SOIL BIOASSAY AS A DIAGNOSTIC TOOL IN THE DETECTION OF SOIL-BORNE PATHOGENS OF COMMON BEAN (*Phaseolus vulgaris*) IN WESTERN KENYA

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DECLARATION

Declaration by the Student

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DEDICATION

This work is dedicated to farmers, students, and all individuals related with research

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ABSTRACT

Lack of accurate and precise diagnostic tools and methods for detecting soil pathogens and their impacts on crop yields hinders the productivity of various crops, including beans. This highlights the need to link field disease severities to bioassay results to ease not only the accuracy in detection but also ensure the reliability of the predictions of crop performance. The study was set to evaluate the effectiveness of existing soil bioassays for screening, detecting, and quantifying soil-borne pathogens (*Fusarium spp., Pythium spp., and root-knot nematode*) and predicting impacts on grain yields and severity levels in smallholder fields. The study was conducted in 50 farmer fields in five counties across Western Kenya. Field demonstration trials consisting of four bean varieties planted on the 4 by 4 m treatment plots were established in each of the five sites. At planting, soil samples were collected for physical and biochemical analysis. A subset of the soil samples was bulked at the field level and assessed for bioassays targeting *Fusarium*, *Pythium*, and plant parasitic nematodes. The bean varieties were assessed for disease severities, and agronomic indicators recorded. The study employed descriptive statistics, correlation analysis, and stepwise linear regression using R software. Pearson's correlation coefficient (r) indicated a significant but weak negative relationship between the *Fusarium* stem assay and vascular browning ($r = -0.27$, $p = 0.0091$). In addition, *Pythium* seed assay had no association with field root rot severity, while the root knot nematode assay showed a marginally significant but weak positive relationship with field galling infestation ($r = 0.14$, $p = 0.05$). Also, a strong negative correlation between vascular browning and bean yield ($r = -0.28$, $p < 0.001$), and between disease pressure and yield ($r = -0.22$, $p < 0.001$) was observed. A stepwise linear regression model revealed that the interaction between *Fusarium* stem assay infestation and pH influenced vascular browning ($p < 0.001$) and stand count ($p < 0.01$). Similarly, the interaction of *Pythium* seed assay infestation with POXC significantly affected stand count ($p < 0.001$), and the association between Root knot nematode assay infestation and stand count was influenced by soil clay content ($p < 0.001$). Apart from the Root knot nematode assay, the soil pathogen bioassays tested here were not effective in predicting the field incidence. However, our findings revealed the ability of the fusarium stem assay to predict other symptoms associated with disease pressure, suggesting that the bioassays result together with soil fertility assessment can led to a more effective prediction of early disease pressure and yield.

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ACRONOMYS AND ABBREVIATIONS

CA	-	Conventional Agriculture
ISFM	-	Integrated Soil Fertility Management
PPN	-	Plant Parasitic Nematode
RKN	-	Root Knot Nematode
LSN	-	Lesion Nematode
RRI	-	Root Rot Rating
VBI	-	Vascular Browning Index
FRN	-	Farmer Research Network
WHC	-	Water Holding Capacity
PDA	-	Potato Dextrose Agar

CHAPTER ONE

INTRODUCTION

1.1 Background information

Common bean *(Phaseolus vulgaris*) is a leguminous crop that is widely grown around the world, providing several benefits to the agroecosystems. They are an important source of nutrients such as iron, potassium, magnesium, zinc, and folic acid (Mederos, 2006), and combat malnutrition (Siddiq & Uebersax, 2022), especially in countries with increasing food demand (Beddington & J., 2011). They help by fixing nitrogen (Reinprecht, *et al*., 2020) which can replace other sources of nitrogen (N) fertility in crop rotations, thereby improving soil fertility (Beebe *et al.,* 2013). It also offers a source of income and fodder for livestock for small holder farmers (Katungi, *et al*., 2010). In 2010, bean production worldwide was approximately 23.8 million metric tons, with 17.7% of the production in Africa (FAO., 2014). This increased, together with the area harvested to 27.5 million metric tons from 34.8 million hectares in 2020. Bean production has increased by about 60% since 1990, whereas the area harvested increased by 36% in the same period (Mekouar, 2021). Kenya has an average consumption of 401,880 tons of beans annual (MoA, 2010) but in the past two decades there have been noticeable fluctuations in bean production, In the year 2008, the area under production was 610,428 ha representing a 28% decrease compared to 2007 with also a decline in production from 2.9 million bags compared to 3.5 million bags in 2007 (MoA, 2010). In recent years, bean productivity in smallholder farming systems of Western Kenya, has been on a downward trend due to poor soil health conditions, biotic, abiotic and socioeconomic constraints (Katungi *et al.,* 2009). Declining government budgets together with decreasing donor interests on agricultural

extension services combined with limited access to improved technologies i.e. certified bean seeds also contribute to the lower farm productivity of beans among smallholder farmers (Chemining'wa *et al.,* 2014). Abiotic factors such as drought and excessive rainfall, low soil fertility, heat, and cold stresses leads to low legume production resulting in low yield (Odendo *et al.,* 2004; Waldman *et al.,* 2016), particularly in low-input smallholder farming systems**.** In addition, improper agronomic practices such as late planting, poor weed management i.e. weeding not done in a timely way, and lack of application of organic or inorganic inputs cumulatively result in poor productivity of beans. Past studies focused on addressing these constraints by engaging farmers in evaluating soil fertility management strategies (Agegnehu & Amede, 2017). For instance, practices such as CA and ISFM increased bean yield (Blum, 2005; Hörner & Wollni, 2021), and also the development of resistant varieties (Thompson & Gyatso, 2020). These studies resulted in and contributed to significant improvements in yield productivity in some farms (Mutuku *et al.,* 2020; Vanlauwe & Zingore, 2011). However, poor bean productivity is still evident in some farms, due to the incidence and attack of common beans by soil-borne pathogens, causing diseases (Paparu P, *et al.,* 2018), followed by PPN, which cause severe damage and economic losses on beans (Medvecky *et al.,* 2007). This is largely due to the difficulty of assessing and diagnosing soil-borne pathogens and pests in the field. Soil testing of soil-borne pests and pathogens, which includes visual assessments of soil-borne symptoms and disease, followed by isolation of the pathogens and culturing on selective media, and identification using microscopy, has been a cornerstone of plant pathology and fungal diagnostics but they have a number of setbacks. The tests require skilled staff in making the diagnosis; the methods are time-consuming and sometimes rely on the ability of the

causal organism to be cultured. Recently, a number of simplified soil bioassay tools have been developed and modified to assess the level of pathogen infestation in the soil (Hay, *et al.,* 2016). Therefore, this study sought to 1), To assess the effectiveness of soil bioassays in predicting soil infestation levels of soil-borne pathogenic *Fusarium*, *Pythium* and plant parasitic nematodes, 2) To establish the relationship of the soil bioassays with the performance of the selected common bean varieties in Western Kenya and, 3) to assess how incidence of soil borne pathogens and soil bioassays vary across different farms, bean varieties, and soil physicochemical properties in Western Kenya.

1.2 Statement of the problem

Crop damage in smallholder bean farming systems across Western Kenya and other East African regions is exacerbated by escalating incidences of soil-borne pathogens, such as plant pathogenic fungi, i.e., *Fusarium* and *Pythium*, as well as plant-parasitic nematodes (PPN), i.e., root knot nematodes and root lesion. These pathogens contribute to severe diseases, such as root rot and *Fusarium* wilt, which causes serious damage to crop yields and degrade produce quality (George *et al.,* 2016; Jimenez-Hernandez *et al.,* 2021). Recent studies demonstrated how these pathogens are abundant in agricultural soils (Maina *et al.*, 2015), with their occurrence, degree of damage and impact influenced by agricultural practices, climatic conditions, and regional geographical features. The major challenge associated with soil-borne pathogens and pests is the difficulty in diagnosing their occurrence and symptoms in the field (Dimkpa, *et al.,* 2017). This challenge is heightened by inadequate infrastructure and technical skills required for conducting standard laboratory tests, making pathogen identification both costly and inaccessible to most smallholder farmers. Additionally, delays in delivering results to farmers diminish their practical utility in informing day to day farm management decisions (Dimkpa, *et al.,* 2017).

Furthermore, many farmers in tropical regions lack or have little knowledge and awareness of soil pathogens and their symptoms (Ngoya, *et al.,* 2023), limiting their ability to respond and implement effective mitigation strategies. These challenges underscore the need for a local and rapid soil testing options that utilize accessible materials, are cost-effective, and have the ability to provide rapid insights to farmers regarding the infestation levels of soil-borne pathogens on their farms. Recent studies have adapted and evaluated various soil bioassay methods in comparison to standard laboratory analyses, demonstrating their effectiveness in detecting pathogenic *Fusarium*, *Pythium*, and two types of PPN (root-knot and lesion nematodes) (Mutai, *et al.*, 2024). However, further research is required to assess these bioassay methods against on-farm observations of pathogen incidence, severity, and yield impacts across diverse agricultural management practices and bean genotypes to determine their applicability and reliability in varying soil conditions and farm management contexts.

1.3 Justification of the study

Given the importance of beans in smallholder systems in Western Kenya as a cheap source of protein, there needs to be a continued focus on production constraints for beans. These constraints include biotic stress such a soil borne disease, particularly *Fusarium* wilt, root rot, bean blights, anthracnose, and damping-off seedling disease, among many others. In Western Kenya, insect pests and diseases cause losses of yield up to 100% crop loss when no measures are taken (Laizer, Chacha, & Ndakidemi, 2019). These, coupled with the difficulty in soil testing of many soil pathogens in smallholder farming systems, make it difficult to quantify their damage to crop productivity. An initial study was done (Mutai, *et al.*, 2024) to assess soil-borne pathogens under different agricultural management practices, using different soil bioassays. The bioassays were able to assess soil-borne

pathogens from the different soil management practices and demonstrate some level of pathogenicity, but the relationship between the assays, physicochemical properties of the soil, bean genotype, and disease damage was not established.

Standard laboratory procedures were used to validate the bioassay, where there still exists a need to evaluate the assays across a wide range of different soils of different fertility statuses and examine the relationship to levels of disease damage seen on farms. There is a need to test the effectiveness of the simplified soil bioassays used as tools in assessing and predicting on field incidence of soil pathogens' infestation with different bean genotypes is of necessity.

Therefore, this study aimed to investigate the relationships and interactions between various soil bioassays results and the disease incidences of soil-borne pathogens across different soil physicochemical properties, agroecological zones, and bean genotypes. The goal was to generate soil health data related to pathogens, share this information with farmers, and raise awareness. These resources would help farmers assess soil pathogens in their fields and adopt better strategies for managing pathogen infestations and minimizing their impact on crop production.

1.4 Objectives

1.4.1 Broad objective

To contribute towards increased common bean production through assessment of soil bioassay as a rapid test tool in the detection of soil pathogens of, *Fusarium, Pythium,* and parasitic nematodes in Western Kenya

1.4.2 Specific objectives

1. To evaluate the effectiveness of soil bioassays in predicting soil infestation levels of soil-borne pathogenic *Fusarium*, *Pythium*, and plant parasitic nematodes
2. To determine the relationships of the soil bioassays and field disease symptoms in relation to bean performance across the sites in Western Kenya
3. To assess how field disease symptoms and soil bioassays vary across different farms, bean varieties, and soil physicochemical properties in Western Kenya.

1.5 Research questions

Objective 1:

I. Is there a relationship between the bioassay results for *Fusarium*, *Pythium*, and PPN and the field disease symptoms of these pathogens, as well as bean yield on selected bean varieties?

II. Which species of *Fusarium*, *Pythium,* and Root Knot Nematodes were frequently isolated from sites in different regions of Western Kenya?

Objective 2:

I. Did the relationship between the bioassays and field disease differ among the four selected bean varieties?

Objective 3:

I. How does the relationship between the infestation of bioassays and the field disease symptoms depend on soil physicochemical properties?

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

This section provides a comprehensive review of previous research, ongoing and future opportunities in understanding soil-borne pathogens, their economic importance, and the methodologies used for their assessment and diagnosis at the field level. The first part presents an overview of the production and constraints associated with beans, with a focus on an in-depth understanding of the soil-borne pathogens of *Fusarium*, *Pythium*, and plant-parasitic nematodes (PPN). The final section examines various soil bioassay techniques employed for pathogen detection and diagnosis, their limitations, and potential strategies to address existing knowledge gaps.

2.2 Bean production trends

Beans (*P. vulgaris*) are the most commonly cultivated legume species and the second most cultivated after maize, with an average production of 461,734 tons annually (Ministry of Agriculture, 2013), with 24.4% and 17.7% of the world production in Latin America and Africa, respectively (FAO, 2014). In East Africa, it's grown twice a year, with sowing seasons running from March to April and from September to October (Rukandema, 1981; C. S. Wortmann, 1998b). *P. vulgaris* is produced in a range of cropping systems and environments, where about 74% of the common bean area in Eastern Africa and 57 % of the bean area in southern Africa (C. S. Wortmann, 1998a) are grown under multiple cropping systems. Production of beans in the east and central regions increased by 14% in 2012 from 6.4 million bags of 90 Kg in 2011 to 7.3 million bags in 2012 (MOA, 2013). Kenya is ranked high in bean production, with about 75% of the annual cultivation

occurring in four regions, namely, Rift Valley, Western, and Eastern regions (Katungi et al., 2009). In Western Kenya, 95% of the population grows beans, which are an important addition to their diet, providing of 65% protein intake (Ramaekers *et al.,* 2013). However, in terms of National output, the western region accounts for 22% (Karanja, 2006) with an annual consumption of 66 kg yr^{-1} (R. A. Buruchara, 2006).

