DEVELOPMENT OF MAIZE LETHAL NECROSIS DISEASE IN MAIZE PLANTS GROWN IN SOILS INFESTED WITH PLANT PARASITIC NEMATODES

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DECLARATION

This thesis is my original work and has not been submitted elsewhere for a degree in any other university or institution.

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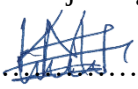
Signature……  …… Date……09/09/2022……..

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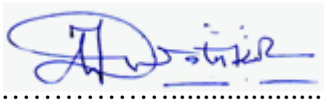
Signature…….  ..... Date….09/09/20212……

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DECLARATION OF ORIGINALITY

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DEDICATION

This study is dedicated to my lovely wife, Teresia Monjero and my lovely children; Grace, Susan, Joana and David for their great love, care, encouragement and perseverence throughout my study period. Above all I exalt The Almighty God for blessing me with life, opportunity and success.

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ABREVIATIONS AND ACRONYMS

BRI	Biotechnology Research Institute
DAS	Double Antibody Sandwich
ELISA	Enzyme-Linked Immunossorbent Assay
KALRO	Kenya Agricultural and Livestock Research Organization
MCMV	Maize chlorotic mottle virus
MLN	Maize Lethal Necrosis
SCMV	Sugarcane mosaic virus
UoN	University of Nairobi
ICIPE	International Centre of Insect Physiology and Ecology

ABSTRACT

Maize (*Zea mays* L.) is ranked as the third most important food crop by production globally, after rice and wheat. Several biotic (diseases, pests) and abiotic (unfavorable climatic conditions) factors affects its production. Maize lethal necrosis (MLN) disease outbreak within East Africa threatens production of maize. Information on interactions of viruses causing MLN with plant parasitic nematodes is lacking. This study was carried out to determine i) the effect interaction of of plant parasitic nematodes with viruses causing MLN on disease development in maize fields and ii) the effect of lesion nematodes (*Pratylenchus* spp.) on MLN disease development in the greenhouse.

For the field study, four counties in Kenya were visited, farms selected at random, MLN scored and both maize leaf and soil samples collected and analyzed for presence of viruses causing MLN disease and for parasitic nematodes. Snowball sampling or chain-referral sampling technique was used to sample MLN infected farms across the selected regions. Variance analysis was used to measure significant differences ($P< 0.05$) in MLN disease incidence and severity due to interaction between viruses and nematode populations. In the greenhouse study, two maize varieties, were used H614D and Emph 1101. Variety H614D is known to be susceptible to both MLN and *Pratylenchus* spp. whereas maize variety Emph 1101 is susceptible to MLN but resistant to *Pratylenchus* nematodes. The two maize varieties were subjected to three distinct treatments: single inoculation with MCMV and SCMV; combined MCMV + SCMV inoculation; the third treatment was the addition of *Pratylenchus* nematodes to the previous two treatments. Disease severity and incidence were recorded weekly over a period of two months.

Survey results indicated no significant effect of combined infestation of parasitic nematodes (*Pratylenchus* spp., *Tylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp.,) on MLN disease severity in the field. However, there was significant effect of Pratylenchus to MLN severity in the greenhouse experiment. The development of MLN disease in maize varieties Emph 1101 and H614D infected with *Pratylenchus* spp. nematodes was studied under a greenhouse experiment. MLN disease severity was higher in H614D than in Emph 1101. Plants inoculated with MLN+*Pratylenchus* recorded a significant difference across the two varieties on area under disease progress curve (AUDPC). There is need for nematodes management even though the field experiment indicated no significant effect of parasitic nematodes on MLN disease developement. There is also need for an open field study to evaluate the effect of Pratylenchus spp. on the development of MLN disease.

