27.2	18.2	36.4
UM3 (n=32)	0	21.8	28.2	50
Mean	7.1	12.3	33.375	47.325

AEZ –Agro-ecological zone

Table 3.4: Percentage (%) of farmers undertaking crop rotation and tillage practices

AEZ	Crop Rotation Practiced on farm		Methods of cultivation		
	Yes	No	Hand Tillage	Oxen plough	Tractor
LM1 (n=7)	28.6	71.4	42.9	57.1	0
LM2 (n=10)	70	30	10	90	0
UM1 (n=11)	27.3	72.7	45.5	54.5	0
UM3 (n=32)	65.6	34.4	3.1	93.8	3.1
Mean	47.9	52.1	25.4	73.9	0.8

AEZ –Agro-ecological zone

3.3.3 Soil characteristics from selected farmer fields in the four agro ecological zones of Western Kenya

The particle size aggregation of soils in the different agro ecological zones were significantly different ($p<0.05$) for clay and sand. Farms in UM3 had soils with the highest percentage of clay (62.4%) while farms in LM1 had soils with the lowest percent clay (37.2%). Percent sand was highest (46.7%) in LM1 farms which was significantly different from soils in LM2 with 25.4% which was the lowest. There was no significant difference across AEZ's for percent silt (Table 3.5).

Soil pH was significantly different ($p < 0.05$) across the four AEZs. Farms in UM3 had the highest pH at 6.01 while farms in LM1 had the lowest pH at 4.59 (Table 3.5). Significant differences ($p < 0.05$) were also observed in percent total Carbon and percent Nitrogen. Total soil N was low in LM2 and UM3 at 0.08% while LM1 had the highest at 0.15% (Table 3.5). Farms in LM1 had the highest percent Carbon (1.9%) whereas those in UM3 had the lowest. The difference in percent total Carbon was however not significant between UM3 and LM2 which was also the case between UM1 and LM1 (Table 3.5)

Table 3.5: Composition of soils from different agro-ecological zones of western Kenya

AEZ	pH ($H_2 0$)	% C	% N	% Clay	%Sand	% Silt
LM1	4.59d	1.90a	0.15a	37.23b	46.73a	16.04a
LM2	5.67b	1.01b	0.08b	60.34a	25.35b	14.31a
UM1	5.06c	1.60a	0.13a	44.78b	38.69a	16.53a
UM3	6.01a	0.98b	0.08b	62.37a	25.69b	11.94a
Mean	5.33	1.2	0.1	30.5	55.9	13.66
LSD ($p \leq 0.05$)	0.31	0.47	0.04	10.19	13.7	4.81
CV %	11.3	46.8	45.7	40	29.4	42.1

Means with same letter(s) within each column are not significantly different at $p \leq 0.05$. AEZ- Agro-ecological zone, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- upper midland zone 1, UM3-upper midland zone 3, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

3.3.4 Soil types and soil mycoflora across the four agro ecological zones of Western Kenya

Six different soil types were found to be infected with root rot causing pathogens as well as other soil inhabiting fungi. There were significant differences ($p \leq 0.05$) in populations of all the root rot fungi isolated from the different soil types. Loamy fine sand had the highest populations of the four root rot fungi in all the six soil types. Populations of *Fusarium* spp and *Rhizoctonia* spp were lowest in clay soils, while *Pythium* spp and *Macrophomina* spp were lowest in sandy clay and clay loam respectively. The isolation of *Trichoderma* spp was highest in Sandy clay soils and lowest in loamy fine sand, the differences being significant ($p<0.05$). Populations of *Aspergillus* spp and *Penicillium* spp were however found not to be significantly different (Table 3.6).

All the soils collected from the four agro-ecological zones in Western Kenya were infected with root rot causing pathogens. The soil borne fungal pathogens isolated were; *Fusarium, Pythium*, *Macrophomina*, *Rhizoctonia* and other soil inhabiting fungi such as *Trichoderma*, *Penicillium* as well as *Aspergillus* spp. Of all the root rot fungi isolated, *Fusarium* spp. was highest across all agro-ecological zones at 61,958 colony forming units (CFU) per gram of soil. *Macrophomina* spp was the least isolated root rot fungi across all AEZ's at 1,829 CFU/g of soil. Significant differences ($p<0.05$) were observed in AEZ's for root rot pathogens namely *Fusarium* spp, *Rhizoctonia* spp and *Macrophomina* spp as well as *Trichoderma* spp. Populations of *Fusarium* spp were highest in LM2, while *Rhizoctonia* spp was highest in UM3 and *Macrophomina* spp was highest in LM1 (Table 3.7; Figure 3.3; Fig 3.4; Fig 3.5 and Fig

3.6). No significant difference was observed for *Pythium* spp*, Aspergillus* spp and *Penicillium* spp (Table 3.7).

Different species of fungi were isolated and identified from soils sampled in western Kenya. The microorganisms of great importance to the study that were identified using cultural and morphological characteristics included: *Fusarium, Rhizoctonia, Pythium, Macrophomina, Trichoderma, Penicillium* and *Aspergillus* spp (Plate 3.1 and 3.2).

Table 3. 6: Populations of soil fungi (x 10^3 CFU/g soil) isolated from different soil types of selected farmer fields in Western Kenya.

Soil types	*Fusarium* spp	*Pythium* spp	*Rhizoctonia* spp	*Macrophomina* spp	*Trichoderma* spp	*Aspergillus* spp	*Penicillium* spp
Loamy fine sand	77.3a	84.3a	69.2a	18.6a	0.6c	22.9	3.5
Sandy Clay	68.7ab	31.3b	40.7bc	2.0bc	12.0a	ND	ND
Sandy loam	64.1ab	40.2b	46.7b	9.4b	2.8bc	ND	ND
Clay loam	59.5b	33.8b	42.5b	0.7c	9.5ab	10.0	8.0
Sandy Clay loam	53.1bc	36.1b	31.5bc	3.9bc	10.6a	28.3	8.5
Clay	42.9c	35.8b	24.9c	8.2bc	1.2c	18.9	2.8
LSD ($p<0.05$)	16.0	12.4	15.8	7.6	7.2	NS	NS
CV %	41.0	45.0	58.0	168.0	185.0	41.0	39.0

Means with same letter(s) within same column are not significantly different at $p \leq 0.05$. ND: Not detected, NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 3. 7: Populations of soil fungi (x 10^3 CFU/g soil) isolated from selected farmer fields in different AEZ's of Western Kenya

AEZ	*Fusarium* spp	*Pythium* spp	*Rhizoctonia* spp	*Macrophomina* spp	*Trichoderma* spp	*Aspergillus* spp	*Penicillium* spp
LM1	44.9b	35.3a	24.6b	12.2a	0.1c	17.1	4.3
LM2	62.0a	39.6a	41.0a	10.0ab	11.0a	23.0	13.3
UM1	44.6b	34.0a	22.5b	1.8c	1.8bc	21.8	2.7
UM3	60.3a	39.2a	42.4a	6.0bc	6.7ab	21.9	3.4
LSD ($p<0.05$)	13.2	10.6	12.9	4.4	5.9	NS	NS
CV %	48.0	55.0	76.0	114.0	234.0	41.0	39.0

Means with same letter(s) within same column are not significantly different at $p \leq 0.05$. AEZ-Agro ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3. NS- No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

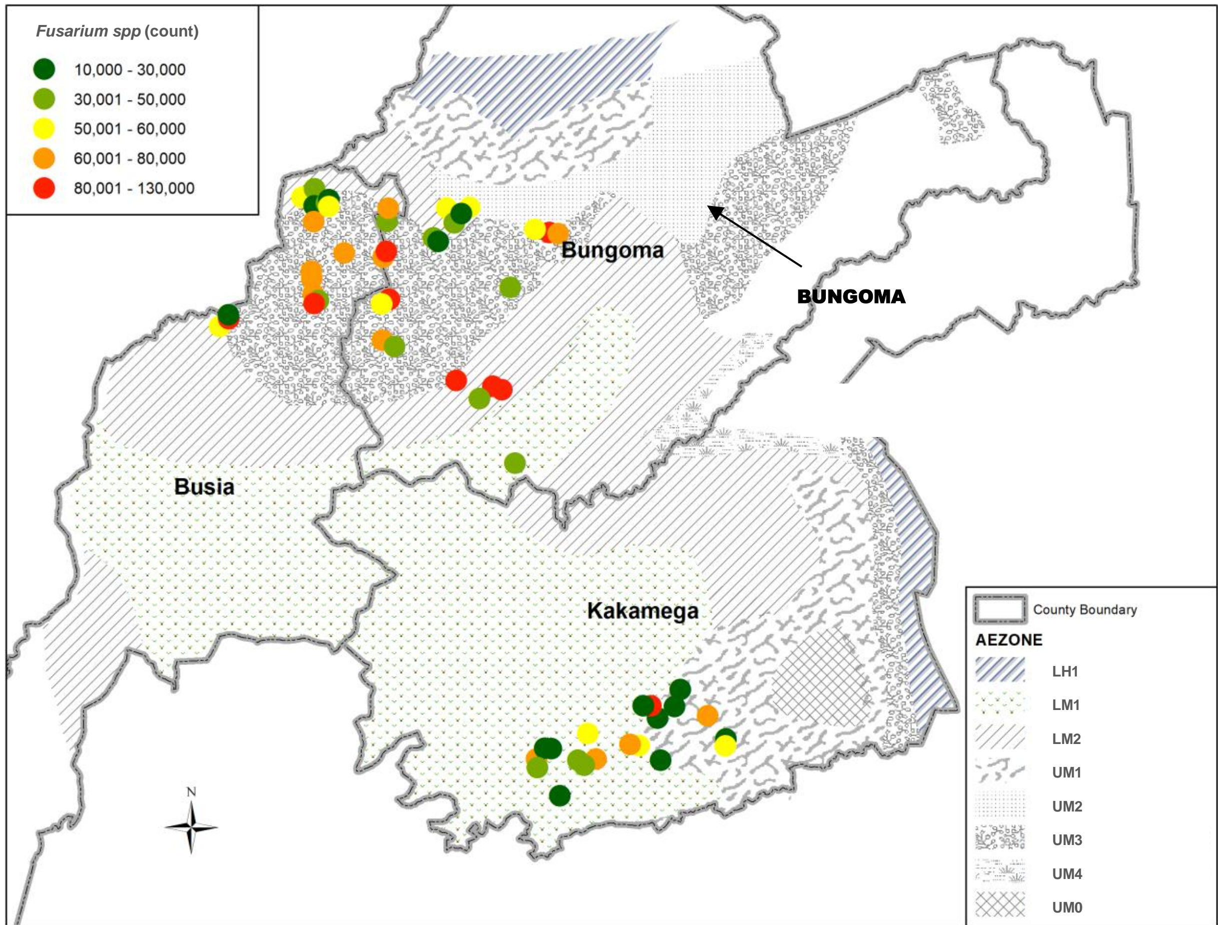


Figure 3.3: Populations of *Fusarium* spp (cfu/g soil) across the four agro ecological zones of Kakamega, Bungoma and Busia counties

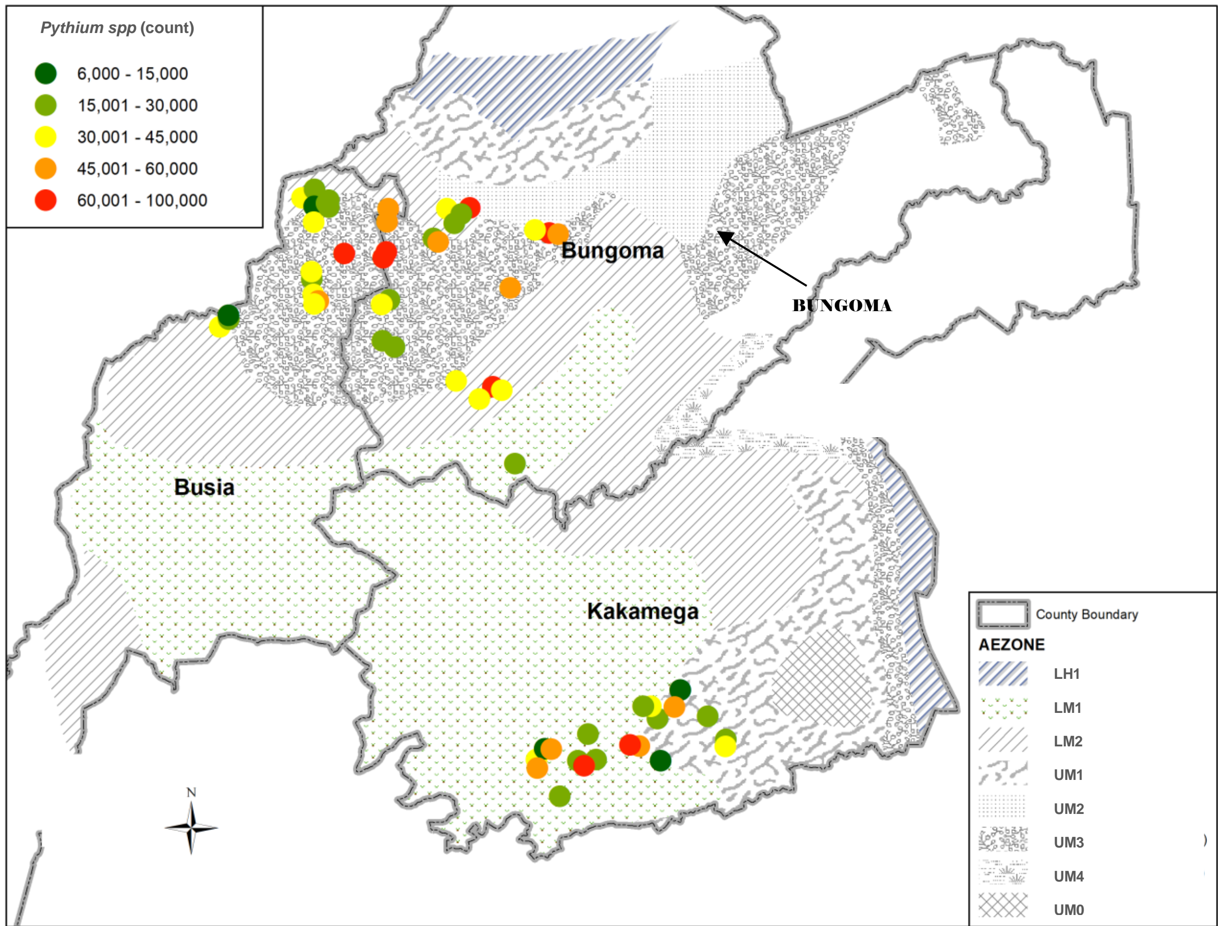


Figure 3.4: Populations of *Pythium* spp (cfu/g soil) across the four agro ecological zones of Kakamega, Bungoma and Busia counties

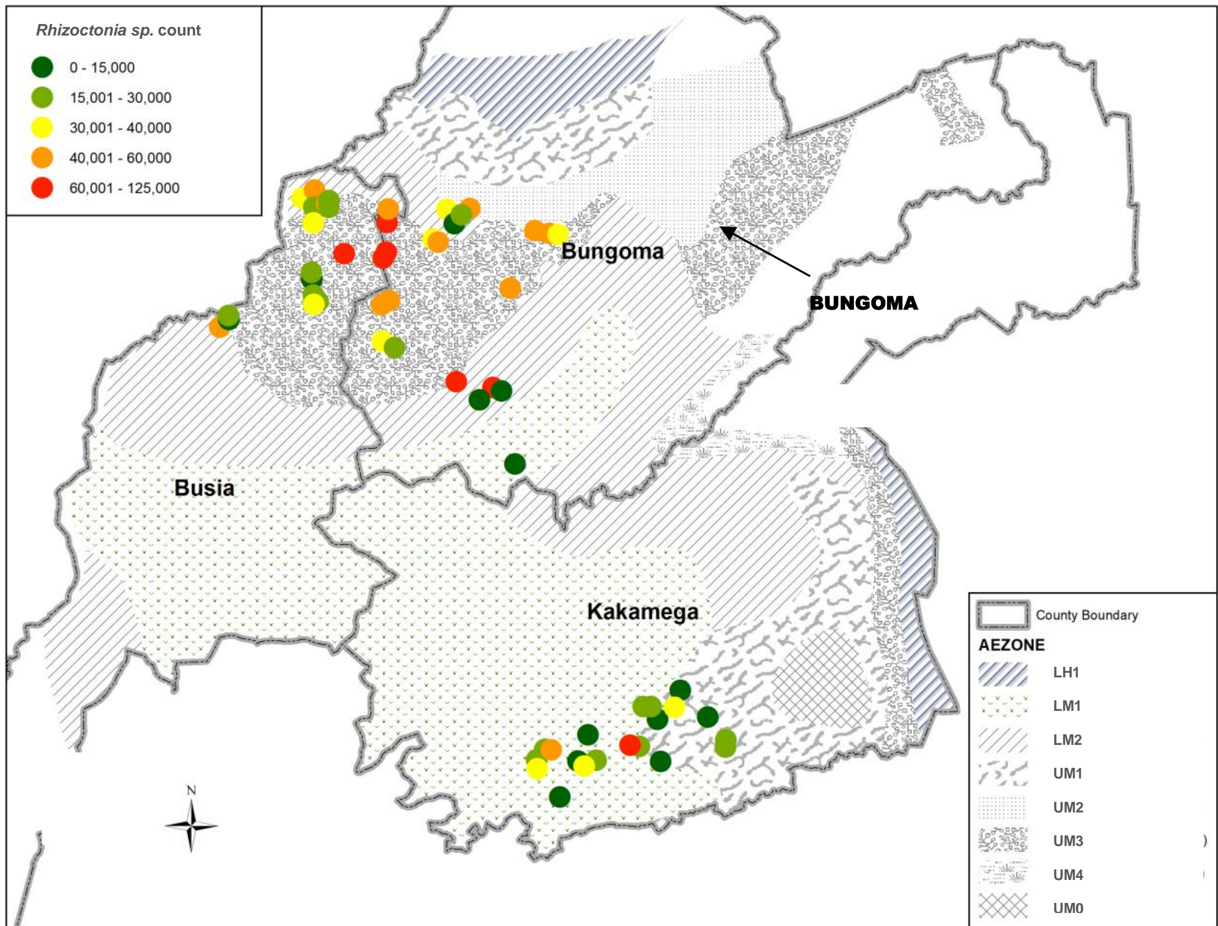


Figure 3.5: Populations of *Rhizoctonia* spp (cfu/g soil) across the four agro ecological zones of Kakamega, Bungoma and Busia counties

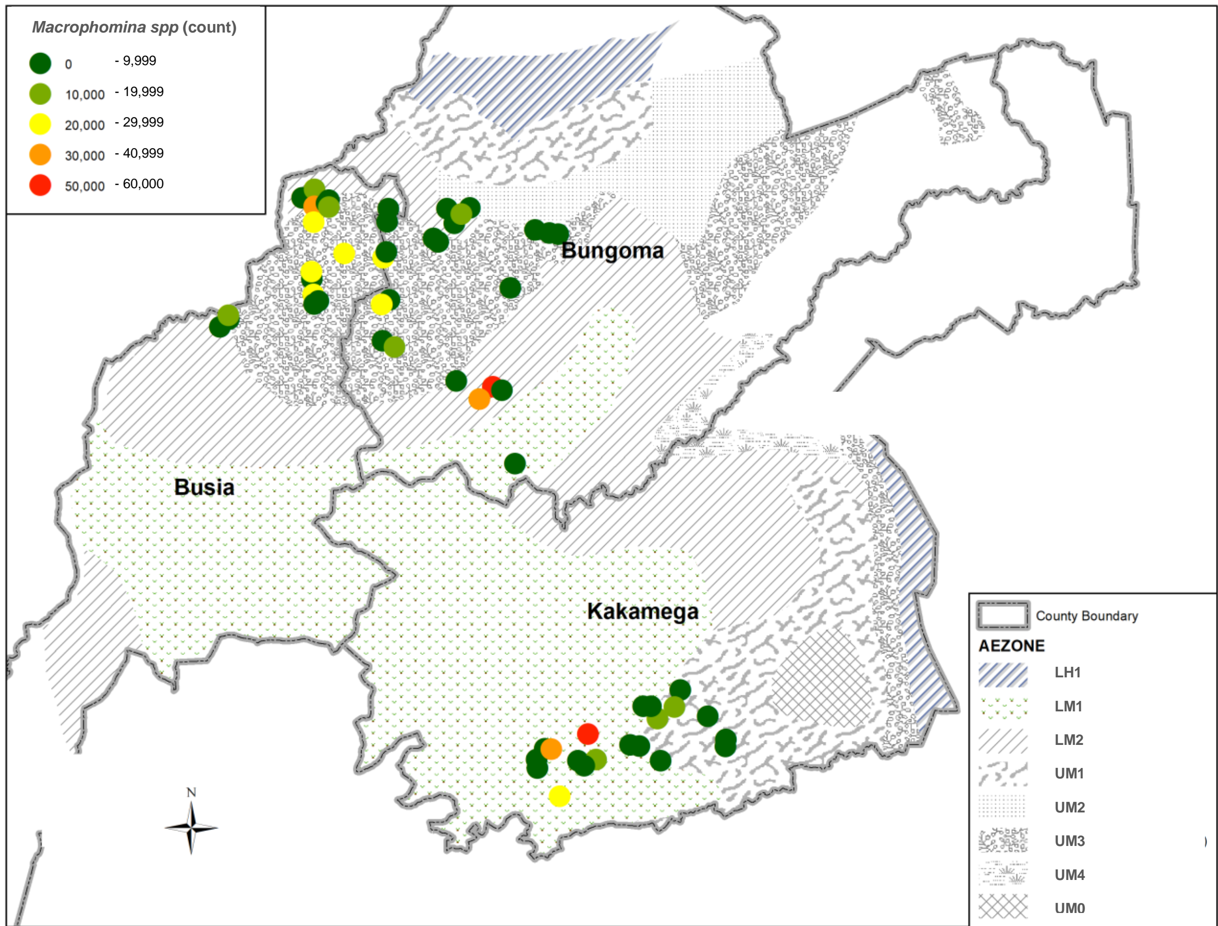
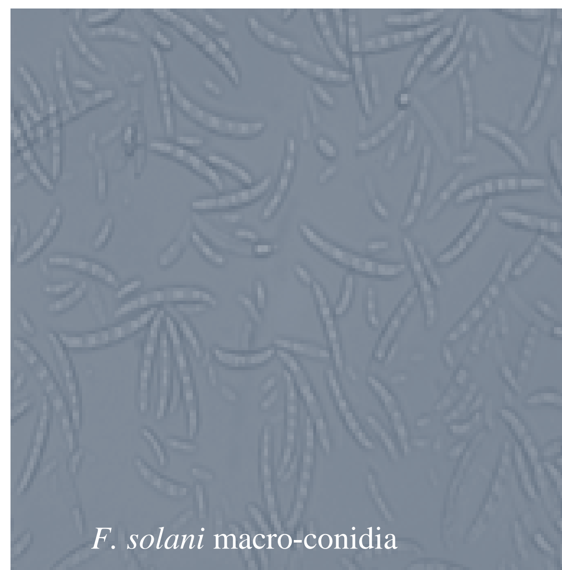


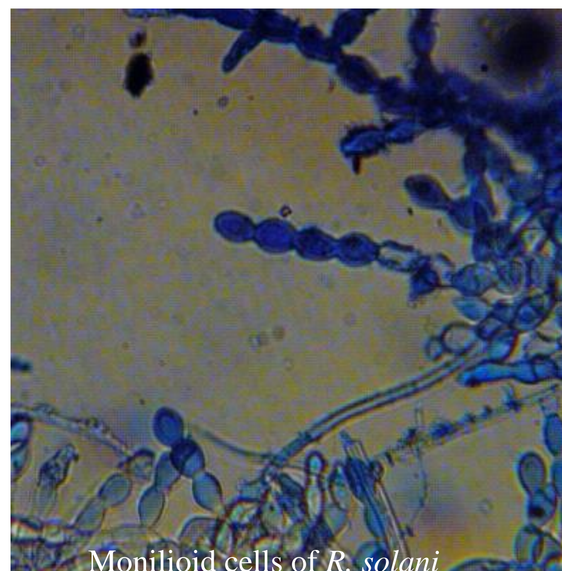
Figure 3.6: Populations of *Macrophomina* spp (cfu/g soil) across the four agro ecological zones of Kakamega, Bungoma and Busia counties

F. solani culture on PDA


F. solani macro-conidia

F. solani micro-conidia

R. solani culture on PDA


Monilioid cells of *R. solani*

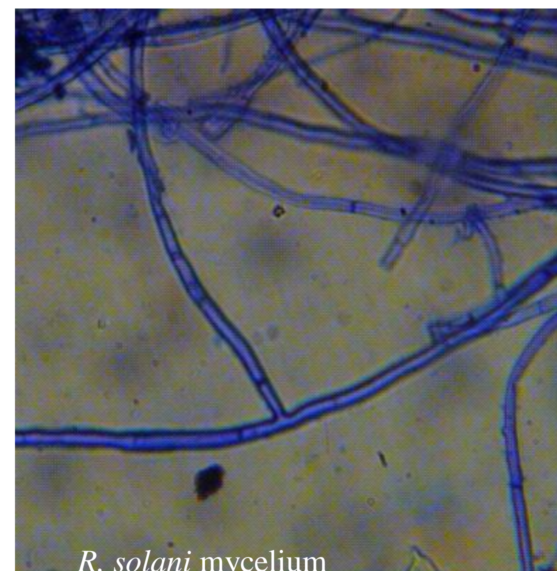
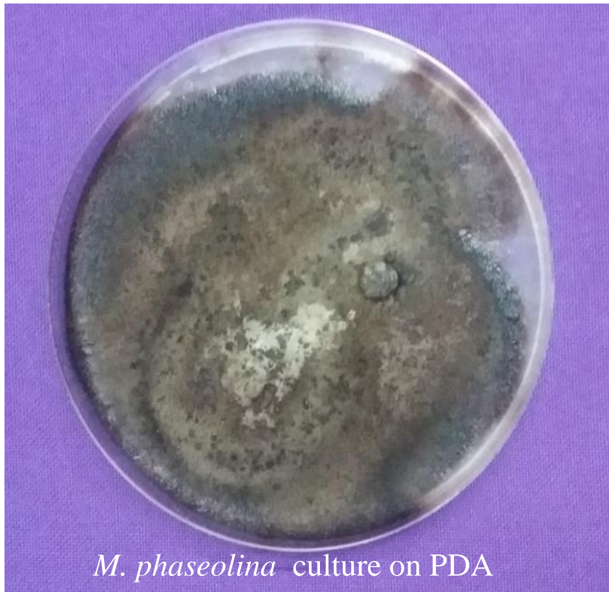
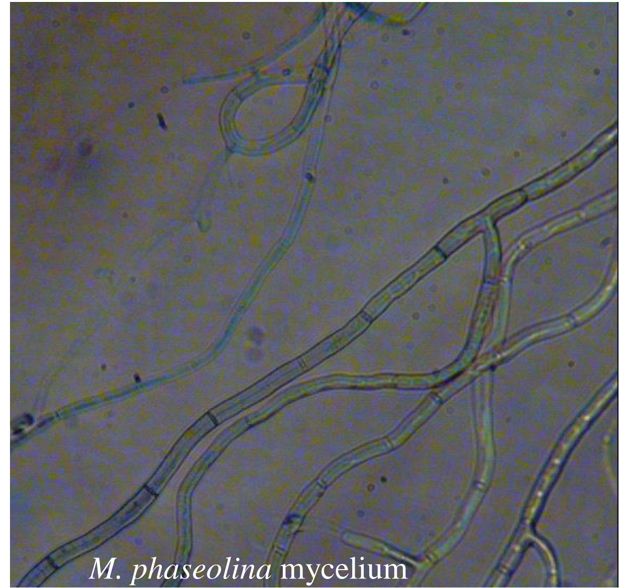
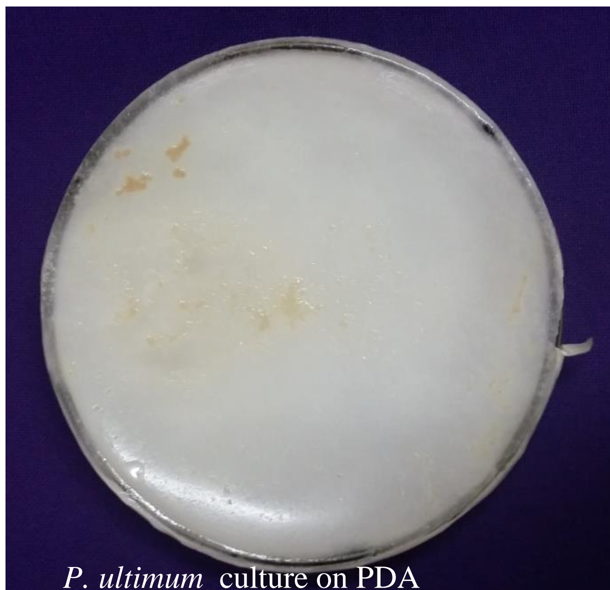

R. solani mycelium

Plate 3. 1: Cultural and morphological characteristics of *F. solani* and *R. solani* isolated from western Kenya soils







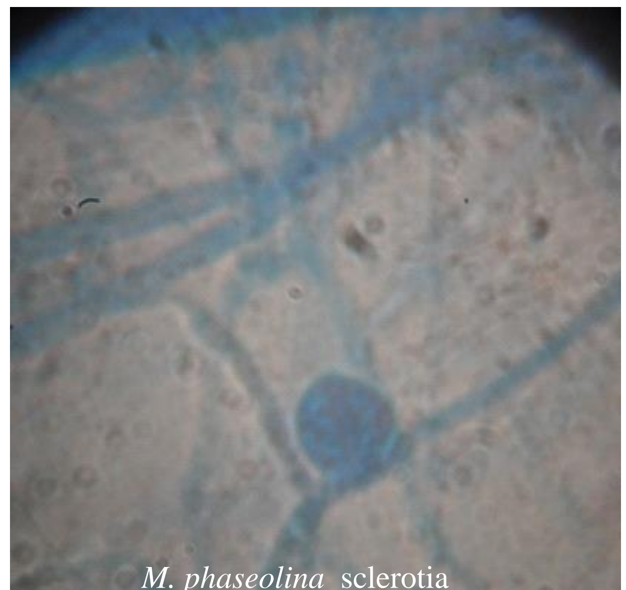


Plate 3. 2: Cultural and morphological characteristics of *M. phaseolina* and *A. oligospora* isolated from western Kenya soils.

3.3.5 Frequency of occurrence of different soil borne fungi in sampled soils and agro ecological zones of Western Kenya

There was a significant difference ($p<0.05$) in the frequency of occurrence of fungal genera across the AEZ's. *Fusarium* spp. had the highest frequency of all fungi from soils while *Trichoderma spp* had the lowest. *Fusarium, Pythium* and *Rhizoctonia* species had the highest frequency recorded in UM1 and the lowest frequency in LM2. *Macrophomina* spp and *Trichoderma* spp on the other hand had the highest frequency in LM1 and LM2 and the lowest was in UM1 and LM1 respectively. No significant difference was observed for *Aspergillus* and *Penicillium* spp across the agro ecological zones (Table 3.8).

With reference to soil types, significant differences ($p\leq0.05$) across the soil types were recorded for all fungi with the exception of species of *Aspergillus* and *Penicillium* (Table 3.9). Of the four root rot pathogens, *Fusarium* spp had the highest frequency of occurrence while *Macrophomina* spp had the lowest occurrence across the six soil types. Highest frequency for *Fusarium* spp was recorded in clay loam and sandy clay soils and lowest in loamy fine sand. *Pythium* spp and *Macrophomina* spp were highest in loamy fine sand and lowest in sandy clay and clay loam respectively whereas *Rhizoctonia* spp was highest in clay loam soil and lowest in clay soils. *Trichoderma* spp was highest in clay loam and lowest in loamy fine sand the differences being significant ($p\leq 0.05$). No significant difference was observed for *Aspergillus* and *Penicillium* spp across the six soil types.