2.3 Challenges of bean production

The cultivation and production of beans by smallholder farmers in Western Kenya face multiple constraints, including one or both abiotic and biotic stresses (E. Katungi *et al.,* 2009; Mourice & Tryphone, 2012) poor agricultural management practices, and limited access to certified and improved seed varieties (Chauhan & Johnson, 2010). Abiotic stresses significantly affect bean growth and productivity (Ye *et al.,* 2017) due to intensifying environmental variations over the decades (Boyer *et al.,* 2021). Key abiotic factors include salinity, heat, and drought stress (HanumanthaRao *et al.,* 2016; Singh *et al.,* 2011), and moisture deficiency (C. S. Wortmann, & Allen, 1994)all of which reduce photosynthesis and subsequently reduce dry matter production in common beans (Akbarabadi, *et al.,* 2015). Additionally, excessive rainfall and declining soil fertility (E. Katungi *et al.*, 2009) have further contributed to reduced bean yields over time (R. Buruchara, *et al.,* 2011; Odendo M. *et al.*, 2004). Biotic constraints to bean production include soil-borne pathogens and field pests, both of which significantly impact crop yields. Also, the interaction of these pathogens with other pathogens may lead to severe plant diseases, i.e., root rot, anthracnose, and common bean mosaic virus, which can drastically reduce productivity (Tusiime, 2003). Major soil pathogens include *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii*, with field pests such as the bean fly (*Ophiomyia phaseoli*) (D. J. Allen, 1996), chafer grub (*Schizonycha* spp.)

(Medvecky, *et al.,* 2007), bean aphids (*Aphis fabae*) (Okwiri *et al.*, 2009), thrips, and various borers (War *et al.*, 2017), can cause significant damage, with potential yield losses reaching up to 100%.

2.4. Soil-borne pathogens limiting bean productivity

Soil pathogens constitute a diverse class of organisms, some of which inhabit the rhizosphere. Key soil pathogens and pests include *Pythium spp., Fusarium spp.,* and PPN (George *et al.,* 2016; Jimenez-Hernandez *et al.,* 2021), among others. The pathogens can survive in agricultural soils for long periods, contributing to soilborne disease, which is regarded as the second most significant constraint to agricultural productivity after low soil fertility. This is a major concern, particularly in small-scale farming systems, where soil-borne pathogen infestations can lead to yield losses of approximately 75–80% (Baysal-Gurel & Kabir, 2018; Brauer *et al.*, 2019). Additionally, these pathogens can form disease complexes involving fungal and oomycete communities, further causing yield reductions. A major characteristic of soil-borne plant diseases is their patchy distribution (Truscott & Gilligan, 2001), with spatial patterns that fluctuate between growing seasons (Schneider *et al.,* 2001). The abundance of soil-borne pathogens is highly influenced by favorable environmental conditions, particularly temperature and moisture levels (Barrett *et al.*, 2009). Pathogen populations tend to increase with rising temperatures, as temperature is integral in regulating pathogen infection processes, including sporulation rates (Kweku *et al.,* 2018), pathogen virulence, and plant defense mechanisms (Sun, *et al.,* 2021). Additionally, elevated moisture levels can enhance the occurrence and severity of soil-borne diseases such as root rot and damping-off (Lamichhane *et al.*, 2024). Similarly, moisture deficiency can heighten plant susceptibility to drought stress, thereby increasing vulnerability to pathogens like *Fusarium* spp. (Maurya, *et al.,* 2022). Furthermore,

environmental stressors, including changes in precipitation and humidity caused by climate change, are expected to increase plant-pathogen interactions (Surówka *et al.,* 2020). These conditions facilitate disease outbreaks, ultimately compromising plant health and increasing mortality rates (Devendra, 2012).

2.4.1 *Fusarium* pathogen

The genus *Fusarium* consists of over 300 distinct species (Balajee *et al.,* 2009), with the most prevalent species belonging to the *F. solani*, *F. oxysporum*, *F. graminearum*, and *F. fujikuroi* complexes, which mostly influence plant diseases (Gagkayeva *et al.*, 2011; Guarro, 2013). Less encountered species include *F. incarnatum-equiseti*, *F. dimerum*, and *F. chlamydosporum* complexes, and *F. sporotrichioides* (O'Donnell *et al.,* 2015). *Fusarium* pathogen is widely distributed and can be recovered across diverse environments, soil types, water, and decomposing organic matter (Summerell *et al.*, 2010a). Pathogenic species are associated with several bean diseases, including *Fusarium* yellow wilt (vascular wilt) caused by *Fusarium oxysporum* (Naseri, 2008), and Fusarium root rot caused by *Fusarium solani*. The hyphae of the *Fusarium* pathogen tend to grow towards the plant roots due to proliferating root exudates on the surface of the roots (Smith, 2007), where they penetrate the roots through wounds inflicted by insects (Zhang *et al*., 2014) or natural openings. Although they are often weak competitors (G. S. Abawi, & Lorbeer, 1972; Marois & Mitchell, 1981) they are quite abundant in cultivated soils (Gomez *et al.,* 2007; Smith, 2007) which is due to the species capability to morph into different lifestyle stages (De Silva *et al.*, 2017) and also the ability to act non-plant hosts (van Diepeningen & de Hoog, 2016). *Fusarium* fungi are disseminated through air, infected plant debris, and soil, facilitating the spread of inoculum (Summerell *et al.*, 2010b). Upon successful penetration of the host crop, *Fusarium* spores invade the developing xylem

tissues (Mace, 1981), leading to vascular discoloration, characterized by a reddish-brown stem, which features as a diagnostic symptom of *Fusarium* infestation (G. Abawi *et al.*, 1990). The infection further induces disease symptoms such as root rot, wilting, chlorosis, and stunted shoot growth (Coleman, 2016). Disease progression is influenced by environmental factors (G. Abawi *et al.,* 1990), particularly drought, which increases disease severity as moisture deficiency and excessive heat increase vulnerability of plants to *Fusarium* pathogen infections (Wegulo *et al.,* 2013). The severities of these infections are heightened under drought conditions. Several studies indicate that high humidity and elevated temperatures promote the growth and development of *Fusarium* spp, leading to increased population densities of the fungi in the soil (Dixit *et al.*, 2024; Hunjan & Lore, 2020). Additionally, disease severity is also led by inadequate crop rotation and soil physicochemical properties, including pH levels, organic matter content, and nutrient availability, which influence the persistence and virulence of *Fusarium* pathogens (Orr & Nelson, 2018).

2.4.2 *Pythium* pathogen

The fungus Pythium is a fungal-like organism, belonging to the species Oomycota (Webster, 2007). It's a cosmopolitan genus found in both natural and agricultural ecosystems (Kageyama, 2014; Senda *et al.,* 2009). Past studies described slightly over 120 species (Dick, 1990) while recent studies conducted by (Ho, 2018; Webster, 2007) believed the genus comprises of 355 recorded species. A few of the *pythium* species are non-pathogenic, i.e. *P. oligandrum,* which does not attack the tissues of crops but occurs at the root crop surface (Dušková, 1995). A few species of *Pythium* that are pathogenic to plants include *P. ultimum. irregulare, P. myriotylum*, and *P. aphanidermatum,* which are found in both temperate and tropical climates (Dick, 1990). These species cause major crop

loss to susceptible crops such as tomatoes, peas, soya beans, and common beans (Weiland *et al.*, 2013), leading to mortality rates of up to 60% during epidemics (González-Pérez *et al.*, 2004). *Pythium* spp are more abundant in cultivated than in uncultivated soil (Nzungize *et al.*, 2012) where the Pathogenic spp produces hyphae that are mostly 5-7 µm wide, sometimes reaching a width of up to 10 µm (van der Plaats-Niterink, 1981) producing swollen digitate regions, called appressoria which enable the fungus to attach and penetrate the host cells (Levesque & De Cock, 2004). This is facilitated by the presence of exudates and other exogenous stimuli from the seeds and roots of the plants in the soil, where they infect the seeds or roots (Whipps & Lumsden, 1991). Once the fungus or the pathogen is in the tissue of the host plant, it induces brownish, water-soaked lesions on the roots of the growing plant (Lamichhane *et al.,* 2017). As the severity and size of the lesions increase, the plant may show varying degrees of stress, such as pre- and post-emergence damping-off of seeds and seedlings (Weiland *et al.,* 2013), followed by leaf chlorosis, defoliation, uneven growth, leading to disease infection, causing lower yield (Schwarz *et al.*, 2007). This causes, depending on the pathogen involved, general root rot symptoms (G. S. Abawi & Widmer, 2000), and seed rot (Agrios, 2005). The presence of the diseases is exacerbated by the continuous buildup of the pathogen inoculum associated with declining soil fertility that is caused by the intensification of land use and poor agronomic practices (G. S. Abawi *et al.,* 2006). In addition, environmental factors, namely excessive soil moisture, lower and higher soil temperatures before and after emergence, respectively (Martin & Loper, 1999), influence the growth and development of the soil pathogen.

2.5 Plant parasitic nematodes (PPN)

PPN are worm-like pests, found in aquatic and soil environments. In cultivated soils, most PPN occur in the upper layers where they spend all or part of their life (Brooks, 2004). They include: *Meloidogyne spp., Pratylenchus spp,* are among the few species associated with PPN (Jones, *et al.,* 2013). Root-knot (*Meloidogyne*) and lesion nematode (*Pratylenchus*) species are the major plant parasitic nematode that reduces crop yields worldwide. They infect all plant organs (root tissues, buds, seeds and tubers) where they feed ecto parasitically or endo-parasitically using their stylet, making it difficult for water and nutrients to be absorbed (Wilcox, 1986), which slows down growth, reduces the productive life and prolong fruiting time of the host crop (M. R. Khan *et al.*, 2021). Also, nematodes serve as vectors by causing mechanical injury (Zalpuri *et al.*, 2013), which increases the susceptibility of the host to other soil-borne pathogens. This causes stunting, chlorosis, leading to low quality of marketable produce`, or even complete crop mortality (George, et al., 2016; Jimenez-Hernandez, *et al.,* 2021), leading to up to 50% yield loss in some farmer farm (Kimenju *et al.*, 2008; Chirchir *et al.*, 2010; Atandi *et al.,* 2017; Maina *et al.,* 2019). PPN are distributed in various agroecosystems (Waceke, 2007; Wangai *et al.,* 2014), where their damage and severity are attenuated by poor agronomic activities and degenerated soils (Nicol *et al.,* 2011). The occurrence of the nematode spp can vary depending on temperatures, with their population usually being higher in warmer areas than in cooler ones (Martin *et al.,* 2023)**.** Their Survival is influenced by their ability to rapidly spread and colonize new localities (Bebber *et al.*, 2014), and a wide distribution of host plant roots. Also, soil conditions such as organic content, aeration, structure, and moisture content (Zalpuri *et al.,* 2013) influence their survival.

The main PPN of particular concern are:

2.5.1 Root-knot nematodes (RKNs)

RKN (*Meloidogyne spp*) are distributed across farms where they ranked as the most economically damaging PPN (Jones, *et al.,* 2013), with over 20 Meloidogyne species occurring (Onkendi *et al.*, 2014). They are endo-parasites with the male having a worm-like feature while the female appears to be pear-shaped in feature (Sherf & MacNab, 1986). The mature females are sedentary (Mai, 2018), where they both cause disease either independently or in association with other fungal pathogens. These infestations can sometimes lead to a 20% yield loss (Valera & Seif, 2004). They have a wide host range, where they attack roots of over 2000 different hosts (Siddiqui & Mashkoor Alam, 1999). Young host plants are most vulnerable to damage by root-knot nematodes (Nicol *et al.,* 2011). A female nematode produces 300 to 800 eggs within 40 days under average conditions, which later hatch into juveniles. The juvenile stage, which is the infective stage, enters the root (Dropkin & Nelson, 1960), where it attacks the cells continuously, leading to growth in the roots known as root galls (CABI, 2014). This impairs the flow of water and other important nutrients from the invaded roots to different parts of the plants, causing the leaves to turn yellow and eventually the plants to wilt. Under heavy infestations, plants are chlorotic, stunted, and necrotic at the leaf margin and show excessive wilting (Melakeberhan, 2004). Root galls is a diagnostic feature associated with damage by root knot nematodes (Wilcox, 1986). Although root-knot movement on its own is limited, their dispersion is mainly through: spread by water, soil adhering on farm implements, man's activities like movement of infested soil and infected plant debris, wind and irrigation (R. G. Allen, 1996) enabling it to rapidly spread and colonize new localities quickly (Bebber, *et al.*, 2014).

2.5.2 Root-lesion nematodes (RLNs)

Root-lesion nematodes *(Pratylenchus* spp) is ranked third only to root-knot and nematode cysts on impacts on crops worldwide (P. Castillo, & N.Vovlas, 2007). A few species have been noted (Janssen *et al.*, 2017), which include *P. mediterraneus, P. neglectus, P. penetrans, P. crenatus, P. pratensis, and P. longicaudatu*s (Di Vito *et al.*, 2002; Greco *et al.*,1992). They are migratory endo parasites that complete their life cycle in the host roots (Perry, 2024), lasting from 45 to 65 days (Smiley, 2010) taking 15-17 days for hatching after the female has laid their eggs inside the root, 15-16 days for the juvenile stage and 15 days as mature adults for egg laying (CABI, 2014), depending on the species and conditions. Some of these species can combine with other soil-borne pathogens and provide entry for fungi, bacteria, and their organisms to generate disease complexes (e.g., wilt disease involving *Fusarium* species) (Saadabi & Yassin, 2007), i.e., an interaction of *Pratylenchus* species and a combination of various agents such as fungi, i.e., *Fusarium* spp., *Pythium*, and *Phytophthora*, causing Apple replant disease (ARD) (Kanfra *et al.*, 2022). They have higher densities in the roots compared to the soil (Askary, 2017) , where they penetrate the growing roots, causing damage to the epidermis, cortex, and root endodermis, leading to distinct necrotic lesions and discolorations of roots that are mostly reddish-brown (P. Castillo & N Vovlas, 2007). The root lesions later coalesce, forming large necrotic areas, causing the infection to occur along the entire length of the root. However, at high population densities, the parasites severely affect the root system, leading to water and nutrient deficiencies, which causes wilting, yellowing and eventual dieback (P. Castillo & N Vovlas, 2007). Several studies demonstrated how plants infected with root-lesion nematodes have lower yield than non-infected plants (DuPont *et al.*, 2018). The severity of these infestations depends on the nematode species' composition, crop rotations,

environmental conditions, and the nematode's pathogenicity (P. Castillo, & N Vovlas, 2007).