CHAPTER ONE

INTRODUCTION

1.1 Global maize production

Maize (*Zea mays* L.) is an important cereal crop grown throughout the world in different agroecological environments, ranking third after wheat and rice in terms of production (Wheeler and Reynolds, 2013). Maize was among the first cultivated plants between 7,000-10,000 years ago, as documented by Mexico archaeological sites of corn cobs and fossil pollen (Piperno and Flannery, 2001; Smith, 2015). Maize distribution from Mexico to other regions of Latin America, Caribbean, United States, Canada, Asia and Africa by European explorers was rapid, leading to its evolution and cultivation for human food and animal feeds (Brown and Darrah, 1985; Gibson and Benson, 2002; Vollbrecht and Sigmon, 2005).

Cereal grains are the main targets from a family of cultivated grasses which provide needed nourishment to humankind more than other foods and accounts for almost half of the total caloric requirement (Ranum *et al.,* 2014). Several cereal crops have been utilized for food; however, maize, rice, and wheat are the ones mainly utilized as human food sources and accounts for the highest consumption (Olugbire *et al.,* 2021). However, maize global human consumption is lower than the stated consumption percentage as a result of wastage, other non-food products usage, and processing as animal feeds (Ranum *et al.,* 2014). Its cultivation cuts across entire Africa making maize the dominant cereal, accounting for about 56% of the total food crops harvest area yearly.

Maize is one of the prefered source of calories, and is used as a primary weaning food for children in more than 20 developing countries globally. The highest percentage of maize is milled and packaged as flour, a process that removes the most nutritious outer layer of maize grains resulting

in the loss of minerals and vitamins (Uchendu *et al.,* 2016). Different maize types, based majorly on colour ranging from yellow to red to black, have been developed and adopted across the world. Yellow maize is of high preferrance in United States while white is mostly utilized in the southern parts of USA, Central America and Africa (Ranum *et al.,* 2014). Regions where white maize is more prefered for food have social status misperception of yellow maize due to it having been associated with food-aid programs for the poor communities while yellow maize is prefered for animal feeds (Louw *et al.,* 2010).

Maize production is dominated by North America and Asia, mainly by 4 countries which account for two thirds of global production; United States, China, Brazil and Argentina (FAOstat, 2014). Sub-Saharan Africa utilize maize as a major staple for income and food to more than 300 million smallholder farmers (Kadjo *et al.,* 2016). In 2021, more than 650 million people consumed an average of 43 kg yr^{-1} of maize, representing a 35% increase since 1960 (Shiferaw *et al.,* 2011).

1.2 Maize production in Kenya

Maize is the main food crop to over 90% of Kenya's population. It accounts for 65% of total staple food intake with an average person consumption of 77 kgs of maize and its products per year (Ariga *et al*., 2010; FAOSTAT, 2014). Maize is mainly produced in the Rift Valley, including Uasin Gishu and Trans Nzoia counties.

Maize production in Kenya fluctuate over years which result in supply shortage, and ensuring sufficent supply of the crop is vital to national food security (Yearbook, 2013). However, there was an increased production in 1994, 2001 and 2003 of which average annual production accounted for 2.3 million tonnes which could not meet annual consumption demand of 2.6 million

tonnes in the same period (Kariuki *et al*., 2018). The country relies on food import due to 40 percent of its population being food insecure (Faostat and Production, 2016; Mutimba *et al.*, 2010). Maize is widely consumed in Kenya's parts of central, Rift Valley, western and eastern regions. Morever, maize has been used in these regions as a source of income, improving the local's living standards. Consequently, factors that threaten maize production inherently impact food security.

Average annual consumption rates of maize in Kenya are amongst the greatest in East Africa which accounts yearly per-capita consumption of approximately 77 kg (Koskei *et al.*, 2020). A survey by world bank in 2015 indicated that 38% of the population in Kenya cultivates maize of which 70% is produced by smallholders for the domestic market, with the remainder produced by large scale, commercial organizations for the export market. The majority of smallholder maize production is for subsistence rather than for income generation, indicating that most families are dependent on maize as their main source of food (Simiyu, 2014). Of the 1.6 million ha of land under annual maize cultivation, 80% is owned by smallholder farmers.