Table 3.8 : Frequency (%) of soil borne fungi in different agro-ecological zones of Western Kenya

AEZ	*Fusarium* spp	*Pythium* spp	*Rhizoctonia* spp	*Macrophomina* spp	*Trichoderma* spp	*Aspergillus* spp	*Penicillium* spp
LM1	36.3ab	25.6a	20.8a	9.2a	0.2c	11.3a	2.6b
LM2	31.5b	20.1b	18.2a	5.2ab	4.6a	12.5a	5.9a
UM1	38.9a	26.6a	23.9a	2.6b	1.5bc	11.5a	1.3b
UM3	34.8ab	21.1b	18.9a	4.2b	3.4ab	11.0a	1.6b
Mean	**35.4**	**23.3**	**20.4**	**5.2**	**2.4**	**11.4**	**2.3**
LSD ($p<0.05$)	7.2	3.7	5.9	4.4	2.6	11.8	2.0
CV %	38.3	30.1	55.4	159.1	208	124.5	201.8

Means with same letter(s) within each column are not significantly different at $p\leq0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3. NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 3.9: Frequency (%) of different soil borne fungi in relation to soil types in Western Kenya

Soil texture	*Fusarium* spp	*Pythium* spp	*Rhizoctonia* spp	*Macrophomina* spp	*Trichoderma* spp	*Aspergillus* spp	*Penicillium* spp
Clay	34.9abc	25.8b	16.6b	6.7ab	0.8b	14.1	1.9
Clay loam	40.2ab	22.7bc	28.7a	0.6b	6.4a	ND	ND
Loamy fine sand	30.9c	32.9a	27.2a	7.3a	0.3b	ND	ND
Sandy Clay	40.2ab	17.4d	28.0a	1.3b	4.9a	5.6	3.0
Sandy Clay loam	31.5abc	20.7cd	21.7ab	4.3ab	5.0a	13.6	3.9
Sandy loam	37.9abc	22.3bc	23.6ab	4.6ab	1.7b	9.3	1.4
Mean	**36.0**	**23.6**	**24.3**	**4.1**	**3.1**	**7.1**	**1.7**
LSD (p<0.05)	8.8	4.5	7.1	5.5	3.1	28.7	10.3
CV %	37.8	29.5	45.7	209.4	154.9	122.7	211.6

Means with same letter(s) within each column are not significantly different at p≤0.05. ND: Not detected, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

3.3.6 Gene sequence of isolated fungi from soils of Western Kenya

Upon successful amplification of the 31 isolates, 25 isolates were identified using gene sequencing (Plate 3.3; Table 3.10; Appendix II). Fourteen isolates were identified as *Fusarium* spp of which eight were *F. oxysporum*, four *F. solani* and two *F. equisetti.* Five *Pythium* spp were identified with three being *P. ultimum* and two *P. irregulare.* Other fungi identified include two *R. solani*, two *M. phaseolina* and two *Paeciliomyces lillacinus.*

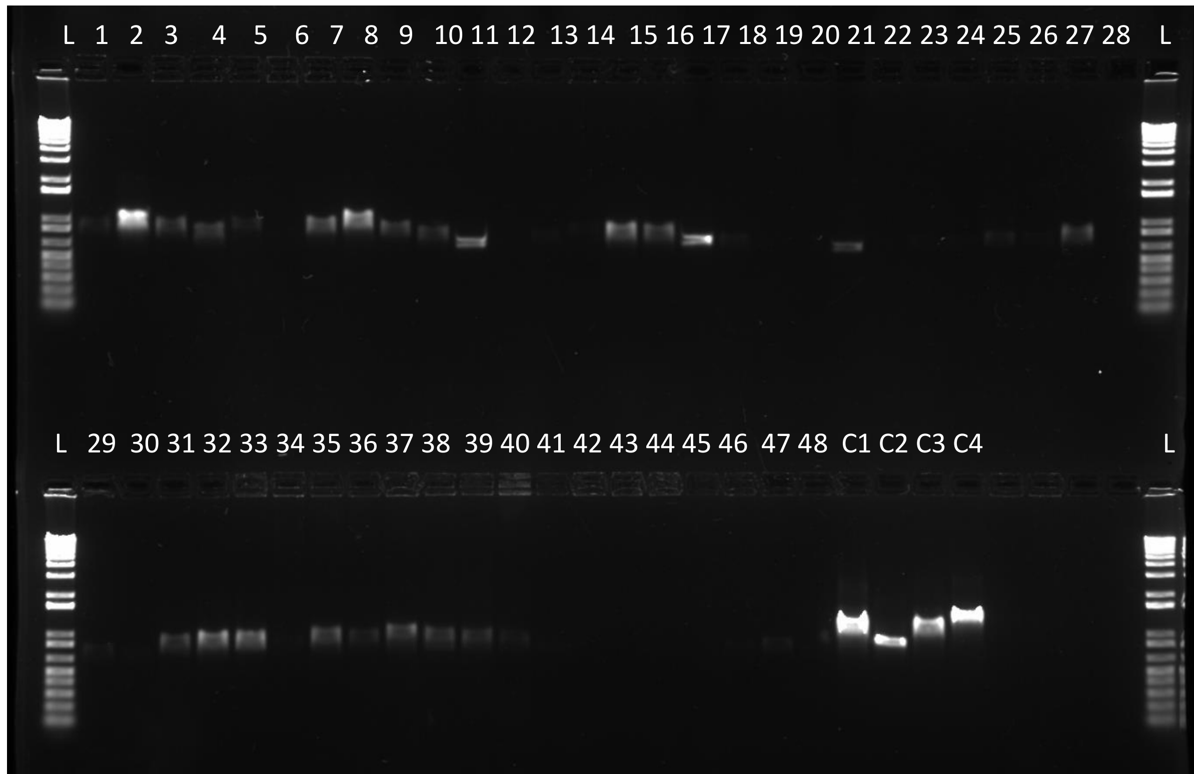


Plate 3. 3: Gel electrophoresis of soil borne fungi isolated from Western Kenyan soils and various agro ecological zones

L- 1Kb Ladder; C1 – Positive control (*F. oxysporum* DNA); C2- positive control (*P. ultimum* P4 DNA); C3 – positive control (*F. solani* DNA), C4 – positive control (*R. solani* DNA)

Table 3.10 Fungal species identified from soils in Western Kenya

Fungal species	Number of species Identified (counts)	Percentage (%) of species identified per Agro ecological Zones			
		LM1	LM2	UM1	UM3
Fusarium equisetti	2	4			4
Fusarium oxysporum	8	12	12	4	4
Fusarium solani	4	4	8		4
Macrophomina phaseolina	2	4			4
Paecilomyces lilacinus	2				8
Pythium irregulare	2	4			4
Pythium ultimum	3	4			8
Rhizoctonia solani	2	4			4
Paecilomyces lilacinus	2				

3.3.7 Total microbial DNA and quantity of soil genomic DNA for bean root rot fungi in Western Kenya

There was no significant difference ($p<0.05$) in total microbial DNA extracted from soil samples across the AEZ's of western Kenya. However, the quantity of genomic DNA was recorded highest for *R. solani* and lowest for *P. ultimum* in all the AEZs. Of the four bean root rot pathogens, only *R. solani* had significant differences ($p<0.05$) recorded across the AEZs (Table 3.11). Upper midland semi humid (UM3) was observed to yield the highest quantity of *R. solani* genomic DNA while the lowest was recorded in UM1. No significant differences in the quantity of genomic DNA were observed for *F. solani, P. ultimum* and *M. phaseolina*.

Table 3.11: Amount of fungal genomic DNA in soils from different AEZs of Western Kenya

AEZ	Total Soil DNA (ug/ml)	*F. solani* (ng/µL)	*P. ultimum* (ng/µL)	*R. solani* (ng/µL)	*M. phaseolina* (ng/µL)
LM 1	13.55a	0.0000251a	0.00001a	0.0168b	0.1485a
LM 2	13.06a	0.000165a	0.01531a	0.3543ab	0.0561a
UM 1	14.95a	0.0000758a	0.00002a	0.0073b	0.0583a
UM 3	10.73a	0.0001641a	0.16225a	1.1339a	0.1854a
MEAN	**13.08**	**0.0001075**	**0.09**	**0.67**	**0.14**
LSD	5.67	0.00021	0.356	0.951	0.415
%CV	132.5	211.4	477.5	170.6	364.5
F. Pr.	0.859	0.568	0.569	0.011	0.837

Means with same letter(s) in each column are not significantly different at $p \leq 0.05$. AEZ - Agro Ecological Zone, LM 1- Lower Midland (Humid), LM 2 - Lower Midland (Sub Humid), UM 1 - Upper Midland (Humid), UM 3 - Upper Midland (Semi humid), LSD: Least significant difference at 5% level, CV: Coefficient of variation.

3.3.8 Correlation between soil properties, farming practices, root rot fungal pathogen population and quantification by molecular techniques

A number of significant relationships ($p \leq 0.05$) were observed between soil properties; populations and quantity of bean root rot pathogen genomic DNA from soil (Table 3.12). Significant positive correlation was observed between percent sand and the *P. ultimum* DNA ($r = 0.256$, $p<0.05$) as well as *R. solani* soil DNA ($r = 0.268$, $p<0.05$). However, the correlation between soil percent sand and *Macrophomina phaseolina* soil DNA was observed to be negative and highly significant ($r = -0.398$, $p<0.001$). Percent clay was observed to have a significant negative correlation with population of *Fusarium sp.* isolated from soils ($r = -0.265$, 0.05) and quantity of *R. solani* DNA in the soil ($r = -0.37$, $p<0.001$). Significant negative correlation was also observed between percent silt and quantity of *R. solani* DNA ($r = -0.366$, $p<0.001$). Soil pH and isolated *Trichoderma* spp was found to have a positive and significant correlation ($r = 0.312$, $p<0.05$). The correlation between *Fusarium* spp populations and *Pythium* spp isolated from the different soils of Western Kenya was found to be positive and highly significant ($r = 0.602$, $p<0.01$). Similar correlation was also observed between *Fusarium* spp population and *R. solani* DNA ($r = 0.256$, $p<0.05$). Significant positive correlations were

also observed between the populations of *Pythium* spp and populations of *Rhizoctonia* spp ($r = 0.342$, $p<0.001$) and the population of *Trichoderma* spp ($r = 0.287$, $p<0.05$). Altitude had a negative significant correlation with DNA of *F. solani* and *R. solani* ($r = -0.321$ and $r = -0.274$, $p<0.05$ respectively). The other correlations like land use and pathogen DNA and populations, Olsen P and pathogen DNA and populations were not significant (Table 3.12).

Table 3.12: Correlation coefficients (r) of soil characteristics, land use, population and DNA of fungal root rot pathogens of common bean

	Altitude	pH	Olsen P	% sand	%Clay	%Silt	Land use	Percent N	Percent C	*Fus*	*Pyth*	*Rhiz*	*Macrop*	*Tricho*	*F.s DNA*	*P. u DNA*	*R. s DNA*	*M.. p DNA*
Altitude	1																	
pH	0.031	1																
Olsen P	0.133	**0.340****	1															
%_Sand	-0.154	**0.394****	0.100	1														
%_Clay	0.146	**-0.367****	-0.120	**-0.608****	1													
%_Silt	0.129	-0.100	0.110	**-0.465****	**0.699****	1												
Land use	-0.116	0.048	0.003	0.012	-0.083	-0.209	1											
Percent N	0.271	-0.135	0.086	**-0.643****	**0.868****	**0.763****	-0.032	1										
Percent C	0.240	-0.149	0.072	**-0.643****	**0.873****	**0.723****	0.01	0.991	1									
Fus	-0.116	0.173	0.162	0.200	**-0.265***	-0.089	0.089	-0.174	-0.176	1								
Pyth	0.212	0.063	0.165	0.087	-0.200	-0.113	0.115	-0.029	-0.032	**0.602****	1							
Rhiz	0.082	0.192	0.155	0.082	-0.107	-0.179	0.187	-0.034	-0.018	0.044	**0.342****	1						
Macrop	-0.012	-0.135	-0.147	-0.056	0.008	-0.028	-0.015	0.094	0.111	-0.132	-0.092	0.088	1					
Tricho	0.064	**0.312***	0.186	0.097	-0.035	0.054	0.053	-0.016	-0.037	0.163	**0.287***	0.213	-0.064	1				
F. s DNA	**-0.321***	0.072	0.216	0.004	0.066	0.044	0.179	-0.041	-0.046	0.121	-0.039	-0.019	-0.125	-0.051	1			
P. u DNA	0.034	0.036	-0.093	**0.256***	-0.218	-0.215	0.038	-0.213	-0.211	0.169	0.230	0.133	0.134	-0.103	-0.096	1		
R.. s DNA	**-0.274***	0.198	0.077	**0.268***	**-0.370****	**-0.366****	0.128	**-0.349****	**-0.338****	**0.256***	0.243	-0.100	0.012	0.093	-0.184	0.204	1	
M.. p DNA	0.052	0.052	0.079	**-0.398****	-0.171	-0.147	0.133	0.051	0.053	0.102	0.057	0.031	0.170	-0.034	-0.066	-0.060	0.165	1

***. Correlation is significant at the 0.05 level.**

****. Correlation is significant at the 0.001 level.**

Duration of land use, *Rhiz- Rhizoctonia* spp , *Pyth- Pythium* spp, *Fus- Fusarium* spp, *Macrop- Macrophomina* spp, *Tricho- Trichoderma* spp, *F.s* DNA - *Fusarium solani* DNA , *P. u* DNA- *Pythium ultimum* DNA, *R. s* DNA-*Rhizoctonia solani* DNA, *M. s DNA Macrophomina phaseolina* DNA , %Clay- Percent clay, %Sand – Percent Sand, %Silt- Percent Silt, pH – Soil pH.

3.4 Discussion

This study demonstrated that bean root rot fungal pathogens were present in all the sixty farms surveyed in the four agro-ecological zones of Western Kenya. More than one root rot pathogen occurred in each farm at different population levels and frequencies and the highest populations occurring in the soil were of *Fusarium spp.* followed by *Pythium spp* and *Rhizoctonia spp* in that order. *Macrophomina spp* was also isolated from the farms though it was not widely spread. This confirms the importance of these root rot fungi in Western Kenya. Otsyula *et al.,* (1998) had earlier reported the importance of *Fusarium solani; Pythium spp* and *Rhizoctonia solani* as the main causal agent of root rot of common beans in Western Kenya. Other root rot fungi such as *Sclerotinia sclerotiorum* were not found to occur along with *Fusarium, Pythium, Rhizoctonia* and *Macrophomina* spp in Western Kenya.

Variations in populations of root rot pathogens occurred in all the AEZs. Upper midland humid (UM3) and LM2 had the highest number while UM1 had the lowest. These AEZs are characterized by mean temperatures of 18.8-20.6^{o}C and rainfall of 550-650mm during long rains and 450-580mm during the short rains. The lower midland sub humid (LM2) which recorded the highest populations of root rot fungi has mean temperatures of 21.4-22.3^{o}C and rainfall of 600-650mm during the long rains and 460-480mm during the short rains (Jaetzold *et al*., 2005). These characteristics result in moderate soil moisture in the farms which influence pathogen populations. The findings are similar to earlier findings by Mwang'ombe *et al.,* (2007) on root rot pathogens of common bean in Embu. They observed that higher fungal pathogen populations occur in areas with moderate soil moisture content which encourages bean root rot establishment. Naseri, (2014) also reported *Fusarium* spp to be a major root rot pathogen at moderate soil moistures, hot weather, acidic and poorly fertilized soil conditions. *Fusarium* spp had the highest isolation frequency in all the AEZ's. In the humid zones (LM1 and UM1), *Pythium* spp was the second highest in frequency of isolation whereas in the lower sub humid (LM2) and upper semi humid (UM3) zones, *Rhizoctonia* spp was second highest followed by *Pythium* spp. Naseri, (2015) reported high frequency of isolation for *Fusarium* spp in soils with high levels of root rot disease of common beans. The findings are similar to those of Okoth *et al.,* (2009) and Sun *et al.,* (2012) who reported that soil moisture and carbon promote growth and populations of *Trichoderma.*

Different soil types were found to have an effect on the soil pathogen populations. *Fusarium, Pythium, Rhizoctonia* and *Macrophomina* spp populations were highest in loamy fine sand

followed by sandy clay soil. The findings concur with Naseri, (2014) who observed high levels of *F. solani* in soils with high sand content. Other findings by Gill *et al.,* (2000) and Bliar, (1943) have also shown the rapid growth of *R. solani* in nutrient deficient sandy soils.

Other soil inhabiting fungi such as species of *Aspergillus, Penicillium* and *Trichoderma* were also isolated in the four AEZ's. *Aspergillus* spp was the highest followed by *Penicillium spp* while *Trichoderma spp* was the least isolated. Lower midland humid (LM1) had the highest populations of the beneficial microorganisms while LM2 had the lowest populations.

Molecular techniques employed in identification of root rot fungi isolated from different AEZ's in western Kenya confirmed the presence of six fungal species of importance in root rot disease development. These were *Fusarium solani, Fusarium oxysporum, Pythium ultimum, Pythium irregulare, Rhizoctonia solani* and *Macrophomina phaseolina.* The same were also positively identified by conventional methods where morphology and cultural characteristics were used.

Molecular quantification of root rot fungi in Western Kenya was observed to reflect similar findings as the conventional quantification methods used. This is in relation to the distribution of each fungus across the agro-ecological zones. The quantity of *F. solani* and *P. ultimum* were highest in LM2 and UM3 respectively while *R. solani* was highest in UM3. Similar findings were recorded for the conventional methods of quantification. However, the two techniques greatly varied in relation to hierarchical quantification of different pathogens in the same AEZ's. The quantity of *Fusarium solani* genomic DNA from soil was the lowest of four root rot fungal pathogens occurring in Western Kenya. The concentrations ranged from 2.51 x 10^{-5} ng/ µL to 16.4 x 10^{-5} ng/ µL of soil DNA. *Rhizoctonia solani* on the other hand had the highest quantity of the genomic DNA from the soils at 113,390 x 10^{-5} ng/ µL which was the highest of the four pathogens. Genomic DNA for *M. phaseolina* was second highest ranging from 5830 x 10^{-5} ng/ µL to 18,540 x 10^{-5} ng/ µL. *Pythium ultimum* was also detected at low concentrations of 1.0 x 10^{-5} ng/ µL to 16,225 x 10^{-5} ng/ µL which were higher than those of *F. solani* in two AEZ's of LM2 and UM3. Lievens *et al.,* (2006) observed that, it was difficult to accurately distinguish target pathogens from non-target pathogens in naturally infested soils using the plating techniques on semi-selective media. They however found that, there was a high correlation between calculated DNA and pathogen density of *F. solani* and *R. solani* in artificially infested soils. This demonstrates how the molecular techniques can accurately quantify occurrence of pathogens in complex samples. Other findings by Fillion *et al.,* (2003) were not able to correlate colony forming units of *F. solani* with qPCR quantification data.

They however demonstrated a consistent expression of *F. solani* DNA to symptom expression in plants which showed that any detection in soil may lead to disease in weakened or stressed plants.

Studies by Lievens *et al.,* (2006) also showed that *R. solani* complex is pathogenic to different hosts largely based on the anastomosis groups (AG). Different AGs of the fungus are usually detected in mixed soil samples. Upon detection in soil, pathogenic capacity of the isolates needs to be tested since not all the AGs of *R. solani* cause disease to all plants. Lievens *et al.,* (2006) also made similar observations for *Pythium* species which are virtually present in all cultivated soils and can be detected easily using the DNA quantification.

The low detection of *F. solani* using molecular quantification techniques as compared to cultural techniques in this study can be attributed to the fact that the method was specific to *F. solani* only and was unable to detect the other *Fusarium* species. At the same time high concentrations of *R. solani* and *M. phaseolina* can be attributed to their presence in the soil in form of mycelium over longer periods. This makes it possible for the pathogens' DNA to be extracted in higher quantities leading to higher quantification. Time of sample collection may also have an impact on the molecular quantification of the pathogens. *Pythium* and *Fusarium* do not thrive in dry soil and form resting spores which may yield lower DNA than their vegetative state. These findings do not however reduce the importance of *Fusarium solani* and *Pythium ultimum* in root rot diseases of common bean but rather emphasizes that even if their genomic DNA is found to be low, they may still cause serious infections, greatly reducing bean yields. This was also observed by Fillion *et al.,* (2003) when working with root rot of beans, who found a consistent statistical trend between expression of symptoms in plants and soil genomic concentration of the *F. solani.* Lievens *et al.,* (2006) while working with wilt of tomato also found that *P. ultimum* was the major cause of root rot disease where it was quantified using molecular techniques.

In this study, root rot fungal populations were observed to be influenced by soil type, AEZ's, and ecological factors in the soil microcosm. Positive and significant ($p<0.05$) correlation was observed between sand, *P. ultimum* DNA and *R. solani* DNA. Correlation between sand and *M. phaseolina* DNA quantity was however observed to be significantly ($p<0.001$) negative. These results confirm previous findings by Gill *et al.,* (2000) who observed that *R. solani* grew more rapidly in well-aerated soil than in moist soil with limited aeration. Blair in 1943 also observed that *R. solani* was more aggressive in nutrient deficient sand. There was also a

significant ($p<0.05$) negative correlation between clay content and populations of *Fusarium spp* in this study. Similar observation was made between clay and *R. solani* DNA. The findings concur with earlier experiments by Naseri, (2014) who observed high levels of *F. solani* in soils having high silt and sand content.

Positive significant ($p<0.05$) correlation in the populations of *Pythium* spp, *Fusarium* spp, and *Rhizoctonia* spp were observed in the study sites. From this study it shows that the pathogens operate synergistically to enhance root rot in the soils. This concurs with observations by Paparu *et al.,* (2017) who reported similar findings in Western Uganda. Abawi and Pastor Corrales (1990) also reported a synergistic interaction between *Fusarium solani* f.sp *phaseoli* and *Pythium ultimum* resulting in higher damage to plants than when each pathogen acts alone.

CHAPTER FOUR

EFFECT OF BIOCHAR AND VERMICOMPOST AS SOIL AMENDMENTS ON ROOT ROT OF COMMON BEAN (*Phaseolous vulgaris* L.)

Abstract

Production of common bean has continued to be constrained by a complex of root rot resulting to losses of up to 70% in Kenya. The aim of this study was to establish the effect of soil amendments biochar and vermicompost on root rot fungal pathogens of common bean in Western Kenya. They also aimed at establishing the residual effect of the amendments on the pathogens. Farmer fields were identified in four agro ecological zones of Western Kenya and treatments of biochar, vermicompost and fertiliser were applied by micro dosing in the furrows prior to planting during the long rains of 2013 and long rains of 2014. No treatment applications were done in the shot rains seasons of 2013 and 2014. Plant emergence and disease incidence was recorded in the field and disease severity determined in the laboratory. Isolation and identification of pathogens was done from treatment plots across all the four agro ecological zones following a 2 weeks and six weeks sampling after planting. Pathogens isolated were identified using morphological characteristics. Soil amendments positively influenced plant emergence. Root rot disease incidence and severity was greatly reduced up to 40% and 60% every season respectively. Biochar and vermicompost treatments reduced the population of fungal pathogens and also influenced the populations of beneficial microorganisms such as *Trichoderma* and *Paecilomyces lilacinus.* Yields were increased by 46% following application of organic amendments. Soil pH and nutrients were also increased by the organic amendments. In conclusion treatment application of vermicompost and biochar reduce root rot disease and improve bean product.

Key words: Root rot, *Fusarium solani, Pythium ultimum, Rhizoctonia solani*, soil amendments, biochar, vermicompost

4.1 Introduction

Common bean production in Kenya is faced by various constraints such as insect pests, reduced soil fertility, environmental stress and diseases which are major constraints. These constraints have led to low production averaging 220-670 kg/ha (Buruchara *et al.,* 2015). Alongside other diseases, root rot is a major constraint to bean production in the tropics. It has been previously

reported to cause total crop failure in western Kenya (Nzungize *et al.,* 2012; Otsyula *et al.,* 2003). Root rots are caused by a complex of soil-borne fungal pathogens including *Pythium ultimum*, *Fusarium solani* f.sp. *phaseoli*, *Macrophomina phaseolina*, and *Rhizoctonia spp* (Nzungize *et al.,* 2012; Mwang'ombe *et al.,* 2008). The root rot fungi persist saprophytically in the soil and on organic matter when there is no host or as resting spores making it difficult to manage the disease complex (Agrios, 2005; Waller and Brayford, 1990).

Options available for managing root rot complex of beans are limited and their effectiveness is often low after planting (Abawi and Pastor-corrales, 1990). Broad range and highly specific fumigants are available to effectively manage root rots. Their use is however limited due to high costs and toxicity to man and environment when not handled well (United Nations 2008; Abawi *et al.,* 2006**;** Nolling 1991). At the same time, efficacy of the available seed dressing chemicals in the market is not sustainable. This emanates from the development of resistance resulting from the multiple genera of pathogens involved in most production locations and their degradation after continued use (Abawi and Pastor Corrales, 1990; Nolling, 1991). Other limitations to conventional methods of managing root rot pathogens include development of resistance by plant pathogens and lack of tolerant or resistant bean varieties to multiple disease causing pathogens (Nzungize *et al.,* 2012).

Agronomic practices such as application of organic amendments have shown positive changes in root disease dynamics and yield increase (Bailey and Lazarovits, 2003). Different types of composts and biochar are recognized to increase soil health and to suppress various soil-borne diseases due to pathogens belonging to diverse genera such as *Fusarium, Pythium, Rhizoctonia* and *Phytophthora* (Mehta *et al.,* 2014; Sohi *et al.,* 2010; Elad *et al.,* 2010). Biochar is the solid co-product of pyrolysis or the thermal degradation of biomass in the absence of oxygen while vermicompost is a humic substance produced through an accelerated composting process by the feeding of earthworms. These amendments are used as soil amendments in management of root rot pathogens. The suppressiveness of vermicompost and biochar may be ascribed to a useful microbial community, an improvement in plant growth and vigour, increased nutrient availability improved nutrition, systemic resistance induction or fungistatic capabilities of the vermicompost and biochar modifications (Bonanomi *et al.,* 2017; Graber *et al.,* 2014). A synergistic effect of biochar and vermicompost has been reported to improve soil fertility, plant growth and beneficial microbial activity in the rhizosphere (Agegnehu *et al.,* 2015; Fischer and Glaser, 2012). However, in some studies different biochar types have been shown to adversely affect crop yield, soil properties and beneficial soil micro biota (Mukherjee and Lal, 2014).

Moreover, it is not known whether the biochar effect will be protective in field situations over a number of seasons since there is no information at all on longevity of these effects for soil borne pathogens (Graber *et al.,* 2014). This study therefore aimed at determining the effect of sugarcane bagasse biochar and vermicompost on root rot diseases of common bean and its residual effects over a period of four growing seasons.

4.2 Materials and methods

4.2.1 Production of soil amendments biochar and vermicompost

Plant residues from sugarcane bagasse were sourced from Kibos Sugar Factory in Kisumu Kenya and sun dried. The bagasse was pyrolised to produce biochar (Laird, 2008; Lehmann, 2007) using a metallic production kiln with a perforation at the base to allow for air flow and a chimney to expel the burning gases. The resultant biochar was weighed and packed into 6 kilograms in gunny bags before application. The biochar was analysed for chemical properties at Crop Nutrition Laboratories Nairobi, Kenya.

Vermicompost was produced at Dudutech, Naivasha, Kenya from vegetable crop residue. The plant debris were chopped, air dried for 7-10 days then placed into 30 centimeter deep rectangular troughs which had an initial population of 6000 earth worms (*Eisinia andrei*) in 40 kilograms of pre decomposed crop material and soil mixture. The crop residue was spread evenly on the surface of the trough where it was decomposed by earth worms feeding on the plant debris for a period of 6 weeks. The resultant worm casting referred to as vermicompost was then analysed for nutrition and chemical content at Dudutech Naivasha, Kenya. The vermicompost was packed in gunny bags and stored before application into the fields.

4.2.2 Study site, experimental design, treatment application and planting

The study was an on farm multi locational trial carried out on sixty farms spread out in three regions of Western Kenya (North Teso, Bungoma and Kakamega) that covered four different agro ecological zones. The zones included Lower midland humid (LM1), Lower midland sub humid (LM2), Upper midland humid (UM1) and Upper midland semi humid (UM3) with an altitude range of 800m to 1900m above sea level (ASL) and temperatures of 18° to 24° C (Jaetzold *et al.,* 2005). All these regions receive a bimodal rainfall consisting of long rains from March to July and short rains from September to November allowing bi annual cropping seasons. The regions have varying soil types which include acrisols, gleysols, regosols,

cambisols, nitisols, vertisols and ferralsols (Ralph *et al.,* 2005). The sixty farms were selected from a sampling frame of 280 small holder bean growers in the three counties of western Kenya that had previously undertaken common bean cultivation the previous season under a technology transfer project. The sample size was calculated following Nassiuma, (2000) formula as described in section 3.2.2 in chapter 3.

Each farmer field measuring 12.5 m by 21.5 m was subdivided into 8 treatment plots each of 6 m by 5 m. A susceptible bean variety to root rot (Rosecoco or GLP2) from CIAT Maseno was used in the trial. Treatments applied were biochar, vermicompost and sympal (NPK 0:23:15) fertiliser (MEA); biochar and vermicompost; biochar and sympal; vermicompost and sympal; biochar, vermicompost together with Sympal and a control where no amendment was applied. Biochar and vermicompost were each applied at a rate of 2000 kgs ha^{-1} and Sympal® fertiliser - N.P.K 0: 23:15 (MEA) was applied at a rate of 300 kg ha^{-1} at planting. Treatments were only applied in the two long rain seasons of 2013 and 2014 prior to planting. Planting in the short rain seasons of 2013 and 2014 were undertaken without application of treatments but maintaining the same plots to assess the residual effect of the treatments on bean root rot. The amendments were applied as a micro dose in the planting furrows then mixed with the soil prior to planting the bean seeds which were then covered with about 2 cm of soil. The bean seed was planted at the rate of 40 kg ha^{-1} at a spacing of 60 cm x 15 cm giving a plant population of 330 plants per treatment plot. The experiment was carried out in a completely randomized design.

4.2.3 Assessment for root rot disease incidence and severity

Root rot disease incidence was recorded as percentage of diseased plants showing root rot symptoms per plot at two and six weeks after seedling emergence. The assessment was undertaken at 2 weeks so as to observe both pre-emergence and post emergence damping off. At six weeks early signs of root were also assessed. Bean plants infected with root rot were identified based on symptoms such as damping off, yellowing of leaves, stunted growth, wilting, brown discolouration on roots and dark brown to red coloured lesions on roots.

Five plants (both symptomatic and asymptomatic) were sampled from each plot at the end of the 2nd and 6th week after emergence and used to determine the disease severity of root rot in each plot. Scoring of disease severity was by visual assessment of necrotic lesions on roots and hypocotyls based on a rating scale of 1-9 as described by Abawi and Pastor-Corrales, (1990). The rating used was 1 = no observable symptoms, 3 = light discoloration without necrotic lesions or 10% of hypocotyl and root tissues covered with lesions, 5 = hypocotyls and root

tissues covered with lesions up to 25% but tissues remain firm, 7 = considerable softening, rotting, and reduction of the root system accompanied by lesions covering approximately 50% of the hypocotyls, and root tissues, 9 = advanced stages of rotting approximately with 75% or more of the root tissues and hypocotyl affected, as well as extensive deterioration of the root system. These scores were then converted to percentage severity index (Assefa *et al.,* 2014).

$$\text{Percent Severity Index} = \frac{Sum\ of\ numerical\ ratings\ X\ 100}{No.\ of\ plants\ scored\ X\ Maximun\ score\ on\ scale}$$

4.2.4 Isolation of root rot fungal pathogens from infected bean roots and rhizosphere soil

Five root tissues from each treatment per farmer field were cleaned of surface soil and other contaminants by washing under running water. Roots were then cut into pieces measuring 1 cm, placed in 1% sodium hypochlorite in 10% ethanol for a period of 3 min to achieve surface sterilisation. It was followed by rinsing thrice in sterile water then blot drying on sterile serviettes. The roots were then plated on PDA amended with 50ppm streptomycin and incubated for 7-14 days at room temperature ranging between 25 ºC and 28ºC.

Rhizosphere soil samples were collected 2 weeks and 6 weeks after emergence and at harvest to determine the fungal flora for each treatment plot following treatment application. Sampling was done at 10 points in each plot in a /\/\/ shape at a spacing of 1.5m between the sampling points. A composite soil sample weighing one kilogram was then taken from the 10 samples, placed in well labeled polythene bag and brought to the laboratory at the University of Nairobi and stored at 4ºC prior to isolation of root rot pathogens.

Three sub samples each weighing 1g were taken from each 1 kilogram of composite soil samples, dissolved in 10ml sterile distilled water in three different universal bottles, mixed by shaking for 1 minute followed by a 10-fold serial dilution series for each sample to achieve a 10^{-4} dilution. One milliliter of 10^{-4} dilution was plated on potato dextrose agar (PDA-HIMEDIA®) medium using pour plate method. The PDA had been amended with 50ppm streptomycin sulphate antibiotic to suppress bacterial growth. Each dilution was replicated three times and incubated at room temperature for 7 days. Different fungal colonies were counted and quantified per gram of soil.

The fungi were then sub cultured on fresh PDA medium and upon identification, different genera of fungi were sub-cultured on different media. *Fusarium spp.* was sub-cultured on

Spezieller Nährstoffarmer agar (SNA) (Nirenberg, 1981) and PDA media. Sporulation of cultures on SNA was achieved by incubation under UV light while those on PDA were incubated under normal 12 hour photo period. All cultures were incubated at 25°C for 14- 21 days to study cultural characteristics of each fungus for their final identification. Based on morphological characteristics, identification of *Fusarium* isolates was done to species level following keys by Nelson *et al.,* (1983) and the *Fusarium* laboratory manual (Leslie and Summerell, 2006). Identification of other fungi was based on morphological and cultural features such as colour of the colony, growth type, colour of mycelia and spore types (Zhou *et al.,* 2010). The colony forming units of each fungal type per gram of soil was also calculated by multiplying the number of colonies with the dilution factor. *Pythium sp.* were sub cultured on corn meal agar to observe the production of sporangia, oogonia and antheridia that were used in identification based on keys by Plaats-Niterink (1981) and Dick (1990).

Relative isolation frequency was calculated for each genus using the formula by Gonzalez *et al.,* (1999). All the fungal isolates were preserved on PDA slants at 4 °C at the University of Nairobi for further identification by gene sequencing.

$$\text{Frequency } (\%) = \frac{\text{number of isolates of a genus}}{\text{total number of all isolates}} \text{ x } 100$$

At the end of the fourth season, soil samples were also analysed using quantitative PCR to establish the pathogen load in comparison with the conventional isolation method.

4.2.5 Effect of biochar and vermicompost on yield of common bean

Harvesting was done from plants in the net plot measuring 22.56 M^2. The crop stand count for each plot was recorded before harvesting. Total fresh weight of pods and hauls at harvest was recorded in the field. Samples were randomly selected from each net plot and the pods per plant counted, separated and weighed. These were later dried at 65°C for 48 h at CIAT Maseno and the weights used to estimate yield parameters such as 100 seed weight per plot and total seed yield per plot and later extrapolated to kg/ha.

4.2.6 Effect of biochar and vermicompost on soil pH and chemical composition

Soil samples collected from field plots at harvest were also used to determine the pH and chemical composition for each treatment plot. The procedure previously described in section

4.2.4 of this chapter with reference to soil sampling was used. From each plot, one kilogram of soil was collected and soil analysis carried out at Crop Nutrition Laboratories Nairobi Kenya to determine the pH and chemical composition following the procedure previously described in Chapter 3 sub-sections 3.2.5.2 and 3.2.5.3.

4.2.7 Data collection and analysis

Data on emergence was recorded fourteen days after planting where the total number of plants that had emerged was counted per treatment plot and expressed as percentages. Disease incidence was determined by counting the number of diseased plants in the net plot. This was then divided by the total number of plants in the net plot multiplied by 100. Data on disease severity was determined after scoring of diseased roots on a scale of 1 to 9 for root rot symptoms. Beans were harvested at physiological maturity from the net plots. Dry grains from each net plot were weighed after drying at 65° C for 24 hours. Data on fungal counts was collected following isolation from the plant and rhizosphere soil samples at 2nd, 6th week and harvest, while other data such as soil particle size percentages, soil pH and soil nutrient content were recorded following laboratory analysis. These data was subjected to analysis of variance (ANOVA) by GENSTAT version 14 and the Tukey test Least Significant difference (LSD) was used for mean separation at 5% level of significance.

4.3 Results

4.3.1 Physical and chemical characteristics of biochar and vermicompost

The two soil amendments analysed varied in their composition. Vermicompost had higher moisture content than biochar. No volatile compounds or ash were found in vermicompost that were present in biochar from sugarcane bagasse (Table 4.1). pH of the two amendments was found to be near neutral with that of vermicompost being higher than sugarcane bagasse (SB) biochar. Electrical conductivity, dry matter content and C:N ratio were higher in SB biochar as compared to vermicompost.