2.6 Soil influence on soil pathogens

Soil is a reservoir for diverse biological communities of organisms, including fungal pathogens, which are consistently present for parts of their life cycle (Oliver & Gregory, 2015). This regulates the potential of pathogens to infect the host (Schlatter*et al.*, 2017), and impact on plants (Janvier, *et al.,* 2007b). Soil environment strongly influences pathogen infestation (Schlatter *et al.,* 2017), a healthy soil ecosystem is characterized by resilience to disturbance or stress (O'Neill, 1986), while soil environments characterized by poor physicochemical (abiotic) properties are most affected. These soil physicochemical properties are considered as indicators of poor soil health ecosystem with a likely link to population density of plant pathogens in soil, and ultimately disease incidence and severity (Van Bruggen & Bolda, 1996). A few reviews (Dordas 2008; H. Höper & Alabouvette, 1996; Janvier *et al.,* 2007a) outlined the interactions and effects of physicochemical characteristics on severity of soil-borne diseases, with more studies required to completely establish and understand these interactions. These physicochemical characteristics are: First, Soil texture (relative proportions of sand, silt, and clay particles) affects pathogen distribution and establishment in the soil i.e., fine-textured clay soil may be more conducive for survival of pathogens (Obayomi, *et al*., 2019), showing the impacts of clay materials on suppression of fungal diseases. Past studies (Domínguez *et al.*, 1996) showed that fusarium wilt is associated with sandy soils with (H. Höper *et al.*, 1995). Secondly, Soil pH influences microbial composition (Rousk *et al.*, 2010), with a positive correlation to fungal diversity and abundance between pH 4 and 8 which is the ideal pH range for most crops. *F. oxysporum* is less likely to meet its metabolic requirements at

higher pH values. Under high soil pH, iron availability is reduced, where the pathogen establishment depends on the ability to acquire and compete for iron, showing that nutrient availability is indirectly influenced by changes in pH (Ghorbani *et al.*, 2008). Generally, most fungal pathogens are able to tolerate a wider range of soil pH (Rousk *et al.,* 2010). A reducing soil environment tends to inhibit nitrification, in turn increasing the concentration of soil ammonium, which increases *F. oxysporum* pathogenicity and fusarium development (Domınguez *et al.*, 2001), also higher available nitrogen can increase disease susceptibility by favoring pathogen growth (Mur *et al.*, 2017). Soil moisture also influences the ability of the pathogen to persist and survive in soil. For instance, excessive moisture results in physico-chemical properties being affected due to oxygen depletion in the soil which in turn has an impact on the activity of soil microorganism (Unger *et al.*, 2009). Conversely, drier soil can impede microbial mobility, limit nutrient availability thereby decreasing microbial growth (Alloway *et al.*, 2013). In some host crop i.e. tomato it was demonstrated how low water content was conducive to fusarium wilt tomato (Ghaemi & Banihashemi, 2011) due to water stress.

2.7 Soil Bioassay

Soil bioassays are tools that include procedures and techniques that involve using soil and pathogen/pest-sensitive plants to detect soil-borne pathogens and pests in the soil. Generally, these soil bioassays use a plant, or plant organ or tissue, to measure and evaluate the initial soil infestation levels that indicate pathogen infestation, thereby identifying fields that may be of concern and potential yield losses. This is useful in that it may allow farmers and other land managers to make informed decisions on management decisions, for example, the choice of cultivar or application of fungicides, to anticipate and prevent the spread of the disease. In addition, the assays are influential in developing disease-

resistant crops, where they help in determining the degree of host susceptibility at different densities of disease propagules in the soil (Vandemark, 2003; Yu *et al.*, 2015). Several assays for the detection of soil-borne fungi have been developed (Bilodeau *et al.*, 2011) and many are currently in use to evaluate disease pressure in soils, validating newly developed or modified detection methods (Sundelin *et al.,* 2010). Previously, (Fink, 1954) developed a greenhouse bioassay method to predict and estimate root rot in pea fields by determining the inoculum level of pea root rot pathogen by planting pea seeds in sampled soils and later rating the infected seedlings using a disease severity index to rate the infestation (G. Abawi *et al.,* 1990) developed an assay for assessment of root health, where susceptible bean seeds are grown in a sampled soil and later rated for symptoms and root damage caused by infection from soil-borne pathogens, i.e., *Fusarium spp.* and *Pythium spp.* The major challenge of the soil bioassays is that they are less specific to precision, and rely on personal judgment of disease symptoms.

2.8 Gaps and way forward

Recent studies focused on polymerase chain reaction (qPCR) based assays as tools for disease risk assessment and molecular identification (Awad A.M. *et al.,* 2019). PCR-based assays have several pros in that they are rapid, fast, and also highly precise. However, they are costly, and the methods are more associated and suited to the academic field rather than local farmers who don't see the utility of these lab-based assays. The method for evaluating soil infestation level includes soil plating onto selective media and counting colonies of the fungus following incubation for several weeks. Though there is little documentation of the different traditional soil assays for diagnosing the three major soil pathogens, there is a need to test and compare different soil bioassay methods in assessing and diagnosing soil-borne pathogens in smallholder systems.

This study aims to use reliable, simplified soil bioassays described by Mutai *et al.,* (2024) in assessing the pressure of soil-borne pests and pathogens, by providing easy-to-understand assessments of disease pressure on farm fields. In addition, this study will provide information on the distribution of pathogens across the different bean genotypes and different soil environments around the different regions in Kenya

CHAPTER THREE

MATERIALS AND METHODS

3.1 Overview

The study was carried out in three major phases, where the first phase involved on-farm screening of the field, disease symptoms associated with soil pathogens, and PPN of common bean in Western Kenya (section 3.2). The second phase involved laboratory assessment of soil samples collected from the Western Kenya region for fungal and nematode infestation levels using different soil bioassays (section 3.3). Finally, the last phase involved testing of the different soil samples collected from the study site for soil physicochemical properties (Section 3.3). This chapter gives details on the materials and methods used in these experiments and the statistical analyses done.

3.2 Screening of field disease symptoms of soil pathogens infestation in western Kenya

3.2.1 Study area description

The study was carried out in Western Kenya, covering the counties of Siaya, Homabay, Kisumu, Vihiga, and Nandi. Siaya County is situated between latitudes 0.4333°N and 0.3°N and longitudes 33.9667°E and -34.55°W, encompassing approximately 2,530 km² with an altitude range of 1,140 to 1,500 m above sea level (KNBS, 2010). Homabay County is located at a latitude of -0.9022° and a longitude of 34.3°E, covering an estimated 4,267.1 km², including water bodies within the county (Obonyo *et al.*, 2012). Kisumu County lies between latitudes -0.3333° and -0.8333°S and longitudes 33.3333° and 35.3333°E, with an altitude of 1,184 m above sea level, an average annual rainfall of 1,464 mm, and mean annual temperature ranges of 25°C to 35°C (maximum) and 9°C to 18°C (minimum) (Musyimi *et al.*, 2018). Vihiga County, covering approximately 531 km², is located at a

latitude of 0.0667°N and a longitude of 34.6667°E, receiving an annual precipitation of about 1,900 mm (Chumba *et al.,* 2024). Nandi County spans approximately 2,884 km², positioned at a latitude of 0.5667°N and a longitude of 35.4167°E, having 1,200 mm of rainfall annually and an average temperature range of 18°C to 22°C (Nandi, 2017).

The study areas exhibit significant variability in soil types. Nandi County is characterized by Nito-rhodic Ferralsols and Vert-mollic Nitisols (Nandi, 2017), while Vihiga County is predominantly composed of Nitisols, with Ferrasols and Acrisols dominating in Siaya County (Jaetzold, Schmidt, Hornetz, & Shisanya, 2009), Homabay County primarily consists of Humic Andosols, Orthic Acrisols, and Plinthic Acrisols (MoALF, 2016). The above regions have two main cropping seasons due to the bimodal rainfall experienced during the long rains (March to May) and the short rains (October to December) (Jaetzold R *et al.*, 2010). Agriculture in these counties is mostly based on smallholder farming systems, with subsistence crop production mainly involving maize (*Zea mays*) intercropped with common bean (*P. vulgaris).*

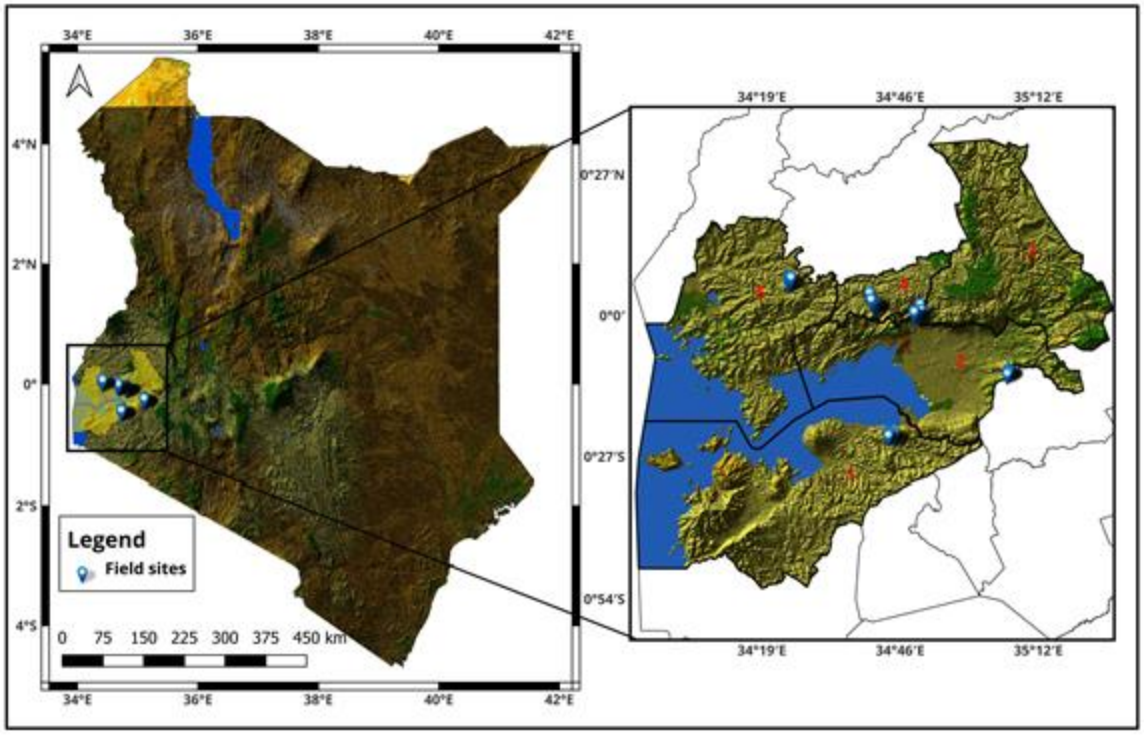

Figure 1: Map showing the counties; 1- Homabay, 2-Kisumu, 3-Nandi, 4-Vihiga, 5-Siaya, and the location of the study sites in Western Kenya.

3.2.2 Farmer and site selection

The experiments were done in collaboration with the Farmer Research Network (FRN-NGO) groups (Nelson *et al.*, 2019), Multipurpose Legume Project (MLP), and Kalro-Kibos in Kisumu, Kenya. A total of fifty farmers were selected across the five regions, with ten farmers per region. The farmers were selected based on their ability and interest in providing fields for the trials, as well as the objective of creating a wide range of soil fertility within each county site, using data on soils acquired during an earlier phase of farmer-led experimentation (unpublished data) and thirdly on past management and production history of the farmer during previous FRN activities.

3.2.3 Soil sampling and preparation

Sampling of soil samples was done using a grid sampling approach (Okalebo, *et al.*, 2002), in the 4 x 4 m trial field plots. In each trial plot, 10 soil cores were sampled at a depth of 20 cm using a soil auger (4 cm diameter) and thoroughly mixed in a bucket to obtain a composite soil sample per plot (~5 kg field moist soil) having a total of 4 samples per field plot totaling to 200 samples for the 50 farmer fields. They were taken to the laboratory and air-dried at room temperature (20°C) and later sieved through a 2.0 mm sieve to obtain fine soil samples. The soil samples were then divided and used for assessment of fungal pathogens and PPN using soil bioassay methods (Section 3.3), and laboratory tests for soil physicochemical properties (Section 3.4).

3.2.4 Experimental layout for the fields

In each farmer field, four trial plots were laid out within a 10 by 10 m field area as shown in (Figure 2) where each treatment plot was 4 x 4 m, with a foot path of 0.5m between the plots and around the outside the plots. In the four treatment plots, two susceptible bean varieties, GLP 2 (Rose coco), and GLP 585 (Red Haricot), and two tolerant bean varieties, KK8 and KK 15, were assigned the four plots randomly, one variety per plot. The beans were susceptible and tolerant to both *Fusarium* and *Pythium* pathogens. In pathways between the experimental plots (0.5 m width), a susceptible bean variety was planted (GLP 2) to act as guard rows during the experiment. Tilling of the field was done before planting the beans. Appropriate management practices for beans, such as timely weeding, were conducted up until harvesting, and in all the trial fields, no pest and disease control was done.