1.3. Maize production constraints

Maize in Kenya is mainly produced under rainfed conditions and, therefore, erratic and lack of rainfall are the principal abiotic causes of low yields (Nyoro *et al.*, 2004). Small scale farm holders dominate most of Kenya's maize production. These farmers are strained by lack of enough resources and therefore cannot afford agricultural inputs like fertilizers and quality seeds. The high cost and inaccessibility of certified seed has affected the adoption of improved varieties leading to poor yields (Shiferaw *et al.*, 2011). An estimate of about 30-1005 bags of maize is lost every year

due to weeds infestation, such as striga, and decline in soil fertility (Jamil *et al.,* 2012; Manyong *et al.,* 2007).

Pests and disease infestation during cultivation and storage are the main biotic factors that limit crop production in Kenya (Pingali *et al.,*2001). Main pests affecting maize production include fall armyworm, stem borers and locusts. Fall armyworm causes 21-53%, stem borers resulting in about 15% of losses every year, while the larger grain borers may cause upto 100% losses of stored maize (Day *et al*., 2017). Diseases affecting maize production include fungal diseases such as Fusarium and Gibberella stalk rots and ear rots both affecting the roots, stalk and ears: others include anthracnose stalk rot, leaf blight and southern rust. Maize production is also affected by diseases caused by viruses including maize streak and the current maize lethal necrosis (MLN) disease (Savary *et al.,* 2019). In the recent years, MLN has emerged as an important viral disease of maize. It is caused by a combination of two viruses, that is, Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV), resulting in significant yield losses in Kenya (Wangai *et al.,* 2012). Nematodes have also been recorded across the globe as major pests of maize. Plant parasitic nematodes infecting maize have been studied across species and level of pathogenicity, correlation of population densities and impact to yields, determination of environmental influence to severity and management strategies (Norton, 1983, Tylka, 2007, Kimenju, 2008 and Bekker *et al*., 2016). Limited research on biotic and abiotic management is amongst constrains affecting maize production. There is need to carry out more research on crop diseases, pests and their interaction as well as developing resistant crops in order to address food security.

1.4 Statement of the problem

Although almost the entire Kenyan population is dependent on maize as main food crop, animal feed, and income generation, the country produced 42.1 million bags in the year 2020 which is less than the national demand of 52 million bags. The deficit is complimented by imports of maize from other countries, such as Uganda and Tanzania (Wamalwa, 2020). Among other factors, MLN poses a high threat to maize production across the country and East African region with over 80% crop loss (Wangai *et al*., 2012). A combination of MCMV and SCMV led to the outbreak of MLN in Kenya (Wangai *et al*., 2012). The loss due to this outbreak was very high, thus calling for more research on its epidemiology and management. Generally, different pathogens are kown to interact and affect disease severity in any given crop (Belval *et al*., 2019), and this is also suspected to be the case for MLN disease. Currently, there is minimal research on synergies between MCMV, SCMV and other pathogens (fungal, bacterial, nematodes and other viruses). There is need to study the role of plant parasitic nematodes associated with maize in the development of MLN disease.

1.5 Justification

Maize lethal necrosis diseases has had a devastating impact locally and globally, thus threatening food security (Wangai *et al*., 2012). Interaction between different pathgens infecting a crop have been shown to lead to increased disease severity or reduced level of disease resistance. Despite the adverse impact of MLN disease, information on the effect of other maize pathogens on MLN development is limited. For instance, decrease in maize yields due to damage caused by parasitic nematode have been documented at a range of 0 to 10% in the United States of America and up to 50% in Kenya (Tylka, 2007; Kimenju, 2008). However, there are no studies to show how the

nematodes may interact with MLN-causing viruses and their effect on infected maize. In addition, MCMV is known to be quite stable in soil (Jiang *et al*., 1992), and there is likelihood that root-infecting nematodes may aid in transfer of the virus into plant roots leading to MLN disease development, but there is no documented evidence. There is therefore need to conduct more research on effects of parasitic nematodes on MLN disease development.