4.3.2 Nutrient composition of biochar and vermicompost

Phosphorus was the highest nutrient in the biochar as compared to other elements. Sugarcane bagasse biochar had higher level of phosphorus than that of vermicompost while Potassium was more in vermicompost than in biochar (Table 4.2). No calcium was found in biochar but vermicompost had 2.5%. Nutrients such as Magnesium, Sulphur, Manganese, Iron and Boron were higher in vermicompost while Sodium, Zinc and Copper were highest in SB biochar.

Table 4.1: Characteristics of vermicompost and biochar

Amendment	MC %	Volatiles (%)	Ash (%)	pH	EC (mS/cm)	DM %	C %	N %	C:N %
Vermicompost	48.2	NIL	NIL	6.92	12	50.8	30.1	3.54	8.51
S. B. biochar	3.10	9.10	9.66	6.83	73.5	96.90	62.87	5.31	11.85

MC- Moisture Content, EC- electrical conductivity, DM- Dry matter, C- Carbon, N- Nitrogen, C:N- Carbon Nitrogen ratio; S. B. biochar Sugarcane bagasse biochar.

Table 4.2: Nutrient analysis of biochar and vermicompost

Amendment	P %	K %	Ca %	Mg %	S %	Mn (ppm)	Fe (ppm)	B (ppm)	Na (ppm)	Zn (ppm)	Cu (ppm)
Vermicompost	0.64	3.31	2.54	0.54	0.4	410.0	6600.0	101.0	1480.0	185.0	17.8
S. B. biochar	1.01	0.73	n/a	0.37	0.03	36.9	485.3	14.4	2668.3	570.2	38.2

P-Phosphorus, K- Potassium, Ca- calcium, Mg- Magnesium, S- Sulphur, Mn- Manganese, Fe- Iron, B- Boron, Na- Sodium, Zn- Zinc, Cu- Copper; S. B. biochar- Sugarcane bagasse biochar; ppm- parts per million; N/A- not available/present

4.3.3 Effect of soil amendments on plant emergence

Significant differences in plant emergence were recorded among treatments in all the four seasons. Interaction between treatments and agro ecological zones resulted to significant differences ($p<0.05$) in LM1 and UM1. The highest emergence was recorded in treatment combination of biochar, vermicompost and fertiliser in LM1 during the long rain season while the lowest was recorded in vermicompost and fertiliser treatments in UM1 (Table 4.3). In the short rain season of 2013, significant differences ($p<0.05$) were recorded for interaction in three AEZ's. Of the three AEZ's, the highest emergence was recorded in vermicompost treated plots in UM1 while the lowest was recorded in the vermicompost and fertiliser treated plots in UM3. Significant differences ($p<0.05$) were also recorded for collective treatments. Treatment combination of biochar, vermicompost and fertiliser had the highest emergence in the long rains of 2013 while the lowest was recorded in non-amended control plots. In the short rains season of 2013, vermicompost treated plots had the highest emergence while the vermicompost and fertiliser treated plots had the lowest emergence, the differences being significant ($p<0.05$).

Significant differences ($p<0.05$) in plant emergence were also observed for treatments and their interactions with AEZ's during the long and short rain season of 2014 (Table 4.4). The highest emergence was recorded in biochar treated plots in LM1 while control and fertiliser treated plots in UM3 had the lowest plant emergence in the long rains of 2014. In the short rains of 2014, highest plant emergence was recorded in biochar and fertiliser treated plots at UM1 while the lowest was recorded in fertilised control plots at LM2. Significant difference ($p<0.05$) in plant emergence was observed for the treatments across the AEZ's both in the 2014 long and short rains season. The highest plant emergence was recorded in vermicompost treated plots in the two seasons. However, the lowest plant emergence was observed in control plots amended with fertiliser in the long rains of 2014 and in plots with a combination of biochar, vermicompost and fertiliser in the short rains of 2014.

Table 4.3: Effect of different treatments on plant emergence (%) in different AEZ's of western Kenya during the long rains and short rains seasons of 2013

Treatments	**Long Rains Season 2013**					**Short Rains Season 2013**				
AEZ	LM1	LM2	UM1	UM3	Means Trt	LM1	LM2	UM1	UM3	Trt Means
Control	38.3c	60.3a	24.2c	60.8a	45.9c	84.1a	73.5a	81.5ab	71.1bc	77.5ab
Fertiliser	46.5bc	60.4a	25.8bc	62.8a	48.8b	88.9a	66.4b	71.4d	67.4bc	73.5c
Biochar	47.8bc	59.7a	34.7ab	66.3a	52.1b	85.7a	70.8ab	73.5cd	72.6ab	75.7bc
Biochar + Fertiliser	40.3bc	63.1a	23.4c	64.0a	47.7bc	85.4a	68.8ab	80.1abc	63.9c	74.6bc
Biochar + Vermicompost	49.9b	62.6a	25.0bc	67.1a	51.2ab	83.5a	70.4ab	82.7ab	69.6bc	76.5b
Biochar + Vermicompost + Fertiliser	63.6a	60.5a	44.1a	62.7a	57.7a	81.5a	69.2ab	77.1bcd	61.9c	72.4c
Vermicompost	46.8bc	58.1a	21.7c	63.3a	47.4bc	81.4a	74.6a	87.3a	79.7a	80.8a
Vermicompost + Fertiliser	40.0bc	63.2a	20.9c	61.9a	46.5c	82.0a	65.0b	78.8bcd	62.2c	72.0c
LSD Interaction Treatment x AEZ	10.3					7.5				
LSD Treatments					5.2					3.7
%CV	40.9					19.5				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.4: Effect of different treatments on plant emergence (%) of common bean in different AEZ's of western Kenya during the long and short rains seasons of 2014

Treatments	Long Rains Season 2014					Short Rains Season 2014				
AEZ	LM1	LM2	UM1	UM3	Trt Means	LM1	LM2	UM1	UM3	Trt Means
Control	90.1a	77.2a	79.6b	78.4a	81.3ab	79.5c	69.1cd	85.9ab	74.6bc	77.3c
Fertiliser	87.0a	68.2b	81.9b	65.4d	75.6d	84.3abc	67.0d	85.5ab	74.1bc	77.7c
Biochar	92.2a	72.9ab	83.7b	77.6ab	81.6ab	88.3a	74.4bc	83.0b	78.3ab	81.0ab
Biochar + Fertiliser	86.0a	77.1a	84.7ab	69.7cd	79.4bc	87.3ab	74.4bc	89.5a	72.8bcd	81.0ab
Vermicompost	87.3a	77.1a	90.1a	76.9ab	82.7a	84.8abc	83.0a	84.0ab	82.6a	83.6a
Vermicompost + Fertiliser	90.6a	67.3b	84.7ab	69.0cd	77.9cd	81.7bc	75.5b	89.1a	67.5d	78.5bc
Biochar + Vermicompost	89.0a	78.3a	86.1a	72.0bc	81.6ab	83.7abc	73.6bc	81.5b	71.4cd	77.6c
Biochar + Vermicompost + Fertiliser	89.1a	72.6ab	85.5ab	69.3cd	79.1bc	80.7c	70.8bcd	83.9ab	68.9cd	76.1c
LSD Interaction Treatment x AEZ	6.2					5.7				
LSD Treatments					3.1					2.8
%CV	15.2					14.1				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

4.3.4 Effect of soil amendments on incidence of root rot in western Kenya

Root rot disease incidence was observed to significantly vary ($p<0.05$) with treatments and interactions between treatments and AEZ's two weeks after planting (Table 4.5). During the short rains season of 2013, the highest incidence of disease among the treatments was recorded in vermicompost amended plots. The lowest incidence was however recorded in biochar and fertiliser treatment combinations as well as in vermicompost and fertiliser treatment combinations though the differences were not significant. The same trend was observed in the short rains season of 2014 with the differences also not being significant. During the long rains of 2014, significant differences ($p<0.05$) were observed among treatments. Plots with treatment combinations of biochar and vermicompost resulted in a 40% reduction in disease incidence when compared to the disease incidence in the control plots. Interaction between the treatments and AEZ's resulted to significant differences ($p<0.05$) in LM2, UM1 and UM3 in three seasons. In the short rains season of 2013, the highest incidence was recorded in vermicompost treated plots in LM2 while the lowest was recorded in biochar and fertiliser treated plots in UMI. The same trend was observed in the short rains season of 2014 though control plots in UM3 had the highest incidence of disease. During the long rains of 2014, the highest disease incidence was recorded in control plots of LM2.

There were significant differences ($p<0.05$) in root rot disease incidence among treatments six weeks after planting beans during the long rains and short rains season of 2013 (Table 4.6) and 2014 (Table 4.7) respectively. The highest disease incidence was recorded in control plots and lowest in vermicompost as well as vermicompost and fertiliser amended plots in all the four seasons. Significant differences ($p<0.05$) were also observed in interaction between treatments and AEZ's. In the long rains of 2013, the highest disease incidence was recorded in control plots of UM1 while the lowest was recorded in biochar and vermicompost amended plots in UM3 and plots treated with vermicompost and fertiliser combination in LM2 (Table 4.6). In the short rains of 2013, control plots in LM1 recorded the highest incidence while vermicompost treated plots in LM1 recorded the lowest disease incidence. However in the long rains of 2014, biochar amended plots in LM1 recorded the highest disease incidence while biochar and vermicompost amended plots had the lowest disease incidence (Table 4.7). Control plots in LM2 recorded the highest incidence of disease during the short rains of 2014 while biochar treated plots in LM1 recorded the lowest disease incidence.

Table 4.5: Effect of different treatments on incidence (%) of bean root rot at two weeks of plant growth in different AEZ's of Western Kenya

Treatments	Short rains season 2013					Long rains season 2014					Short rains season 2014				
	LM1	LM2	UM1	UM3	Trt Mean	LM1	LM2	UM1	UM3	Trt Mean	LM1	LM2	UM1	UM3	Trt Mean
Control	0.6a	1.0b	1.7ab	3.1a	1.6ab	0.6a	2.7a	1.1ab	2.1a	1.6a	0.7a	1.5b	1.2ab	3.9a	1.8ab
Fertiliser	0.3a	1.3b	1.1ab	2.0a	1.2ab	0.4a	2.3ab	1.3a	2.3a	1.6a	0.6a	1.7b	1.2ab	2.3b	1.4ab
Biochar	1.0a	1.4b	2.0a	2.0a	1.6ab	1.0a	2.0b	0.7ab	1.3b	1.2bc	1.2a	2.0ab	2.5a	2.5ab	2.1a
Biochar + Fertiliser	0.6a	1.1b	0.4b	1.5b	0.9b	0.4a	1.8bc	0.7ab	2.2a	1.3ab	0.8a	1.3b	0.6b	2.1b	1.2b
Vermicompost	1.1a	3.5a	0.8ab	1.5b	1.7a	0.4a	1.5c	0.7ab	1.4b	1.0bc	1.4a	3.3a	1.6ab	2.1b	2.1a
Vermicompost + Fertiliser	0.4a	1.2b	0.6b	1.2b	0.9b	0.7a	2.0b	1.1ab	0.9b	1.2bc	0.5a	1.8b	0.9b	1.9b	1.3b
Biochar + Vermicompost	1.3a	0.5b	1.7ab	2.3ab	1.4ab	0.6a	1.3c	0.5b	1.1b	0.9c	1.9a	1.0b	1.9ab	2.9ab	1.9ab
Biochar + Vermicompost + Fertiliser	1.5a	0.7b	0.7ab	1.8ab	1.2ab	0.4a	1.8bc	1.2a	1.3b	1.2bc	1.9a	1.3b	1.0b	2.5ab	1.7ab
LSD Inter Trt x AEZ	1.3					0.6					1.4				
LSD Treatments					0.7					0.3					0.7
%CV	195.3					98.7					160.3				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.6: Effect of different treatments on incidence (%) of bean root rot at six weeks after plant emergence in different AEZ's of Western Kenya in the long rains and short rains season of 2013

Treatments	**Week 6 Long rains season 2013**					**Week 6 Short rains season 2013**				
	LM1	**LM2**	**UM1**	**UM3**	**Means of Treatments**	**LM1**	**LM2**	**UM1**	**UM3**	**Means of Treatments**
Control	5.7a	5.0b	7.0a	5.1a	3.5a	10.8a	9.3a	8.2a	9.7a	9.5a
Fertiliser	5.4a	6.8a	6.4ab	2.8b	3.4a	9.1b	8.6a	6.1b	7.4b	7.8b
Biochar	2.5b	1.7c	3.8cd	1.9bc	1.4bc	5.7c	5.9bc	3.4d	5.0c	5.0c
Biochar + Fertiliser	1.8b	1.3c	3.0de	1.2c	1.0bc	4.3de	6.2bc	3.7cd	4.4c	4.7c
Vermicompost	2.7b	1.2c	5.3bc	1.6bc	0.8bc	3.0de	6.7b	3.4d	5.0c	4.5c
Vermicompost +Fertiliser	2.0b	1.1c	3.4d	1.7bc	0.8bc	4.5cd	5.7bc	4.8bc	4.3c	4.8c
Biochar + Vermicompost	2.6b	1.5c	5.1bc	1.1c	1.8b	4.9cd	5.8bc	3.4d	5.2c	4.8c
Biochar + Vermicompost + Fertiliser	1.8b	1.7c	1.7e	2.1bc	1.5bc	5.1cd	5.3c	3.4d	5.4c	4.8c
LSD Inter Treatment x AEZ	1.5					1.3				
LSD Treatments					0.8					0.6
%CV	171.3					42.9				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4. 7: Effect of different treatments on incidence (%) of bean root rot at six weeks of plant growth in different AEZ's of Western Kenya in the long rains and short rains season of 2014

Treatments	**Week 6 Long rains season 2014**					**Week 6 Short rains season 2014**				
	LM1	**LM2**	**UM1**	**UM3**	**Means Trt**	**LM1**	**LM2**	**UM1**	**UM3**	**Means Trt**
Control	3.5b	1.0b	1.0a	0.9a	1.6a	3.4a	4.6b	2.5a	6.8a	4.3a
Fertiliser	1.0c	3.3a	0.7a	1.0a	1.5ab	2.4abc	6.3a	2.1a	6.1a	4.2a
Biochar	5.0a	1.2b	0.5a	0.6a	1.8a	1.1c	4.4bc	1.8a	2.8b	2.5b
Biochar+Fertiliser	1.0c	0.9b	0.5a	1.1a	0.9bc	1.4bc	3.5bcd	2.0a	3.0b	2.5b
Vermicompost	1.1c	1.0b	0.4a	0.6a	0.8c	2.2abc	2.2d	1.7a	2.8b	2.2b
Vermicompost+Fertiliser	0.3c	0.6b	0.3a	1.0a	0.6c	1.3bc	3.7bc	1.4a	3.9b	2.6b
Biochar+Vermicompost	0.3c	1.3b	0.3a	0.5a	0.6c	2.1abc	3.4bcd	2.3a	3.1b	2.7b
Biochar+Vermicompost+Fertiliser	0.7c	1.2b	0.7a	0.8a	0.9c	2.5ab	3.1cd	1.5a	3.7b	2.7b
LSD Inter Trt x AEZ	1.1					1.3				
LSD Treatments					0.6					0.6
%CV	202.4					85.9				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

4.3.5 Effect of soil amendments on bean root rot severity in western Kenya

Addition of soil amendments had an effect on the root rot disease severity at two weeks, six weeks and at harvest. Significant differences ($p<0.05$) were observed in percent severity index (PSI) among the treatments and their interaction with AEZ's two weeks after planting in three rain seasons (Table 4.8). In the short rain season of 2013, the highest PSI among treatments was recorded in control plots and the lowest was recorded in vermicompost treated plots. The same was observed among treatments during the long rains season of 2014 and short rains of 2014 with the lowest PSI recorded in plots amended with a combination of biochar and vermicompost. Treatment interaction with AEZ's had the highest PSI recorded in control plots of UM3 while amendments with biochar and vermicompost resulted in 30% reduction in severity in the short rains season of 2014. During the long rains of 2014 and the short rain season of 2014, PSI was significantly reduced ($p<0.05$) in plots amended with biochar and vermicompost or their combinations. In LR of 2014, disease severity was reduced by 39% to 46% while in the SR of 2014 it was reduced by only 20% to 29%. Control plots had the highest PSI in the second week after planting in all three seasons.

Significant differences ($p<0.05$) were observed in disease severity 6th week of plant growth among treatments and in their interaction with AEZ's during the long rains and short rains season of 2013 (Table 4.9). The highest PSI were recorded in the control plots while the biochar treatment resulted in a 52% and 31% reduction in disease severity during the long and short rains seasons respectively. Interaction between treatments and AEZ's had the highest PSI recorded in control plots in the two seasons. Plots amended with biochar resulted in a reduction of the PSI of 48% to 67% in LR 2013. In the SR of 2103 biochar amended plots had a reduced PSI of between 23% and 43% (Table 4.9).

During the long rains season of 2014, significant differences ($p<0.05$) were observed among treatments and their interaction with AEZ's (Table 4.10). However in the short rains season of 2014, significant differences ($p<0.05$) were observed in the interaction between treatments and AEZ's but non among the treatments alone. Percent severity was highest in the control plots across the AEZ's and treatments. Treatment amendments of biochar, vermicompost and their combinations resulted in PSI reductions of between 40% and 54% in the LR of 2014 and 23% and 30% in the SR of 2014 across the AEZ's (Table 4.10).

Table 4.8: Effect of different treatment on bean root rot severity (%) two weeks after planting in the long and short rains seasons of 2013 and 2014 in the four AEZs

Treatments	Short rains season 2013					Long rains season 2014					Short rains season 2014				
	LM1	LM2	UM1	UM3	Trt	LM1	LM2	UM1	UM3	Trt	LM1	LM2	UM1	UM3	Trt
Control	47.6a	45.5a	52.1a	53.5a	49.7a	47.3a	58.9a	51.9b	53.7a	52.9a	53.3a	55.5a	49.3b	54.9a	53.2a
Fertiliser	42.7ab	35.0bcd	39.8bc	44.2b	40.4b	44.8a	42.2b	58.0a	48.3b	48.3b	47.4b	50.0b	58.0a	45.7b	50.3b
Biochar	45.7a	42.8a	36.8cde	39.6bc	41.2b	36.5bc	31.7d	32.5c	33.8c	33.6c	46.8b	41.5c	42.4c	41.4bc	43.0c
Biochar + Fertiliser	32.9c	31.7d	42.7b	40.4bc	36.9c	32.1cd	37.2c	34.3c	35.0c	34.6c	47.8b	42.9c	40.8c	42.8bc	43.6c
Vermicompost	33.4c	39.5b	33.9d	37.2c	36.0c	27.0d	35.6cd	36.0c	33.1c	32.9c	39.3c	50.0b	43.4c	46.0b	44.7c
Vermicompost + Fertiliser	38.3bc	36.1bcd	36.8cde	38.2c	37.4c	37.1b	34.5cd	32.5c	31.0c	33.8c	39.7c	43.2c	43.3c	43.4bc	42.4c
Biochar + Vermicompost	34.8c	32.3cd	39.3bcd	39.5bc	36.5c	32.1cd	35.0cd	31.6c	31.7c	32.6c	40.5c	45.3bc	42.7c	40.4c	42.2c
Biochar + Vermicompost + Fertiliser	37.3bc	37.8bc	31.9e	40.0bc	36.7c	33.3bcd	33.9cd	31.6c	34.7c	33.4c	39.9c	41.7c	44.9bc	43.0bc	42.4c
LSD Inter Trt x AEZ	5.5					4.8					5.2				
LSD Treatments					2.7					2.4					2.6
%CV	27.5					25.2					22.5				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.9: Effect of different treatments on bean root rot severity (%) six weeks after planting in the long and the short rain seasons of 2013 in the four AEZs

Treatments	**Long rains season 2013**					**Short rains season 2013**				
	LM1	**LM2**	**UM1**	**UM3**	**Means of Treatments**	**LM1**	**LM2**	**UM1**	**UM3**	**Means of Treatments**
Control	52.6a	64.4a	56.8a	52.9a	56.7a	50.5a	51.6a	49.4a	53.2a	51.2a
Fertiliser	55.6a	51.1b	49.5b	52.2a	52.1b	53.2a	41.7b	38.3bc	45.2b	44.6b
Biochar	26.7d	34.5c	18.7f	27.3c	26.8e	35.0bc	39.4b	31.7c	34.8c	35.3c
Biochar + Fertiliser	32.6c	33.3cd	34.8cd	27.6c	32.1d	36.1bc	42.2b	32.8bc	33.5c	36.2c
Vermicompost	28.9d	33.3cd	33.4de	33.1b	32.2d	30.1c	45.0ab	40.0b	38.0bc	38.3c
Vermicompost + Fertiliser	32.6c	32.2cd	30.4e	34.4b	32.4d	38.3b	43.9b	32.8bc	34.0c	37.3c
Biochar + Vermicompost	36.3b	31.1d	37.8c	32.2b	34.4c	34.5bc	39.4b	33.4bc	35.9c	35.8c
Biochar + Vermicompost + Fertiliser	36.3b	25.6e	30.4e	31.8b	31.0d	35.6bc	45.0ab	36.7bc	34.8c	38.0c
LSD Inter Trt x AEZ	3.2					7.2				
LSD Treatments					1.6					3.6
%CV	16.8					38.5				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM3-upper midland zone 3, Trt-Treatment. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.10: Effect of different treatments on bean root rot severity (%) six weeks after planting in the long and the short rain seasons of 2014 in the four AEZs

Treatments	**Long rains season 2014**					**Short rains season 2014**				
	LM1	**LM2**	**UM1**	**UM3**	**Means of Treatments**	**LM1**	**LM2**	**UM1**	**UM3**	**Means of Treatments**
Control	60.6a	48.9a	53.6a	51.4a	53.6a	45.2a	51.0a	48.3a	50.6a	48.8a
Fertiliser	45.4b	51.7a	46.6b	47.6a	47.8b	43.0ab	49.6a	43.1b	49.0a	46.2a
Biochar	30.8de	25.6d	36.9c	33.8bc	31.8cd	34.9cd	35.6c	37.8cd	38.7b	36.8c
Biochar + Fertiliser	31.4de	32.8c	26.3e	31.2bc	30.5cd	34.9cd	38.5bc	42.2bc	38.7b	38.6bc
Vermicompost	34.6cd	35.6bc	29.0de	34.0bc	33.3c	31.9d	43.0b	40.5bcd	38.5b	38.5bc
Vermicompost + Fertiliser	27.6e	35.6bc	36.0c	35.4b	33.7c	40.7ab	37.8c	38.7bcd	41.3b	39.6b
Biochar + Vermicompost	29.5e	38.3b	29.9de	32.9bc	32.7cd	37.1bc	39.3bc	38.7bcd	39.4b	38.6bc
Biochar + Vermicompost + Fertiliser	39.1c	31.1c	33.4cd	30.5c	33.5c	32.7cd	37.1c	36.1d	40.4b	36.5c
LSD Inter Trt x AEZ	4.5					5.1				
LSD Treatments					2.2					2.6
%CV	23.6					24.9				

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. AEZ-Agro-ecological zones, LM1- lower midland zone 1, LM2- lower midland zone 2, UM1- Upper midland zone 1, UM 3-upper midland zone 3. Trt-Treatment, LSD: Least significant difference at 5% level, CV: Coefficient of variation

4.3.6 Effect of soil amendments on populations of root rot fungal pathogens two weeks after planting common bean in 2013

Soil amendments had a significant effect ($p<0.05$) on the population of fungi isolated from the soils two weeks after planting of common bean in the short rain season of 2013 (Table 4.11). *Fusarium* spp was the most abundant fungi isolated across all treatments while the lowest populations isolated were those of *Macrophomina* spp. Significant differences ($p<0.05$) were observed in the populations of *Fusarium* spp with different treatments. Control plots had the highest populations while plots amended with vermicompost and fertiliser resulted in a 38% reduction. Biochar and vermicompost treatments also resulted in a 30% reduction in the populations of *Pythium* and *Rhizoctonia* spp when compared to control plots. Biochar and fertiliser treatments were observed to result in a 60% and 30% increase in populations of *Trichoderma* and *Aspergillus* spp respectively when compared to control. The highest populations of *Penicillium* spp were found in plots treated with a combination of biochar, vermicompost and fertiliser which was 64% higher than the control which had the lowest populations.

4.3.7 Effect of soil amendments on population of root rot fungal pathogens six weeks after planting common bean in 2013

Significant differences were observed in the population of fungi isolated from the soil rhizosphere; six weeks after planting during the long rains season of 2013 (Table 4.12). *Fusarium* spp populations were found highest across all treatments while *Macrophomina* spp was the least isolated. The highest population of *Fusarium* spp was recorded in control plots whereas biochar and vermicompost amendments caused a 50% reduction in the populations of *Fusarium* spp. Biochar treatments resulted in a 54% and 49% reduction in the populations of *Rhizoctonia* and *Pythium* spp respectively. Control plots also had the highest populations of these fungi. Biochar and vermicompost treatments resulted in the highest populations of beneficial fungi including *Trichoderma* spp and *Aspergillus* spp whereas plots treated with vermicompost alone had the highest populations of *Penicillium* spp. The lowest populations of *Trichoderma* spp and *Aspergillus* spp were recorded in fertiliser treated plots, with significant differences ($p<0.05$) when compared to control. The same trend was observed during the short rains season of 2013 six weeks after planting (Table 4.13). There was, however, no significant difference in the populations of *Macrophomina, Trichoderma* and *Penicillium* spp during the short rains of 2013.

Table 4.11: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) two weeks after planting common bean in the short rains season of 2013

Treatments	Fungal colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Trichoderma spp*	*Aspergillus spp*	*Penicillium spp*
Control	134.1a	46.3a	35.3a	33.0a	2.9ab	3.5bc	8.1bcd	6.7bc
Fertiliser	133.8a	40.6b	37.3a	31.0a	3.7a	1.3c	8.8abcd	11.3bc
Biochar + Fertiliser	116.0b	29.3c	29.7b	23.7b	1.2b	8.9a	11.9a	14.2ab
Vermicompost	114.6b	30.3c	27.7b	24.0b	1.9ab	4.4b	9.0abcd	16.4ab
Biochar + Vermicompost + Fertiliser	111.3b	30.6c	26.4b	23.6b	2.2ab	3.1bc	6.1d	18.7a
Biochar	110.1b	31.5c	26.3b	23.1b	2.0ab	4.6b	6.5cd	14.8ab
Vermicompost + Fertiliser	109.9b	28.7c	26.2b	24.7b	2.3ab	4.2b	9.4abc	11.9bc
Biochar + Vermicompost	108.5b	30.7c	25.7b	22.3b	3.3ab	4.4b	11.2ab	11.3bc
LSD	11.3	3.7	4.5	4.2	2.4	2.2	3.2	5.9
%CV	39.1	44.2	59.8	63.0	434.1	204.1	143.3	175.7
Fpr	<0.001	<0.001	<0.001	<0.001	0.329	0.016	0.004	0.004

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

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Table 4.12: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) six weeks after planting common bean in the long rains season of 2013

Treatments	Fungal colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Trichoderma spp*	*Aspergillus spp*	*Penicillium spp*
Control	154.2a	49.9a	31.8a	38.0a	1.9abc	0.8c	26.1b	5.6de
Control + Fertiliser	152.8a	44.3a	35.6a	35.9a	3.1a	0.5c	26.3b	3.7e
Biochar + Vermicompost + Fertiliser	115.0a	24.4b	17.7c	20.8bc	2.1ab	2.9b	47.8a	6.7cde
Biochar + Fertiliser	114.8a	24.4b	18.3bc	21.4bc	0.5bc	1.7bc	30.0b	8.0bcd
Verm + Fertiliser	114.5a	28.9b	22.8b	24.0b	0.8bc	2.6b	27.4b	10.8b
Biochar	114.2a	24.8b	16.1c	19.1c	0.3c	1.4bc	26.9b	6.3de
Biochar + Vermicompost	108.3a	24.1b	20.3bc	21.1bc	0.9bc	3.1b	24.6b	9.7bc
Vermicompost	105.7a	24.9b	17.8c	20.5bc	0.2c	11.3a	18.1b	15.7a
LSD	NS	9.9	4.5	4.0	1.7	1.8	11.7	3.0
%CV	50.7	63.7	77.2	62.4	421.0	227.4	162.5	143.4
Fpr	0.07	0.001	<0.001	0.001	<0.001	<.001	<.001	<.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp, NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.13: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) six weeks after planting common bean in the short rains season of 2013.

Treatments	Fungal colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Trichoderma spp*	*Aspergillus spp*	*Penicillium spp*
Control	171.2a	54.2a	33.2a	44.7a	4.2a	5.9a	13.1a	1.0d
Control +Fertiliser	150.3b	47.9b	29.8ab	32.0b	4.5a	9.5a	14.8a	8.1abc
Biochar	136.5c	36.9c	21.3d	25.7c	1.8a	9.1a	16.6a	11.2a
Biochar + Fertiliser	130.6cd	35.6c	24.1cd	24.5c	2.3a	6.9a	16.2a	9.6ab
Biochar + Vermicompost + Fertiliser	128.8cd	33.2c	23.7cd	23.9c	4.2a	6.5a	13.1a	11.4a
Biochar + Vermicompost	126.3cd	35.6c	24.7cd	23.7c	3.3a	6.5a	13.5a	6.5bc
Vermicompost + Fertiliser	123.5d	32.6c	26.8bc	23.6c	3.1a	5.0a	16.6a	5.0cd
Vermicompost	122.3d	35.9c	22.7cd	25.0c	2.0a	7.5a	7.8b	11.4a
LSD	12.5	4.9	4.3	3.9	2.8	4.0	4.6	4.4
%CV	36.3	49.8	65.4	55.9	325.8	222.9	129.4	188.4
Fpr	<0.001	0.057	<0.001	<0.001	0.519	0.326	0.003	0.024

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

4.3.8 Effect of soil amendments on population of root rot fungal pathogens at harvest of common bean during the long rains of 2013

Soil amendments were observed to have an effect on root rot pathogens and other soil inhabiting fungi at the time of bean harvest after the long rains season of 2013 (Table 4.14). *Fusarium* spp were highly prevalent among all the fungi across all treatments while *Macrophomina* spp was the least of all fungi. Significant differences ($p \leq 0.05$) were found in population of all fungi across the treatments except for *Aspergillus* spp where no significant differences were recorded. Treatment combinations of biochar, vermicompost and fertiliser resulted in the reduction of *Fusarium* spp population by 67% when compared to control. Vermicompost and fertiliser combination reduced *Fusarium* by 63%. The population of *Pythium* spp was significantly lower in biochar and fertiliser treatment translating to a 60% population reduction. Populations of *Rhizoctonia* were lowest in biochar and vermicompost treatment combination while the highest populations were recorded in the non-amended control plots. Vermicompost and fertiliser treatment combination at the same time resulted in elevated population of *Trichoderma* spp which were lowest in biochar treatment. Vermicompost standalone treatments resulted in significantly ($p<0.05$) high populations of *Penicillium* spp which were lowest in the control plots (Table 4.14).

4.3.9 Effect of soil amendments on population of root rot fungal pathogens two weeks after planting of common bean in the long rain season of 2014

Soil amendments were observed to have a significant effect ($p<0.05$) on the population of bean root rot two weeks after planting in 2014 (Table 4.15). *Fusarium* spp were most abundant across all treatments while the lowest populations were of *Macrophomina* spp. Populations of *Fusarium* spp were significantly different ($p<0.05$) across the six treatments. The highest populations were found in the control plots while soils amended with vermicompost had a 59% reduction in populations (Table 4.15). Vermicompost treatment resulted in a 52% reduction of *Pythium* spp populations. Combination of vermicompost and fertiliser reduced *R. solani* populations by 48%. Biochar treatments were observed to reduce all root rot pathogens by 40% margin. The control plots recorded the highest populations of all root rot pathogens. Consequently, the populations of *Penicillium*, *Aspergillus, Paecilomyces, Athrobotrys* and *Trichoderma* spp were highest in vermicompost treatments in the range of 60% to 90%. Biochar resulted in an increase of between 50% and 80% of these fungi. Similar observations were made in the short rains season of 2014, though the effect of the treatments was observed to have reduced by a margin of 20% (Table 4.16).