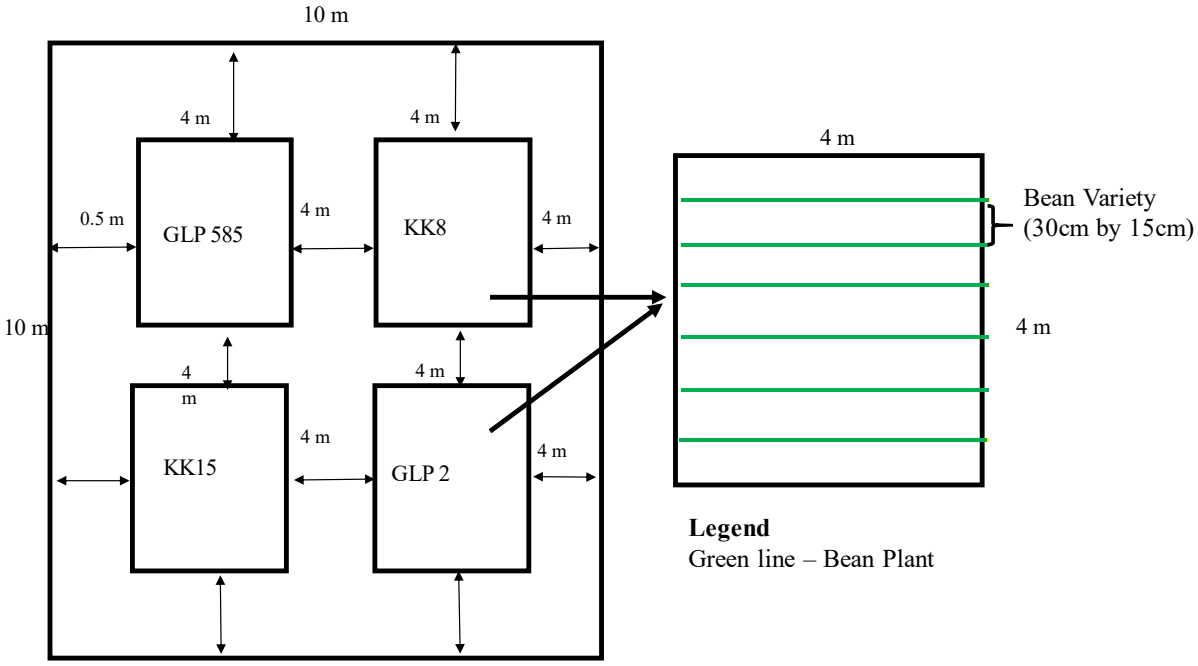

Figure 2: Experimental layout for the field trial (the green lines represent the bean planted in each row while the guard rows were planted along the pathways between the

3.2.5 Disease severity and yield assessments

After planting, the bean varieties were monitored and assessed. Plant stand count was recorded 14 days after planting to evaluate seedling establishment. At the flowering to pod formation stage of the bean crop (Van Schoonhoven & Pastor-Corrales, 1987), destructive root sampling along delimited areas of the plot was done using a quadrat measuring 0.5 by 0.5m ($0.25m^2$) replicated four times per plot. The number of plants in each quadrat, wilting, and chlorosis (yellowing), were observed and recorded. In each laid quadrat, four bean plants were randomly selected, uprooted, washed, and rinsed carefully to free the roots of the soil in each trial plot for field assessment of Fusarium, Pythium, and PPN (Root knot nematode and lesion nematodes).

3.2.5.1 Evaluation of *Fusarium* spp. severity

The washed plants from the field were cut above the hypocotyl region and separated from the above-ground biomass. The stem was dissected and assessed for the discoloration of the vascular browning of the inner stem (diagnostic feature for Fusarium infestation). The vascular browning was rated to assesses the severity of fusarium infestation on a scale where: 0 = no vascular discoloration; 1 = discoloration restricted to base of stem only; 2 = discoloration of the 'internode 0' (hypocotyl) region of the stem below the cotyledons; 3 = discoloration of stem above the cotyledons; 4 = complete vascular discoloration of stem; and 5 = plant dead as described by (Becerra Lopez-Lavalle *et al.*, 2012). This was done on all the sampled plants. The above-ground biomass was collected and stored inside khaki bags (size 5) and dried in the oven, and their weight was recorded.

3.2.5.2 Evaluation of *Pythium* spp. severity

Additionally, the four plants uprooted through quadrat sampling were assessed for *Pythium* infestation by rating root rot severity, caused by *Pythium spp*., using a modified 0–5 scale developed by (Schoonhoven & Pastor Corrales, 1987). The scale was based on the percentage of root tissue showing discoloration, lesions, or rotting: 0 = no detectable symptoms; 1 = light discoloration or 1–10% lesion coverage; 2 = heavy discoloration, 10–25% lesion coverage, or light rotting; 3 = 25–50% lesion or rot coverage; 4 = 50–75% lesion or rot coverage; and 5 = 75–100% root rot with plant death occurring prematurely.

3.2.5.3 Assessment of root-knot and lesion nematode

The plants uprooted from the quadrat were also assessed for Root-knot using a galling severity scale (RGS) ranging from 1-5 was used to quantify infestation: RGS 1 = no galls, RGS 2 = 1–4 galls, RGS 3 = 5–12 galls, RGS 4 = 13–40 galls, and RGS 5 = more than 40 galls per plant.

Lesion Nematode was visually assessed and number of lesions counted where possible and recorded.

3.2.5.4 Grain yield assessment

Harvesting of bean grain was done when over 95% pods were brown (Keino *et al*., 2015). This was done inside an effective area measuring 3 m by 3 m in each plot, leaving an area of 1 m and 1 m, which included the edge rows. In each plot, the total number of pods inside the effective was manually counted and recorded. Ten bean plants were selected randomly from each plot and their pods were counted. The seed weight of the ten plants was weighed and 1000-seed weight (g) data obtained (Zaimoglu Onat, 2004) for yield estimation on each

trial plot. To estimate yield from the whole plot, the dry bean weight from the 10-plant sample was scaled up to the entire plot using the following formulae:

$$Esimatedyield(kg/ha) = \left(\frac{Dryseedweight\ (g)\ of the\ 10\ plants * T.podweightoftheentireplot}{Podno.of the\ 10\ selectedplants * effectiveareaoftheplot\ (9m^2}\right) * 10kg/ha$$

3.3 Evaluation of the soil bioassays in assessing pathogens

3.3.1 Bioassay procedures and analysis

For the Bioassay experiments of (*Fusarium, Pythium*), the four soil samples from each farmer's field were bulked together as one sample, totaling 50 samples across all the trial sites. For the nematode bioassay assessment, the soil samples in the 10 x 10m field were sampled using a grid sampling approach, with a soil auger, and 2kg of the soil sample was placed in a sealed plastic bags and stored at 4°C until processing and bioassay initiation (preferably within 2 days of sampling).

3.3.2 Root lesion nematode bioassay

A modified bioassay based on (Gugino, *et al.,* 2008) was used to assess soil pathogen infestation. Sampled soil was divided into two 500 g aliquots for laboratory replicates and placed in separate pots (8 cm in diameter and 12 cm in height) with drainage holes at the bottom, where three soybean seeds were planted within a greenhouse environment and watered daily. Three weeks' post-germination, the young seedlings were carefully uprooted and washed thoroughly with water to free the roots of soil. Infestation levels and pathogen pressure were evaluated through visual examination of the roots for diagnostic lesions, characterized by elongated dark brown markings or lesions. The total number of lesions observed across the root systems of the three plants per pot was recorded.

3.3.3 Root-knot nematode bioassay

Two sub-samples of bulked soil (500 g each) were weighed and placed in separate pots (8 cm in diameter and 12 cm in height) with drainage holes at the bottom. Three tomato seeds, known to be susceptible to root-knot nematodes (RKN), were planted in each pot under greenhouse conditions with daily watering. Five weeks' post-germination, the seedlings were carefully uprooted, and the roots were carefully and thoroughly washed to remove soil. Root galling, a key diagnostic feature of RKN infestation, was assessed using the galling severity scale (RGS, discussed in Chapter3.2.5.3) to quantify the level and severity of nematode infestation.

3.3.4 *Fusarium* Assay using bean stem

A susceptible bean variety (GLP 2) was first planted in a soilless medium (free from the concerned soil pathogen) and grown in the dark (to avoid photosynthesis) for 14 days at room temperature (~ 25°C) as described by Furuya *et al.* (1999). Long, etiolated, white bean stems were obtained (> 25 cm) and cut into 6 cm long segments, which easily show *Fusarium* infestation. Two sub-samples (lab replicate), 500g of each of the 2 mm sieved air-dried, bulked soil, were weighed and placed inside plastic containers with lids (11 cm diameter, 12 cm high). WHC of each soil sample was determined by a method described by (Muratore & Knorr, 2025), where ten white bean stems (6 cm segments) were buried in the soil in the container and evenly spread. The water content was adjusted to 50% WHC, and the container was stored at room temperature (~ 25°C). After 4 days the buried bean stems were removed from the bulked soils and rinsed thoroughly with clean water to free from soil. The number of lesions associated with fusarium infestation were assessed, counted, and recorded. Later the lesions from the beam stems were isolated and incubated for identification of fusarium spp. The lesions were cut from the bean stems and surface-

sterilized using 1% sodium hypochlorite solution (NAOCL) for 30 seconds and rinsed three times in sterile water, where it was then plated on sterile PDA. PDA was prepared by adding 39g of PDA into a volume of 1000ml of distilled water. The media was then swirled, autoclaved and sterilized at 121 °C for 15 minutes (Pardley & Sharma, 2010). Before plating, the sterilized lesions were dried of excess water using clean serviette (Suwandi, 2012), followed by plating the lesion tissues on solidified PDA plate with 0.1% streptomycin sulfate. The plates were later placed inside a laminar flow at room temperatures under continuous white fluorescent light, alternating at 12 h near-UV light and 12 h dark conditions (Were, 2018) for 7-10 days for sporulation. After the set days, the number of growing fungal colonies was differentiated based on colony color, growth type, and color of mycelia. The different colonies were later sub-cultured onto freshly prepared sterile PDA and incubated for another 7-14 days in similar conditions as described above to obtain clean and pure isolates (Suwandi, 2012). For identification of the pure isolates, we used morphological characteristics of the isolates on the PDA, using standard identification keys such as colony color for both front and reverse side, both micro and macro conidial shape and size were used to identify the different isolates.

3.3.5 *Pythium* assay

Two sub-samples (lab replicate), 150g of each of the 2 mm sieved air dried, bulked soil were weighed and placed inside plastic containers (same as the *fusarium* assay). Measured water was added to the soil to bring it to 75% WHC as described (Chapter 3.3.4), which favors *pythium* growth. Ten bean seeds, susceptible variety (GLP2), were buried in the moistened soil and the container closed and incubated in the dark at 21 ˚C. After 3 days, the seeds were removed from the soil, washed with de-ionized water, sterilized using 1% sodium hypochlorite solution for 30 seconds, and rinsed three times in sterile distilled

water. We used a *Pythium* selective medium known as PARP, as described by Jeffers & Martin (1986), to plate the seeds to detect *Pythium* spp. 17g of Cornmeal agar was added to a liter of deionized water. This mixture was swirled slowly and then autoclaved for 15 minutes at 121 psi (pounds per square inch). The sterilized media was allowed to cool in the laminar flow air system at about 45^0C. The following were then added, 0.1 g of 10 mg of rifampicin (antibiotic) dissolved in 10ml Dimethyl sulfoxide (DMSO, solvent),0.1 g (100 mg of) PCNB (fungicide) dissolved in 50 ml ethanol in 70^0C water bath, 250 mg of ampicillin powder (antibiotic), and 5 mg of pimaricin, forming the PARP selective media. This media was then dispensed onto sterile medium sized glass Petri-dishes and allowed to cool under a laminar flow air system before use (Kipkogei, 2019). The seeds were then plated on the solidified media and the cultures were then incubated in the dark at room temperature (22-28°C) for 48-72 hrs. in the incubator. The mycelia and colonies that grew were observed and recorded for pythium infestation. Some of the mycelia were sub-cultured for identification of the *pythium* spp.

3.4 Assessing soil physicochemical properties

3.4.1 Laboratory soil tests

Samples collected from the 200 trial plots were tested for different soil parameters using a simplified soil health analysis tool kit (Nyamasoka-Magonziwa *et al.*, 2020) in Kalro-Kibos, Kisumu. These soil parameters were soil pH, Olsen Phosphorus (Olsen method), Permanganate-Oxidizable Soil Carbon (POXC), POM, and Amino Sugar N described by (S. A. Khan, *et al.*, 2001). In addition, part of the soil was also taken to the University of Eldoret for laboratory soil tests as described by (Okalebo, *et al.*, 2002). These tests, conducted in the university lab setting, were soil pH (water), total soil nitrogen (Kjeldahl

method), and available P (Olsen method). In addition, TOC was determined by the wet ashing method (Walkey-Black method)following the standard soil test procedures described by (Okalebo, *et al.*, 2002). Soil particle size (soil texture) was also determined on 50 bulked soil samples (i.e., bulked across the four experimental plots) using the Hydrometer method as described by (Bouyoucos, 1962).

3.4.1.1 Soil pH

10g of soil was added in 20 ml of deionised water in 1.2 ratios to measure the soil pH. The mixture was then stirred for a minute and allowed to settle for 2 minutes before taking the pH measurements while gently swirling the mixture occasionally using a portable pH meter (Extech model PH110).

3.4.1.2 Potassium Oxidizable Carbon (POXC)

The POXC test was carried out using a method adapted from (R. R. Weil, *et al.*, 2003), based on the oxidation of labile SOC by potassium permanganate ($KMnO_4$). 2.5 g of soil was mixed with 20 mL of a digestion solution containing 0.015 M $KMnO_4$ and 0.1 M $CaCl_2$, with the mixture being shaken for 2 minutes using a mechanical shaker and allowed to settle for 10 minutes.0.5 mL of the settled solution was diluted in 30 mL of deionized water and then placed in a vial. A 100% $KMnO_4$ blank solution (control for absorbance), was also prepared using the same dilution method without soil, and the absorbance was measured using a field colorimeter (Hanna model HI-717 phosphate high-range colorimeter).

3.4.1.3 Particulate Organic Matter (POM)

POM was determined using density flotation with tap water. 100 g of the dried soil sample was placed on a 2 mm sieve in a half-filled basin. The sieve and soil were then submerged, and the soil left to soak for 10–20 seconds for slaking before lifting it in and out of the

water gently. Materials sieved through the 2mm sieve were then transferred to a 250 μm sieve to capture coarse sand and POM. After gentle lifting, the remaining materials were rinsed into a beaker and decanted into a basin with POM suspended in water and denser particles at the bottom of the beaker. POM suspended in water was decanted (Continue decanting until the suspension is clear of floating POM) and later transferred to a pre-weighed cloth using a rinse bottle. The POM was then dried and weighed on a microbalance with a precision of 0.1mg.

3.4.1.4 Particle analysis and textural classification

Soil particle sizes of sand, silt, and clay, together with their textural class, were determined by the hydrometer method (Bouyoucos, 1962) after dispersion with Calgon solution (sodium hexametaphosphate). 50 g of soil was put into a beaker (400 ml) and saturated with distilled water. 10 ml of 10% sodium hexametaphosphate was added, with the mixture being stirred for 2 minutes using an electric stirrer and allowed to settle for 10 minutes. After 40 seconds, the hydrometer was gently placed into the suspension, where the first hydrometer and temperature reading were recorded (silt and clay suspension), after 2 hrs. The second reading was recorded (amount of clay suspension). Temperature readings are used for correcting hydrometer readings. Later, the soil sample is assigned a textural class as described by (Okalebo, *et al.*, 2002).

3.4.1.5 Total nitrogen analysis

Total nitrogen in the soil was determined using the Kjeldahl method as described by (Okalebo, *et al.*, 2002). First, 3.5 g of Selenium (Se) was dissolved in 1 L of concentrated H_2SO_4 (a digestion solution), and the mixture was heated at 300°C until it developed a clear light yellow color, then 0.32 M salicylic acid in the Se- H_2SO_4 mixture.0.3 g (0.25-

mm sieved) of soil was digested with 2.5 ml of the digestive solution (Se and salicylic acid in H_2SO_4) at 110°C on a block digester for 1hr with the addition of 1 ml of hydrogen peroxide three times. Heating continued, raising the temperature to 330^{O}C until white sand (colorless solution) is obtained. The digested sample was cooled and brought to 50 ml with distilled water to dissolve all the sediments. 5 ml of each of N 1 and N 2 reagents were added to 0.2ml of the digest and allowed to stand for 2hrs, for color to develop, before absorbency was measured using a spectrophotometer (Model T80 UV/VIS).

3.4.1.6 Total organic carbon analysis

Total organic C was determined using the Walkey-Black method (Okalebo, *et al*., 2002). The soil sample (2mm sieved) was sieved using another sieve (0.25 mm sieve). 0.3g was added to a solution of sulphuric acid and potassium dichromate ($K_2Cr_2O_{7)}$ at 155 °C for 30 minutes for the oxidation process to occur. After complete oxidation, the unused $K_2Cr_2O_7$ was titrated against ferrous ammonium sulphate until the solution turned the color from greenish to brown (endpoint). The measure of organic carbon content was measured as the difference between added and unused $K_2Cr_2O_7$.

3.4.2 Statistical analysis

To understand the ability of soil bioassays to provide a reliable and effective measure of soil pathogen infestation in the field. A simple linear regression model was used to explore the bivariate correlation between soil bioassays and diagnostic disease incidences in different bean genotypes in the field to understand their effectiveness in predicting disease incidence in the field. Data for regression analysis (models) were checked for adherence to model assumptions (e.g., using residual plots vs fitted values for homogeneity of variance, normal Q-Q plot for normality of residuals) and transformed as needed. Additionally, more

trends and relationships between soil bioassay and field disease incidence were assessed through a Pearson correlation analysis, multidimensional principal component analysis (PCA) and scatter plots. To explore relationships and interactions between soil bioassays and soil physico-chemical variables to understand potential predictors of soil-borne pathogen pressure, was done using stepwise linear regression. All analyses were conducted using R version 4.5.0 with the above packages (“ggplot2", "dplyr", "ggpubr and “mlogit”)

CHAPTER FOUR

RESULTS

4.1 Field Symptomatology of the diagnostic feature of the fungal pathogens

The severity of disease symptoms caused by *Fusarium*, *Pythium*, and root-knot nematodes was observed across all five study regions. To assess different disease symptoms in the field, four bean plants were sampled from a 1x1 quadrat between 14 and 21 weeks, where the following symptoms were observed

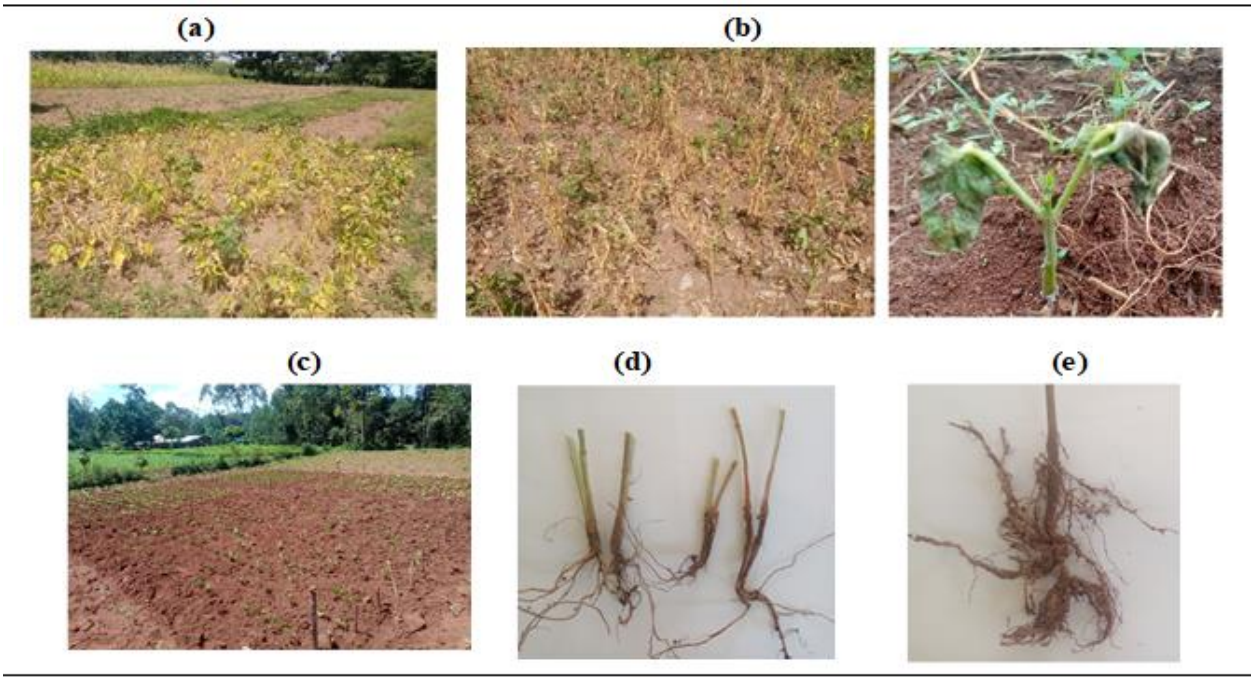

Figure 3: Bean plants parts showing field disease symptoms a) yellowing of bean plants b) wilting of bean plants, c) reduced plant stand count, d) Root rot infestation, and e) Root galling infestation

4.2 *Fusarium, Pythium,* and RKN bioassays

The Fusarium and pythium, and RKN assay results were as follows:

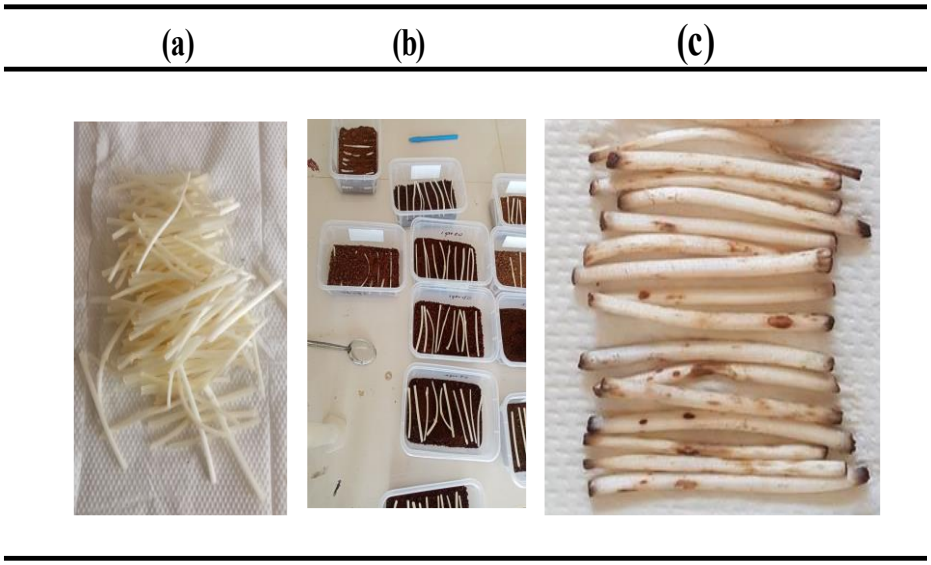

Figure 4: Stem lesions counted for Fusarium stem assay infection, a) white stems of beans after growing in darkness for 16 days, b) ~ 6cm long sections of bean stems buried in infested soil, c) lesion development on bean stems after 4 days incubated in the infested

For the pythium seed assay, the buried bean seeds in soil were washed, sterilized, and later plated in pythium selective media, PARP (Fig 5), which was used in the confirmation of soil pythium.

4.2.1 Identification and characterization of Fusarium and Pythium spp from the soil bioassay tests

Figure 5: *Pythium* infestation assessed by assessing the presence/absence of mycelia, a) Bean seeds removed from infested soil, b) Seeds placed on a *Pythium*-selective medium and incubated for 3 days at 21 ˚C, c) white mycelia from the medium are placed in a Petri dish and also incubated for 7 days at 21 ˚C.

Culturing of the lesions (Fig.1c) from the stem assay was done to validate the bioassay by identifying, and understanding the diversity of the fungal pathogens across the study sites. The analysis consistently identified two species of *Fusarium*, believed to be *F. oxysporum* and *F. solani* (Fig. 2). However, other fungal strains were not identified.

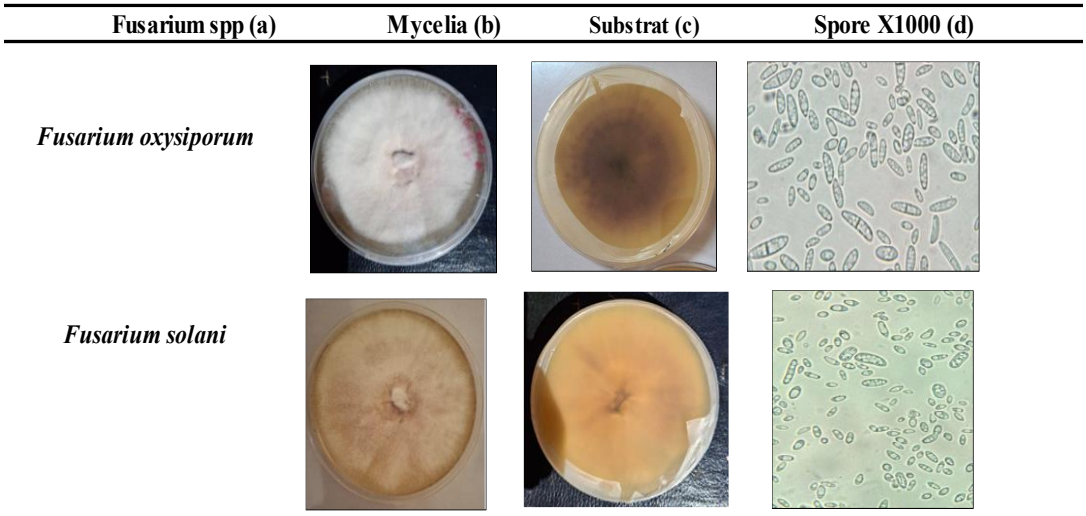

Figure 6: *Fusarium* species isolated from white lesions from the fusarium stem assay consistent with two known fungal characteristics: a) *Fusarium spp* (*Fusarium oxysporum* and *Fusarium solani*), b) Cultural and morphological features of the isolated *fusarium*

4.2.2 *Fusarium* bioassay infection predicting vascular browning infection

The approach used was based on Pearson correlation analysis, where the *Fusarium* stem assay infection and vascular browning severity showed a negative and statistically significant correlation ($r = -0.19$, $p < 0.001$, Fig.7a). When the bean varieties were grouped by genotype, *Fusarium* stem assay infection and vascular browning severity in susceptible varieties showed a negative and significant association ($r = -0.27$, $p < 0.001$, Fig 7b), with tolerant varieties showing a non-significant correlation.

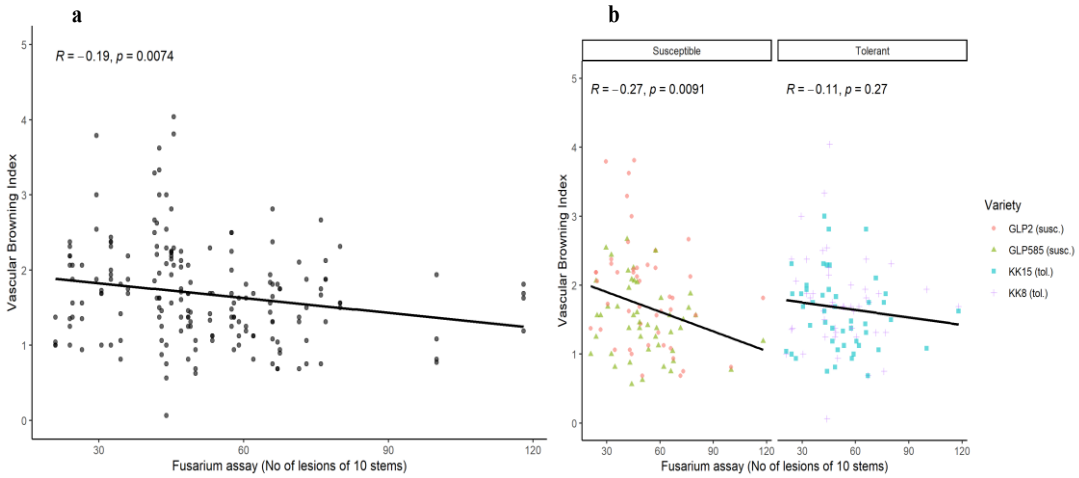

Figure 7: Plot showing relationship between a). *Fusarium* stem assay with vascular browning b). *Fusarium* stems assay and vascular browning grouped by susceptible and tolerant bean varieties

The procedure to examine the VBI involved visually examining the external stem at the soil line and dissecting it to check for internal vascular tissues for browning caused by *Fusarium* infection in the field (Figure 9).

Figure 8: Examples of dissected bean stems showing vascular discoloration for *fusarium* infestation

4.2.3 *Pythium* bioassay infection predicting Root rot infection

A Pearson correlation analysis showed a non-significant relationship between *Pythium* seed assay results on farmers' soils and root rot rating from farmers' fields (Figure 10a). When the genotype was added to the model, no significant relationship was observed (Figure 10b).