Table 4.14: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) at harvest of common bean in the long rains season of 2013

Treatments	Fungal colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Trichoderma spp*	*Aspergillus spp*	*Penicillium spp*
Control	161.3a	63.6a	15.1b	42.8a	3.6a	10.0ab	19.5a	11.4c
Fertiliser	120.5b	44.3b	19.6a	36.5b	3.0a	11.7ab	15.7abc	5.8d
Biochar	86.9cd	25.5c	8.5c	21.1c	1.0bc	2.5c	13.7bc	13.1bc
Biochar + Fertiliser	75.7d	23.5c	5.9d	20.6cd	0.4bc	9.1abc	18.3ab	10.4cd
Vermicompost	117.1b	27.1c	7.1d	20.2cd	0.02c	13.5a	18.4ab	20.8a
Vermicompost + Fertiliser	100.2bc	23.7c	10.7c	21.8c	0.01c	13.9a	13.1c	13.4bc
Biochar + Vermicompost	101.7bc	25.4c	9.1cd	16.8d	1.1b	11.3ab	18.9a	14.6bc
Biochar +Vermicompost + Fertiliser	98.8bcd	20.6c	7.4d	19.6cd	0.2bc	5.6bc	20.5a	17.8ab
LSD	24.3	8.2	3.2	4.2	1	6.9	4.9	5.5
%CV	43.9	54.1	106.1	64.7	319.6	278.6	111.5	161.8
Fpr	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	0.018	<.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.15: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) two weeks after planting common bean in the long rains season of 2014

Treatments	**Fungal Colonies**	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paecilomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	140.2a	52.9a	38.4	31.7a	4.5a	0.8d	3.6d	3.8bc	0.3c	4.1e
Fertiliser	132.5a	45.9b	37.9	34.6a	3.3ab	1.6d	2.4d	1.3d	0.3c	5.3de
Biochar	95.3bc	31.1c	18.5	19.4b	0.5d	5.6bc	9.5ab	1.9cd	0.4c	8.4ab
Biochar + Fertiliser	97.7bc	26.7de	22.1	18.9b	1.0cd	7.2ab	7.6bc	4.4ab	2.3b	7.7bc
Vermicompost	104.8b	21.4f	18.3	18.9b	1.7bcd	10.0a	11.9a	6.7a	5.7a	10.3a
Vermicompost + Fert	91.0c	22.5f	19.3	16.4b	2.1bcd	9.5a	5.1cd	4.9ab	5.6a	5.7c
Biochar +Verm	95.2bc	27.2d	18.5	19.7b	3.3ab	3.8cd	7.6bc	5.6ab	3.0b	6.4bcd
Biochar + Verm + Fert	90.2c	23.3ef	19.1	17.7b	2.3bc	6.2bc	6.7bc	5.6ab	4.7a	4.6d
LSD	10.2	3.7	NS	3.8	1.7	3.2	2.8	2.4	1.4	2.0
%CV	37.8	48.6	64.3	67.9	261.6	180.7	145.3	239.3	200.2	146.4
Fpr	<0.001	<0.001	0.074	<0.001	0.05	0.05	<0.001	<0.001	<0.001	<0.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp. Fert: Fertiliser, Verm: Vermicompost, NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.16: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) two weeks after planting of common bean during short rains season of 2014

Treatments	Fungal Colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paeciliomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	139.1a	52.0a	36.7a	28.9ab	1.3ab	4.7bc	5.4bc	4.9cde	0.01d	5.1d
Fertiliser	130.5ab	46.7b	39.0a	32.3a	3.3a	1.9c	2.8c	2.1e	0.4cd	1.9e
Biochar	129.9ab	39.2c	31.1b	24.6bc	1.8ab	7.4ab	6.6b	3.0de	1.7bc	14.6a
Biochar + Fertiliser	122.4bc	38.2c	29.3bc	25.4bc	0.8b	4.8bc	5.5bc	6.3bc	2.9b	9.2b
Vermicompost	130.5ab	35.2cd	28.8bc	25.8bc	1.3ab	9.2a	11.8a	5.6cd	4.4a	8.5bc
Vermicompost + Fert	115.7c	32.1d	25.2c	23.9c	1.8ab	8.9ab	6.8b	11.2a	0.01d	5.7d
Biochar + Verm	121.7bc	37.3c	27.3bc	25.8bc	1.0ab	7.7ab	8.0b	6.2bc	2.1b	6.4cd
Biochar + Verm + Fert	122.7bc	34.8cd	29.0bc	24.6bc	0.7b	6.9ab	7.3b	9.2ab	0.4cd	9.8b
LSD	11.7	4.7	4.1	4.6	2.3	4.3	3.5	3.1	1.4	2.6
%CV	37.1	49.0	53.9	67.6	467.4	202.3	185.6	214.3	367.1	153.9
Fpr	<0.001	<0.001	0.002	<0.001	<0.001	≤0.05	<0.001	0.004	0.001	<0.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp, Fert: Fertiliser, Verm: Vermicompost, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

4.3.10 Effect of soil amendments on populations of root rot fungal pathogens six weeks after planting common bean during the long rain season of 2014

Significant differences were observed in the population of root rot fungi isolated from the soils of treated plots six weeks after planting in the long rains season of 2014 (Table 4.17). *Fusarium* spp was the most prevalent of all the fungi across all treatments while *Macrophomina* spp was the least. Vermicompost treatment and the combinations of biochar and fertiliser were observed to cause a 40 to 50% reduction in the populations of *Fusarium* spp when compared to control. Biochar and fertiliser amendments also resulted in a 32% reduction of *Pythium* populations and a 42% reduction of *Rhizoctonia* populations. Control plots had the highest populations of all the root rot fungi. Vermicompost treated plots were observed to have the highest population of *Penicillium* spp representing a 55% difference from the control plots which had the lowest populations. *Paecilomyces* spp*, Trichoderma* spp and *Aspergillus* spp were positively affected by biochar treatments. *Athrobotrys* spp population was highest in plots treated with a combination of biochar, vermicompost and fertiliser whereas the control plots had the lowest population.

Similar trends in reduction of root rot populations were observed in the short rains season of 2014 but at lower percentages (Table 4.18). Significant differences ($p<0.05$) were observed for all root rot fungi. Vermicompost treatments resulted in a reduction of between 32% and 37% for *Fusarium, Pythium* and *Rhizoctonia spp* while control plots recorded the highest population of the root rot fungi. Treatment combination of biochar, vermicompost and fertiliser resulted in 50% and 89% increase in the populations of *Paeciliomyces* spp and *Athrobotrys spp.* Biochar and fertiliser on the other hand resulted in a 54% increase in the populations of *Aspergillus spp* with the control plots recording the lowest populations of *Aspergillus* and *Athrobotrys* spp.

Table 4.17: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) six weeks after planting common bean in the long rains season of 2014

Treatments	**Fungal Colonies**	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paeciliomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	158.2a	50.4a	29.7a	32.0a	6.3abc	6.8c	15.3c	7.8a	1.8c	8.1ab
Fertiliser	152.3a	46.2a	30.3a	27.6a	8.2a	8.4bc	17.1bc	8.0a	2.6c	3.8c
Biochar	133.9b	28.5b	20.4b	19.8b	3.8c	8.6bc	22.5b	10.5a	9.5a	10.2a
Biochar + Fertiliser	132.7bc	27.6b	20.2b	18.4b	6.7abc	10.7bc	29.5a	8.6a	4.1bc	6.8b
Vermicompost	132.9bc	29.4b	21.5b	17.7b	5.2bc	19.7a	19.2bc	10.2a	4.1bc	5.9bc
Vermicompost + Fertiliser	121.9c	25.6b	22.9b	20.3b	4.2c	11.6b	19.2bc	9.0a	2.5c	6.6b
Biochar + Vermicompost	126.4bc	30.4b	20.9b	19.3b	7.4ab	10.9bc	16.9bc	10.9a	5.8b	3.8c
Biochar + Vermicompost + Fertiliser	129.9bc	29.3b	20.3b	21.7b	4.1c	9.5bc	18.1bc	9.1a	11.5a	6.3b
LSD	11.8	5.2	4.3	4.6	2.9	4.2	6.0	4.5	2.8	2.3
%CV	34.7	62.2	71.4	80.9	190.9	163.1	123.8	191.1	238.4	156.5
Fpr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.07	0.009	<0.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. Nonpathogenic fungi – *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp. LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.18: The residual effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) six weeks after planting common bean in the short rains season of 2014

Treatments	Fungal Colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paeciliomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	160.9a	50.0a	33.9a	36.6a	5.2a	9.7b	15.5b	5.1bc	0.5c	5.6ab
Fertiliser	155.4ab	44.8b	30.7a	33.7a	5.9a	5.6c	19.7b	8.5ab	2.7abc	3.6bc
Biochar	138.7cde	37.7c	26.1b	27.4b	4.7a	7.4bc	19.9b	9.6a	2.1bc	3.8bc
Biochar + Fertiliser	146.8bc	36.1c	26.2b	27.7b	6.2a	7.0bc	32.9a	4.7bc	2.8abc	3.3c
Vermicompost	134.5de	33.3c	25.8b	22.8c	6.9a	13.6a	19.2b	5.0bc	3.7ab	4.1bc
Vermicompost + Fertiliser	127.7e	34.3c	22.9b	27.6b	5.1a	8.1bc	18.5b	3.9c	2.0bc	5.2bc
Biochar + Vermicompost	143.1cd	35.0c	26.5b	28.5b	4.7a	9.1bc	17.8b	9.9a	4.3ab	7.4a
Biochar + Vermicompost + Fertiliser	137.5cde	33.8c	25.0b	29.0b	3.6a	10.3ab	17.0b	10.2a	4.8a	3.9bc
LSD	11.3	4.9	3.7	4.2	NS	3.8	6.6	4.2	2.3	2.1
%CV	31	51.1	54.3	58.2	213.8	164.4	126.9	230.2	334.9	161.3
Fpr	<0.001	<0.001	<0.001	<0.001	0.10	<0.001	0.01	0.016	0.003	0.002

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. LSD: Least significant difference at 5% level, CV: Coefficient of variation, NS- No significant difference.

4.3.11 Effect of soil amendments on population of root rot fungal pathogens at harvest of common bean during the long rain season of 2014

During the harvest period of long rains season of 2014, soil amendments were observed to have an effect on root rot pathogens and other soil inhabiting fungi (Table 4.19). *Fusarium* spp. was most isolated of all the fungi in all treatments while *Macrophomina* spp was the least isolated. Significant differences ($p \leq 0.05$) were observed in population of all fungi across the treatments. Treatment combination of biochar, vermicompost and fertiliser resulted in the reduction of *Fusarium* spp population by 39% and the highest populations being recorded in control plots. The population of *Pythium* spp was significantly lower ($p<0.05$) in biochar and fertiliser treatment translating to a 40% reduction in population. *Rhizoctonia* was also observed to be lowest in biochar and fertiliser treatment combinations while the highest populations were recorded in the control plots. Biochar and fertiliser treatment combination at the same time resulted in elevated population of *Penicillium* spp, *Aspergillus* spp and *Trichoderma* spp. The population of these three genera was observed to be lowest in the control plots. Similar trends were observed for root rot pathogen as well as other soil inhabiting fungi in the short rains season of 2014 though the percentage reduction in populations was 10 percent lower than in the long rains season (Table 4.20).

Table 4.19: Effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) at harvest of common bean in the long rains season of 2014

Treatments	**Fungal Colonies**	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paecilomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	159.5ab	50.6a	25.3a	34.1b	7.5b	8.9b	17.5	9.0ab	1.7e	4.8c
Fertiliser	164.8a	47.8a	27.5a	39.1a	11.0a	8.9b	17.5	6.7b	2.1e	4.3c
Biochar	146.7c	31.0b	16.5b	21.7cd	4.7c	14.2a	25.8	8.8b	13.2a	10.8ab
Biochar + Fertiliser	147.7bc	31.2b	17.7b	19.5d	4.7c	16.0a	30.4	9.5ab	6.7c	12.1a
Vermicompost	132.2d	30.7b	18.5b	21.2cd	4.9bc	13.6ab	21.0	7.7b	5.7cd	9.0b
Vermicompost + Fertiliser	139.9cd	32.5b	17.5b	25.0c	5.3bc	12.3ab	26.5	8.7b	3.4de	8.6b
Biochar + Vermicompost	136.1cd	31.2b	17.4b	20.0d	7.5b	12.5ab	18.3	12.8a	7.0bc	9.4ab
Biochar + Vermicompost + Fertiliser	139.4cd	28.4b	18.4b	22.9cd	4.0c	13.1ab	23.6	8.2b	9.6b	11.2ab
LSD	12.4	5.2	3.2	4.6	2.7	4.7	NS	3.8	2.8	2.7
%CV	34.1	58.3	65.6	71.3	171.6	152.0	107.1	173.6	193.9	124.9
Fpr	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.065	<0.001	<0.001	<0.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 4.20: The residual effect of biochar and vermicompost on fungal populations (x 10^3 CFU/g soil) at harvest of common bean in the short rains season of 2014

Treatments	Fungal Colonies	*Fusarium spp*	*Pythium spp*	*Rhizoctonia spp*	*Macrophomina spp*	*Penicillium spp*	*Aspergillus spp*	*Paeciliomyces spp*	*Athrobotrys spp*	*Trichoderma spp*
Control	155.5a	49.8a	27.8a	38.0a	4.2a	6.9cd	16.7b	5.1bcd	0.9c	5.5bc
Fertiliser	141.5b	43.9b	25.0abc	29.7b	4.5a	6.0d	18.9b	8.5a	2.8bc	3.6c
Biochar	137.4b	37.8c	19.9c	25.8bcd	6.5a	5.8d	20.3b	8.7a	5.8a	7.5ab
Biochar + Fertiliser	137.5b	37.1c	21.9bc	22.6d	6.6a	9.0bcd	30.4a	3.3cd	1.7bc	4.5c
Vermicompost	134.0b	36.4c	22.2bc	22.7d	5.3a	15.7a	18.8b	5.8abcd	3.1b	3.8c
Vermicompost + Fertiliser	131.9b	36.1c	25.3ab	23.1cd	5.9a	12.1ab	18.1b	2.7d	2.3bc	7.7ab
Biochar + Vermicompost	141.0b	35.1c	23.2bc	24.7cd	6.3a	10.4bc	19.7b	8.0ab	2.5bc	9.0a
Biochar + Vermicompost + Fertiliser	134.8b	34.6c	22.8bc	26.8bc	4.6a	8.0bcd	18.3b	6.6abc	3.6b	9.2a
LSD	11.2	4.9	3.4	4.0	NS	4.1	6.4	3.3	1.9	2.7
%CV	31.9	50.4	57.3	59.1	208.3	178.9	127.2	188,5	272.9	133.8
Fpr	<0.001	<0.001	<0.001	<0.001	0.09	<0.001	0.006	0.018	<.001	0.001

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

4.3.12 Effect of biochar and vermicompost on yield and 100 seed weight of common bean

Bean grain yield was significantly affected ($p \leq 0.05$) by the treatments in all the seasons except the short rains season of 2014 where the differences were not significant (Table 4.21). The long rains season of 2013 recorded the highest average yield across all treatments. The yields were observed to be 17% higher than the long rains season of 2014 which ranked second. There was however a significant drop of 45% in yield from the long rains season of 2013 into the short rains season of the same year. This trend was reversed in the long rains season of 2014 recording a 30% to 50% increase in yield across all treatments. Vermicompost and fertiliser treatments had the highest grain yield in the long rains and short rains of 2013 as well as in the long rains of 2014. In the long rains of 2013, the yield was observed to be 81% higher in vermicompost and fertiliser treatment and 46% higher in biochar, vermicompost and fertiliser treatment plots. These were in comparison to the non-amended control plots. During the short rains of 2013, plots that were amended with solitary biochar treatments recorded the lowest grain yield as was the case during the long rains of 2013. There was no significant difference in bean yield in the short rains season of 2014 where the yields were greatly reduced. Treatment combinations of vermicompost and fertiliser still recorded the highest grain yield while biochar and vermicompost plots had the lowest yield.

Bean seed weight was affected by the soil amendment treatments in all the seasons with differences being significant ($p \leq 0.05$) in all the seasons (Table 4.21). Vermicompost and fertiliser amended treatment plots had the highest 100 seed weight in three seasons averaging 8% to 20% change in g/100 seeds. Biochar vermicompost and fertiliser amended treatment plots had the second highest seed quality which was 10% higher than the control plots in the long rains of 2013. In the subsequent short rain season of 2013, biochar treated plots recorded the lowest seed quality though it was observed to only be significantly different ($p < 0.05$) from the vermicompost and fertiliser treated plots from which the highest seed quality was recorded. In the short rains season of 2014, the highest seed quality was in biochar and fertiliser treatment combinations. This was 48% higher than in vermicompost amended treatment plots which had the lowest seed quality the differences being significant ($p \leq 0.05$).

Table 4.21: Effect of biochar and vermicompost on common bean yields (kg/Ha) and seed quality (weight per 100 seeds) across all the four seasons in 2013 and 2014

Treatment	Common bean grain yield (Kg/Ha)				Common bean seed quality (g/100 seeds)			
	Long rains 2013	Short rains 2013	Long rains 2014	Short rains 2014	Long rains 2013	Short rains 2013	Long rains 2014	Short rains 2014
Vermicompost+Fertiliser	565.2a	306.3a	481.1a	64.7a	33.3a	32.1a	37.3a	17.5b
Biochar +Vermicompost +Fertiliser	489.7ab	282.3ab	445.9ab	46.8a	32.1a	29.2a	36.3a	17.9b
Vermicompost	455.0abc	252.6ab	333.5bc	48.6a	31.9a	29.5a	36.1a	14.5c
Biochar +Fertiliser	433.8bcd	254.0ab	380.9abc	51.0a	32.7a	28.2a	36.5a	21.4a
Biochar +Vermicompost	413.3bcd	220.7b	456.5ab	41.1a	31.9a	29.4a	36.3a	16.4bc
Control + Fertiliser	377.7bcd	239abc	319.5c	57.2a	30.6a	28.5a	36.2a	16.8bc
Biochar	353.9cd	172.8c	259.1c	52.8a	30.4a	26.2a	36.1a	16.9bc
Control	311.7d	271.5ab	350.5bc	44.3a	29.7a	28.2a	34.8a	19.1ab
LSD	126.0	76.9	124.3	NS	NS	NS	NS	2.8
%CV	54.1	62.4	71.7	117	14.2	34.7	12.3	28.4
F.pr	0.004	0.037	0.005	0.88	0.065	0.524	0.532	0.002

Means with same letter(s) within the same column are not significantly different at $p \leq 0.05$. NS: No significant difference, LSD: Least significant difference at 5% level; CV: Coefficient of variation.

4.3.13 Effect of soil amendments on soil pH and chemical composition in the four agro-ecological zones

Soil pH was observed to be significantly different ($p<0.05$) only in LM2. The soil pH was observed to be highest in farms in LM2 and lowest in UM1 (Table 4.22). All plots amended with biochar had the highest pH while the control plots had the lowest pH in three AEZs. In upper midland humid zone, treatment combinations of biochar and vermicompost had the lowest pH though the differences were not significant. Electrical conductivity was recorded highest in biochar amended plots in LM2 and UM3 while vermicompost and control plots recorded the highest EC in LM1 and UM1 respectively though the differences were not significant. No significant differences were observed in the AEZ's except for LM1 where significant differences ($p<0.05$) were observed with vermicompost having the highest percent OM of 3.24%.

Significant differences in the elements NPK were observed to be affected by the interaction between the AEZ's and treatments (Table 4.22). Nitrogen was recorded highest in vermicompost treatment plots in LM1 while the lowest was recorded in UM1 in vermicompost amended plots. The highest percent P was recorded in biochar and vermicompost treatment combinations in UM3 while the lowest percent P was in LM1 at 6.3% in vermicompost amended plots. Potassium content was highest in vermicompost amended plots in LM2 while the lowest was recorded in control plots the difference being significant ($p<0.05$) in LM2. There was no significant differences ($p<0.05$) for zinc in three AEZs except for LM1 where combination of biochar and vermicompost had the highest concentration and control plots had the lowest concentration. Concentration of copper was significantly different ($p<0.05$) in LM1 and UM1 with vermicompost treatment plots recording highest concentration. Boron was significantly different ($p<0.05$) in LM1 and UM1. The highest concentration was in biochar and vermicompost in UM3 at 0.34ppm and the lowest in LM1at 0.09 ppm.

Table 4.22: Effect of different treatments on pH and selected chemical properties of soils from smallholder farms following two applications of biochar and vermicompost in Western Kenya

Treatment	Lower midland humid (LM1)									
	pH	OM	EC	CEC	N	P	K	Zn	CU	B
Biochar	5.08a	3.09b	79.20ab	7.40b	0.18b	10.60a	73.59a	4.03bc	3.43b	0.11b
Biochar +Verm	5.06a	3.07c	74.90ab	6.40c	0.19ab	8.60ab	70.66a	4.81a	3.63b	0.29a
Control	5.01a	3.18ab	72.00b	6.60c	0.18b	6.60b	61.49a	3.46c	3.67ab	0.09b
Vermicompost	5.05a	3.24a	83.10a	8.10a	0.20a	6.30b	75.51a	4.23ab	3.93a	0.15b
LSD	0.09	0.10	8.36	0.60	0.01	3.36	14.05	0.73	0.28	0.12
%CV	2.30	4.00	14.10	11.10	3.50	54.50	26.10	23.00	10.00	97.10
	Lower midland sub humid (LM2)									
Biochar	6.06a	2.40b	108.36a	10.31a	0.13a	19.12b	206.50ab	3.80b	3.18a	0.175a
Biochar +Verm	5.89ab	2.89a	73.64b	7.24b	0.14a	15.22b	158.90bc	4.96a	3.01a	0.159a
Control	5.60c	2.92a	71.36b	8.44ab	0.14a	24.62b	142.70c	4.56ab	3.39a	0.141a
Vermicompost	5.74bc	2.80ab	92.00b	8.69ab	0.14a	45.84a	234.20a	3.87b	3.17a	0.173a
LSD	0.20	0.43	32.74	2.31	0.01	10.93	52.08	1.07	0.47	0.05
%CV	4.50	20.50	49.60	34.90	13.80	54.50	36.70	32.70	19.50	39.40
	Upper midland humid (UM1)									
Biochar	5.05a	2.33a	57.71a	4.62c	0.134a	11.50b	62.78a	2.90a	4.48b	0.10ab
Biochar +Verm	4.92b	2.32a	60.71a	4.94bc	0.131ab	13.40ab	57.14a	3.10a	4.34b	0.12a
Control	4.96b	2.37a	66.07a	5.42b	0.134a	17.90a	60.23a	3.10a	4.45b	0.09b

Vermicompost	5.02b	2.34a	56.21a	6.03a	0.129b	10.60b	54.97a	3.70a	5.07a	0.12a
LSD	0.11	0.07	11.97	0.56	0.003	5.90	11.72	0.80	0.19	0.02
%CV	2.90	4.10	29.00	13.9.0	3.10	57.90	26.10	32.70	5.30	24.40
Upper midland semi humid (UM3)										
Biochar	5.97a	2.88ab	79.50a	7.96a	0.15ab	28.78b	159.60ab	5.09a	2.55a	0.21a
Biochar +Verm	5.95a	2.78b	75.20a	8.40a	0.14b	46.48a	168.80a	6.32a	2.52a	0.34a
Control	5.74a	2.88ab	70.60a	7.91a	0.15ab	40.21ab	159.10ab	4.92a	2.41a	0.21a
Vermicompost	5.81a	3.07a	68.80a	6.64a	0.16a	33.37ab	138.00b	5.12a	1.58a	0.17a
LSD	0.26	0.24	18.29	1.83	0.01	13.97	43.85	2.17	1.13	0.18
%CV	5.80	10.60	32.50	30.90	7.10	49.10	36.70	53.00	65.20	101.20

Means with different letter(s) within each column are significantly different at $p \leq 0.05$. C-control, Verm- vermicompost, OM –organic matter, N-nitrogen, P-phosphorus, K-potassium, Zn-zinc, Cu-copper, B-boron, EC-electric conductivity, CEC-cation exchange capacity, LSD: Least significant difference at 5% level, CV: Coefficient of variation

4.4 Discussion

4.4.1 Effect of soil amendments on plant emergence

Plant emergence was affected by the application of individual treatments of biochar and vermicompost as well as their combinations. Soil amendments positively influenced the plant emergence. Treatment combinations of biochar and vermicompost had the highest emergence immediately after application and the subsequent season when amendments were not applied. Ievinsh *et al.,* (2017) and Arancon *et al.,* (2012) also observed increased germination of hemp seeds and cucumber seeds treated with vermicompost. Solaiman *et al.,* (2011) also reported an increase in mung bean germination with biochar treatment. The results from this study also confirm the presence of positive residual effect of biochar and vermicompost on plant emergence in short rain seasons of 2013 and 2014 which has not been previously reported.

Plant emergence was also observed to be influenced by the AEZ's. Lower midland humid (LM1) and upper midland humid (UM1) were observed to have higher emergence in the long rains season of 2014 and the two short rain seasons. However, in the long rains season of 2013 UM3 and LM2 were observed to have significantly higher emergence. This can be attributed to the distribution of the rainfall at the time of planting. Upper midland zone 3 (Kakamega region) recorded highest precipitation at 712 mm in the three growing months and lowest in LM1 (N. Teso sub county) at 447 mm for the three months of growth (Appendix IV). Plant emergence is of great importance since the plant population would eventually affect the final yield.

4.4.2 Effect of soil amendments on root rot disease incidence in western Kenya

Different treatments of biochar and vermicompost and their interaction with AEZ's reduced bean root rot incidence. The findings also point to the influence of AEZ's on the effectiveness of soil amendments in suppressing root rot disease in common bean. Disease incidence was reduced by 60% in both the long rain seasons when the treatments were applied and 40% in the short rain seasons with no treatment application but with residual effect. Treatment combinations of biochar and vermicompost greatly reduced root rot incidence after application. These plots had the lowest disease incidence showing a synergy at play while those that received one amendment alone had a higher disease incidence which was however significantly ($p<0.05$) lower than the control plots. This finding corroborate previous findings by Chaoui *et al.,* (2002) and Edwards and Arancon (2004b) who reported on suppression of root rots in

strawberry using vermicompost. Jaswal *et al.,* (2013) also reported on root rot disease suppression in cucumber using biochar.

During the period of this research, rainfall amounts varied between 143mm and 712mm in the four different seasons in the months of March to July; September to November of 2013 and 2014. Disease incidence was lower in the long rains season after application of soil amendments. This was observed both at two weeks and six weeks after planting where the disease incidence was reduced by as much as 60% as compared to that in the control plots. In the long rains season, the highest incidence was in LM1 while UM3 recorded the lowest. This corresponds with previous studies by Mwang'ombe *et al.,* (2007) and Hall and Philips (1992) while working on bean root rots in Embu, Kenya and South Western Uganda respectively. They observed that elevated rainfall stimulated root infection. In turn this would lead to accumulation of inoculum to higher levels in the root tissues. The impact of the inoculum build up is then felt in the short rains season with elevated root rot incidences where no rotation is practiced. However in this study, findings show that amendments with biochar and vermicompost prevented development of inoculum resulting to reduced disease incidence. Similar findings have been reported by Warnock *et al.,* (2007) and Ameloot *et al.,* (2013) that biochar can be used as a source of energy or mineral nutrients which may induce changes in community composition.

In the subsequent season, disease incidence was observed to be higher in the plots where no inorganic fertiliser sympal® (N.P.K 0:23:15) had been applied. This implies the importance of the phosphorus in root development and in turn disease suppression. Similar findings were reported Yamato *et al.,* (2006) who stated that biochars antifungal potential was due to its important properties among them increased nutrient retention, increased soil cation exchange capacity and effects on Phosphorus. Da Silva Ceroz and Fitzsimmons, (2016) and Cichy *et al.,* (2007) observed that disease severity may reduce through new growth resulting from improved crop vigour as a result of phosphorus nutrition.

4.4.3 Effect of soil amendments on root rot disease severity in western Kenya

Root rot disease severity was greatly reduced by as much as 60% following application of biochar and vermicompost soil amendments across all seasons and growth stages. In the subsequent seasons when no amendments were applied, disease severity was reduced by 30%. Treatment combinations of biochar and vermicompost with addition of sympal® fertiliser had

the lowest disease severity than with amendments alone. Similar findings were reported by Matsubara *et al.,* (2002) who observed reduced *Fusarium* wilt disease in Asparagus following application of biochar. Jaiswal *et al.,* (2014) also reported reduction in damping off disease caused by *Rhizoctonia solani* in cucumber and beans following addition of 0.5% wt/wt of greenhouse waste biochar. Other findings by Jack (2012) also showed disease suppression in cucumber caused by *Pythium aphanidermatum* following application of vermicompost extract.

The control plots recorded the highest severity in all seasons across the AEZ's. This can be attributed to the continuous planting of beans with no rotation period. Disease severity did not however vary greatly across the agro-ecological zones though LM2 appeared to have the highest severity while the lowest severity was recorded in UM1. These levels of severity can also be linked to the rainfall received in different agro-ecological zones. Similar findings have been reported by Mwang'ombe *et al.,* (2007) working on bean root rots in Embu. They observed that increased rainfall leads to high soil moisture which favours root rot pathogens such as species of *Pythium* and *Rhizoctonia*.

4.4.4 Effect of soil amendments on fungal populations isolated from soils planted with common bean

Treatments with biochar, vermicompost and in combination were found to greatly impact soil fungal populations. Vermicompost treatment resulted in significant ($p<0.05$) reduction of *Pythium* spp populations across the agro-ecological zones. Vermicompost treatments also resulted in the highest reduction of *Fusarium* spp. populations at the second week of plant growth. With the progression of the cropping season, biochar treatments as well as in combination with vermicompost resulted in significant reduction of *Fusarium* spp and *Rhizoctonia* spp. These findings are similar to those of Jack, (2012) and Scheuerell *et al.,* (2005) who observed significant suppression of *P. aphanidermatum* and *P. ultimum* populations in soils treated with vermicompost in cucumber and beans respectively. Graber *et al.,* (2010) attributed the reduction of detrimental fungal populations to chemical compounds in the residual tars found on biochar. They identified several biochar compounds known to have detrimental effects on growth and survival of pathogenic microorganisms. In low levels, these compounds can suppress sensitive components of the soil microorganisms and result in a proliferation of resistant microbial communities that are beneficial to plant growth. This phenomenon was observed in biochar treatments which resulted to an increase in population

of beneficial microorganisms such as *Trichoderma* spp, *Paeciliomyces* spp and *Athrobotrys* spp. Similarly vermicompost treatments were also observed to result in an increase of *Penicillium spp* and *Aspergillus spp* after application and also as a residual effect when no amendments were applied.

4.4.5 Effect of biochar and vermicompost on yield and seed weight of common bean

Yields of common bean were significantly ($p \leq 0.05$) influenced by the treatments in all the seasons other than the short rains season of 2014 where the differences were not significant. Higher grain yield was recorded in plots amended with vermicompost and sympal® fertiliser treatments as well as in the biochar, vermicompost and fertiliser amended plots. The amendments resulted in yield increase of between 46% and 81%. Similar findings have also been reported in previous studies by Guerena *et al.,* (2015) and Lin *et al.,* (2015). They observed an increase in bean biomass and grain yield following the application of biochar and vermicompost. This study also showed an increase in yield when biochar was combined with fertiliser than in individual application of biochar or Sympal fertiliser. Similar results were reported earlier by Liang *et al.,* (2014) and Oram *et al.,* (2014) who reported improved yield following application of biochar and organic/inorganic fertilisers together. This was attributed to an increase in nutrient resource to plants. Liard *et al*. (2010) on the other hand demonstrated heightened nutrient preservation in soils amended with biochar. This explains why biochar stand-alone treatments posted low yields which were only higher than the control treatments without inorganic fertiliser in the first season and lowest in the subsequent seasons.

Seed weight was highest in vermicompost and fertiliser amended treatment plots ranging between 33.3g and 37.3g 100^{-1} seeds followed by biochar and fertiliser amended treatment plots ranging between 32.65 and 36.49g 100^{-1} seed. Biochar standalone treatment plots recorded low 100 seed weight in subsequent seasons when no amendments were added. The non-amended control treatment plots recorded the lowest seed weight of 29.7g 100^{-1} seeds.

4.4.6 Effect of biochar and vermicompost on soil pH and chemical composition in the four agro-ecological zones

The variable effects of biochar and vermicompost incorporation on crop production may be due to changes in soil physiognomies and/or the accessibility of nutrients. This study observed that additions of biochar affected the soil pH by raising it. Even though this was not significant from the initial soil pH at the beginning of the study, the differences with the other treatments shows great improvement. Addition of the organic amendments increased the levels of these

nutrients that are of importance in crop improvement. Previous studies by Rajkovich *et al.* (2012) and Yuan *et al.* (2011) reported the ash content and soluble cations of biochar having an effect on soil pH and nutrients respectively. Studies by Jouquet *et al.* (2011) also demonstrated the increase in soil pH and nutrient content following application of vermicompost. Biochar and vermicompost additions also influenced the nitrogen and phosphorus content in the soils. The organic amendments were however not observed to influence the organic matter content of the soil since there were significant differences across the treatments. These findings contradicted those of Nelissen *et al.* (2015) and Jouquet *et al.* (2011) who observed increased C:N ratio and soil organic matter following application of biochar vermicompost respectively.

CHAPTER FIVE

EFFECT OF VERMICOMPOST AND DIFFERENT BIOCHARS IN THE MANAGEMENT OF ROOT ROT DISEASE OF COMMON BEAN (*P. vulgaris* L.)

Abstract

Root rot of common beans has continued to increase in importance and in some instances lead to 100% yield loss especially in intensified monocultures. The pathogens broad host range and survival on crop residue as well as in the soil under different conditions posse a challenge in their management. Soil amendments have been known to influence plant growth and also impact on soil borne pathogens. Effect of vermicompost and biochars from different feed stocks on bean root rot was assessed in a greenhouse study. Soils were amended with vermicompost and two different biochars at a rate of 1:1 v/v. The experiment was laid out in a completely randomised design with 6 soil treatment applications. The treatments applied were vermicompost, sugarcane bagasse biochar, rice husks biochar, combinations of sugarcane bagasse and vermicompost, rice husks and vermicompost and a control with no amendments. Five grams of infected sorghum grain with spore strength of 10^7 CFUs/g of sorghum was then used to inoculate each pot by mixing with the top 10cm of the soil. Soils were then incubated for two weeks. Five bean seeds were planted in the inoculated soils and assayed for germination, shoot height; root weight and root rot severity at the end of the study. Treatment combinations of biochar and vermicompost had a positive impact on plant emergence. Amendment with rice husks biochar resulted in the highest shoot height while biochar from sugarcane bagasse had the greatest root length. Combination of rice husks and vermicompost had the greatest dry shoot and root weight. Plants in soils amended with the two biochars had 9% lower root rot severity than plants in vermicompost amended soils and 25% less than the non-amended soils. Rice husks biochar had greater impact on plant growth whereas sugarcane bagasse biochar greater effect on root rots severity.

Key words: Root rot, *Fusarium solani, Pythium ultimum, Rhizoctonia solani*, soil amendments, biochar, vermicompost.

5.1 Introduction

Root rot diseases greatly affect bean production when plants are grown typically under monoculture with reduced or no fallow periods (Katan, 2002). Soil borne root rot pathogens can survive actively on host, plant residues and organic materials as saprophytes. They can also survive in soil in the form of chlamydospores, oospores, sclerotia and or melanised mycelium until they are triggered in to germination by the presence of a suitable host (Waller and Brayford, 1990; Koike *et al.,* 2003).

Losses due to soil borne pathogens have been assessed to be 10–20% of the achievable yield or 100% crop loss for many crops when not managed (Nderitu *et al.,* 1997). There are however limited efficient options for management of soil borne diseases (Abawi and Pastor Coralles, 1990). Most of the options in use rarely result in complete disease control. Furthermore, some of the measures employed can have negative significant impacts that far surpass the impacts of the disease to the producer and consumers. It is therefore important to consider the effects of the management strategies will have on both environment as well as the human population in the area of application.

Disease management strategies such as soil amendments have been known for their influence on plant development and efficiency in management of soil-borne diseases from the time they were suggested (Noble and Coventry, 2005). Their use has continued to be encouraged following the increased awareness on food safety concerns and environmental pollution as a result of indiscriminate use of agro-chemicals (Nolling 1991**;** United Nations, 2008). Studies on application of soil amendments to encourage plant development and biological control agents in soil have shown them to have great potential in root rot disease management (Atiyeh *et al.,* 2000; Graber *et al.,* 2014). Disease suppression due to application of soil amendments such as vermicompost and biochar have been reported in case of damping-off caused by *Pythium* species in cucumber (Edwards and Arancon, 2004b), *Rhizoctonia* root rot in cucumber and beans (Jaiswal *et al.,* 2014) and *Fusarium* wilts in asparagus (Matsubara *et al.,* 2002).