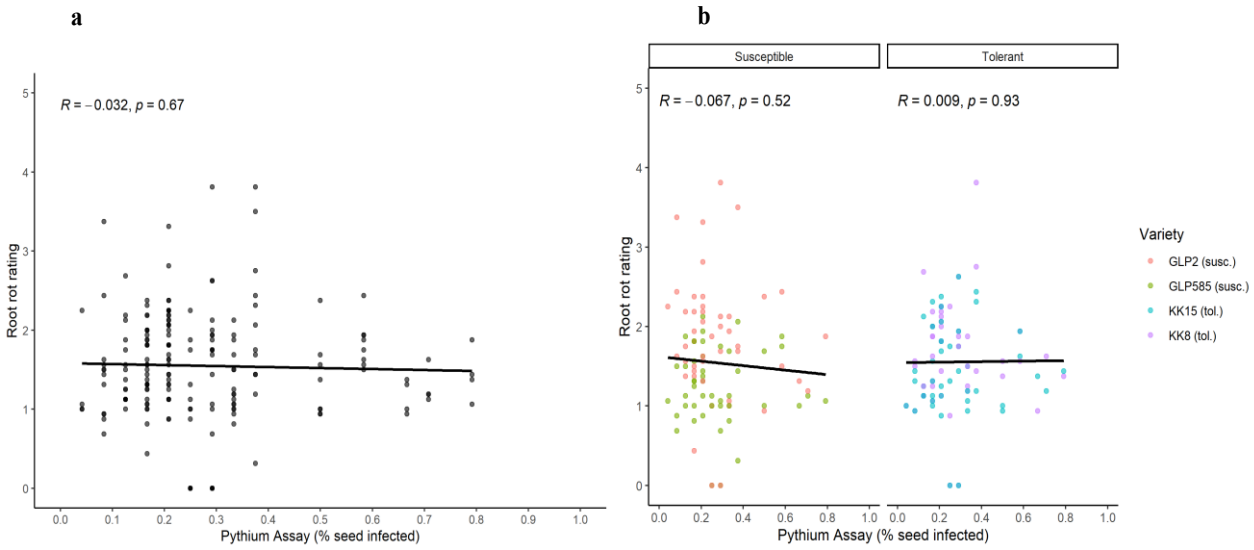

Figure 9: Plot showing the relationship between a). *Pythium* seed assay and root rot infection b). *Pythium* seed assay and root rot infection grouped by susceptible and tolerant bean

The procedure to examine the RRI involved visually examining the external root systems for rots caused by *pythium* infection in the field (Figure 11)

Figure 10: Examples of bean roots assessed for root rot symptoms, assessed for *Pythium* infection

4.2.4 Root rot Nematode bioassay infection predicting galling infestation

Pearson analysis showed a positive, significant (marginally) correlation between RKN assay and RKN field rating (gall rating) (r = 0.14, p = 0.063, Figure 11).

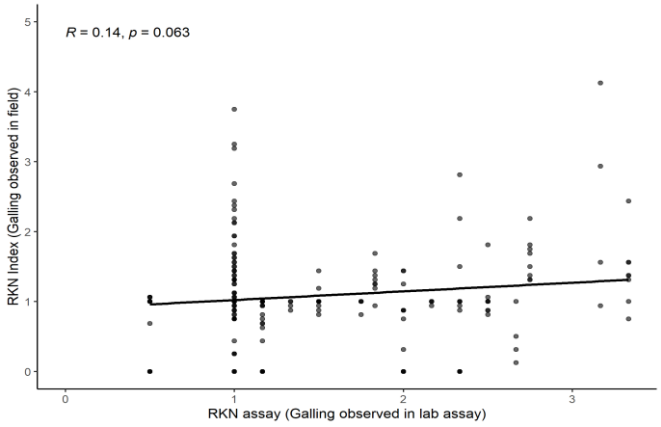

Figure 11: Plot showing the relationship between RKN bioassay and the RKN field infestation

The RKN field infestation was examined by visually observing the galling infestation of the bean roots caused by RKN in the field (Fig 13)

Figure 12: Root-galling severity of the sampled bean plants for RKN infestation

4.3 Relationship of the soil bioassays, field disease severity and soil physicochemical properties across the 50 farm sites in five counties of western Kenya

4.3.1 Performance (aboveground biomass and bean yield) for Fusarium susceptible and tolerant bean varieties across the 50 farm sites in five counties of western Kenya

The highest-yielding sites were Kisumu with an estimated grain yield of 282.0 kg/ha and Siaya with a 230.5kg/ha, with no significant difference in their average yields (Figure 14a). The yields from Homabay and Nandi were the lowest, statistically similar but lower than in Kisumu, with an estimated grain yield of 153.3 kg/ha and 103.8kg/ha, respectively. Furthermore, the sites with the highest average yields also exhibited the greatest variability in bean yield from one plot to another. (Appendix 2). Additionally, the different bean varieties across the sites performed differently, with KK8 having 221.4 kg/ha been highest average yield (Figure 14b), but statistically similar to GLP 2 and KK 15, which have an estimated yield of 218.3 kg/ha and 213.0 kg/ha, respectively. These varieties also showed

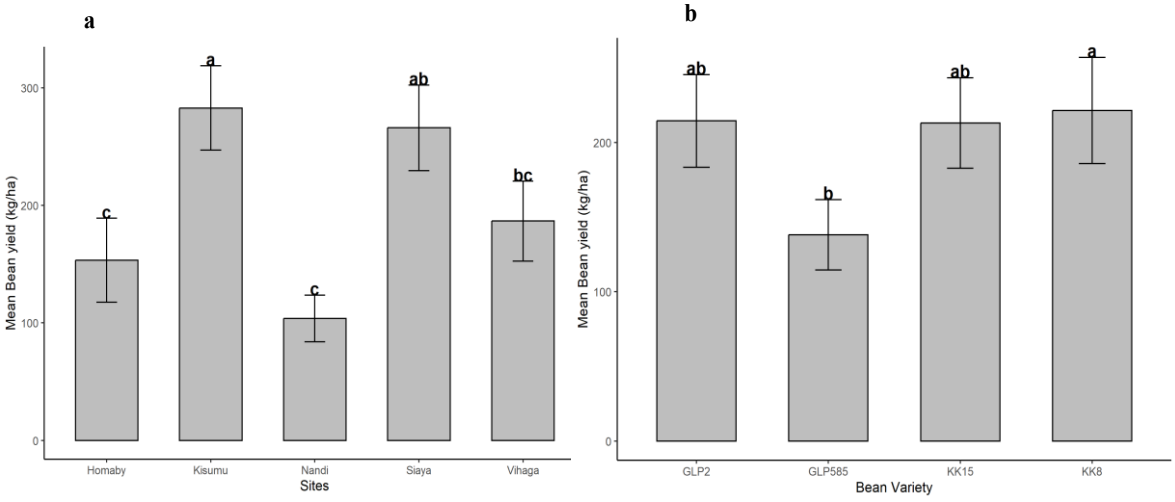

Figure 13: Chart showing comparison of mean bean yield across a) sites, and b) bean varieties incorporating statistical analysis through LSD and error bars

more variability in their yield performance across the sites. GLP 585 135.8 kg/ha had the lowest average yield, significantly lower than KK8 (Appendix 2)

For the above ground biomass in (Figure 15a), Kisumu had the highest average above-ground biomass**,** though not statistically different from Homabay site with greater variability in their results. Nandi and Vihiga sites produced the lowest above ground biomass, having no significant difference between them. Additionally, the above ground biomass of the different bean varieties among them, KK15 had the highest mean above-ground biomass (Figure 15b), significantly outperforming GLP 2, and KK8 which were the lowest performing varieties.

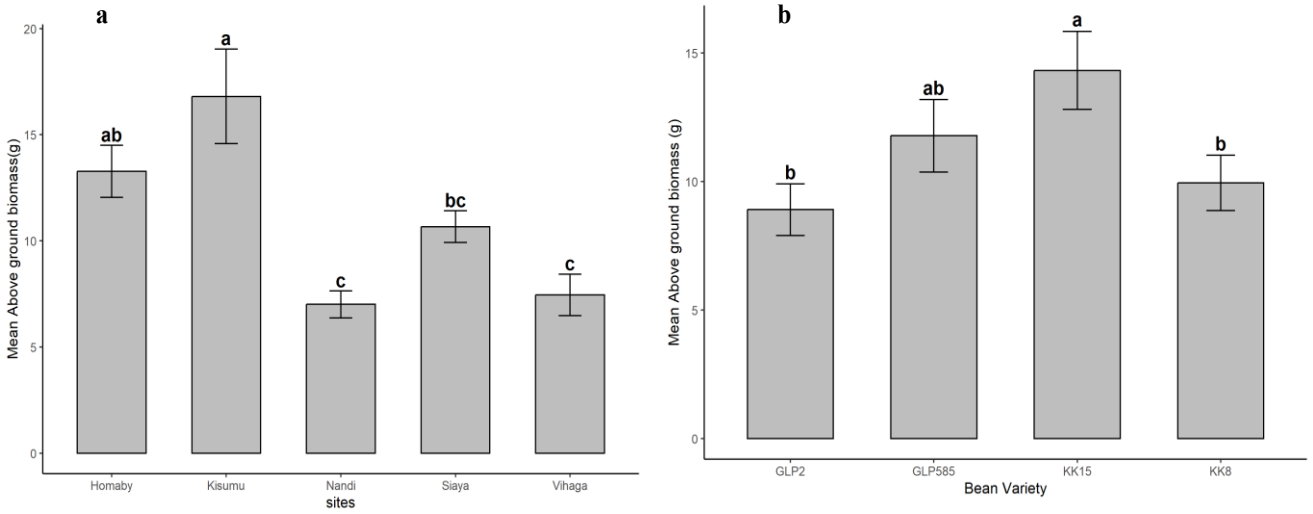

Figure 14: Comparison of mean above-ground biomass across a) sites, and b) bean varieties incorporating statistical analysis through LSD and error bars

4.3.2 Relationship between Soil bioassays and disease field severity

Other relationships between the three soil bioassays and disease symptoms were shown by the Pearson correlation matrix (Table 1). Apart from the previously mentioned relationships between bioassay and field disease symptoms for specific fungal pathogens, there was a significant correlation between the *Fusarium* stem assay and plant yellowing ($r = 0.19$, $p < 0.01$), with a positive relationship between plant yellowing and wilting ($r = 0.57$, $p < 0.001$). Furthermore, there was a negative association between the *Fusarium* stem assay and stand count ($r = -0.19$, $p < 0.01$). Moreover, a negative correlation between the vascular browning and the RKN bioassay ($r = -0.27$, $p < 0.001$) and the *Pythium* seed assay ($r = -0.15$, $p < 0.05$) was also observed.

4.3.3 Relationship between the standardized Disease index, bioassays, and bean yield

The Pearson correlation analysis linked the relationship between the standardized disease index with the bioassays and bean yield. The disease index combines multiple disease symptoms that measure the health of plant roots. A negative and significant relationship between bean yield and vascular browning and disease pressure was found ($r = -0.28$, $p < 0.001$, $r = -0.21$, $p < 0.001$, Figure 16).

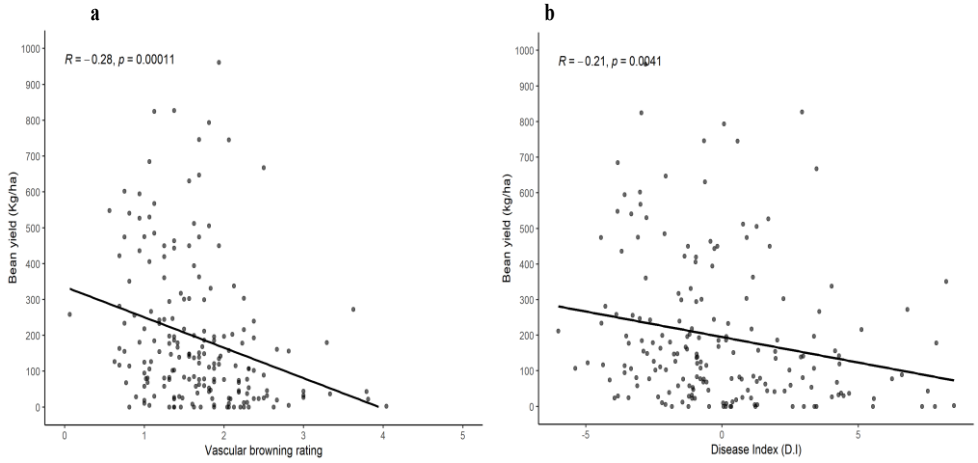

Figure 15: Plot showing relationship between a) Vascular browning and estimated bean yield b) disease index and estimated bean yield

When the *Fusarium* stem assay and disease index were correlated, it showed a statistically significant positive relationship *(r = 0.16, p<0.05).*

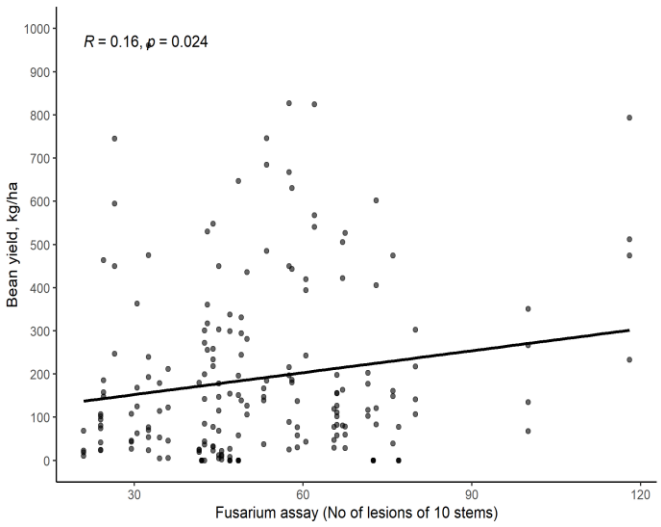

Figure 16: Plot showing relationship between disease index and *Fusarium* stem

4.3.4 Associated effects between different disease symptoms across the study sites

The PCA shed light on the differences in overall plant performance characteristics and disease field occurrence between study sites. Together, principal components (PC1) and (PC2) accounted for 29.8% and 20.4%, with a combined variation of 50.1 % in the data explained, respectively. VBI, RRI, and No. of plants with root rot were negatively associated with PC1; in contrast, RKN field rating (galling infestation), stand count, above-ground biomass, and estimated bean yield (kg/ha) were positively associated with PC1. Along PC2, plant wilting and yellowing were positively correlated. Variability in disease symptoms and plant performance was demonstrated by the relative overlap vs. clustering of the various sites (Homabay, Kisumu, Nandi, Siaya, and Vihiga) over the PCA space. While Homabay and Siaya sites are more dispersed toward disease-related factors, Kisumu tended toward higher above-ground biomass and yield, while Nandi site gathered in the center.

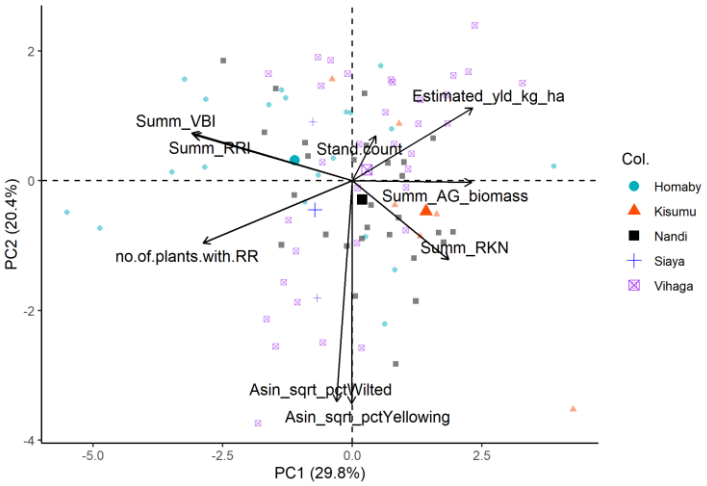

Figure 17: A biplot showing the distribution of field incidence based on different sites

4.4 Correlations between soil bioassays, disease symptoms, and soil physicochemical properties across the sites, Western Kenya.

4.4.1 Correlation between soil bioassays and physicochemical properties

According to the Pearson correlation matrix results on the relationship between soil bioassays and soil physicochemical properties (Table 1), the number of lesions evaluated in the *Fusarium* stem assay results was positively correlated with POM results, POXC levels, soil pH, and Amino N Sugar levels. Additionally, a negative relationship between *Fusarium* stem assay results and the percent sand and available phosphorus was observed. (Table 1). *Pythium* seed assay results showed a negative association with POM levels, available phosphorus, and sand content, whereas a positive association was observed with amino N sugar levels and percent clay. Simultaneously, the RKN assay findings showed a positive correlation with POXC levels and percent clay in soils (Table 1), while demonstrating a negative relationship with available phosphorus and percent sand in soils.

4.4.2 Correlation between soil physicochemical properties and field disease severity

The correlations and relationships between field disease occurrences and soil physicochemical parameters were demonstrated using a Pearson correlation matrix. VBI was positively associated with available phosphorus and percent sand, but negatively with soil pH, amino N sugar, percent clay, and POXC. Stand count correlated positively with amino N Sugar, and percent clay, but POM levels, POXC, pH, available phosphorus, and percent sand were negatively correlated. RRI was positively associated with soil pH and had a marginally significant relationship with POXC. Finally, RKN (galling rating) was

positively connected with POXC, pH, available phosphorus, percent sand, and POM, while negatively correlated with amino N (Table 1).

Table 1: Pearson correlation coefficients of soil physicochemical properties, disease incidence, and soil bioassay measurements across 50 farm sites with four bean varieties in Western Kenya

	POM	POXC	Soil Ph	Amino N	Av. P	%Sand	%Clay	Stand count	Yellowing	Wilting	VBI	RRI	RKN	F.A	P.A	R.A
POM (g kg−1)	1															
POXC (mg C kg-1)	**0.36*** **	1														
Soil pH	**0.5*** **	**0.19** **	1													
Amino Nv (mg N kg-1)	**0.15***	**0.66*** **	**-0.26*** **	1												
Av. P (mg P kg-1)	**0.34*** **	**-0.12+**	**0.61*** **	**- 0.41*** **	1											
Sand (%)	**0.31** **	**-0.19** **	**0.54*** **	**-0.48** **	**0.50*** **	1										
Clay (%)	**-0.35*** **	**>0.001**	**-0.65*** **	**0.40*** **	**-0.51*** **	**-0.83*** **	1									
Stand count	**-0.20** **	**-0.17***	**-0.51*** **	**0.15***	**-0.21** **	**-0.25*** **	**0.35*** **	1								
Yellowing	-0.034	-0.064	-0.075	-0.082	0.014	-0.011	0.089	-0.088	1							
Wilting	-0.034	**-0.17***	-0.035	-0.11	-0.044	0.11	-0.011	-0.053	**0.57*** **	1						
VBI	-0.008	**-0.18***	**-0.29*** **	**-0.39*** **	**0.29*** **	**0.29*** **	**-0.37*** **	-0.073	-0.027	0.1	1					
RRI	0.083	0.14+	**0.18***	0.1	-0.068	-0.064	-0.084	0.1	-0.047	-0.021	**0.49*** **	1				
RKN	**0.12+**	**-0.20** **	**0.16***	**-0.17***	**0.22** **	**0.22** **	-0.045	**0.041**	0.05	**0.24*** **	-0.091	**0.0007**	1			
F.A	**0.14***	**0.46*** **	**0.22** **	**0.31*** **	**-0.16***	-0.16*	-0.04	**-0.19** **	**0.19** **	**0.13+**	**-0.19** **	**0.12+**	0.06	1		
P.A	**-0.19** **	0.064	**-0.34*** **	**0.23** **	**-0.42*** **	**-0.42*** **	**0.50*** **	-0.04	-0.04	-0.075	**-0.15***	-0.032	**-0.20** **	-0.088	1	
R.A	-0.078	**0.20** **	-0.071	**0.12+**	**-0.19** **	**-0.19** **	**0.12+**	-0.015	-0.015	0.05	**-0.27*** **	-0.019	**0.14+**	**0.26*** **	-0.051	1

P-value: (+ marginal significance, $p < 0.05$; = $p < 0.01$; $p < 0.001$; ns., not significant) and Coeff: Model Coefficient

VBI: Vascular browning; RKN: Root knot nematodes field rating; RRI: Root rot rating; F.A: Fusarium stem assay; P.A: Pythium seed assay; R.A: Root knot nematodes plant bioassay; POM: Particulate Organic Matter; POXC; Permanganate Oxidizable Carbon: Av. P; Available Phosphorus: RRI; Root Rot rating

4.5 Linear regression models between *Fusarium* stem assay, bean varieties and soil physicochemical properties

4.5.1 Stepwise linear regression models relating disease incidence to bean variety, soil bioassay, soil physicochemical properties, and their interactions

Stepwise linear models sought to explore the relationship of the soil bioassays along with soil physicochemical predictors at each of the treatment plots to the disease symptoms, as aligned with the direct correlation findings (Table 1). The model indicated that the VBI was negatively associated with the Fusarium stem assay. Furthermore, VBI also showed negative associations with POM, percent clay, and Amino N sugar (Table 2). However, VBI had a positive association with soil pH, and the interaction between fusarium stem assay results and soil pH being strongly significant, revealing that the relationship between VBI and the *Fusarium* stem assay was negative at higher (more neutral) pH levels, while in acidic soils (below 5.3) this relationship became the expected positive relationship between VBI and *Fusarium* stem assay. Meanwhile, stand count showed an expected negative relationship to the stem assay results and soil pH, with the interaction between the stem assay and soil pH to stand count, and overall disease index being significant (Figure 10b, 10c). Symptoms of yellowing and wilting of beans in the field both showed an expected positive association with *Fusarium* stem assay results, though the relationship was only marginally associated with wilting. Bean variety had a significant association with VBI, stand count, and overall disease pressure.

Table 2: Regression models showing the relationship and interactions among the disease severity, bean variety, *Fusarium* assay, and soil physicochemical across the 50 sites in Western Kenya

	VBI		Stand Count		Yellowing		Wilting		D.I	
Predictors	**Coeff**	**P value**	**Coeff**	**P value**	**Coeff**	**P value**	**Coeff**	**Pvalue**	**Coeff**	**P value**
F.A	-0.006	**< 0.01**	-0.222	**< 0.001**	0.002	**< 0.01**	0.002	**0.08+**	0.029	**< 0.01**
Bean Variety	-	**< 0.01**	-	**< 0.001**	-	ns	-	ns	-	**< 0.01**
pH	0.050	**< 0.001**	-47.129	**< 0.001**	-	ns	-	ns	0.85	**< 0.01**
POM (g kg−1)	-0.470	**< 0.01**	-	ns	-	ns	-	ns	-	ns
Clay (%)	-0.011	**< 0.001**	-	ns	-	ns	-	ns	-	ns
AminoN (mg N kg-1)	-0.004	**0.069+**	-	ns	-	ns	-	ns	-0.021	**< 0.001**
POXC (mg C kg-1)	-	ns	-	ns	-0.0002	**< 0.05**	-0.0003	**< 0.01**	-	ns
F.A X pH	-0.015	**< 0.001**	-1.066	**< 0.01**	-	ns	-	ns	-0.043	**< 0.05**

P-value: (+ marginal significance, $p < 0.05$; = $p < 0.01$; $p < 0.001$; ns., not significant) and Coeff: Model Coefficient.

The times symbol (X) refers to the interaction between Predictor variables, F.A, Fusarium stem Assay, POXC, Permanganate oxidizable carbon, POM, Particulate Organic Matter

4.5.2 Relationship between *Pythium* seed assay, RKN assay, bean varieties, and soil physicochemical properties

A few significant relationships between the *Pythium* seed assay and field bean disease symptoms were observed. The stepwise linear regression showed an unexpected positive association between the *Pythium* seed assay with stand count, also capturing the varietal differences in stand count. The stand count showed a negative association with soil pH, and although an unexpected association between pythium assay and stand was observed, the interaction between pythium seed assay and POXC to stand count was positively significant. Finally, the RKN field (galling) rating was positively significant with the RKN assay. Soil pH, clay, however, it was negatively associated with POXC level (Table 3).

Table 3: Regression models showing the relationship and interactions among the disease incidence, bean variety, Pythium seed assay, RKN assay, and soil physicochemical properties across the 50 sites in Western Kenya

a) Responses	Stand Count		b) Responses	RKN field rating	
Predictors	**Coeff**	**P value**	**Predictors**	**Coeff**	**P value**
P.A	35.01	**< 0.001**	**R.A**	0.25	**< 0.05**
Bean Variety	-	**< 0.001**	**Bean Variety**	-	ns
pH	-45.55	**< 0.001**	**pH**	0.18	**< 0.001**
POMV (g kg−1	-	ns	**POXC (mg C l**	-0.002	**< 0.001**
POXC (mg C k	-0.01	0.3741	**Clay (%)**	0.99	**< 0.05**
P.A X POXC (r	0.67	**< 0.001**			

P-value: (+ marginal significance, $p < 0.05$; $p < 0.01$; $p < 0.001$; ns., not significant) and Coeff: Model Coefficient.

The times symbol (**x**) refers to the interaction between Predictor variables, P.A., Pythium seed assay, R.A, Root knot nematode assay, POXC, Permanganate oxidizable carbon, POM, Particulate Organic Matter

CHAPTER FIVE

DISCUSSION

5.1 Overview

This study aimed to assess the effectiveness and practical value of soil laboratory-based assays in detecting changes within agricultural systems, as suggested by Terekhova, (2011). Additionally, it sought to provide insights into the pressure from soil-borne pathogens, as well as the performance and disease challenges affecting beans in smallholder farming systems in Western Kenya. This was achieved by examining the relationships and interactions between various soil bioassays and the field disease symptoms of soil pathogens, *Fusarium, Pythium,* and PPNs in varying soil fertility levels across multiple agroecological zones. Western Kenya served as an appropriate setting for the study due to its considerable variation in soil properties, as revealed by the research, and its significance as a bean-producing region. Overall, the findings indicated that tolerant bean varieties yielded higher grain output and above-ground biomass, while showing lower levels of infestation compared to susceptible varieties across the different study locations.

5.2 Effectiveness of the bioassay to predict disease symptoms under different soil physicochemical conditions

The study found that Fusarium stem assay infection was positively linked to various field-observed bean disease symptoms such as reduced stand count, yellowing, and wilting. In contrast to our initial hypothesis, significant negative associations between Fusarium stem assay infection and vascular browning severity in susceptible bean varieties were observed.

This suggests that high levels of *Fusarium* stem assay infection were associated with low levels of VBI in the field. This could be due to the genotype-dependent nature of vascular discoloration and the capability of the *fusarium* stem assay to capture multiple *fusarium* species, either as parasites or saprophytes as described by Mutai, *et al.,* (2024). However, the study revealed a significant interaction between the Fusarium stem assay infections and soil pH in relation to VBI. This indicated that the interaction between the stem assay infection and VBI was strongly negative at higher (near-neutral) pH levels, while at lower (more acidic) pH levels, the relationship shifted to

a positive one, implying that the influence of Fusarium stem assay infection on vascular browning may be pronounced under acidic soil conditions. Furthermore, soil pH was also associated with vascular browning infection, indicating that pH plays a vital role in modulating disease pressure in the field. Differences in soil pH might alter Fusarium spp growth and inoculum density, with studies reporting the most suitable pH ranges for Fusarium growth is around 3.5 to 7 (Gupta *et al.*, 2010). Rousk, *et al.*, (2010), reported a positive correlation between fungal composition and pH ranges between pH 4 and 8, which is the ideal pH range for most crops which might contribute to the expression of disease symptoms (Schuerger & Mitchell, 1992) indicating the potential of the stem assay to predict disease symptoms in different soil pH ranges. Similarly, plant stand count declined with increasing levels of Fusarium stem assay infection, particularly under moderate to high soil pH conditions. This has been linked to damping-off disease (Gordon *et al.*, 2015),especially in young seedlings (Stewart *et al.,* 2006) ultimately impacting plant populations in the field, which aligned with the findings of our study.