Common soil borne pathogens like *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* cause diseases in common bean. They have numerous hosts, high degree of specificity and enduring resting structures. This in combination with their saprophytic nature makes their management difficult (Agrios, 2005). There is therefore a growing requirement for effective

approaches for management of soil-borne diseases, more so on small holder farms with intensive farming.

Therefore, this study is to explore the prospective suppression effect of vermicompost from vegetable waste and biochars produced from rice husks and sugarcane bagasse against bean root rot caused by a complex of fungi like *Pythium ultimum*, *Fusarium solani* and *R. solani* in common bean.

5.2 Materials and methods

5.2.1 Production of biochar and vermicompost

The method of producing the two biochars for this study slightly differed from the one used for previous experiments (Chapter 4 sections 4.2.1), by controlling the pyrolysis temperature. Two types of biochars from sugarcane bagasse and rice husk feedstock's were used in this study. The sugarcane bagasse was obtained from Kibos Sugar Co., Kisumu, Kenya. The rice husks were sourced from Riceland Food Co., Stuttgart, AR, USA. The feedstock's were dried for 24 h at 75°C and ground in a hammer mill with a 4 mm screen. Biochar was produced by pyrolysing the feed stocks at 400°C using a charcolator at Cornell University (Ithaca, NY, USA) in Johannes Lehmans Laboratory in 2015. The resulting biochars were stored in 1 kg sterile glass jars for 5 d, 10 d and 15 d before being used. Vermicompost was produced was produced as described in Chapter 4, section 4.2.1 of this thesis.

5.2.2 Characterisation of biochar and vermicompost

Biochars were air-dried, ground with mortar and pestle and sieved to reach a particle size ranging from149 to 850µm before analyses. Method of Chemical analysis for wood charcoal based on ASTM D1762-84 was used to determine the proximate analysis with modification in order to accommodate reactivity of biochars (Enders *et al.,* 2012). Elemental analyses were done after sieved biochars were ground using a ball mill to achieve a fine homogenous powder. Dumas combustion was used to determine total carbon (C_{tot}) and nitrogen of the biochars. The pH of both biochars was measured after 1 g of each char was weighed directly in to a 60-mL glass vial. Twenty milliliters of 1M KCl prepared using deionized water was then added to the vials. The vials were then placed on a mechanical shaker and agitated for one and half hours. The biochar water mixture was continually mixed while the pH was measured (Enders *et al.,* 2012).

Vermicompost was left for incubation for one month after production before the determination of its physical and chemical characteristics. A two millimeter strainer was used to sieve the vermicompost followed by air drying for 24 hours at room temperature before analyses for nutrients was carried out. Determination of organic matter content was done following the method of Kacar (1994). Ten grams of vermicompost was oven dried at 55°C for a period of 24 hours and the difference in weights used to determine the moisture content. Ten grams were then placed in a dry porcelain pot which was then heated in a combustion oven at 550°C for 8 hours. Percentage ash was then calculated by the formular:

Ash (%) = [(W3 - W1) / (W2 - W1)] x 100 and

Organic matter (%) = 100 – ash %

where W1 = the weight of the empty, dry crucible; W2 = the weight of the dry crucible containing vermicompost; and W3 = the weight of the dry crucible containing vermicompost following ignition. Weight of the ash = W3 - W1.

An EC and pH meter was used to determine the electrical conductivity and pH of vermicompost in a 1:5 and 1:2.5 v:v of vermicompost to 1 M KCl mixtures, respectively.

Total soil nitrogen was determined using the Kjeldahl method as described by Kacar (1994). Cation exchange capacity of vermicompost was determined by the ammonium acetate method defined by Kacar (1994). Filtrates from triple repeats of the above described procedure were collected and used in flame photometer reading for Na. Bacterial load in vermicompost was also determined by plate count technique (Szczech, 1999).

5.3 Effect of vermicompost and biochars on plant emergence, growth and disease development

5.3.1 Growth media preparation and mixing of amendments and experimental set up

A two millimeter mesh was used to sieve garden soil and sand which were then autoclaved for 30 min at a temperature of 121°C and 1.5 bars pressure. The autoclaving was repeated three times on consecutive days. The sand and soil mixture were used for potting at the ratio of 1:2 (v:v). Mixing of the soil and amendments of vermicompost, sugarcane bagasse biochar and rice husks biochar was done at a ratio of 1% (v/v) for each amendment per treatment. The mixing was done aseptically in buckets previously surface sterilised with Green shield. These mixtures of amendments and growth media were then transferred in to pots measuring 1650mL (6 inch diameter) and filled to 1 cm shy of the brim to allow for irrigation without having overflow and then labeled accordingly. The treatments were set up in a completely randomized

design with three replications for each treatment laid in triplicates. The treatment combinations used were biochar (sugarcane bagasse, rice husks), vermicompost, sugarcane bagasse biochar + vermicompost, rice husks biochar + vermicompost and a control making a total of 270 pots. The pots were then placed on plates to contain any water percolating from the pots.

5.3.2 Inoculum preparation and inoculation of plants

Inoculum was prepared following the procedure described by Mueller *et al.,* (2003) with minor modifications. One hundred grams of sorghum seeds were soaked in water in 500-ml conical flask overnight. Debris and floating seeds were removed after soaking. The seeds were washed three times with water and the excess water drained. The seeds were then autoclaved for 60 minutes at 121 ºC on 2 consecutive days. Each flask containing sterilized sorghum seeds was separately inoculated with an individual isolate of *F. oxysporum, F. solani, P. ultimum* and *R. solani* by transferring five 5-mm-diameter plugs from the edge of 7 day old cultures on potato dextrose agar. The inoculated flasks were incubated at 25ºC and shaken on alternate days to promote uniform growth of fungi. After 14 days of incubation, inocula were air dried for 24 hours in a laminar flow hood, crushed using a mortar and pestle then used for both colony-forming units (CFU) assay and later for inoculation in the greenhouse.

The CFU assay of the sorghum grain inoculum was conducted following the procedure by Li *et al.,* (2008). One gram of infected sorghum seed was soaked in a 250-ml conical flask containing 100 ml of sterile distilled water followed by a 30 min shaking at 150 rpm on a mechanical shaker. The resultant inoculum suspension was subjected to a tenfold serial dilution in sterile distilled water to attain dilutions of 10^{-3} and 10^{-4} for each pathogen. Pour plating was undertaken for these dilutions using molten PDA amended with 50ppm streptomycin antibiotic in a 9mm petri dish and incubated for 7days at 25 ºC. They were replicated three times for each inoculum dilution. After incubation the colonies developed in each plate of each fungal pathogen were counted to determine the CFUs per gram of sorghum seed for each pathogen. The inoculum quantity was determined and adjusted to 10^7 CFUs/gram of sorghum by mixing infected sorghum grain with non-infected sterile sorghum grain (w/w). Five grams of infected sorghum grain with spore strength of 10^7 CFUs/g of infected sorghum was then used to inoculate each pot by mixing with the top 10cm of the soil. The soils were then irrigated to water holding capacity and incubated in the greenhouse for two weeks to ensure colonization of the soil by the fungi prior to planting bean seeds.

5.3.3 Seedling establishment, determination of plant emergence, crop vigour and disease rating

Five Rosecoco seeds were seeded in each pot to a depth of 2.5cm and slightly covered with the same soil-gravel treatment mixture within the pot. The plants were then irrigated with 100mL sterile water per pot. Irrigation of the bean plants continued on alternate days using sterile water.

Seedling emergence was determined 14 days after planting and the number of seedlings emerging per treatment was recorded. Disease incidence was determined at seedling stage and every two weeks after germination. This was done by visually assessing the plants for root rot symptoms and scoring using a scale of 0-5 as described by Fillion *et al.,* (2003) where, 0=healthy plants, 1 = initial signs of wilting (water loss in plant leaves and stems where affected plant parts lose their turgidity and droop), 2 = up to 25% of the leaves with wilting symptoms, 3 = < 25% up to 50% of the leaves wilting, 4 = < 50% up to 75% of the leaves with wilting symptoms, 5 = plants dead. Crop development was determined following procedure by Marcos-Filho, (2015) with adjustments on the time of taking measurements. Plant shoot and root lengths were recorded after 6 weeks of plant growth when plants were uprooted and later dried at 65ºC for 24 hours to determine their dry weights which were also recorded. The average of these parameters was then used to determine the crop vigour in each treatment.

Root rot severity was assessed 42 days after planting. All five plants were carefully uprooted and washed to remove excess soil from the roots. Drying was done on paper towels after which the roots were rated for severity by visual assessment of necrotic lesions on the roots using a scale of 1-9 (as outlined in chapter 4 section 4.2.3 of this thesis) as described by Abawi and Pastor-Corrales (1990). Re-isolation of the pathogens from diseased plants was undertaken to confirm the cause of disease and pathogenicity of test samples.

5.4 Data collection and analysis

Data on plant emergence was recorded fourteen days after planting for a period of 5 days where the total number of seedlings that had emerged was counted per treatment. Percent disease incidence was determined by counting the number of diseased plants in all the 9 pots of each treatment totaling to 45 plants per treatment and a total of 1,350 plants for the whole experiment. The number of diseased plants were then divided by the total number of plants in the treatment and multiplied by 100. Data on plant height, root length and disease severity was determined at six weeks after planting. The shoots and roots were measured from the soil level

to the tip. Disease severity scoring on roots was done based on a scale of 1 to 9 (Abawi and Pastor-Corrales, 1990). Dry shoot and root weights were measured after drying at 65°C for 24 hours. The data of measured variables were analyzed by ANOVA ($p < 0.05$) using Genstat 15 edition. The means were separated by the least significant difference using the Tukeys range test.

5.5 Results

5.5.1 Characteristics of biochar and vermicompost

All the three soil amendments analysed varied in their composition. Vermicompost had the highest moisture content while rice hull biochar (RH biochar) had the least (Table 5.1). No volatile compounds were found in vermicompost but were highest in rice husks biochar as compared to sugarcane bagasse biochar (SB biochar). Ash content was also high in RH biochar and low in SB biochar. The pH in rice husks biochar was found to be alkaline while that of vermicompost and SB biochar were observed to be near neutral. Electrical conductivity (EC) was found to be very high in RH biochar as compared to SB biochar. Vermicompost had the lowest EC of all the amendments used. Dry matter content was recorded highest in RH biochar and lowest in vermicompost. The C:N ratio was also highest in RH biochar and lowest in vermicompost.

5.5.2 Nutrient composition of biochar and vermicompost

Phosphorus was the nutrient observed to be highest in the biochars as compared to other nutrients (Table 5.2). RH biochar had the highest level of phosphorus as compared to SB biochar and vermicompost. Potassium was recorded highest in vermicompost while the lowest percentage was recorded in SB biochar. No calcium was found in the two biochars but vermicompost had 2.5%. Rice husks biochar was found to have highest level of magnesium which was more than 58% higher than in vermicompost and SB biochar. Conversely Sulphur was highest in vermicompost and RH biochar as compared to SB biochar. Iron was the highest micro nutrient in all the soil amendments which was at 6600ppm recorded in vermicompost while the lowest micro nutrient was Boron at 14.4ppm recorded in SB biochar. Other micro nutrients available in the amendments including sodium, zinc, copper and manganese were higher in RH biochar as compared to SB biochar.

Table 5.1: Characteristics of different biochars and vermicompost

Amendment	MC %	Volatiles (%)	Ash (%)	pH	EC (mS/cm)	DM %	C %	N %	C:N %
Vermicompost	48.2	-	-	6.92	12.0	50.8	30.1	3.5	8.5
Rice husks biochar	1.7	18.4	54.8	11.92	1978.5	98.3	53.2	2.8	18.7
S. bagasse biochar	3.1	9.1	9.7	6.83	73.5	96.9	62.9	5.3	11.9

MC- Moisture Content, EC- electrical conductivity, DM- Dry matter, C- Carbon, N- Nitrogen, C:N- Carbon Nitrogen ratio; S. bagasse- Sugarcane bagasse.

Table 5.2: Chemical composition of different biochars and vermicompost

Amendment	P %	K %	Ca %	Mg %	S %	Mn (ppm)	Fe (ppm)	B (ppm)	Na (ppm)	Zn (ppm)	Cu (ppm)
Vermicompost	0.6	3.3	2.5	0.5	0.40	410.0	6600.0	101.0	1480.0	185.0	17.8
Rice husks biochar	4.7	1.2	n/a	1.3	0.40	188.1	4191.4	53.0	3865.9	3520.6	263.5
S. bagasse biochar	1.0	0.7	n/a	0.4	0.03	36.9	485.3	14.4	2668.3	570.2	38.2

P-Phosphorus, K- Potassium, Ca- calcium, Mg- Magnesium, S- Sulphur, Mn- Manganese, Fe- Iron, B- Boron, Na- Sodium, Zn- Zinc, Cu- Copper; S. bagasse- Sugarcane bagasse; ppm- parts per million; N/A- not available/present

5.5.3 Effect of soil amendments on seedling emergence

There was no significant difference in seedling emergence among the treatments in the greenhouse (Table 5.3). However in the control plots, rice husks and sugar cane bagasse biochar in combination with vermicompost recorded higher emergence than the non-amended soils. Similar observations were made for soils inoculated with the root rot pathogens with the exception of *P. ultimum* inoculated pots. The lowest emergence was recorded in the non-amended soils inoculated with *F. solani*.

5.5.4 Effect of biochar and vermicompost on growth and development of common bean inoculated with different root rot pathogens

Significant differences ($p<0.05$) were recorded for plant shoot lengths in across all treatments (Table 5.4). Plants in pots amended with RH biochar in combination with vermicompost and inoculated with *F. oxysporum* had the highest shoot height while the least was recorded for plants in non-amended soils challenged with the mixture of the four pathogens. The non-amended soils were observed to have shoot length which was 17% to 27% lower across all treatments.

There was a significant difference ($p<0.05$) in root length across all the treatments (Table 5.5). Plants inoculated with *R. solani* in soils amended with sugarcane bagasse biochar had the longest roots. These were 53% longer than plants challenged with *R. solani* in soils amended with vermicompost which were observed to have the shortest roots. The same trend was observed in other plants challenged with different root rot pathogens. Plants in vermicompost amended soils were however observed to have the greatest root length in the non-challenged plants.

Table 5.3: Effect of different biochars and vermicompost on plant emergence (%) in presence of root rot pathogens

Treatment	Percentage emergence (%)						
	Control	*Fo*	*FS*	*Pu*	*Rs*	Mix	Treatment means
Non amended soil	83a	64a	67a	77a	56a	56a	67.1a
Vermicompost	97a	87a	87a	77a	87a	80a	85.8a
R.H biochar	100a	77a	84a	80a	84a	80a	84.2a
R.H Vermicompost	90a	87a	84a	77a	77a	83a	83.0a
S.B biochar	87a	87a	74a	77a	84a	87a	82.7a
S.B biochar Vermicompost	94a	77a	73a	77a	73a	76a	78.3a
LSD treatment x pathogen	25.7						
LSD treatment							10.5
%CV	15.8						
F. Pr	0.991						

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. RH-rice husks; SB- sugarcane bagasse; *Fo- F. oxysporum, Fs- F. solani, Pu- P. ultimum, Rs- R. solani,* Pat Mix – mixture of the four pathogens. LSD: Least significant difference at 5% level, CV: Coefficient of variation

Table 5.4: Effect of vermicompost and different biochars on bean shoot length (cm) in soils inoculated with root rot pathogens

Treatment	Shoot height (cm)						
	Control	*Fo*	*FS*	*Pu*	*Rs*	Pat Mix	Treatment means
Non amended soil	19.7d	18.2d	18.5c	18.3c	18.0b	17.8c	18.4d
Vermicompost	20.7cd	20.3c	21.6b	20.7b	20.9a	20.6b	20.8c
RH biochar	23.0a	22.1b	21.8ab	20.9ab	21.4a	21.8ab	21.8ab
RH biochar Vermicompost	21.6bc	23.5a	21.8ab	21.6ab	21.5a	22.4a	22.1a
SB biochar	22.6ab	21.3bc	23.0a	20.9ab	21.6a	21.3ab	21.8ab
SB biochar Vermicompost	22.7ab	21.5bc	21.1b	22.1ab	20.4a	20.5b	21.4b
LSD treatment x pathogen	1.23						
LSD treatment							0.5
%CV	2.9						
F. Pr	0.013						

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. RH-rice husks; SB- sugarcane bagasse; *Fo- F. oxysporum, Fs- F. solani, Pu- P. ultimum, Rs- R. solani,* Pat Mix – mixture of the four pathogens. LSD: Least significant difference at 5% level, CV: Coefficient of variation

Table 5. 5: Effect of vermicompost and different biochars on bean root length (cm) in soils inoculated with root rot pathogens

Treatment	Root length (cm)						
	Control	*Fo*	*FS*	*Pu*	*Rs*	Pat Mix	Treatment Means
Non amended soils	24.6c	28.6b	29.1a	25.0bc	22.4c	25.0c	25.8c
Vermicompost	31.6a	19.9d	21.2c	19.2d	18.3d	19.1d	21.5e
R.H biochar	28.1b	22.9d	22.5c	23.6c	26.2b	24.6c	24.6d
RH biochar Vermicompost	24.9c	31.8a	29.6a	26.1b	22.7c	27.0b	27.0b
SB biochar	27.8b	28.4b	30.7a	33.2a	39.7a	33.9a	32.3a
SB biochar Vermicompost	31.2a	25.3c	24.8b	26.6b	25.1b	24.7c	26.3bc
LSD treatment x pathogen	1.63						
LSD treatment							0.67
%CV	3.1						
F. Pr	<0.001						

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. RH-rice husks; SB- sugarcane bagasse; *Fo- F. oxysporum, Fs- F. solani, Pu- P. ultimum, Rs- R. solani,* Pat Mix – mixture of the four pathogens, Control – No pathogen. LSD: Least significant difference at 5% level, CV: Coefficient of variation

5.5.5 Effect of biochar and vermicompost amendments on dry shoot and root weights of common bean inoculated with different root rot fungi

Significant differences ($p<0.05$) in dry shoot weight were observed across all treatments (Table 5.6). Plants challenged with the four root rot pathogens in rice husks biochar in combination with vermicompost amended pots recorded the highest dry shoot weight. This was followed by plants inoculated with *F. Oxysporum* in rice husks and vermicompost amended pot while those inoculated *R. solani* in vermicompost amended pots were third highest. The lowest was recorded from control plants in non-amended soil translating to a 91% difference when compared to plant in rice husks biochar amended pots.

Significant differences ($p<0.05$) in dry root weight was observed in plants across all treatments (Table 5.7). The highest dry root weight was recorded from *R. solani* challenged plants in soils amended with sugarcane bagasse. This was followed by non-challenged plants in soils amended with rice husks biochar in combination with vermicompost. Sugarcane bagasse biochar amendments resulted in an 80% increase in dry root weight of plants challenged with root rot pathogens. The lowest dry root weight was recorded from control plants in non-amended pots.

Table 5. 6: Effect of biochar and vermicompost on dry shoot and root weights of common bean inoculated with root rot pathogens

Treatment	Dry Shoot weight (g) Experiment 1						
	Control	*Fo*	*FS*	*Pu*	*Rs*	Pat Mix	Treatment Means
Non amended soil	0.08c	0.17c	0.17c	0.21d	0.30c	0.19d	0.18d
Vermicompost	0.29ab	0.40b	0.43b	0.73a	0.79a	0.55b	0.53b
R.H biochar	0.21b	0.44b	0.44b	0.45bc	0.30c	0.34c	0.36c
R.H biochar vermicompost	0.36a	0.86a	0.80a	0.52b	0.78a	0.87a	0.69a
S.B biochar	0.35a	0.26c	0.28c	0.42bc	0.54b	0.38c	0.37c
S.B biochar Vermicompost	0.33a	0.45b	0.44b	0.40c	0.36c	0.36c	0.39c
LSD treatment x pathogen	0.11						
LSD treatment							0.04
%CV	12.4						
F. Pr	<0.001						

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. RH-rice husks; SB- sugarcane bagasse; *Fo- F. oxysporum, Fs- F. solani, Pu- P. ultimum, Rs- R. solani,* Pat Mix – mixture of the four pathogens. LSD: Least significant difference at 5% level, CV: Coefficient of variation

Table 5. 7: Effect of different biochars and vermicompost on dry root weights of common bean inoculated with root rot pathogens

Treatment	Dry root weights (g)						
	Control	*Fo*	*FS*	*Pu*	*Rs*	Pat Mix	Treatment Means
Non amended soils	0.11d	0.16c	0.16c	0.14d	0.24c	0.17c	0.16e
Vermicompost	0.21c	0.32b	0.34b	0.26c	0.39b	0.26b	0.29c
R.H biochar	0.30b	0.31b	0.33b	0.27c	0.24c	0.28b	0.29c
RH biochar Vermicompost	0.64a	0.57a	0.53a	0.57a	0.43b	0.52a	0.54a
S.B biochar	0.28bc	0.38b	0.41b	0.45b	0.86a	0.58a	0.49b
SB biochar Vermicompost	0.23bc	0.22c	0.24c	0.23c	0.29c	0.24bc	0.23d
LSD treatment x pathogen	0.08						
LSD treatment							0.03
%CV	12.1						
F. Pr	<0.001						

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. RH-rice husks; SB- sugarcane bagasse; *Fo- F. oxysporum, Fs- F. solani, Pu- P. ultimum, Rs- R. solani,* Pat Mix – mixture of the four pathogens. LSD: Least significant difference at 5% level, CV: Coefficient of variation

5.5.6 Effect of biochar and vermicompost on root rot severity in common bean inoculated with different root rot fungi

There was a significant ($p<0.05$) difference in disease severity across the treatments following inoculation with root rot pathogens in the first experiment (Figure 5.1). The highest percent severity index was observed in the non-amended soils inoculated with all four root rot pathogens and *R. solani* at 77.8% each during the first trial. The lowest severity was recorded in sugarcane bagasse biochar amended plots challenged with a mixture of pathogens at 33.3 % and in treatment combination of SB biochar and vermicompost amended pots challenged with *R. solani* at 33.3%.

During the repeat trials, significant differences ($p<0.001$) in percent disease severity was also observed in all the treatments (Figure 5.2). The highest disease severity rating of 85.2% was observed in the non-amended pots challenged with a mixture of root rot pathogens with the differences being significant at $p\leq0.05$. This was followed by *F. oxysporum* inoculated plants in non-amended soils at 77.8%. The lowest severity was recorded in treatment combinations of biochar and vermicompost challenged with *R. solani* at 33.3%. Non-inoculated pots recorded a percentage of between 11.1% and 28.9% PSI (Figure 5.3 and Figure 5.4).

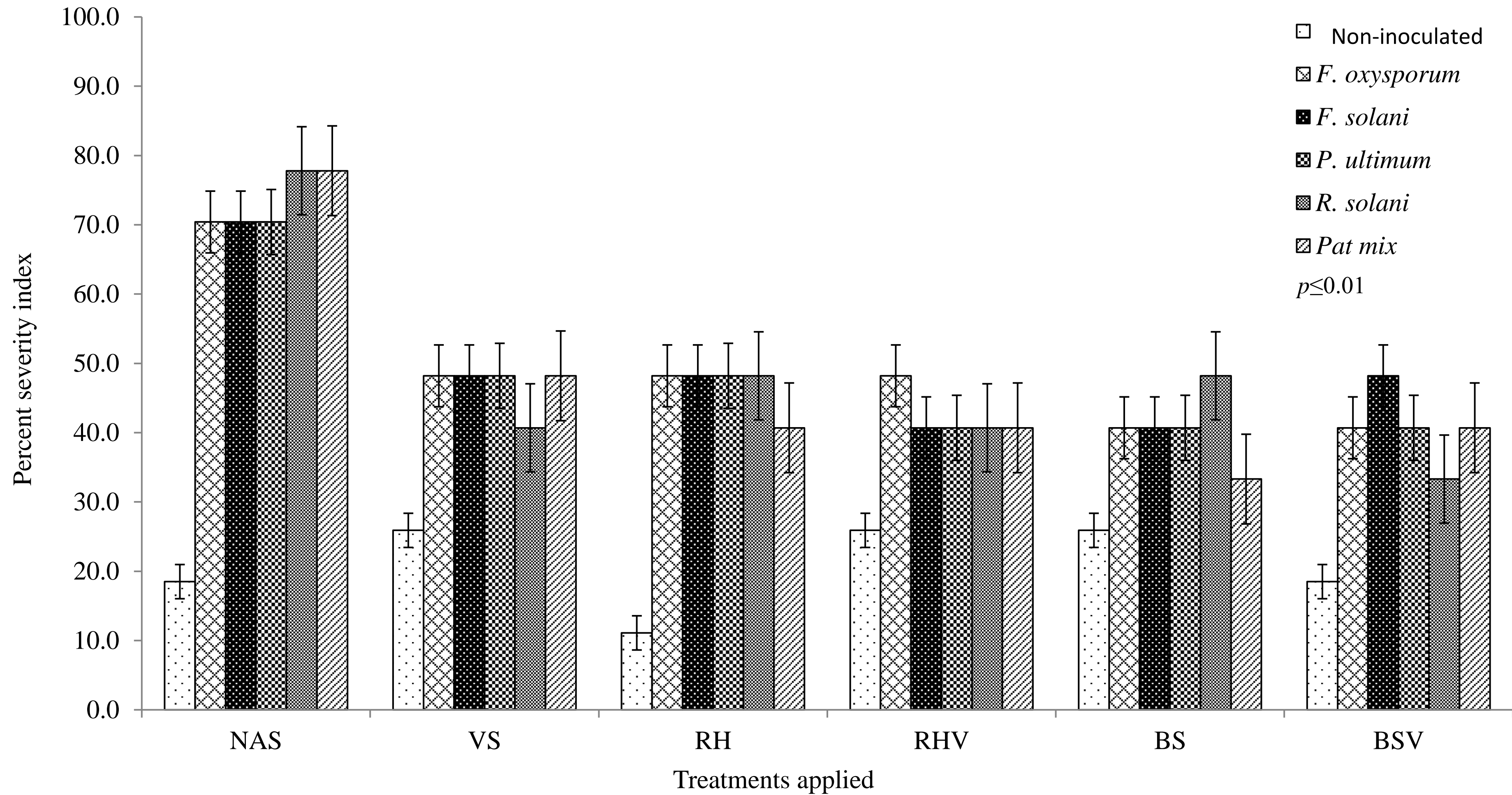

Figure 5.1: Effect of biochar and vermicompost on disease severity of common bean - experiment I.

NAS-non amended soil; VS- vermicompost amended soil; RH- rice husks biochar; BS - sugarcane bagasse biochar; BSV- sugarcane bagasse biochar + vermicompost; RHV - rice husks biochar + vermicompost; Pat mix- mixture of all four root rot pathogens.

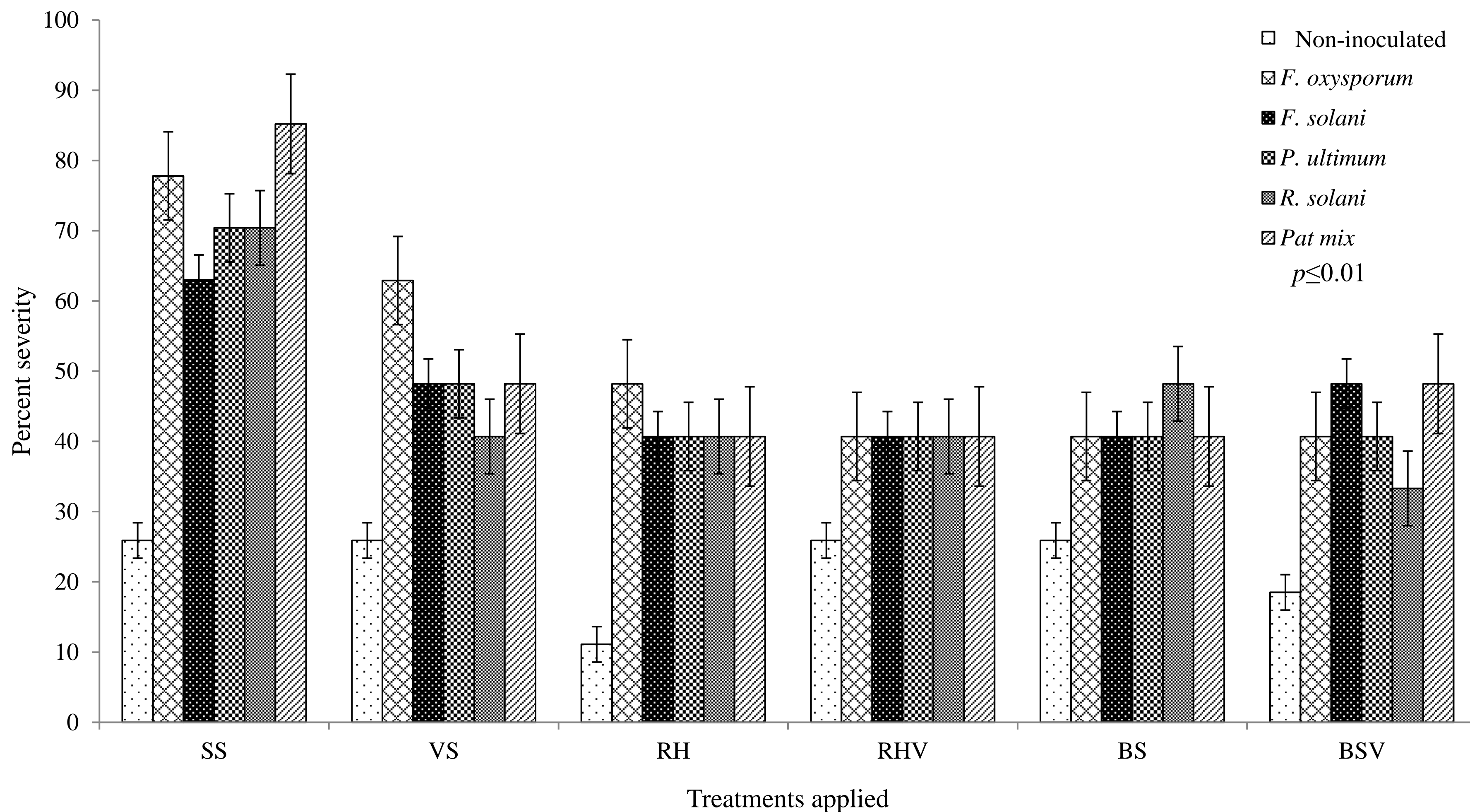

Figure 5.2: Effect of biochar and vermicompost on disease severity of common bean - experiment II.

NAS-non amended soil; VS- vermicompost amended soil; RH- rice husks biochar; BS - sugarcane bagasse biochar; BSV- sugarcane bagasse biochar + vermicompost; RHV - rice husks biochar + vermicompost; Pat mix- mixture of all four root rot pathogens.

5.6 Discussion

5.6.1 Characteristics of biochar and vermicompost

In this study, biochar that was produced from different feed stocks using similar pyrolitic conditions varied in their composition. The highest variability was noted for ash, volatile matter contents, pH and carbon. Sugarcane bagasse biochar had low ash content and higher fixed carbon content than rice husks biochar. Consequently, rice husks biochar which had high ash content was observed to have 53% of carbon content. Similar results were previously reported by Mitchell *et al.* (2013) who observed strong negative correlation between ash and fixed carbon content. The findings, however, slightly depart from the findings by Enders *et al.* (2012) who concluded that fixed carbon contents for biochar with greater than 35% ash content were limited to below 30%. The vermicompost produced from crop residue using *Eisinia andrei* worms did not greatly vary from the sugarcane bagasse biochar in the pH. It, however, had lower dry matter, carbon content and C:N ratio than the two biochars.

5.6.2 Effect of soil amendments on seedling emergence

This study observed that application of vermicompost and the two biochars in pots resulted to slight differences in common bean germination. Sugarcane bagasse biochar and rice husks biochar had high germination percentage of above 72% in the first trial while vermicompost had the lowest germination of 56%. The results point to moderate toxicity of biochar to germination in the first trial but this was observed in the second trial when it recorded a germination of over 96 %. Vermicompost was observed to have higher toxicity during the first trial with a germination of 56% but the effects were lower in the second trial with a germination of 88%. According to Zucconi *et al.* (1985) and Emino and Warman (2004), germination index (GI) values <50% suggest a high phytotoxicity; 50–80% suggest moderate phytotoxicity and GI values of 80% suggest no phytotoxicity. When GI exceeds 80%, the material can be considered as a phytonutrient or phytostimulant.

5.6.3 Effect of biochar and vermicompost soil amendments on growth and development in common bean plants inoculated with *F. oxysporum, F. solani, P. ultimum* and *R. solani*

In this study, plant height was significantly enhanced with the addition of biochar to the soil medium. SB biochar and RH biochar significantly ($p<0.005$) influenced plant height both in control and in challenged plants. In subsequent experiments, plants challenged with *F. solani* and *R. solani* had the highest plant height in comparison to non-challenged plants in controls

with the same treatments. The same trend was observed with root length and dry root weight. Similar observations were previously reported by Jaiswal *et al.* (2014) who observed reduced sensitivity of plant growth parameters to biochar dose in the absence of the disease causing pathogen than when it was present. Other findings by Guerena *et al.* (2015) reported increased crop biomass of common bean following application of sugarcane bagasse biochar. Increase in root length in biochar treated plants has also been reported by Atiyeh *et al.,* (2001) and Gutierrez-Miceli *et al.,* (2007) when working with tomato. This can be explained by the fact that biochar applications are known to stimulate plant growth thereby increasing demand for nutrients and water as was previously reported by Biederman and Harpole, (2013). Clough *et al.* (2013) and Prendergast-Miller *et al.*(2011) also reported that the nutrient ions including inorganic nitrogen that are absorbed by biochar led to nutrient deficiencies which resulted in enhanced root lengths of wheat.

Treatment combinations of RH biochar and vermicompost had a fivefold increment in dry weight of roots as compared to the non-amended pots which had the lowest dry root weight. The findings were replicated in all the subsequent trials. These results are in agreement with previous findings by Rondon *et al.,* (2007) who observed a 39% increase in common bean crop biomass following vermicompost amendments. Other studies by Roy *et al.,* (2010) and Valdez-Perez *et al.,* (2011) also reported significant increase of 20% in total shoot; root and pod dry biomass of the common bean in vermicompost treatment in comparison to control plots.

5.6.4 Effect of biochar and vermicompost soil amendments on root rot diseases severity in common bean inoculated with *F. oxysporum, F. solani, P. ultimum* and *R. solani*

Bean root rot severity was significantly reduced due to the application of soil amendments in this study. Sugarcane bagasse biochar amendment which had high fixed carbon of 62% recorded lower disease severity as compared to rice husks biochar and vermicompost. These findings suggest that biochars ability to influence soil fungal pathogens is related to recalcitrant forms of Carbon which was earlier suggested by Graber *et al.,* (2010). Similar findings on effect of fixed carbon on disease severity have been observed by Jaiswal *et al.,* (2014). They observed high incidence of *Rhizoctonia* damping off disease in cucumber following application of comparatively low C content (40.2% and 13.2 % C) glasshouse waste biochars. In the same study they reported low disease incidence following application of biochars with relatively high C content (69.3% and 76.7 % C). Gasco *et al.* (2016) also obtained similar results when

studying the influence of biochar on sudden syndrome disease caused by *Fusarium virguliforme* on Soy bean.

Percent severity index was significantly different ($p<0.05$) across all the treatments. Greatest suppression was observed in SB biochar treated pots which had been inoculated with root rot pathogens. Pots amended with SB biochar and challenged with *F. solani* had the lowest root rot disease severity index as well as those challenged with *R. solani*. The same trends were observed in the subsequent trials. The superiority of SB biochar over RH biochar and vermicompost may be attributed to its higher fixed carbon and C:N ratio. In earlier studies Mutitu *et al.* (1988) observed that organic amendments high in C:N ratio resulted in reduction of severity of *Fusarium* yellows severity on beans. Other studies have shown soils poor in organic matter resulted in high severity of root rot. Addition of biochar rich in fixed carbon to soils may alleviate root rot severity. However the recommended rates of application should be observed in order to avoid the hormesis effect of biochar as earlier reported (Graber *et al.,* 2010; Jaiswal *et al.,* 2014). This is in relation to the organic compounds contained in biochar that at lower doses, they result in beneficial effects, but may result in making plants susceptible to diseases and retard growth at higher doses.

CHAPTER SIX

MECHANISMS OF BIOCHAR AND VERMICOMPOST IN SUPPRESSION OF ROOT ROT FUNGAL DISEASE OF COMMON BEAN (*Phaseolus vulgaris* L)

Abstract

Organic amendments influence root rot pathogen in diverse ways following their application. The study sought to determine the mechanisms by which biochar and vermicompost affect root rot pathogens. Biochar and vermicompost were tested for their effect on spore and sporangia germination as well as on growth of mycelia *Fusarium solani* and *Pythium ultimum*. Germination of *Pythium ultimum* sporangia as well as *Fusarium solani* macro conidia was significantly inhibited by different concentration of the water extractible substances from the biochars and the vermicompost. Significant difference ($p<0.05$) were observed in growth of *P. ultimum* and *F. solani* colonies when inoculated PDA plates were inverted over biochars exposed to air over different periods of time. Tests conducted to assess biochars and vermicompost ability to adsorb signaling molecules was conducted by filtering bean seed and root exudates through pre irrigated and drained organic amendments. No phytochemicals were observed after filtering the exudates through the biochars and vermicompost. The filtered exudates lost their ability to induce sporangial and conidial germination of *Pythium ultimum* and *Fusarium solani* respectively. The study showed that biochar and vermicompost effectively inhibited the germination of root rot propagules by their water extracts as well as through adsorption of signal molecules from bean seeds. Freshly produced biochar also inhibited the growth of the *Fusarium solani* and *Pythium ultimum* cultures on PDA when incubated in close proximity. In conclusion from the results, biochar and vermicompost adversely affect root rot pathogens. This study has been able to identify some of the mechanisms by which biochar and vermicompost suppress root rots in common bean as adsorption of molecules that trigger germination of pathogens and inhibition of germination by the water extractible substances on the surface of biochar.

6.1 Introduction

Bean root rot has been cited as a major constraint in the production of common bean leading to production losses of up to 70% in some bean varieties such as Rosecoco in Kenya (Mutitu 1988; Otsyula *et al.,* 2003; Miklas *et al.,* 2006; MOA, 2011). This has led to the lagging behind

of the production despite the increase in demand due to the ever growing population (Kambewa, 1997; Katungi *et al.,* 2009).

There are various options available for managing the root rot complex of beans but some are of questionable efficacy after planting (Abawi and Pastor-corrales, 1990). The use of options known to be highly effective in control of root rot such as the broad spectrum soil fumigants including metham sodium are restricted by their high costs and toxicity to man and environment (Nolling, 1991**;** United Nations ,2008). Applications of organic amendments such as compost, farmyard manure and biochar have been shown to have positive effects to root disease dynamics and yield increase (Jaiswal, 2013; Ruano-Rosa and Mercado-Blanco 2015). They reduce disease inoculum density in the soil as well as creating conditions favourable for development of microorganisms that are antagonistic to plant pathogens. Antagonistic microorganisms identified include *Trichoderma harzianum*, *Penicillium* spp*., Bacillus* spp. and *Pseudomonas fluorescens* (Meenu *et al*., 2010).

Composts and biochar are also known to improve the soil health and suppress various soil-borne diseases caused by fungal pathogens belonging to diverse genera such as *Fusarium, Pythium, Rhizoctonia* and *Phytophthora* (Mehta *et al.,* 2014; Sohi *et al*., 2010; Elad *et al.,* 2010). The suppressiveness of compost and biochar has been attributed to a number of factors which include: promotion of beneficial microbial communities, improvement in plant growth and vigour, increased nutrient availability, the induction of systemic resistance and /or fungistatic abilities that compost and biochar amendments may express (Meller Harel *et al.,* 2012; Graber *et al*., 2014). In earlier field experiments conducted in chapter four of this thesis, populations of *Trichoderma* and *Penicillium* spp were observed to increase following application of biochar and vermicompost. These two fungi have also been reported to have antagonistic effect on root rot fungal pathogens (Sreevidya and Gopalakrishnan, 2016 *and* El-Sheshtawi *et al.,* 2014)

This study therefore was conducted to establish the mechanisms involved in suppression of bean root rot when biochar and vermicompost were used as amendments. To determine these mechanisms, this study hypothesized that (i) vermicompost and biochar-derived water extracts inhibit germination and growth of root rot pathogens (*Fusarium spp., Pythium spp* and *Rhizoctonia spp.*); (ii) biochar and vermicompost adsorbs and alters availability of plant root exudates leading to reduced spore germination resulting to reduced infection of beans by

pathogenic soil-borne fungi and (iii) duration of exposure of biochar to air has an effect on its ability to suppress the growth of root rot pathogens.

6.2 Materials and methods

6.2.1 Production and characterisation of vermicompost and biochar from different feed stocks

A similar method for production of biochar and vermicompost described in Chapter 5 sections 5.2.1.1 and 5.2.2.2 were used in production of the soil amendments for these mechanism experiments.

Physical, chemical, and biological characteristics of the vermicompost were determined following the procedure described earlier in Chapter Five section 5.2.2 of this document.

6.3 *In vitro* fungal antagonism

6.3.1 Antagonistic activity of *Trichoderma harzianum* and *Penicillium* spp against *root rot* pathogens

Trichoderma harzianum and *Penicillium* spp were tested against the isolated pathogenic fungi of *F. solani, F. oxysporum, P. ultimum* and *M. phaseolina in vitro* using the dual culture technique. Each pathogen was inoculated on sterilized PDA and grown for a period of seven days at a temperature of 25°C. The antagonist was inoculated on the plate with the established pathogen using a 5 mm culture disc. Plates inoculated with pure cultures of root rot pathogens were used as controls in the experiment. The plates were then incubated at room temperature for a period of nine days. Each treatment was replicated three times with the pure culture of the pathogen being the control. Observations of the plates was done daily and the growth antagonism ratings were recorded using the modified scale of class 1 to 5 (Bell *et al.,* 1982) where; Class 1 (R1) = the antagonist completely overgrows the pathogen. Class 2 (R2) = the antagonist overgrows at least ¾ of pathogen surface. Class 3 (R3) = the antagonist colonized at least half of the pathogen. Class 4 (R4) = the pathogen locked at point of contact with the antagonist. Class 5 = the antagonist overgrown by the pathogen.

Percent inhibition on growth of the pathogenic fungi by the antagonist was then calculated after measuring the radial growth of the test pathogens in control, as well as in dual culture plates. The formular used was:-

$$\text{Percent Inhibition of radial growth of pathogen (\%)} = \frac{(C-T)}{C} X100$$

Where C is radial growth of the pathogen (mm) in control; T is Radial growth of the pathogen (mm) in the treatment

6.3.2 Antagonistic effect of *Trichoderma harzianum* and *Penicillium* spp against root rot pathogens on a slide culture

A clean slide was placed on a v-shaped glass rod and autoclaved. Inside a 9-cm petri dish, a thin film of molten water agar was poured and spread evenly leaving one end of the slide free of medium to facilitate handling. The slide with the medium was inoculated at a distance of 1cm with the pathogen and a bio control agent separately following the technique by Sivakumar *et al.* (2000). Sterile water was then added to the petri dish at a rate 1 mL to prevent drying during incubation at 25°C for seven days. On incubation, the regions where the two fungi met was observed under a light microscope at the end of the incubation period and pictures taken of wall disintegration of the pathogen which was indicated by crumbling of the mycelium or coiling structures produced by the antagonist. The treatments were replicated three times.

6.4 Effect of water extracts of biochar and vermicompost on root rot pathogens

6.4.1 Extraction, quantification and pH of water-extracts from biochars and vermicompost

Extraction of water-extractable substances (BWES) from biochar was undertaken according to the methodology proposed by Smith *et al.* (2012). Fifty grams of each biochar were soaked separately in 200 mL of nano pure water in 500mL conical flasks. The biochar and water mixture were shaken at 100 rpm for 24 h on a horizontal mechanical shaker. The mixture was transferred to 12-cm diameter Buchner funnel lined with Whatman Grade-1 qualitative filter paper (11 µm pore size) and vacuum filtered. The collected filtrate was frozen and lyophilized to obtain and determine the volume of biochar water-extractable substances (BWES) in a dry state. This also was to enable preparation of known concentrations of stock solutions. The remaining biochar solid was stored for further testing for adsorption of seed exudates. The biochar water-extractable substances were re-dissolved in sterile nano pure water to make the stock solution from which different concentrations of total BWES were made and used for experiment.

Vermicompost extract was obtained after mixing 50 g vermicompost with 100 mL of sterile distilled water. The mixture was then shaken for 5 min on a mechanical shaker and left to stand at room temperature for an hour. This extract was then obtained by filtration using a Whatman filter paper, frozen and then lyophilized to determine the volume of the extract in dry state. The filtrate was stored at -20°C until it was used for the assay on their effect on spore germination and mycelial growth. pH of the water extracts of both biochar and vermicompost was measured at the aqueous state of the extracts which was done in triplicates following filtration.

6.4.2 Effect of water-extracts of biochar and vermicompost on spore germination of root rot pathogens

Bioassays of the effect of BWES on spore germination were conducted for *Fusarium solani* and *Pythium ultimum* on water agar and SM+ Lecithin discs respectively due to their ability to produce spores. The germination experiments were conducted with BWES from the two biochars (sugarcane bagasse and rice husks). The spore and sporangia germination for the two fungi was done using the different concentrations of BWES (0%, 0.5%, 1%, 3%, 10% and 20%). A solution of root exudates collected from bean plants (extraction has been described below) and sterile water were used as the positive and negative control respectively. A volume of 200 µL of BWES was added to 200 µL of fungal spore suspension adjusted to 1 x 10^7 spores' ml^{-1} containing both macro and micro conidia of *F. solani* and sporangia of *P. ultimum* on SM+L discs were used for the assay. These were incubated at 25°C in a moist chamber. The experiment was conducted twice with five replicates. After 24 h, lactophenol cotton blue and 0.03% acid fuchsin in 85% lactic acid were used to stain the conidia and sporangia so as to determine the germination microscopically by counting 200 microconidia per slide (Steinkellner *et al.*, 2008) and 200 sporangia on SM+L discs. A conidium/sporangium was considered germinated if the germ tube is visible under the microscope. The spore/sporangial germination/inhibition was calculated using the formula given below:

$$\text{Percent Spore Germination} = \frac{\text{Number of spore germinated}}{\text{Totoal number of spores examined}} \text{ X } 100$$

$$\text{Percent inhibition in spore germination} = \frac{\text{SG}}{\text{SG} - \text{TSG}} \text{ X } 100$$

Where, SG = number of spores germinated in the positive control

TSG = total number of spores germinated in treatment

6.4.3 Effect of water extracts of vermicompost and biochar on mycelial growth of root rot pathogens

Four wells were cut in PDA media using a 5 mm diameter cork borer before introduction of 1ml of biochar water extracts and vermicompost water extracts into two opposite 5mm wells in separate plates. One milliliter of sterile distilled water which served as the negative control was introduced into the other two wells on each plate. A disc measuring 5mm cut from a 5-day old culture of *F. solani, P. ultimum* and *R. solani* were placed at center of PDA plates separately. Evaluation of the plates was done after incubation of the five replicates for 7 days at 25℃. Vermicompost and biochar extracts filtered using 0.2µm pore Syringe filters were also evaluated for their effects on mycelium growth of *F. solani, P. ultimum* and *R. solani* based on the method by Szczech, (1999) with minor modifications where biochar and vermicompost extracts were introduced into 5mm cored wells on the PDA as opposed to addition flooding the surface of the media with the extracts. The effect of water extracts was determined by the presence of an inhibition zone around the wells infused with the extracts. Diameters of the zones of inhibition were measured using a vernier caliper.

6.4.4 Effect of time of maturation of biochars on fungal growth

Biochars produced from sugarcane bagasse and rice husks were used together with sand at a ratio of 1:1 w/w for this assay translating to 2.5grams of biochar mixed with 2 grams of sand. Biochars produced as described above were left to mature for 5 days, 10 days and 15 days at room temperature in glass jars under sterile conditions. Sand was wet sieved to 0.5 to 1 mm diameter, oven dried at 65°C for 24 h, then autoclaved at 121°C for 30 min at 15psi. Five treatments were set up as outlined below to test both *F. solani* and *P. ultimum* for inhibition or promotion of mycelial growth when exposed to biochar. Treatments of biochar; biochar moistened with 5mL of water; biochar mixed with sand; biochar and sand moistened; sand; moistened sand and PDA. These treatments were used for each batch of biochar exposed for varying period of time to air. All the treatments were replicated three times. Five grams of biochar was placed on a petri dish lid for the biochar and sand individual treatments while 2.5 grams of sand and 2.5g of biochar for biochar and sand treatments. Five millimeter agar plugs from vigorously growing 3 to 5 d-old-cultures of *F. solani* and *P. ultimum* were inoculated on petri plates with PDA media. Each inoculated plate was inverted and incubated for 8 days at a temperature of 25°C in sterile incubation boxes. The germinating cultures were observed and the diameters of the developing cultures were measured every 2 days from the day of plating

up to the 8th day. Completely randomised design was used for the experiment which was run twice with three replications.

6.5 Effect of filtering bean seed and root exudates through biochar and vermicompost on root rot pathogen spore germination

6.5.1 Extraction of bean seed and root exudates

Rosecoco bean seeds were used to extract seed exudates based on the method by Tambalo *et al.* (2014) with minor modifications. Seeds were sorted and only those with no visible cracks utilized for the experiment. A total of eighty seeds were weighed separately in four batches of twenty. Each batch of seeds was surface sterilized in 2.5% Sodium hypochlorite for 3 min followed by washing with 70% ethanol for 3 min. The seeds were rinsed with sterile Nano pure water 3 times.

To each set of twenty (20) surface sterilised seeds in a 100mL conical flask, sterile Nano pure water was added at a ratio of 2 mL g^{-1} of seed. These were then shaken on a rotary shaker (120 rpm) at a temperature of 22°C±2 for 8 hours. Seed exudates from different batches were then collected separately. The seeds were then transferred into 150 cm^3 sterile 2mm acid washed glass beads at 60% moisture content for an additional 48 h before being removed from the glass bead matrix. Harvesting of the root exudates was carried out by rinsing with 400mL of sterile Nano pure water, and strained through 4 layers of sterile cheesecloth or whatman filter paper No.1 and then lyophilized. The lyophilized exudates were weighed, reconstituted in 15 mL sterile water then filtered using a 2 µm with sterile cellulose acetate syringe filters and relyophilised for a second time. The resulting powder was stored at -80°C and reconstituted prior to use in the bioassays described below.

Spore germination was conducted as described in section 6.4.2 of thesis using filtrates from the biochars and vermicompost with water, the seed and root exudates being the control.

6.5.2 Adsorption of bean seed and root exudates by biochar and vermicompost

Adsorption of bean seed and root exudates by biochar was done by filling 25 g of each biochar and vermicompost as substrates into separate 12 cm diameter Buchner funnels. Sterile distilled water was added to the substrates in the funnels up to field capacity and the excess water let to drain for 2 hours. Bean seed exudates were then added to the moist substrates in the funnels at a volume of 50 mL. The biochars and vermicompost were then covered with a perforated

parafilm to create moist chamber conditions in the funnel. These were then incubated for 12 h. The substrates were then flooded with sterile distilled water and left to sit for 15 min after which the funnels were drained. The water collected was strained through whatman filter paper No.1 and lyophilized. The bean seed and root exudates were assessed separately for presence of phytochemicals in the fresh exudates and in the filtrate collected after straining the exudate through biochar and vermicompost to determine presence of alkaloids, flavonoids, fatty acids, phenols and amino acids.

Alkaloids were tested by mixing 2mL of each test sample with 1mL dilute hydrochloric acid and 1mL Mayer's reagent. The formation of a white precipitate indicated the presence of alkaloids. Presence of flavonoids was tested by mixing 2mL of the extracts with magnesium granules (100 mg) in 0.5 mL of concentrated HCl. Presence of the flavonoids was denoted by the appearance of a red colour within two minutes of mixing all the reagents (Markham, 1982). The exudates were tested for phenols by adding a few drops of ferric chloride solution to 2 mL to the test solution. A bluish green or red colour indicated the presence of phenols (Kardong *et al.,* 2013).

6.6 Data collection and analysis

Data on inhibition of fungal growth (mm) and spore germination was collected ten days after incubation using a vernier caliper and counting microscope respectively. Dry weight of water extracts of biochar and vermicompost as well as that of seed and root exudates was measured after their lyophilisation. Counts on pathogen spore and sporangial germination was recorded at intervals of 12 hours. The presence or absence of phytochemicals in the seed and root exudates was determined by the colour change in the test samples. The data of all variables measured were averaged and the ANOVA ($P < 0.05$) conducted using GENTSTAT 15th Edition. Means for treatments were separated by LSD (Least Significant Difference) following Tukey multiple dispersal range.

6.7 Results

6.7.1 Antagonism of *Trichoderma harzianum* and *Penicillium* spp on root rot pathogens of common bean

There was a significant difference ($p<0.05$) in inhibition of mycelial growth of all the test fungi in dual culture with *Trichoderma harzianum* and *Penicillium* spp (Table 6.1; Plate 6.1, 6.3, 6.5, 6.7). The highest inhibition was recorded for *T. harzianum* against *F. oxysporum* while the lowest inhibition was observed for *Penicillium* spp against *R. solani*. Significant difference (p<.0.05) was observed in percent inhibition of *Trichoderma* spp against all the test pathogens. *T. harzianum* recorded the highest inhibition against *F. oxysporum* at 67.7% while the lowest inhibition by *T. harzianum* was recorded in *M. phaseolina* at 45.3%. Percent inhibition in *Penicillium* spp dual plates was highest on *M. phaseolina* at 39.9% and the lowest on *R. solani* at 35.8% though the differences were not significant (Table 6.1; Plate 6.1, 6.2, 6.3, 6.4, 6.5, and 6.6).

There was a significant difference ($p<0.05$) in the time taken for the antagonists to contact the pathogens in the dual plates. The shortest period to establish contact was recorded for *T. harzianum* against *R. solani* while the longest period was recorded for *Penicillium spp* against the two *Fusarium* spp (Table 6.2). *Penicillium* spp were observed to be locked with all the test pathogens upon contact after 10 days of incubation thereby being rated R4 according to the ranking by Bell for all the test pathogens (Table 6.2; Plate 6.1, 6.3 and 6.5). *T. harzianum* on the other hand was observed to overlap the test pathogens upon contact and achieved 75% overgrowth on *F. solani, F. oxysporum* and *R. solani* as a result being ranked R2. *T. harzianum* antagonism was however ranked R3 against *P. ultimum* when it was observed to achieve 50% overgrowth following 10 days of incubation. It was however observed to be locked upon contact with *M. phaseolina* and was rated as R4.

Observations made from the slide culture showed *Trichoderma harzianum* coiling around the mycelium of *Macrophomina phaseolina* and production of appresorium after five days of incubation (Plate 6.8). The disintegration of the pathogen was observed indicated by crumbling of the mycelial walls.

Table 6.1: Antagonism of *Trichoderma harzianum* and *Penicillium* spp on root rot pathogens of common bean

Pathogen	Radial mycelial growth (mm)			Percent inhibition (%)	
	Control	*T. h*	*P. s*	*T. h*	*P. s*
F. oxysporum	68.4b	22.1d	43.3b	67.7a	36.6a
F. solani	67.1b	31.8c	42.9b	52.7b	36.1a
M. phaseolina	66.6b	36.3b	41.8b	45.3c	39.9a
P. ultimum	85.9a	39.1a	52.1a	54.4b	39.4a
R. solani	79.3a	35.0b	49.4a	55.8b	35.8a
LSD	6.5	2.0	2.8	5.3	4.8
%CV	4.7	3.2	3.3	5.1	6.8
F.Pr	<.001	<.001	<.001	<.001	0.247

Means with same letter(s) within a column not are significantly different at $p \leq 0.05$. *T. h- Trichoderma harzianum, P. s- Penicillium* spp, LSD- Least significant difference at 5% level, CV: Coefficient of variation:

Table 6.2: Effect of *Trichoderma harzianum* and *Penicillium* spp on mycelial growth of bean root rot pathogens

Pathogen	Time taken to contact (days)		Time taken to overlap (days)		Bell's Ranking (Class)	
	T. h	*P. s*	*T. h*	*P. s*	*T. h*	*P. s*
F. oxysporum	3.3a	4.7a	4.3a	Loc	R2	R4
F. solani	3.3a	4.7a	4.3a	Loc	R2	R4
M. phaseolina	3.0a	0.0c	0.0c (Loc)	Loc	R4	R4
P. ultimum	2.3ab	3.3b	2.3b	Loc	R3	R4
R. solani	1.3b	3.7ab	3.7ab	Loc	R2	R4
LSD	1.3	1.0	1.1			
%CV	25.6	15.8	19.2			
F.Pr	0.031	<.001	<.001			

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. *T. h- Trichoderma harzianum, P. s- Penicillium* spp, Loc: Locked at point of contact; LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Plate 6.1: Dual plate culture of *F. solani* and *Penicillium* sp. on PDA

Plate 6.3: Dual plate culture of *Fusarium oxysporum* and *Pencillium* sp on PDA

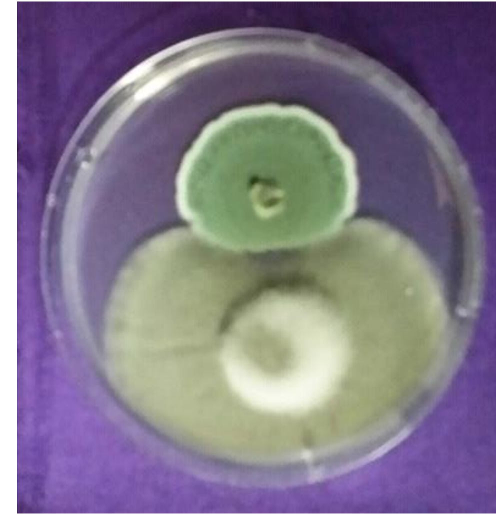

Plate 6.5: Dual plate culture of *Macrophomina phaseolina* and *Penicillium* spp on PDA

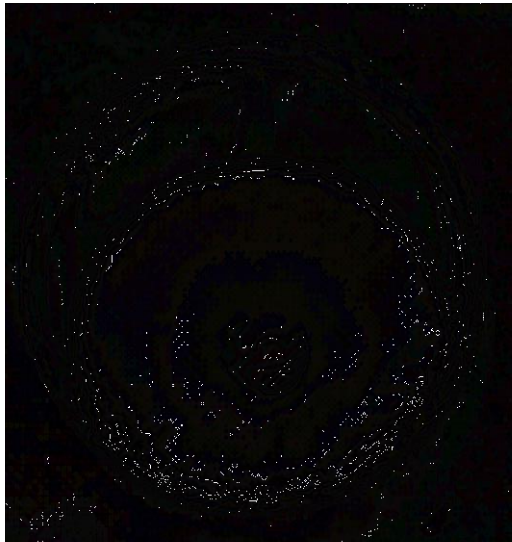

Plate 6.2: Mycelial growth of *Fusarium solani* on PDA (control)

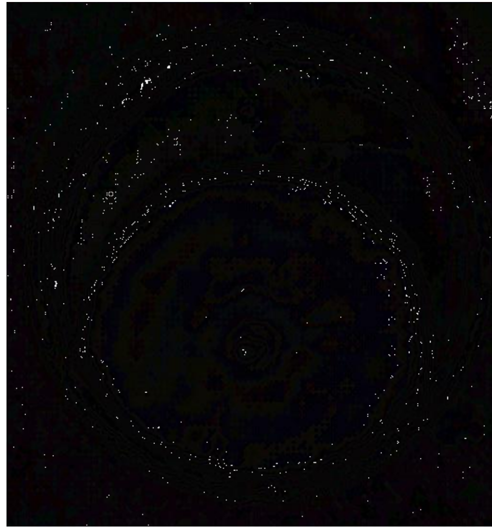

Plate 6.4: Mycelial growth of *Fusarium oxysporum* on PDA (control)

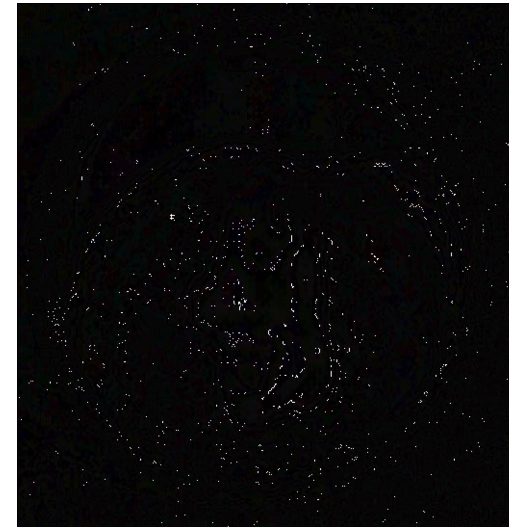

Plate 6.6: Mycelial growth of *Macrophomina. phaseolina* on PDA (control)

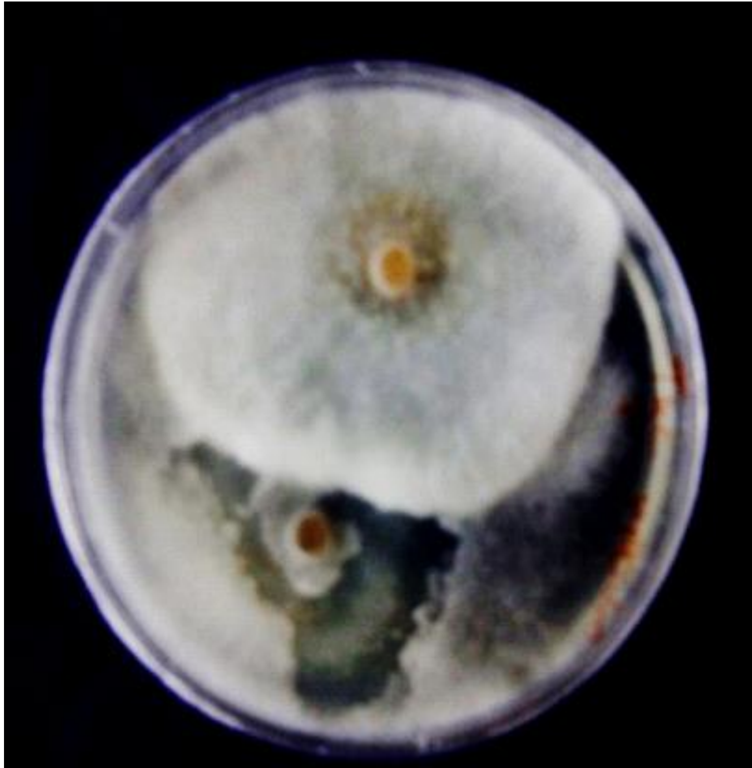

Plate 6.7: Dual plate culture of *P. ultimum* and *Trichoderma harzianum* (Magnification X100)

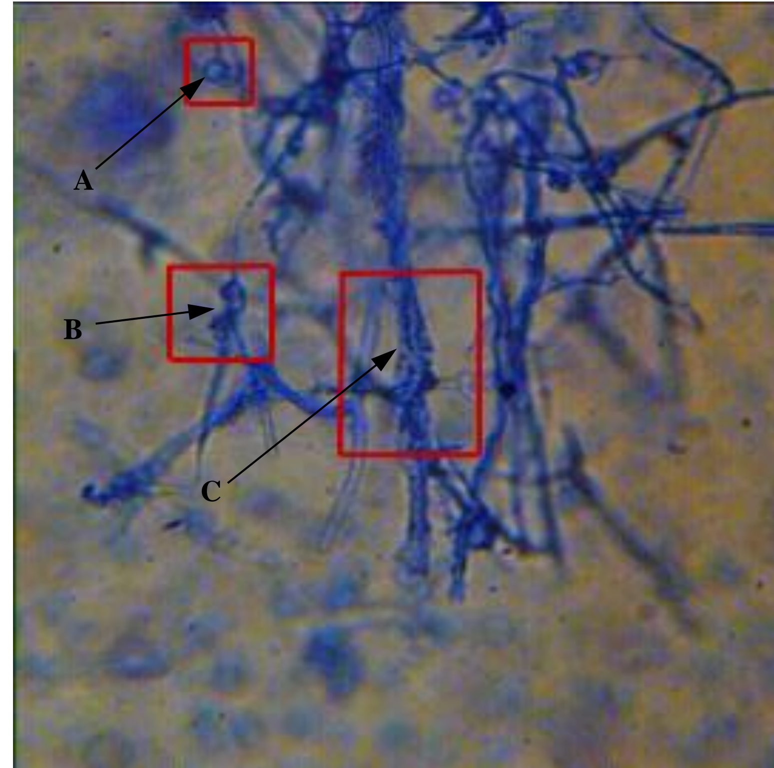

Plate 6.8: Mycelia of *Trichoderma harzianum* parasitising *M. phaseolina* A: Production of an appresorium by *Trichoderma harzianum* upon contact with *M. phaseolina*, B: *Trichoderma spp* mycelium twisting around *M. phaseolina* mycelium, C: Lysed mycelium of *M. phaseiolina* after parasitisation. (Magnification X100)

6.7.2 Quantity and pH of water-extracts from biochar and vermicompost

Significant differences ($p<0.05$) were observed in the water extractible substances from the organic amendments both in trial one and two (Table 6.3). Sugarcane bagasse biochar BWES and rice husks BWES had alkaline pH though the sugarcane bagasse was highest of the two. Vermicompost on the other hand had a near neutral pH. Concentration of water extractable substances was significantly different ($p<0.001$) across all the three samples. Vermicompost had the highest concentration in both experiment 1 and 2 translating to 35% higher than rice husks BWES and 20% higher than sugarcane bagasse BWES.

Table 6.3: Quantity, concentration and pH of water extracts of different biochars and vermicompost

Sample	Experiment 1			Experiment 2		
	pH	WES wt/ 250g (gms)	Conc. WES (gm/L)	pH	WES wt/ 250g (gms)	Conc. WES (gm/L)
Sugarcane bagasse biochar	9.33a	0.06b	0.64b	9.30a	0.07a	0.70b
Rice husks Biochar	8.47b	0.05c	0.52c	8.48b	0.05b	0.50c
Vermicompost	6.92c	0.08a	0.81a	6.84c	0.08a	0.80a
MEAN	**8.24**	**0.07**	**0.66**	**8.21**	**0.07**	**0.67**
LSD	0.16	0.01	0.08	0.18	0.01	0.08
%CV	1.6	9.7	9.7	1.8	10.2	10.2
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different letter(s) within each column are significantly different at $p \leq 0.05$. Conc. WES- concentration of water extractable substances, SB-sugarcane bagasse, RH-rice husks, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

6.7.3 Effect of water extracts of vermicompost and biochars on spore germination

There was a significant difference ($p<0.001$) in the germination of *F. solani* spores (Plate 6.9) and *Pythium ultimum* sporangia (Plate 6.10) across all the concentrations of water extractable substances and seed exudates (Table 6.4). Spores of *F. solani* incubated in seed exudates recorded the highest germination percentage in seed exudates (SE). The highest was achieved in 20% concentration of SE. Significant inhibition of *F. solani* spores was observed in biochar water extractible substances (BWES) across all concentration gradients. Highest inhibition was observed in 20% rice husks BWES where 100% inhibition was achieved. However these differences in germination inhibition of *F. solani* spores in rice husks BWES were not significant from sugarcane bagasse BWES and vermicompost water extract. Similar observations were made for *F. solani* spore inhibition during the second experiment (Table 6.4).

Significant differences ($p<0.05$) were also observed in sporangial germination of *P. ultimum* germination during the first experiment (Table 6.4). *Pythium ultimum* sporangia incubated in seed exudates had the highest germination which was more than 80% higher than other treatments. Root exudates on the other hand resulted in 50% higher germination of *P. ultimum* sporangia than water, vermicompost and biochar WES. The greatest inhibition in sporangia germination was observed in the rice husks BWES. It represented an almost 100% inhibition in 20% concentration of rice husks BWES. This was however not significantly different from sugarcane bagasse BWES and vermicompost WES which also inhibited sporangia germination significantly ($p<0.05$) at all the concentration gradients. Similar observations were made in the repeat experiments where biochar and vermicompost WES significantly ($p<0.05$) inhibited *F. solani* spore and *P. ultimum* sporangia germination (Table 6.4). Consequently, seed exudates did enhance the germination of the spores and sporangia significantly ($p<0.05$) in all the concentrations by between 57% and 79%.