Despite expectations of a positive relationship, *Pythium* seed assay infection with field root rot incidence showed a non-significant interaction. This finding concurs with previous outcomes by Martin & Loper (1999) , who noted the challenges of predicting field outcomes from controlled seed assays due to variable seedling responses and environmental influences. However, an interaction between *Pythium* seed assay infection and POXC levels to plant stand count was observed. Specifically, higher *Pythium* infestation was associated with lower stand counts, particularly at low POXC levels. POXC (labile carbon), a sensitive indicator of soil organic carbon (SOC) influenced by land management (Culman *et al.,* 2013; Hurisso *et al.,* 2016) affects soil microbial dynamics (R. R. Weil & Magdoff, 2004). These low-carbon dynamics may foster competitive interactions that support the presence and activity of *Pythium* species in agricultural soils (Broders *et al.,* 2009). Additionally, several studies demonstrated that repeated application of organic amendments positively influences SOM content (Bonanomi, *et al.,* 2020), thereby enhancing the soil's ability to suppress plant diseases (Bonanomi et al., 2018). These support our findings, which showed that the interaction between the *Pythium* seed assay infection and high POXC levels corresponded with greater plant stand counts, suggesting soils rich in organic matter exhibit suppressive characteristics, supporting healthier plant populations compared to soils with low organic levels, which had reduced stand counts. Additionally, the RKN assay effectively predicted field galling severity, indicating its potential as a useful tool for assessing nematode pressure in soils where beans or other RKN host crops are cultivated. Nonetheless, to improve the predictive reliability of the bioassays, further comprehensive testing that incorporates soil physicochemical properties and genotype responses is recommended.

5.3 Potential of the bioassay to predict disease pressure and bean yield

The soil lab assay results were promising under natural conditions, although field disease expression is influenced by environmental variables such as temperature and moisture (Jat & Ahir, 2013), adding complexity to field assessments. Despite these challenges, the study found that overall disease pressure was positively associated with the Fusarium stem assay infection, supporting its potential as an early indicator of disease risk in bean production. Furthermore, a significant negative correlation was observed between vascular browning severity and bean yield, suggesting that increased vascular damage is associated with reduced yield. Similarly, overall disease pressure was negatively associated with bean yield, reinforcing the trend that higher disease pressure leads to lower bean productivity. The study further demonstrated that disease pressure remained consistent across different levels of *Fusarium* stem assay infection at average soil pH. However, at lower pH levels, disease pressure intensified with increased *Fusarium* infestation, while at higher pH levels, there was a modest rise in disease pressure with increasing infection levels. These outcomes support those of (Huber & Watson, 1974), who demonstrated the effects of soil pH in disease severity, and (Otten & Gilligan, 2006), who emphasized the complex interactions between soil environment and disease development. The same observation was reported by (Tegg *et al.,* 2005), that poor root health often driven by fungal disease can substantially reduce field yields. Additionally, the results also suggest that VBI may be a practical proxy for *Fusarium* stem assays in estimating early disease pressure and forecasting potential yield loss in bean crops. However, the Fusarium stem assay still holds distinct value, particularly in its capacity to screen large numbers of soil samples under controlled conditions. The inverse relationship observed between the Vascular Browning Index and bean yield, further substantiated by the stem assay's ability to predict elements

of disease pressure in beans, strongly indicates that *Fusarium* bioassays have a stronger predictive potential than *Pythium* or RKN assays in the specific ago ecological context. It also shows the major role played by *Fusarium spp.* in reducing plant root health and consequently diminishing bean productivity in Western Kenya, with the findings of the study highlighting *Fusarium* as a key pathogen impacting agricultural outcomes in Western Kenya region

CHAPTER SIX

CONCLUSION, RECOMMENDATION, AND WAY FORWARD

6.1 Conclusions

1. A significant negative association between *Fusarium* stem assay infection and vascular browning severity in susceptible bean varieties was observed, contrary to our hypothesis. Despite expectations of a positive relationship, *Pythium* seed assay infection with field root rot incidence showed a non-significant interaction, and lastly, RKN bioassay correlated moderately with gall ratings, suggesting it can help detect nematode pressure.

2. Among the three tested bioassays only the *Fusarium* stem assay showed great potential in predicting disease symptoms and bean yield in the field, with *Fusarium* stem assay infection being positively linked to various field-observed bean disease symptoms, such as reduced stand count, yellowing, and wilting, with the overall disease pressure being positively associated with the *Fusarium* stem assay infection.

3. The study showed that plant growth is mediated by soil fertility, which influences pathogen prevalence, with the study showing a significant interaction between the *Fusarium* stem assay infections and soil pH in relation to VBI. Similarly, plant stand count declined with increasing levels of *Fusarium* stem assay infection,

particularly under moderate to high soil pH conditions. Additionally, an interaction between *Pythium* seed assay infection and POXC levels to plant stand count was observed.

6.2 Recommendation

The soil lab assay results were promising under natural conditions, with the expression been influenced by environmental factors adding complexity to field assessments but integrating bioassay results with soil physicochemical properties results provides a deeper understanding of soil pathogen infestation levels in the field, leading to more effective disease management.

6.3 Way forward

To improve the predictive power of the bioassays in predicting pathogen pressure in potential soils, further comprehensive and detailed testing that incorporates bioassay results with soil physicochemical properties assessment and genotype responses is needed.

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APPENDICES

APPENDIX I: Summary of the mean, standard deviation, minimum, and maximum for the different variables of soil physicochemical properties and disease symptoms

Variables	Mean	SD	Min	Max
Soil pH	5.97	0.64	4.90	7.84
POXC (mg C kg^{-1})	444.72	145.11	112.50	89.00
Amino N (mg N kg-1)	85.09	40.98	14.50	210.00
POM (g kg^{-1})	0.21	0.14	0.05	1.07
Available P (mg P kg^{-1})	3.93	4.24	1.00	27.50
Nitrates (mg kg-1)	34.57	18.06	9.13	153.04
Ammonia (mg kg-1)	7.06	6.82	0.70	45.61
Total Nitrogen (%)	0.06	0.03	0.02	0.19
Total Organic Carbon	0.06	0.03	0.02	0.19
Stand count	244.10	57.32	79.00	334.00
Above ground biomass (g)	11.23	9.15	0.79	68.20
Bean Yield	197.12	216.64	0.00	1019.71

APPENDIX II: General bean performance (above-ground biomass and bean yield) across the 50 study sites

		Above-ground Biomass (g)			Bean yield (kg/ha)		
County	**Bean genotype**	**medium**	**mean**	**sd**	**mean**	**median**	**sd**
Homabay	**Susceptible**	14.14	12.57	7.38	142.30	75.37	173.56
Homabay	**Tolerant**	10.77	13.98	8.18	164.29	60.26	272.24
Kisumu	**Susceptible**	12.38	16.74	13.40	306.81	267.56	255.69
Kisumu	**Tolerant**	12.59	16.87	15.10	258.82	201.12	197.61
Nandi	**Susceptible**	7.29	6.14	3.36	76.35	64.20	64.70
Nandi	**Tolerant**	8.96	7.87	4.48	131.23	73.19	162.18
Siaya	**Susceptible**	9.57	10.16	4.42	243.14	162.67	225.14
Siaya	**Tolerant**	9.45	11.18	5.07	288.81	276.24	239.95
Vihiga	**Susceptible**	3.34	5.20	3.75	123.22	71.76	140.23
Vihiga	**Tolerant**	7.68	9.71	7.35	249.97	148.11	257.81

[1] **Tolerance and Susceptibility of varieties to *Fusarium spp***

APPENDIX IIII: Summary of the mean, standard deviation of the above-ground biomass (g), and bean yield across the four varieties

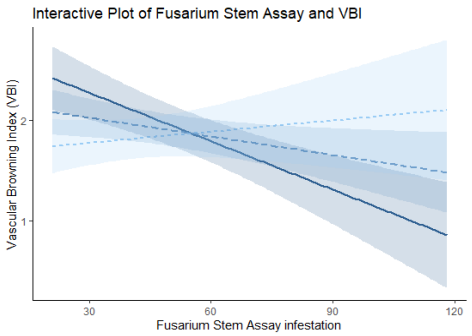
	Above ground biomass (g)				Estimated bean yield (kg/ha)			
Farmer sites	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Homaby	13.3	7.7	2.1	29.9	153.3	225.6	2.3	1019.7
Kisumu	16.8	14.1	2.2	68.2	282.8	226.9	0.0	793.7
Nandi	7.0	3.99	0.8	13.7	103.8	125.0	0.0	646.6
Siaya	10.7	4.71	3.5	23.7	266.0	230.5	0.0	1013.5
Vihaga	7.5	6.2	1.1	27.6	186.6	214.7	0.0	961.0

APPENDIX IV: Summary of the mean, standard deviation of the above-ground biomass (g), and bean yield across the 50 study sites

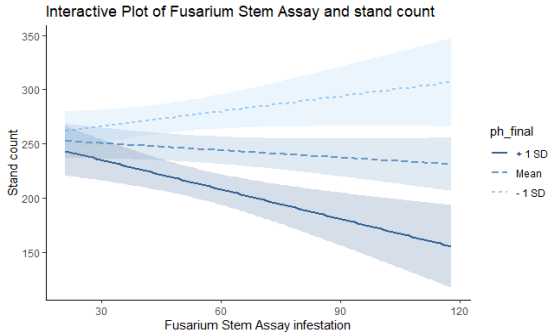
	Above ground biomass (g)		Estimated bean yield (kg/ha)	
Variety	**Mean**	**SD**	**Mean**	**SD**
GLP 2	8.9	7.7	218.3	222.1
GLP 585	11.8	9.9	135.8	164.2
KK 15	14.3	10.8	213.0	214.7
KK8	9.9	7.6	221.4	251.2

Appendix IV: Interaction plots between fusarium stem assay and pH in predicting a) Vascular browning b) Stand count c) Diseases pressure, d) interaction plot between pythium seed assay and POXC in predicting Stand count

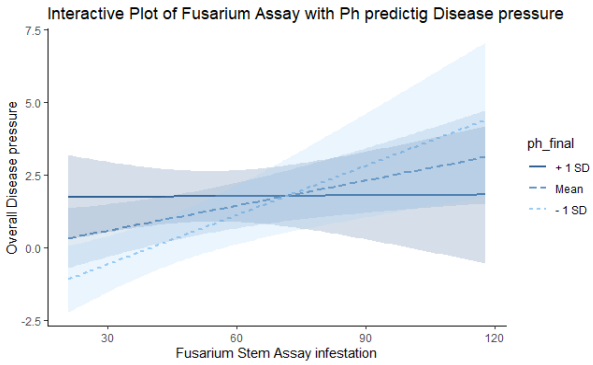
a



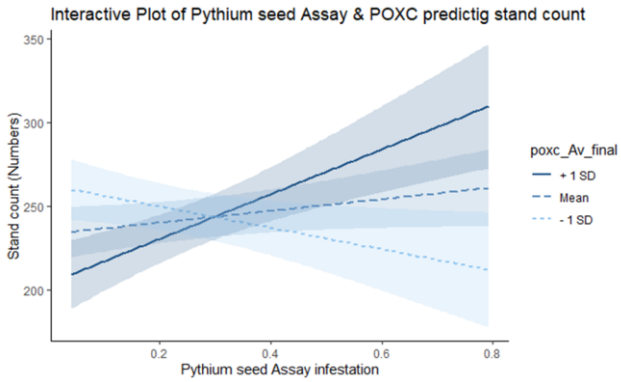
b



c



d



Appendix VI: Field activities carried out during the research across the study sites

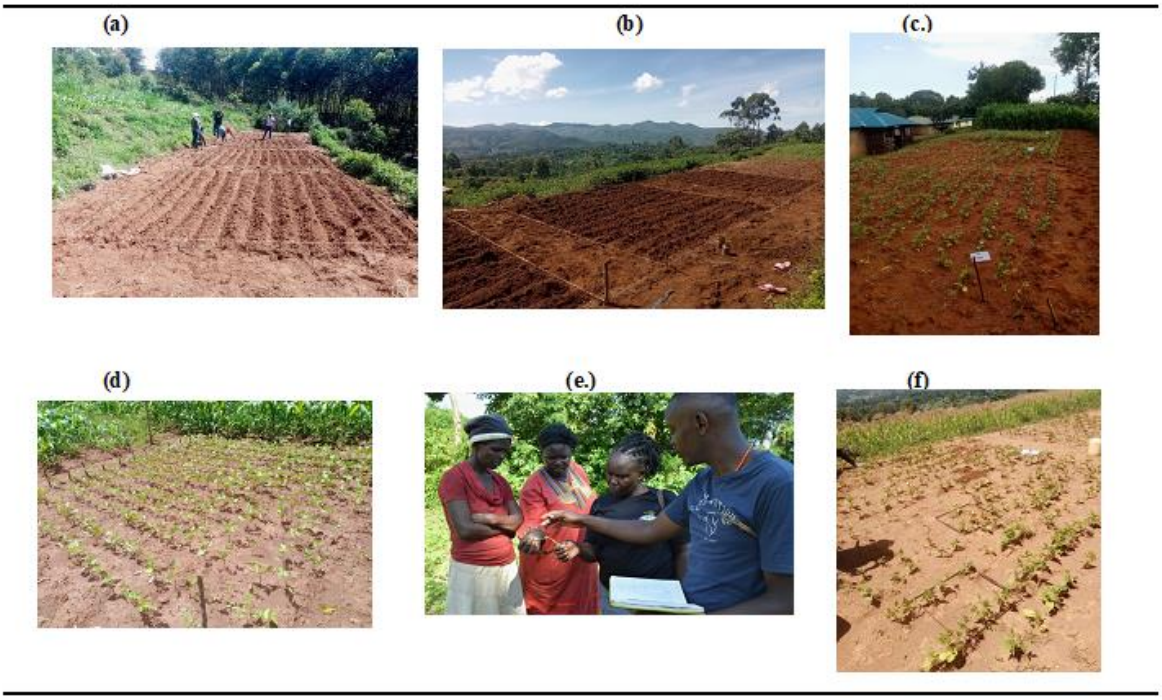


Figure 18: Field activities a) Soil sampling and field preparations, b) planting, c) Demarcating the treatment plots, d) Weeding, e) data collection, f) quadrat method along the transect in the treatment plot

Appendix VII: Sensitization and capacity building of farmers on the results attained across the study sites

Appendix VIII: Similarity Report

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