Table 6.4: Effect of different water extracts of biochars, vermicompost and bean root and seed exudates on spore germination of *F. solani* and *P. ultimum*

Treatment	Experiment 1						Experiment 2					
	F. solani spores			*P. ultimum* sporangia			*F. solani spores*			*P. ultimum sporangia*		
Conc.	3%	10%	20%	3%	10%	20%	3%	10%	20%	3%	10%	20%
R. husks biochar WES	1.0d	0.3d	0.0d	0.4d	0.3d	0.1d	1.3c	0.5c	0.2d	0.9c	0.7d	0.6d
Root exudates	18.9b	29.9b	36.4b	13.1b	24.9b	34.3b	16.3b	27.4b	34.3b	12.7b	26.9b	35.2b
S. bagasse biochar WES	0.2d	0.1d	0.1d	0.9d	0.4d	0.2d	2.9bc	1.0c	1.2d	1.7c	0.9d	0.4d
Seed exudate	53.8a	59.2a	69.5a	68.6a	72.1a	84.3a	56.3a	63.2a	78.2a	63.2a	70.1a	83.5a
Vermicompost WES	1.7d	0.8d	0.1d	1.0d	0.9d	0.3d	2.1c	0.6c	0.4d	1.1c	0.4d	0.2d
Water	8.2c	7.5c	8.0c	6.5c	6.1c	6.1c	16.7b	16.5b	16.1c	12.7b	12.0c	11.6c
LSD	4.5	3.5	6.0	5.0	5.0	5.2	13.9	14.2	14.1	9.7	8.8	10.3
%CV	24.8	16.4	24.2	25.2	22.1	19.1	66.9	60.0	49.5	48.3	36.3	35.9
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. Conc. WES- concentration of water extractable substances, S bagasse WES-sugarcane bagasse biochar water extractable substances, R husks WES-rice husks biochar water extractable substances, Vermicompost WES- Vermicompost water extractable substances, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

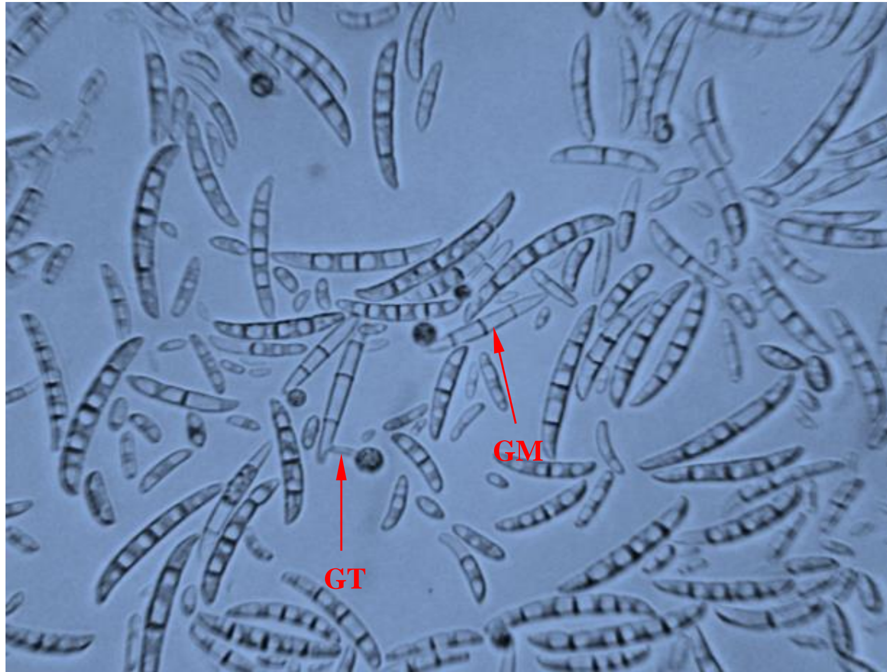

Plate 6.9: Germinating Macro conidia of *Fusarium solani* in seed exudates X100
GM: Germinating macro-conidium; GT: germ tube of *F. solani*

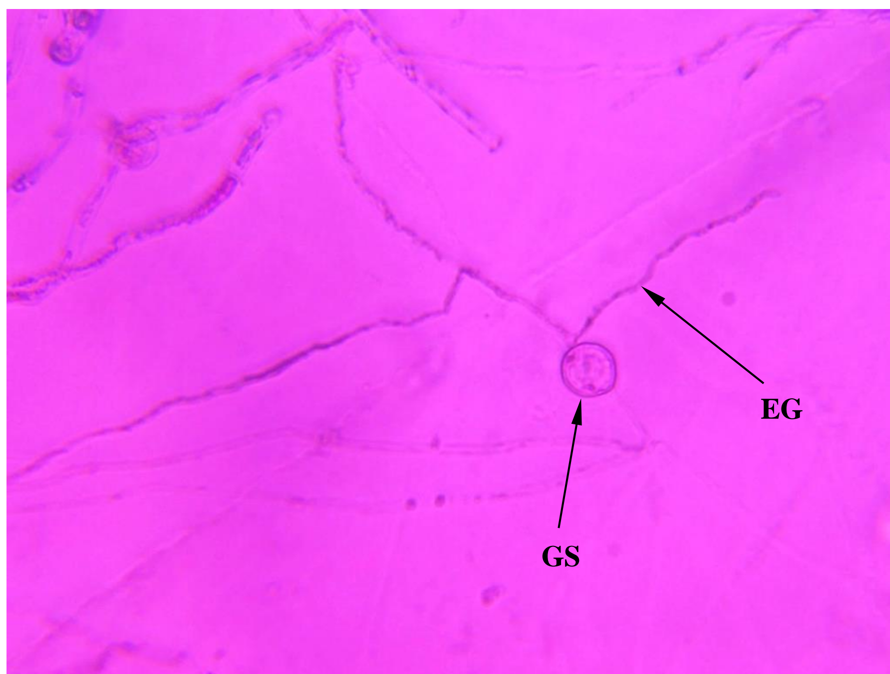

Plate 6.10: Sporangia of *Pythium ultimum* germinating in seed exudate X100
GS: Germinating sporangium of *P. ultimum*; EG: Elongating germ tube of *P. ultimum*

6.7.4 Effect of water extracts of vermicompost and biochars on mycelial growth of root rot pathogens

Sterile vermicompost extract did not show any inhibition of fungal growth in *R. solani* and *F. solani* (Plate 6.11). There was also no inhibition in mycelium growth of *F. solani, P. ultimum* and *R. solani* on plates with wells infused with sugarcane bagasse BWES. Consequently, rice husks BWES did not cause any inhibition of growth on the fungal colonies of *F. solani* and *R. solani* at different concentrations.

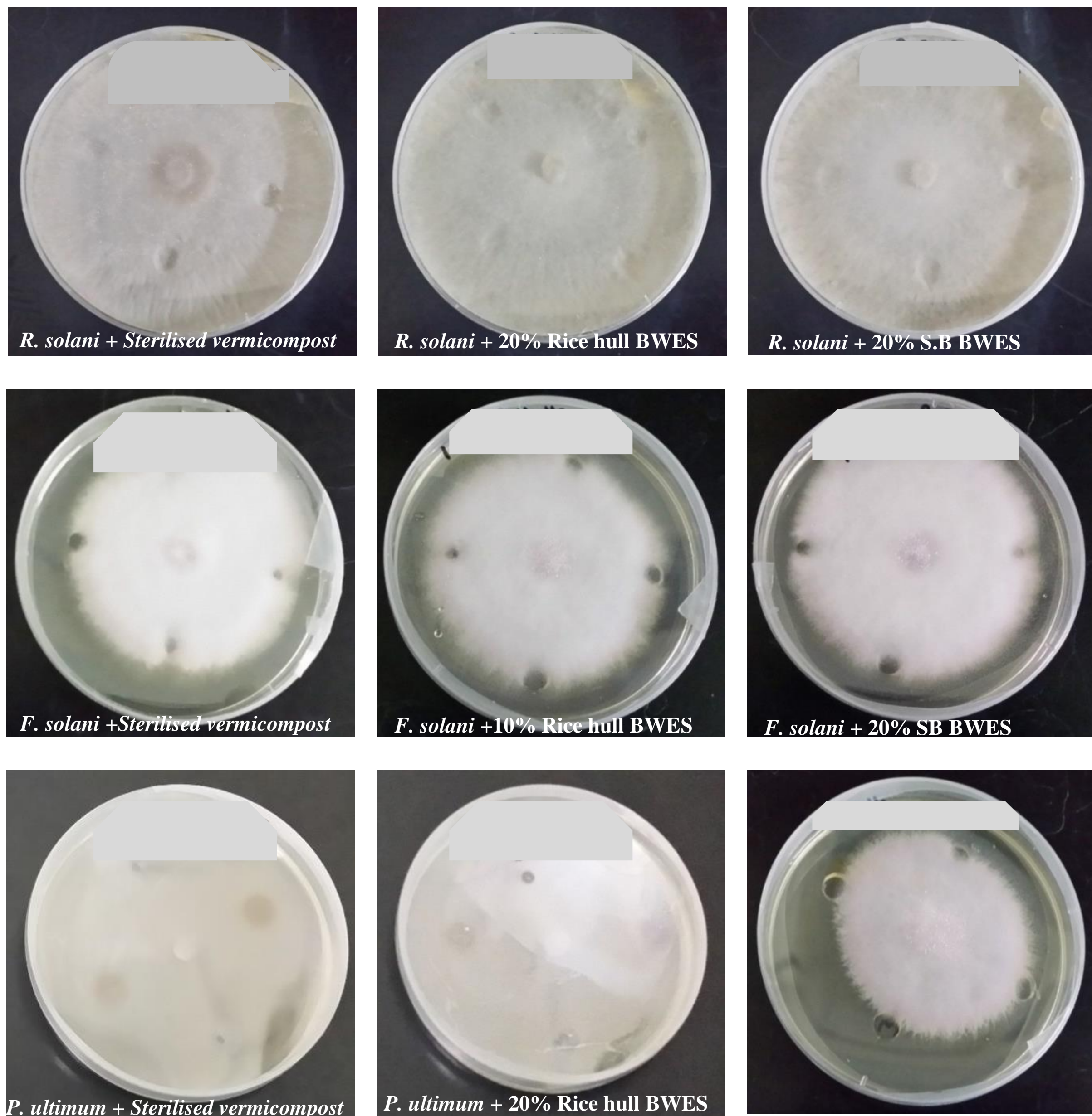

Plate 6.11: Growth of fungal mycelium on PDA with biochar and vermicompost water extractible substances.

6.7.5 Effect of time of maturation of sugarcane bagasse biochar on fungal growth

Colony growth of *F. solani* and *P. ultimum* were affected when grown inverted above biochar exposed to air over different periods of time after production. There was a significant difference ($p<0.05$) in the growth of *F. solani* across all the treatments of sugarcane bagasse (SB) biochar (Table 6.5). The SB biochar exposed to air for 15 days and slightly moistened had the highest colony growth on the second day after incubation. The lowest growth at the same period was recorded in SB biochar exposed to air for 5 days that was moistened representing a 26% inhibition of growth. After eight days of incubation, the greatest inhibition of *F. solani* growth was observed in SB biochar exposed to air for 5 days as compared to the one exposed for 15 days with the differences being significant ($p<0.05$). Similar observations were made in the second experiment where the 5 day exposed biochar resulted in a 14% inhibition of *F. solani* growth (Table 6.6).

There was a significant difference ($p<0.001$) in growth of *P. ultimum* colonies over the eight day period when incubated inverted over plates with SB biochar (Table 6.5). The greatest inhibition was observed in SB biochar exposed to air for 5 days. This represented a 12% inhibition of *P. ultimum.* Biochar exposed to air for 15 days resulted in the lowest inhibition of 1% which was not significantly different from the control. Similar observations were made in the repeat experiments where 15% inhibition of *P. ultimum* growth was observed in freshly produced SB biochar (Table 6.6).

Table 6.5: Effect of time of maturation of sugarcane bagasse biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 1

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
PDA (Control)	13.4def	31.2abc	43.0ab	53.6a	16.6a	34.3a	53.9a	78.8a
Sand	13.4def	32.2a	44.1a	52.3bcd	16.2ab	33.7ab	53.6a	77.4ab
Sand + W	13.3def	31.8ab	43.6a	52.4bc	15.7ab	33.5abc	53.7a	78.2a
Sugarcane bagasse biochar fpd	12.5f	27.4fg	38.3g	48.9g	10.3f	24.5ij	44.4de	69.0d
Sugarcane bagasse biochar +W fpd	13.7def	27.2g	38.4g	49.6fg	11.7def	25.1hij	44.7d	70.2d
Sugarcane bagasse biochar +S fpd	12.3g	27.2g	40.6de	51.3de	11.9def	25.9ghi	42.1e	70.4cd
Sugarcane bagasse biochar +S+W fpd	12.0g	29.5cde	40.2def	51.4cde	13.4cd	23.8j	43.7d	68.6d
Sugarcane bagasse biochar 10d	13.3def	27.6fg	38.8fg	49.4fg	10.6f	26.3fgh	48.0b	74.1bc
Sugarcane bagasse biochar +W 10d	15.6ab	29.5cde	40.0efg	49.6fg	13.2cd	30.8d	45.6cd	76.3ab
Sugarcane bagasse biochar +S 10d	12.9efg	29.0def	41.3cde	52.2bcd	12.7cde	30.6d	45.7bcd	76.5ab
Sugarcane bagasse biochar +S+W 10d	14.1cd	30.1bcd	41.3cde	51.8bcd	14.4bc	27.4efg	45.4cd	77.5ab
Sugarcane bagasse biochar 15d	14.6c	28.3efg	39.7efg	49.9fg	11.2ef	27.7ef	52.6a	77.3ab
Sugarcane bagasse biochar +W 15d	16.4a	30.0cde	41.2cde	50.4ef	13.5cd	32.0bcd	48.0b	77.1ab
Sugarcane bagasse biochar +S 15d	14.7bc	30.1bcd	42.6abc	52.4bc	14.2c	31.8cd	47.4bc	78.1a
Sugarcane bagasse biochar +S+W 15d	14.9bc	30.9abc	41.7bcd	52.6ab	15.7ab	28.1e	47.1bc	77.3ab
LSD	0.9	1.7	1.6	1.0	1.9	1.7	2.3	3.8
%CV	3.9	3.4	2.3	1.2	8.4	3.5	2.9	3.1
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with same letter(s) within a column are significantly different at $p \leq 0.05$. fpd-freshly produced biochar, 10d- exposed to air for 10 days after production, 15d exposed to air for 15 days after production, +S- addition of sand, +W- addition of water, PDA- potato dextrose agar, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 6.6: Effect of time of maturation of sugarcane bagasse biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 2

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
PDA (Control)	18.3abc	31.9a	43.2a	55.0a	17.6a	36.7a	52.3ab	76.2a
Sand	18.5ab	31.6a	42.1ab	51.2bc	18.5a	31.6bcd	52.4ab	75.8ab
Sand +W	18.6a	29.8abc	42.0ab	51.4bc	18.6a	29.8cde	53.0a	75.4abc
Sugarcane bagasse biochar fpd	13.5gh	27.1bc	39.3de	48.9de	11.2e	22.8gh	46.4d	69.3fg
Sugarcane bagasse biochar +W fpd	14.1g	26.9c	39.7cd	50.3cd	12.2de	26.1efg	45.4de	69.3fg
Sugarcane bagasse biochar +S fpd	12.7h	29.5abc	39.9cd	47.3e	12.9cd	24.8fgh	37.0g	67.9g
Sugarcane bagasse biochar +S+W fpd	13.7gh	27.9bc	37.8e	47.0e	13.1cd	22.1h	37.7fg	64.2h
Sugarcane bagasse biochar 10d	14.9efg	27.8bc	40.3bcd	50.4bcd	12.6de	28.9de	48.3bcd	73.0cde
Sugarcane bagasse biochar +W 10d	14.9efg	28.2bc	40.4bcd	51.2bc	13.4cd	30.0cd	48.0cd	72.3de
Sugarcane bagasse biochar +S 10d	15.9de	29.0bc	40.9bcd	50.5bcd	13.7cd	28.5def	38.7fg	70.5ef
Sugarcane bagasse biochar +S+W 10d	14.8fg	30.1ab	41.3bc	50.4bcd	14.3bc	26.1efg	39.4fg	70.6ef
Sugarcane bagasse biochar 15d	15.8def	28.3bc	42.1ab	51.6bc	13.8cd	34.7ab	51.9abc	75.9ab
Sugarcane bagasse biochar +W 15d	16.4de	29.7abc	41.8ab	52.4b	14.3bc	33.4abc	51.1abc	74.7abcd
Sugarcane bagasse biochar +S 15d	17.1abc	29.4abc	41.9ab	51.1bc	15.6b	30.1cd	41.4ef	73.4bcd
Sugarcane bagasse biochar +S+W 15d	16.9cd	27.6bc	41.8ab	51.6bc	15.9b	29.3de	41.1fg	72.6de
LSD	1.5	3.0	1.8	2.0	1.6	3.7	4.1	2.5
%CV	5.9	6.2	2.7	2.4	6.5	7.8	5.4	2.1
F pr.	<0.001	0.045	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different letter(s) within each column are significantly different at $p \leq 0.05$. fpd- freshly produced biochar, 10d- exposed to air for 10 days after production, 15d exposed to air for 15 days after production, + S- addition of sand, + W- addition of water, PDA- potato dextrose agar, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

6.7.6 Effect of time of maturation rice husks biochar on growth of *Fusarium solani* and *Pythium ultimum*

Significant differences ($p<0.05$) in growth of *F. solani* and *P. ultimum* were also observed in the trials with rice husks (RH) biochar (Table 6.7). Significant difference ($p<0.05$) in the diameter of *F. solani* colonies was observed across all the treatments with the exception of the fourth day after incubation. At the sixth day of growth, the highest growth was recorded in plates incubated over sand while the lowest was recorded in plates incubated over freshly produced RH biochar. Upon termination of the experiment on the eighth day, the lowest growth was recorded in freshly produced RH biochar while colonies incubated over RH biochar exposed for 15 days recorded the highest growth. This translated to a growth inhibition of 19%. Similar observations were made in the repeat experiment where significant differences ($p<0.05$) were observed in *F. solani* colonies across all treatments (Table 6.10). *Fusarium solani* colonies that were incubated inverted over freshly produced RH biochar had the least growth. The highest growth of *F. solani* colony was recorded in RH biochar that had been exposed to air for 15 days. The reduction reflects an 18% growth inhibition.

Significant differences ($p<0.05$) were also recorded in the growth of *P. ultimum* colonies across all treatments (Table 6.7). Control plates with PDA recorded the highest growth across the 8 day period with diameters ranging from 16.5mm to 78.8mm. These were not significantly different from the growth of colonies incubated over RH biochar exposed for 15 days to air. The least growth was however recorded in plates inverted over RH biochar exposed to air for 5days, being significantly different ($p<0.05$) from those grown over RH biochar exposed for 10 days and 15 days. This indicated a growth inhibition of 12% for colonies incubated over freshly produced RH biochar. Similar observations were made during the follow up experiments where significant differences ($p<0.05$) were recorded across all the treatments (Table 6.8). The lowest growth was recorded for plates incubated over freshly produced RH biochar while those incubated over RH biochar exposed for 15 days had the highest diameter. This difference in growth represents an inhibition of 33%.

Table 6.7: Effect of time of maturation rice husks biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 1

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	DAY2	**DAY4**	**DAY6**	**DAY8**	**DAY2**	**DAY4**	**DAY6**	**DAY8**
PDA (control)	13.4	31.2	43.0ab	53.6ab	16.6a	34.3a	53.9a	78.8a
Sand	13.4	32.2	44.1a	52.3bcd	16.2a	33.7a	53.6a	77.4ab
Sand + W	13.3	31.8	43.6a	52.4bcd	15.7ab	33.5a	53.7a	78.2a
Rice husks biochar fpd	12.1	24.7	32.0g	43.9g	10.8de	27.0e	45.9cde	71.3cd
Rice husks biochar +W fpd	12.0	28.4	35.5f	45.2g	12.1cde	27.1e	44.5e	70.8cd
Rice husks biochar + Sand fpd	12.6	27.0	39.3e	50.9cdef	10.5e	24.6f	43.7e	71.3cd
Rice husks biochar + Sand +W fpd	12.3	26.5	39.4e	51.8bcde	11.3cde	23.6f	42.7e	68.8d
Rice husks biochar 10d	13.0	28.8	40.7bcde	48.9f	12.7bc	28.9cd	48.9bc	73.6bc
Rice husks biochar +W 10d	12.4	26.3	40.5cde	49.6ef	13.0bc	28.6de	51.9ab	73.7bc
Rice husks biochar + Sand 10d	13.0	30.5	41.2bcde	52.2bcd	12.4bcd	28.3de	44.8e	74.7abc
Rice husks biochar + Sand +W 10d	12.7	29.1	40.2de	52.0bcd	12.6bc	30.5bc	44.9e	73.2bc
Rice husks biochar 15d	13.2	29.2	41.2bcde	50.2def	13.0bc	30.8bc	52.2ab	77.3ab
Rice husks biochar +W 15d	12.8	28.5	41.0bcde	52.0bcd	14.0b	30.5bc	52.7a	76.2ab
Rice husks biochar + Sand 15d	13.3	31.2	42.8abc	54.7a	13.0bc	29.6cd	45.3de	76.9ab
Rice husks biochar + Sand +W 15d	13.2	31.0	41.9abcd	53.1abc	12.9bc	31.8b	48.4cd	75.7ab
LSD	NS	NS	2.3	2.2	1.7	1.7	3.4	4.2
%CV	4.7	13.2	3.5	2.5	7.7	3.4	4.3	3.4
F pr.	0.1	0.469	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. fpd- freshly produce biochar, 10d- exposed to air for 10-days after production, 15d- exposed to air for 15 days after production, +W- addition of water, PDA- potato dextrose agar, NS: No significant difference, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 6.8: Effect of time of maturation rice husks biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 2

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	DAY2	**DAY4**	**DAY6**	**DAY8**	**DAY2**	**DAY4**	**DAY6**	**DAY8**
PDA (Control)	18.3a	31.9a	43.2a	55.0a	17.6ab	36.7a	52.3a	76.2a
Sand	18.5a	31.6a	42.1ab	51.2bcd	18.5ab	31.6bcd	52.4a	75.8a
Sand +W	18.6a	29.8ab	42.0ab	51.4bcd	18.6a	29.8cde	53.0a	75.4ab
Rice husks biochar fpd	12.1c	24.9d	37.0ef	44.6h	10.4i	27.7def	36.4g	67.0g
Rice husks biochar +W fpd	12.0c	26.7bcd	36.2f	46.9g	12.1fghi	27.4efg	37.0fg	69.6ef
Rice husks biochar +Sand fpd	12.3c	26.4bcd	39.6bcde	48.9ef	11.2hi	21.9h	39.4ef	59.9h
Rice husks biochar +Sand +W fpd	12.3c	25.2cd	37.4def	46.7g	11.3ghi	21.5h	40.5e	50.5i
Rice husks biochar 10d	13.8c	29.5ab	39.9bcd	50.2de	12.1fghi	29.9cde	43.9d	72.9bcd
Rice husks biochar +W 10d	12.5c	28.3abcd	38.8cde	48.3fg	13.1efg	29.6cde	43.6d	72.5cd
Rice husks biochar +Sand 10d	13.8c	29.3abc	41.6a	50.8cd	13.4def	24.6fgh	43.4d	66.0g
Rice husks biochar +Sand +W 10d	13.1c	28.1abcd	40.2bc	50.6de	13.0efgh	23.6gh	43.3d	67.1fg
Rice husks biochar 15d	14.2bc	30.9a	41.0abc	52.5bc	14.0de	31.8bc	48.9b	75.2ab
Rice husks biochar +W 15d	14.4bc	30.2ab	40.6abc	51.4bcd	15.2cd	34.1ab	47.4bc	75.0abc
Rice husks biochar +Sand 15d	16.3ab	31.0a	41.9ab	53.0b	16.7bc	29.6cde	45.6cd	68.9ef
Rice husks biochar +Sand +W 15d	13.4c	29.6ab	42.1ab	51.4bcd	16.9abc	28.2cdef	48.4b	71.1de
LSD	2.4	4.1	2.7	1.8	1.8	3.9	2.6	2.6
%CV	9.9	8.5	4.0	2.1	7.7	8.2	3.5	2.2
F pr.	<0.001	0.018	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different letter(s) within each column are significantly different at $p \leq 0.05$. fpd- freshly produced biochar, 10d- exposed to air for 10 days after production, 15d exposed to air for 15 days after production, +S- addition of sand, +W- addition of water, PDA- potato dextrose agar, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

6.7.7 Adsorption of bean seed exudates by biochar and vermicompost

Quantities of seed and root exudates varied amongst the different seed weights and seed sets of Rose coco beans though the differences were not significant (Table 6.9). The highest yield was recorded as 7.8mg of seed exudate and 6.7 mg of root exudates per gram seed though the difference was not significant($p<0.05$). Phytochemical analysis of the seed exudates indicated high concentration of flavonoids and phenols in bean seed exudates (Table 6.10; Plate 6:13; Plate 6.14). Only phenols were detected in the root exudates. Other phytochemicals such as alkaloids, fatty acids and amino acids were not detected in both the seed and root exudates (Plate 6.12; Plate 6.15). Upon passing the exudates through moistened biochar and vermicompost, there was no detection of flavonoids and phenols for exudates passed through SB biochar and RH biochar. However phenols were detected in root exudates passed through vermicompost (Table 6.10; Plate 6.14). These results indicated that the phytochemicals had not passed through the substrates (Table 6.10).

Table 6.9: Quantities of seed and root exudates of different batches of Rosecoco bean seeds

Seed batch	**Weight of seed (g)**	**Liquid seed exudate (mL)**	**Lyophilized seed exudate (g)**	**Liquid root exudates mL**	**Lyophilized root exudate (g)**
1	9.32a	9.90a	0.06a	18.06a	0.06a
2	8.99a	9.40a	0.07a	18.18a	0.06a
3	8.02a	8.83a	0.05a	15.55a	0.05a
4	9.03a	9.63a	0.06a	17.24a	0.06a
LSD	2.29	1.69	0.03	4.28	0.05
%CV	8.1	5.6	15.8	7.8	26.9
F pr.	0.433	0.382	0.314	0.349	0.858

Means with the same letter(s) within each column are not significantly different at $p \leq 0.05$.
LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 6.10: Different phytochemicals in bean seed and root exudates

Treatment	Alkaloids	Flavonoids	Phenols	Fatty acids	Amino acids
Seed exudate (SE)	-	++	++	-	-
Root exudates (RE)	-	-	+	-	-
Water	-	-	-	-	-
SE through S. bagasse	-	-	-	-	-
SE through R. husks	-	-	-	-	-
SE through Vermi	-	-	-	-	-
RE through S. bagasse	-	-	-	-	-
RE through R. husks	-	-	-	-	-
RE through Vermicompost	-	-	+	-	-

= Absent, + =Present, ++ Present in high concentration

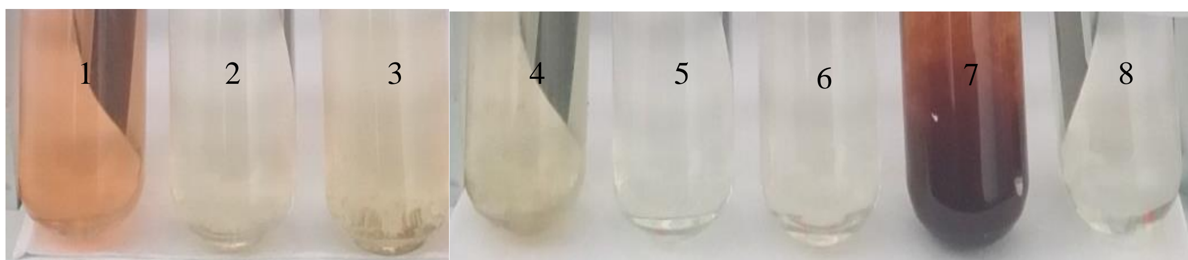

Plate 6.12: Presence of alkaloids in bean seed and root exudates

1- fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3- seed exudates passed through sugarcane bagasse biochar; 4- fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; 8- blank

Plate 6.13: Presence of flavonoids in bean seed and root exudates

1- fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3- seed exudates passed through sugarcane bagasse biochar; 4- fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; 8- blank

Plate 6.14:Presence of phenols in bean seed and root exudates

1- fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3- seed exudates passed through sugarcane bagasse biochar; 4- fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; 8- water

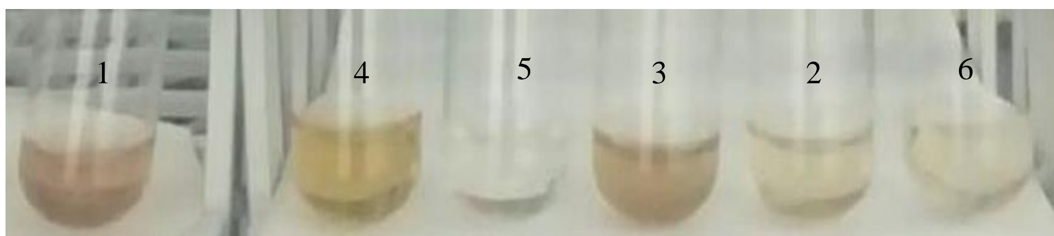

Plate 6.15: Presence of amino acids in bean seed and root exudates

1- fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3- seed exudates passed through sugarcane bagasse biochar; 4- fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar

6.7.8 Effect of filtered bean seed exudates through biochar and vermicompost on spore germination of root rot pathogens

Significant differences ($p<0.05$) were observed in conidial and sporangial germination of *F. solani* and *P. ultimum* respectively in the root and seed exudates filtered through the SB biochar, RH biochar and vermicompost (Table 6.11). The highest percent spore germination was observed in *F. solani* incubated in fresh seed exudates while the lowest was recorded in vermicompost strained seed exudates. There was a 96% to 99% and 49% to 97% inhibition of germination *F. solani* for seed and for root exudates respectively. Similar observations were made in the repeat experiment.

Germination of sporangia of *P. ultimum* in seed exudates recorded the highest percentage which was significantly different ($p<0.05$) from other treatments (Table 6.12). Filtering the exudates through vermicompost and biochar resulted in significant inhibition of germination. Highest inhibition to germination was observed in seed exudates filtered through vermicompost at 99% inhibition. This was however not significantly different from filtering through the two biochars which resulted in a 97% inhibition of sporangial germination. Filtering of exudates resulted in a germination inhibition of between 50% and 80%, the greatest inhibition being observed in seed exudates strained through vermicompost. Similar observations were in the repeat experiments (Table 6.11).

Table 6.11: Effect of filtering bean seed and root exudates through vermicompost, sugarcane bagasse and rice husks biochars on spore germination of *F. solani* and *P. ultimum*

Treatment	Experiment 1 (% germination)		Experiment 2 (% germination)	
	F. solani spores (%)	*P. ultimum* sporangia (%)	*F. solani* spores (%)	*P. ultimum* sporangia (%)
Seed Exudate (control)	53.8a	68.6a	56.3a	63.2a
Seed Exudate through vermicompost	0.3d	0.5d	0.4c	0.6c
Seed Exudate through RH biochar	1.9d	2.0d	1.0c	1.5c
Seed Exudate through SB biochar	1.3d	1.4d	1.0c	0.8c
Root Exudates (control)	18.9b	13.1b	16.3b	12.7b
Root Exudates through vermicompost	9.6c	6.6c	11.4b	8.5b
Root Exudates through RH biochar	0.4d	1.5d	0.5c	0.9c
Root Exudates through SB biochar	0.5d	1.1d	0.6c	0.8c
LSD	7.9	8.4	12.3	9.5
%CV	28.1	27.6	43.6	33
Fpr	<0.001	<0.001	<0.001	<0.001

Means with different letter(s) within each column are significantly different at $p \leq 0.05$. % - percent germination, SB Biochar-sugarcane bagasse biochar, RH Biochar -rice husks biochar, LSD: Least significance difference at 5% level, CV: Coefficient of variation.

6.8 Discussion

6.8.1 Effect of *in vitro* antagonism of *Trichoderma* and *Penicillium* spp on root rot fungi of common bean

The increase in number of soil beneficial microorganisms following application of biochar and vermicompost in this study contributed to the decline in population of pathogenic fungi. The microorganisms that were observed to increase in number included *Trichoderma, Penicillium, Aspergillus, Paeciliomyces.* and *Athrobotrys* spp. The decline was probably due to competition for space as well as the production of toxic metabolites including antibiotics by the beneficial microorganisms.

In vitro inhibition of the bean root rot fungi by antagonistic *Trichoderma spp* and *Penicillium spp* in dual culture pointed to different mechanisms for the two antagonists. These include production of fungal growth inhibitory substance(s) by the *Penicillium spp* (Sreevidya and Gopalakrishnan, 2016) and mycoparasitism and direct competition by *Trichoderma* spp (El-Sheshtawi *et al.,* 2014). Growth inhibitory substances produced by the antagonists may diffuse through the media affecting growth and sporulation of the pathogen. This was observed in the plates with *Penicillium* spp which reduced the growth of *F. solani, F. Oxysporum, P. ultimum, M. phaseolina* and *R. solani*. This resulted in a 35 to 40% growth inhibition of all the five test pathogens. This has been observed in previous studies by Graber *et al.* (2014) and Kolton *et al.* (2011) who reported on soil microorganisms that are promoted by biochar additions and thrive on biochar residues. They observed that these microorganisms compete with pathogens for resources, produce compounds that are toxic to pathogens, or parasitize pathogens. Graber *et al.* (2010) also observed that beneficial microorganisms promoted by biochar enhance plant growth directly, thus affecting plant susceptibility or resistance to disease. The findings of this study are similar to previous findings by Sreevidya and Gopalakrishnan, (2016) reported on the control of *Macrophomina phaseolina* in sorghum using *Penicillium citrinum.* They observed that citrinin which is produced by many species of *Penicillium* was responsible for growth inhibition of *M. phaseolina*. Other studies by Melouk and Akem, (1987) reported on the antagonistic activity of citrinin against soil borne plant pathogenic fungi such as *Rhizoctonia solani.*

Other mechanisms such as mycoparasitism and competition were also observed in this study and may be responsible for pathogen growth inhibition by the antagonists. The observed interaction between the mycelia of *Trichoderma* spp and *M. phaseolina* gives credence to

mycoparasitism mechanism. In this study, *Trichoderma* spp mycelium was observed twisting around the mycelia of *M. phaseolina.* It was also observed to grow over test pathogens upon contact covering 75% of colony surfaces of *F. solani, F. oxysporum* and *R. solani*. Similar observations have been made in previous studies by El-Sheshtawi *et al.* (2014) who reported 50% reduction of *F. solani* f.sp *cucurbitae* colony diameter by *Trichoderma* spp. Ahmad and Baker (1987) and Boughalleb *et al.* (2018) have also pointed to direct parasitism of *Trichoderma* on hyphae of other fungi. They observed that control of many soil borne plant pathogens occurs by production of extracellular lytic enzymes which degrade pathogen cell walls. Ramaraju *et al.* (2016) also reported on the mechanism of *Trichoderma* antagonism by coiling around fungal mycelia which they parasitise. They eventually produce penetration pegs that are haustoria like knobs which penetrate the host dissolving the protoplasm. This in turn may lead to shrinking of mycelia and eventual lysis thus achieving control.

6.8.2 Effect of biochar and vermicompost water-extracts on spore, sporangia and mycelial growth of the pathogenic fungi

Fusarium solani spores and *P. ultimum* sporangia germination were greatly inhibited by three concentrations of biochar and vermicompost water extracts as compared to seed exudates and root exudates. The water extractible substances reduced the germination of *F. solani* conidia and *P. ultimum* sporangia by more than 80%. The results are similar to those of Smith *et al.* (2016) who reported on growth inhibition of blue−green algae (*Synechococcus elongates*) and the eukaryotic alga (*Desmodesmus*) in biochar water extracts derived from pine wood biochar. Jack, (2012) also reported the inhibition of *Pythium aphanidermatum* zoospores in vermicompost extract. The antifungal potential of biochar could be due to the water soluble organic compounds found in biochar which can affect soil and aquatic microorganisms (Graber *et al.,* 2014; Fabbri *et al.,* 2012). Graber *et al.* (2010) identified these compounds known to adversely affect microbial growth and survival as ethylene glycol and propylene glycol, hydroxypropionic and hydroxybutyric acids, benzoic acid and *o*-cresol, quinones and 2-phenoxyethanol. Klinke *et al.* (2004) also reported of other biochar associated compounds known to inhibit microbial activity to include carboxylic acids, furans and ketones.

When the biochar and vermicompost water extractable substances were tested on mycelia growth using the well diffusion technique, no inhibition of growth was observed at all the three concentrations. This pointed to the fact that the biochar water extracts are effective in inhibiting spores and sporangia germination but not on the somatic growth of the pathogens.

Consequently, sterile vermicompost extract was also not found to have an effect on mycelia growth. Similar findings have been reported by Ersahin *et al.* (2009) on the loss of vermicomposts' inhibitory effect on mycelial growth following sterilization.

6.8.3 Effect of biochar maturation on spore germination and fungal colony growth

This study tested the effect of exposing biochar to air at staggered durations after production. This was then tested for its effect on colony growth of *F. solani* and *P. ultimum.* Radial growths of the microorganisms were significantly ($p<0.05$) reduced when they were incubated over biochar. Freshly produced sugarcane bagasse biochar slightly moistened recorded significantly lower radial growth of *P. ultimum* as did the freshly produced rice husks biochar on growth of *F. solani* colony. This was in comparison to biochar exposed to air for 10 days and 15 days. When the freshly produced rice husks biochar was mixed with sand and slightly moistened it also significantly reduced radial growth of *P. ultimum.* Similar findings were observed in the repeat experiments. These findings point to production of compounds biochar that have inhibitory effects on fungal colony growth. This study showed that the effectiveness of these compounds wanes with prolonged exposure of biochar after production. No previous findings have reported on this phenomenon of the effect on fungal pathogens in biochar. However previous studies by Ghidotti *et al.* (2016) profiled the volatile organic compounds in biochar and reported of some harmful compounds such as benzene, ethylbenzene, xylenes and polycyclic aromatic hydrocarbons present in biochar. They reported on the compound not being released at ambient temperature when biochar is produced at temperatures greater than 400ºC. These volatile organic compounds were however observed not be harmful to cress seed germination.

6.8.4 Biochar adsorption of bean seed and root exudates

This study established adsorption of phytochemicals such as flavonoids and phenols present in seed exudates by vermicompost, rice husks and sugarcane bagasse biochar. This was achieved by straining seed exudates and root exudates through pre moistened biochar. When the rinsate was passed through biochars and vermicompost negative results for all the phytochemicals tested were obtained save for phenols which were positive in rinsate through vermicompost.

When the rinsate were tested for their effect on germination of *Pythium* sporangia and *Fusarium* conidia, it was observed to be greatly reduced as compared to that of fresh seed and root exudates. These findings point to the fact that the pathogen germination signaling

molecules of *Pythium* and *Fusarium* were not present in the rinsates. These results give credence to suppositions by Masiello *et al.* (2015) and Graber *et al.* (2014) of fractional adsorption of signaling molecules by biochar. This in turn modifies the communication among soil biota leading to disease suppression by biochar. It is also important to note that biochar may have possibly changed the chemistry of the root exudates resulting in reduced spore and sporangia germination. According to findings by Silber *et al.* (2010), increased adsorption may be linked to the cation exchange capacity (CEC) of the biochar surface.

In this study, flavonoids and phenols were observed to be adsorbed by biochar and vermicompost. This is of importance since flavonoids and phenols have been shown to be efficient anti-microbial agents which inhibit several root pathogens, especially fungal ones (Makoi *et al.,* 2007). Importance of flavonoids in development of plant resistance to pathogenic microorganisms has been previously stated by Mierziak *et al.* (2014). They reported that these molecules' anti pathogenic activities can be nonspecific but result from their antioxidative properties. Dai *et al.* (1996) and Blount *et a.l.* (1992) also observed that these molecules lead to quenching of reactive oxygen species generated by both the pathogens and plants as a result of infection leading to control of disease.

By biochar and vermicompost exhibiting adsorption capabilities of the phytochemicals including flavonoids and phenols, points to enhanced concentration of these phytochemicals around the germinating plants. These may in turn enhance the protection of the plants both in a specific and a nonspecific manner. Previous studies by Beckman, (2000) and Skadhauge *et al.* (1997) have also reported that flavonoids may contribute to the tightening of the plant tissues and structures by controlling the activity of auxin (IAA) in plants. This may lead to the differentiation of tissues and promotion of tylose, callus formation and closure of vascular system preventing pathogen infection. They also reported on other mechanisms such as inhibition of pathogen xylanases, pectinases and cellulases. Other studies by Truetter, (2005) have shown that these phytochemical compounds may directly inhibit pathogen enzymes that digest plant cell wall. This they do by chelating metals that are required for the enzyme activity. Previous studies by Matern and Kneusel, (1988) suggested that the rapid accumulation of phenols at infection site serves to slow and halt the growth of pathogens. This therefore acts as the first line of plant defense thereby allowing the plant to activate secondary mechanisms of pathogen control.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

This study found bean root rot fungal pathogens to be occurring in all the farms and agro ecological zones of western Kenya. Six main root rot pathogens were identified to be occurring in Western Kenya based on cultural and morphological characteristics as well as using molecular techniques. They include *Fusarium solani, Fusarium oxysporum, Pythium ultimum, Pythium irregulare, Rhizoctonia solani* and *Macrophomina phaseolina*. This confirmed the importance of these root rot fungi in Western Kenya as earlier reported by Otsyula *et al.* (1998). However, root rot pathogen populations varied greatly based on the AEZs where UM3 and LM2 had the highest populations. These agro ecological zones are characterized by temperature and rainfall characteristics which result in moderate soil moisture in the farms. *Fusarium spp* had the highest isolation frequency in all the AEZ's. In the humid zones (LM1 and UM1), *Pythium spp* was the second highest in frequency of isolation whereas in the sub humid (LM2) and semi humid (UM3) zones, *Rhizoctonia sp.* was second highest followed by *Pythium sp.* The findings confirm previous studies by Otsyula *et al.* (1997) and Medvecky *et al.* (2007), who observed that *Fusarium* and *Pythium* were some of most important fungal pathogens causing reduction of bean yields in Western Kenya. Naseri, (2014) also reported *Fusarium spp* to be a major root rot pathogen at moderate soil moistures, hot weather, acidic and poorly fertilized soil conditions.

In this study, root rot fungal populations were observed to be influenced by soil type. This is due to the significant ($p<0.05$) positive correlation between sand, *P. ultimum* DNA and *R. solani* DNA. The correlation was however significantly negative between sand and *M. phaseolina* DNA. The results confirm previous findings by Gill *et al.* (2000) who observed that *R. solani* grew more rapidly in well-aerated soil than in moist soil with limited aeration. Blair, (1943) also observed that *R. solani* was more aggressive in nutrient deficient sand. There was also a significant ($p<0.05$) negative correlation between clay content and populations of *Fusarium spp* in this study. Similar observation was also made between clay and *R. solani* DNA. The findings concur with earlier experiments by Naseri, (2014) who observed high levels of *F. solani* in soils having high silt and sand content.

This study also observed a positive significant ($p<0.05$) correlation in the population of *Pythium* spp., *Fusarium* spp., *and Rhizoctonia* spp. This pointed to the fact that the pathogens

operate synergistically to enhance root rot in the soils. Other studies by Paparu *et al.* (2017) and Abawi and Pastor Corrales, (1990) have also reported of a synergistic interaction between *Fusarium solani* f.sp *phaseoli* and *Pythium ultimum* resulting in higher damage to plants than when each pathogen acts alone.

Beneficial microorganisms isolated from the soil included *Aspergillus* spp *Penicillium* spp and *Trichoderma* spp prior to application of amendments. They however increased to include *Paecilomyces lillacinus* and *Athrobotrys spp* in the subsequent seasons. *Aspergillus spp* was the highest followed by *Penicillium spp* while *Trichoderma spp* was the least isolated. Lower midland humid (LM1) had the highest populations of the beneficial microorganisms while LM2 had the lowest populations. The findings are similar to Okoth *et al.* (2009) and Sun *et al.* (2012) who reported that soil moisture and carbon promote growth and populations of *Trichoderma.*

Quantitative PCR was found to be an important tool that can be used for rapid detection of pathogen DNA in a soil sample. It was observed to reflect similar findings as the conventional quantification methods. This can in turn be used to quantify the pathogen propagules with specificity and rapidly hence necessitating management to be undertaken early enough. This has previously been reported by Lievens *et al.* (2006) who observed that it was difficult to accurately distinguish target pathogens from non-target pathogens in naturally infested soils using the plating techniques on semi-selective medium. They however found that there was a high correlation between calculated DNA and inoculum density of *F. solani* and *R. solani* in artificially infested soils. This demonstrates how the technique can substantially quantify occurrence of pathogens in complex samples.

In this study, the application of individual treatments and combination of biochar and vermicompost positively influenced plant emergence. Combination of the treatments had the highest emergence both as an immediate effect as well as a residual effect. These findings confirm previous findings by Ievinsh *et al.* (2017) and Solaiman *et al.* (2011) who reported increase in germination of hemp and mung bean seeds following application of vermicompost and biochar separately. Findings from this study showed slight improvement in germination from the combination of the amendments in comparison to individual applications. They were however not significantly different from the individual treatment applications. Agro ecological zones also influenced plant emergence with the highest emergence being observed in LM1 and

UM1. This can be attributed to the distribution of the rainfall during the growing period and was lowest in these zones at the time of planting.

Findings from this study showed that root rot disease incidence was greatly reduced following application of biochar and vermicompost. Disease incidence was reduced by 60% immediately after the application of the soil amendments. In the subsequent season where no amendments were applied, a 40% reduction in disease incidence was recorded. This pointed to the residual effect of the treatments in management of root rot. It is also worth noting that treatment combinations of biochar and vermicompost resulted in the lowest disease incidence as compared to individual treatment applications. This finding points to a synergistic effect of the amendments while also confirming the importance of individual treatment applications. Shoaf, (2014) reported on synergistic effect of biochar and vermicompost treatments when applied as a combination. This was observed when studying their impact on crop productivity in vegetable cropping systems. Findings of this study also brought to light the importance of phosphorus in root rot disease management. This was evident in the subsequent seasons when, disease incidence was observed to be higher in the plots where no inorganic phosphate fertiliser had been applied. The findings from this study confirm Yamato *et al.* (2006) previous findings which showed that biochars antifungal potential was due to its important properties among them increased nutrient retention, increased soil cation exchange capacity and effects on phosphorus. They also confirm Prabhu *et al.* (2007) findings that phosphorus nutrition improves crop vigour and may decrease severity of diseases through new growth.

Application of soil amendments greatly reduced the root rot severity by as much as 60% across all seasons and growth stages in this study. During the following season when no amendments were added, disease severity was observed to have reduced by 30%. This further confirmed the presence of residual effect of the amendments in root rot management. Treatment combinations of biochar and vermicompost also reflected a synergistic effect by having the lowest disease severity. This was however not significantly different from standalone applications of vermicompost and biochar. The finding thus shed light on the importance of individual amendments in management of root rot disease as well as their combinations. The findings corroborate other findings by Jaiswal *et al.* (2014) who also reported reduction in damping off disease caused by *Rhizoctonia solani* in cucumber and beans following addition of 0.5% wt/wt of greenhouse waste biochar. Other findings by Jack, (2012) also reported disease suppression in cucumber caused by *Pythium aphanidermatum* following vermicompost extract application.

Soil amendments were also observed to have an impact on fungal populations where root rot pathogens were greatly reduced in this study. These findings point to the fact that the amendments of biochar and vermicompost prevent development of inoculum resulting to reduced disease incidence and severity. The reduction of fungal populations detrimental to common bean growth can be attributed to toxic effects of chemical compounds in the residual tars found on biochar. This has been previously attributed to by Graber *et al.* (2010) who identified a number of biochar compounds that are known to adversely affect microbial growth and survival. The compounds include ethylene glycol and propylene glycol, hydroxy-propionic and butyric acids, benzoic acid and o-cresol, quinones and 2-phe¬noxyethanol. At the same time the soil amendments of vermicompost and biochar were found to result in the increase of *Penicillium* and *Aspergillus* spp as well as *Trichoderma, Paeciliomyces* and *Athrobotrys* spp. The finding confirms Laborda *et al.* (1999) previous observation that *Trichoderma* and *Penicillium* spp contribute to depolymerisation of coal via production of enzymes such as Mn-peroxidase and phenoloxidase.

Grain yield and 100 seed weight were also significantly affected by application of biochar and vermicompost. Grain yield was higher in vermicompost and sympal fertiliser treatments in the two long rain seasons and one short rains season. This study also showed an increase in yield when biochar was combined with fertiliser than individual application of biochar or Sympal fertiliser. Similar results have been reported earlier by Liang *et al.* (2014) and Oram *et al.* (2014) who reported of improved yield following application of biochar and organic/inorganic fertilisers together. This was attributed to an increase in nutrient resource to plants. Liard *et al*. (2010) on the other hand demonstrated heightened nutrient preservation in soils amended with biochar. This explains why biochar stand-alone treatments posted low yields which were only higher than the control treatments without application of inorganic fertiliser.

In subsequent studies for comparison of biochars from different feedstock's, it was observed that sugarcane bagasse biochar which had high fixed carbon of 62% recorded lower disease severity compared to rice husks biochar and vermicompost. These findings suggest that biochars ability to influence soil fungal pathogens is related to recalcitrant forms of carbon which was earlier suggested by Graber *et al.* (2010). Similar findings on effect of fixed carbon on disease severity have also been observed by Jaiswal *et al.* (2014). The study consequently observed that sugarcane bagasse biochar and rice husks biochar significantly ($p<0.05$) influenced plant height both in control and in challenged plants. It was however observed that there was reduced sensitivity of plant growth parameters to biochar dose in the absence of the

disease causing pathogen than when the plants had been challenged by introduction of pathogens. Similar observations were previously reported by Jaiswal *et al.* (2014) who observed reduced sensitivity of plant growth parameters to biochar dose in the absence of the disease causing pathogen than when it was present.

This study observed that water extractable substances from biochar and vermicompost greatly inhibited *F. solani* spore and *P. ultimum* sporangia germination. Previous studies by Jack, (2012) also reported the inhibition of *Pythium aphanidermatum* zoospores germination in vermicompost extract. Graber *et al.* (2014) and Fabbri *et al.* (2012) attributed the antifungal potential of biochar to the water soluble organic compounds which can affect soil and aquatic microorganisms. This study also established that biochars' efficacy to suppress microbial growth is greatly affected by exposure to air. It was observed that biochar that had been freshly produced had greater inhibitory effects to growth of fungi as compared to the one exposed for 15 days.

Biochar and vermicompost were also observed to adsorb phytochemicals from seed and root exudates. Flavonoids and phenols present in seed and root exudates were adsorbed on to the rice husks and sugarcane bagasse biochars as well as vermicompost. The rinsate passed through the biochars posted negative results for flavonoids and phenols whereas vermicompost was negative for flavonoids but was positive for phenols. Germination of *Pythium* sporangia and *Fusarium* conidia in the rinsate was greatly inhibited. These findings point to the fact that the signaling molecules were not present in the rinsate. This gives credence to suppositions by Masiello *et al*. (2015) and Graber *et al.* (2014) of fractional adsorption of signaling molecules hence modifying the communication among soil biota as being one of the mechanisms of disease suppression by biochar and vermicompost.

7.2 Conclusions

This study confirmed that bean root rot is caused by all the four pathogens *Fusarium spp, Pythium spp, Rhizoctonia spp* and *Macrophomina spp.* They form important bean root rot complexes in western Kenya. Of all the four pathogens, *Fusarium spp.* occurs in high populations in the bean growing areas of western Kenya.

Applications of biochar and vermicompost greatly inhibited the growth of root rot fungi. They also inhibit the spore germination and growth of root rot pathogens hence protecting the plants from pathogenic attack. The soil amendments therefore have the potential to suppress soil

borne pathogenic microorganisms directly and also induce multiplication of resistant microbial communities that are beneficial to plant growth and also suppress pathogens in the soil environment. The addition of amendments as a combination or standalone treatments resulted in reduction of incidence and severity of root rot. This in turn led to increased common bean productivity.

A combination of biochar and vermicompost had a better effect on soil properties, which can explain its effects on plant growth and grain yield. These amendments increase soil pH, electrical conductivity and extractable phosphate. However, since biochar does not provide nutrients to the soil, it is important to incorporate a source of nutrient supplement in soils that are deficient. A combination of biochar and vermicompost can improve bean yield with the environmental benefits of improving soil nutrient status.

Production of biochar meant for use in management of soil borne disease should be in tandem with the growing season so as to avoid storing it over a long period of time. Prolonged storage leads to reduction of its effectiveness against pathogen development.

7.3 Recommendations

1. Soil amendment, biochar, should be used in combination with nutrient providing amendments such as vermicompost so as to reap maximum benefit from the amendments. This is because biochar is usually nutrient deficient but will tend to adsorb nutrients and make them available to the plant over long periods of time.
2. Application of biochar should be done in moist soils since relative moisture is required to dissolve the water extractable substances occurring on biochar that were observed to inhibit sporangia and spore germination.
3. Sustainable mechanisms for production of biochar and vermicompost should be developed at farm level. This will enable the farmer's utilize organic waste from their farms in a sustainable manner and reduce field infections.
4. The tonnage of biochar application of 2000 tons ha^{-1}used in this study may be bulky to produce and labour intensive to apply in large areas therefore spot application of biochar may be used.
5. Future studies should consider a chemical check against biochar and vermicompost treatments as well as in combination. This will enable the development of an integrated control of root rot encompassing all the strategies.

6. Capacity building for farmers on the importance of utilising resources available on their farms in soil health management. This will build up soil organic matter; improve soil fertility and mange soil borne pathogens on their farms by utilising organic wastes.

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APPENDICES

Appendix I: Semi structured Questionnaire on farming systems in the study area

Survey code: ____________________ Date of Interview: ____________________

Enumerator: ____________________ County: ____________________

Sub county: ____________________ Division: ____________________

Location: ____________________ Village: ____________________

Farm code: ____________________ Altitude: ____________________

Latitude: ____________________ Longitude: ____________________

Name of Farmer: ______________________________ Gender: M ( ) F ( )

Acreage/ Size of Farm: (Acres/ Ha) ______________________________

Duration of Land Use [Years]: … **[1]** <5 **[2]** 5-10 **[3]** 11-15 **[4]** 16-20 **[5]** 21-25 **[6]** 26-30 **[7]** 31>

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

Others Specify 1 ……………………………………………………………………

2……………………………………………………………………

No. of Household Members……………………….

Main Source of house hold income …….. [1] Sale of farm products [2] Permanent Employment [3] Business (specify) [4] Donations [5] Casual labour

Highest Level of Education for the Household Head *[Tick appropriate check box]*

[1] = Illiterate **[2]** = Finished Primary **[3]** = Finished Secondary **[4]** = Finished Tertiary **[5]** = Finished University

Source of labour …… [1] Family labour [2] Hired labor

If hired labour, how many people per week …… [1] 1-3 [2] 4-6 [3] 7-10 [4] >10

Is any house hold spouse a member of a group ……. [1] Farmer group [2] Women group [3] CBO [4] Other (specify).

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

Last crop grown	Acreage	Production Period
1.		
2.		
3.		

Type of Cropping System Practiced and Crops Grown on the Farm

Block e.g. 1	Number of Plots e.g. 1, 2	Cropping System (Code A)	Main Crop			Intercropped Crops		
			Crop	Season	Yield (Code B)	Crop	Season	Yield (Code B)
				S1			S1	
							S2	
				S2			S1	
							S2	
				S1			S1	
							S2	
				S2			S1	
							S2	
				S1			S1	
							S2	
				S2			S1	
							S2	

CODE A
1 = Monocropping
2 = Intercropping
3 = Relay Cropping
4 = Strip Cropping
5 = Rotational Cropping
6 = Others (Specify)

CODE B
One 90kg Sack without cobs = 90Kg
One Debe/Gorogoro = 15Kg

CODE A	CODE B	Code C
1 = DAP 2 = NPK 3 =CAN 4 = Foliar feed 5 =Urea 6 = Cow manure 7 = Chicken manure 8 = Lime 9 = Other (Specify)	***Dry compost*** 1 = Debe – 6kg 2 = 90kg sack or a wheel barrow – 30 kg 3 = Oxcart – 150 kg 4 = Pick up – 450kg ***Green/Wet Compost*** 5 = Debe – 7kg 6 = 90 kg sack or wheel barrow – 35kg 7 = Oxcart – 175 kg 8 = Pick up – 525kg	1 = Per Season 2 = Once Annually

If **NO**/ don't apply manure, Why (***Tick Appropriately***)

[1] = Lack of Livestock [**2**] **=** Lack of Capital to Buy [**3**] **=** Beliefs and Culture [**4**] **=** Lack of Information **[5] =** Others (Specify) ………………………………………………

General Farm Practices

Do you Practice Crop Rotation [1] Yes **[2]** No

If Yes Why? ……………………………………………………..

How Often do you Rotate [1] Per Season **[2]** Annually **[3]** Other………………

How long have you Practiced Crop Rotation (Y*ears*)…………………

[1] <5 Years **[2**] 5-10 Years **[3]** > 10 Years

If NO, why don't you Rotate Your Crops *(Tick Appropriately)*

[1] Size of Farm **[2]** Limited Crop Diversity **[3]** Lack of Information **[4]** Others (Specify)

1 ………………………………………………………

2 ……………………………………………………………..

3…………………………….. .. ………………………………..

Methods of cultivation indicate …….. [1] Hand tillage [2] Oxen Plough [3] Machine tillage [4] Slash & burn [5] Other (Specify)

Do you Apply Farm Amendments to your Farm [1] *Yes* **[2]** *No* …………..

If Yes, which one(s) and why (specify) ……………………………………

Reason 1 ……………………………………………………

Reason 2 ……………………………………………………….

TYPE OF AMENDMENT (Code A)	QUANTITY (Kg) (Code B)		HOW OFTEN (Code C)
	Season 1	Season 2	

Pest and Disease Management Practices:

Crops (Code A)	Pest/disease (Code B)	Identification Methods	Crop stage it Occurs (Code C)	% Loss	Management Practices(Code D)	Information Source (Code E)

Other Specify ………………………………………………………………………………

…………………………………………………………………………………………

CODE A	Code B	Code C	Code D	Code E
1 = Maize	1 = Stem Borer	1= Germination	1= Cultural	1.Ministry of Agriculture
2 = Sorghum	2 = Termites	2= 2-3 weeks	2= Chemical	2.Agro-chemical Agent
3 = Beans	3 = Cutworms	3= Vegetative	3= Biological	3..Non-Govermental Organisations
4 = Pigeon Peas	4 = Aphids	4= Flowering stage	4= None	4. Family/Friends
5 = Brassicas	5 = Whiteflies			5. Neighbours
6 = Cassava	6 = Thrips			6. Radio
7 = Fruits	7 = Leaf miner			7.Newspapers, Leaflets
8 = Cucurbits	8 = Leaf spots			8. Exchange Visits
9 = Sweet potato	9 = Wilting			9.Field Demonstration
10 = Irish Potato	10 = Pod spots			10. Agricultural Shows
11= Millet	11 = Bean fly			11. Internet
12= Other	12 = Nematodes			12. Other (Specify)
	13 = Weeds			

Constraints to farming/ crop production (Most important to the least)

……………………………………………………..

……………………………………………………

Appendix II: DNA Sequences of fungi isolated from soil and bean roots from Western Kenya

KU527803.2 *Fusarium oxysporum* isolate MC-22-F internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CCCAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGAC
CCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGAC
TCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGG
TAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAG
CATATCAATAAGCGGAGGAAA

MH161270.1 *Fusarium solani* strain T21 SAB5-f internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

TCGGCGGGAACAGACGGCCCTGTAACAACGGGCCGCCCCCGCCAGAGGACCCCTAACTCTGTTTTTATAATGTTTTTCTG
AGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCCTGTGGGCACACGC
CGTCCCTCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGG
CCATGCCGTAAAACACCCAACTTCTGAATGTTGAC

KR094457.1 *Fusarium equiseti* strain G388 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

ACTCCCAAACCCCTGTGAACATACCTATACGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAAAGGGACGGCCCGCCCG
AGGACCCCTAAACTCTGTTTTTAGTGGAACTTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGATCTCTT
GGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTGTT
GGGACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACACCTCGT
TACTGGTAATCGTCGCGGCCACGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA
CTTAAGCATATCAATAAGCGGAGGAAA

KR012878.1 *Macrophomina phaseolina* voucher CIAT519 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CGATCCTCCCACCCTTTGTATACCTACCTCTGTTGCTTTGGCGGGCCGCGGTCTTCCGCGGCCGCCCCCCGATTTTGGGG
GGTGGCTAGTGCCCGCCAGAGGACTATCAAACTCCAGTCAGTAAACGTTGCAGTCTGAAAAAAATATTAAATAAACTAAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAAC

CCTCAAGCTCTGCTTGGTATTGGGCACCGTCCTTTGCGGGCGCGCCTCAAAGACCTCGGCGGTGGCGTCTTGCCTCAAGC
GTAGTAGAATACACCTCGCTTCGGAGCGTAAGGCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

MH023384.1 *Pythium ultimum* isolate P2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

AGTCATCGAAATTTTGAACGCATATTGCACTTTCGGGTTATGCCTGGAAGTATGTCTGTATCAGTGTCCGTAAATCAAAC
TTGCCTTTCTTTTTCTGTGTAGTCAGGGATGGAATGTGCAGATGTGAAGTGTCTCGCATGGTTGCGTTCGTTTTTTCGAT
CGAGAATCTGTCGAGTCCTTTTAAATGGACACGGTCTTTTCTATGGTTTCTATGAAGTGTAATGGTTGGAAGGCAGTGAT
TTTCGGATTGCTGGCGGCTTTTGGCGACTTCGGTATGAACGTATGGAGACTAGCTCAATTCGTGGTATGTTAGGCTTCGG
CTCGACAATGTTGCGTAATTGTGTGTGGTCTTTGTTTGTGCCTTGAGGTGTACTAGAGGTTGTCGGTTTGAACCGTAAGT
GATTGTTTAGTAGAGCATTTTCACGATGTATGGAGACGCTGCATTTAGTTGCGTAGAGAGATTGATTTGGGAAATTTTGT
ATCATTGTCAATTGCAAGATTGTGTATGGTA

KP862949.1 *Pythium irregulare* isolate B1-19 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CTTTCCACGTGAACTGTCGTTATTTGTTGTGTGTGTGCGTGTTGGTAGCATGCGTGTTTGCTTACGCTTTGGTGTTTGCG
AGTGTGTGTGTTGTCGGTGCGCAGACTGAACGAAGGTCGTGTGTTGCTGTGTGCCTGCTGCACTGCTGACTTTGCATTGA
TTTGCATGGTGTTGGCGGAGCGGCGGGTGCTGTTGCGTGCGCGGCTGACCTATTTTTTTCAAACCCCATACCTAAATGAC
TGATTATACTGTGAGAACGAAAGTTCTTGCTTTTAACTAGATAACAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCG
ATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGAATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCA
CTTCCGGGTTATGCCTGGAAGTATGTCTGTATCAGTGTCCGTAAATCAAACTTGCGTTTCTTCCTTCCGTGTAGTCGGTG
GAGGAGAGTTGCAGATGTGAAGTGTCTCGCTGT

KT692550.1 *Rhizoctonia solani* strain V1E9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

TGCACGCCTGTCTCATCCACTCTCAACTTCTGTGCACTTTTCATAGGCCGGCTTGTGGGTGCGTTCGCGCACTTGTAGGT
GTCGGGCTTATGCTTTATTACAAACGATTCAGTTTTAGAATGTCATACTTTGCTATAACGCAATTTATATACAACTTTCA
GCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
TCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTCTCATGGAATTCTCAACC
TTCAGCTTTATTGATGAAGGCTTGGACTTGGAGGTCGTGCCGGCTCTCGTAGTCGGCTCCTCTGAAATGCATTAGTGCGA
ACGTTACCAG

KR012878.1 *Macrophomina phaseolina* voucher CIAT519 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CGATCCTCCCACCCTTTGTATACCTACCTCTGTTGCTTTGGCGGGCCGCGGTCTTCCGCGGCCGCCCCCCGATTTTGGGG
GGTGGCTAGTGCCCGCCAGAGGACTATCAAACTCCAGTCAGTAAACGTTGCAGTCTGAAAAAAATATTAAATAAACTAAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAAC
CCTCAAGCTCTGCTTGGTATTGGGCACCGTCCTTTGCGGGCGCGCCTCAAAGACCTCGGCGGTGGCGTCTTGCCTCAAGC
GTAGTAGAATACACCTCGCTTCGGAGCGTAAGGCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

EF411236.1 *Paecilomyces lilacinus* strain BCC 15610 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

AACCCACTGTGAACCTTACCTCAGTTGCCTCGGCGGGAACGCCCCGGCCGCCTGCCCCCGCGCCGGCGCCGGACCCAGGC
GCCCGCCGCAGGGACCCCAAACTCTCTTGCATTACGCCCAGCGGGCGGAATTTCTTCTCTGAGTTGCACAAGCAAAAACA
AATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGC
GTCATTTCAACCCTCGAGCCCCCCCGGGGGCCTCGGTGTTGGGGGACGGCACACCAGCCGCCCCCGAAATGCAGTGGCGA
CCCCGCCGCAGCCTCCCCTGCGTAGTAGCACACACCTCGCACCGGAGCGCGGAGGCGGTCACGCCGTAAAACGCCCAACT
TTCTTAGAGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

Appendix III: Leaching Buffer: For the production of sporangia of *Pythium* (1 L)

(Chen, D. W. and Zentmyer, G. A. (1970) — Production of sporangia by *Phytophthora cinnamomi* in axenic culture. Mycologia 62: 397–402.)

In to a 1 L media bottle add the following ingredients

1. $Ca(NO_3) \cdot 4H_2O$ (0.01M) 2.362g
2. $Mg\ SO_4 \cdot 7H_2O$ (0.004M) 0.9858g
3. KNO_3 (0.005M) 0.5058g

To these ingredients add 995ml Milli-Q Water

Adjust the solution to a pH of 5.8

Apendix IV: Weather Data for Kakamega; Bungoma and Busia for Year 2013 and 2014

Appendix IV (a) Data on Temperature, Relative Humidity and Rainfall for Kakamega, Bungoma and Busia the year 2013

AREA	**PARAMETER**	**MONTH (2013)**											
		JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
KAKAMEGA	Temp (ºC)	21.3	22.1	24.0	21.3	20.9	20.7	20.2	20.2	20.6	21.2	21.1	21.0
BUNGOMA	Temp (ºC)	24.5	21.7	22.4	21.1	20.8	20.1	19.9	20.1	20.5	21.0	20.8	20.7
BUSIA	Temp (ºC)	25.0	27.0	25.0	23.0	22.0	21.0	22.0	23.0	25.0	25.0	24.0	25.0
KAKAMEGA	Humidity (%)	49.0	40.0	65.0	68.0	59.0	59.0	53.0	58.0	59.0	63.0	63.0	55.0
BUNGOMA	Humidity (%)	49.3	44.4	53.5	65.0	63.3	65.1	60.6	64.3	62.6	59.4	58.0	54.2
BUSIA	Humidity (%)	63.0	54.0	68.0	82.0	84.0	83.0	75.0	80.0	77.0	77.0	78.0	73.0
KAKAMEGA	Rainfall (mm)	109.0	32.5	262.4	376.6	201.4	134.4	89.3	262.6	231.0	173.6	116.2	153.5
BUNGOMA	Rainfall (mm)	30.5	13.5	268.9	257.5	277.5	135.9	65.5	90.6	107.5	173.6	186.5	113.8
BUSIA	Rainfall (mm)	18.0	34.5	96.5	127.2	245.9	109.4	124.4	185.0	176.1	140.5	176.0	80.9

Appendix IV (b): Data on Temperature, Relative Humidity and Rainfall for Kakamega, Bungoma and Busia the year 2014

AREA	PARAMETER	MONTH (2014)											
		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Kakamega	Temp (ºC)	21.6	21.8	22.5	21.4	21.4	21.3	20.7	20.4	21.0	21.2	21.4	24.0
Bungoma	Temp (ºC)	21.5	21.5	22.2	21.7	21.0	20.4	20.0	19.8	20.3	20.8	21.0	20.9
Busia	Temp (ºC)	26.0	27.0	27.0	26.0	24.0	23.0	23.0	23.0	25.0	25.0	25.0	26.0
Kakamega	Humidity (%)	43.0	42.0	46.0	52.0	65.0	61.0	56.0	60.0	58.0	66.0	59.0	67.0
Bungoma	Humidity (%)	48.0	50.8	50.6	54.9	63.5	63.7	65.4	65.5	62.1	62.8	59.8	54.6
Busia	Humidity (%)	60.0	56.0	63.0	74.0	84.0	84.0	80.0	81.0	80.0	81.0	78.0	71.0
Kakamega	Rainfall (mm)	45.2	583.0	74.9	174.0	244.3	282.4	1293.0	317.4	211.9	164.1	197.2	46.5
Bungoma	Rainfall (mm)	62.8	48.8	199.6	74.9	446.1	108.9	143.8	90.0	178.2	210.5	138.6	31.0
Busia	Rainfall (mm)	38.0	52.2	130.8	147.5	298.8	209.7	174.0	266.0	259.0	274.9	185.9	87.2

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