This study was carried out to determine the effects of common plant parasitic nematodes on MLN disease development on infected maize plants in the field and how different plant parasitic nematode species affect individual viruses causing MLN disease. The study results will add on to the understanding of MLN disease epidemiology, leading to more effective MLN disease mangement, thus improving maize yields, and resulting in increased income, animal feeds and food for current and future population.

1.6 Objectives

1.6.1 Broad objective

To enhance management of Maize lethal necrosis (MLN) disease by determining the role of plant parasitic nematodes in disease development

1.6.2 Specific objectives

i. To determine the effect of interaction of plant parasitic nematodes with viruses causing Maize lethal necrosis on disease development in maize fields in major maize growing regions of Kenya

ii. To determine the effect of lession nematode infestation on severity of Maize lethal necrosis disease.

1.7 Hypotheses

i. High infestation of maize by plant parasitic nematodes in the field results in increased level of MLN disease severity.

ii. Lesion nematode (*Pratylenchus* spp.) infestation significantly increase maize lethal necrosis disease severity in maize.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of maize cultivation

Maize originated from South America and was taken to Europe by Christopher Columbus in the 15th Century and since then it has been dispersed to the rest of the world, including Asia and Africa (Purseglove, 1976). In Kenya, it was first produced during the 15th Century by the Portuguese along the coast (McCann, 2001). Today, maize is an important cereal crop worldwide and is cultivated globally across diverse agro-ecological zones. Maize is a tall plant (up to 3 m, depending on variety and altitude) belonging to the grass family (Poaceae), and is cultivated between 58 North and 40 South latitudes and to an altitudes above 3000 meters above sea level, and in regions that experiemce an annual rainfall of between 250 to more than 5000mm (Jakhar *et al.*, 2017).

2.1.1 Maize production in Kenya

Kenya relies on maize as a significant staple and food security crop, with approximately 90% of its population depending on maize for food, employment and income (Mohajan, 2014). It is cultivated in the Rift Valley, part of central, western and eastern provinces. However, biotic and abiotic factors contsrains its production. Ecological conditions, including drought and soil fertility, are key abiotic factors that contribute to low yields, as are low rates of utilization of new technologies, such as use of hybrid seeds and other improved agronomic practices. Infestation by weeds, insect pests and diseeases are among the critical biotic factors straining maize production in Kenya leading to up to 30% yield losses annually. Losses due to plant-parasitic nematodes may go up to 50% on maize in Kenya, with lesion nematodes being rated as the most significant

nematode species with highest impact (Kimenju *et al.,* 1998). Due to continuous cropping systems as a common practice in small-scale farming, nematodes population build-up is high. Maize is mainly affected by root-knot (*Meloidogyne* spp.) nematodes and lesion nematodes (*Pratylenchus* spp.) which cause a significant decline to crop production (Coyne *et al*., 2018).

2.2 Maize lethal necrosis disease

Maize lethal necrosis (MLN) disease or Corn lethal necrosis (CLN) disease is as a result of a combined infection of maize by Maize chlorotic mottle virus (MCMV) (*Machlomovirus*, *Tombusviridae*), and Sugarcane mosaic virus (SCMV, potyvirus; Potyviridae) or alternate cereal Potyvirivuses such as Wheat streak mosaic virus (WSMV) and Maize dwarf mosaic virus (MDMV). Maize lethal necrosis was first reported in Kansas, United States. The disease was later discovered in Nebraska, Hawaii, china and in Kenya (Niblett and Claflin, 1978; Uyemoto *et al*., 1980; Wangai *et al.,* 2012). The outbreak in Kenya was in 2011 and has since been reported across all maize growing regions in East Africa (Adams *et al*., 2014). While MCMV is a new pathogen in the region, SCMV has been historically prevalent in many parts of Kenya and East Africa and affects most cereals (Chambers *et al.,* 2014).

2.2.1 Etiology of Maize lethal necrotic disease

In East Africa, field infection of maize by MLN is mainly due to synergistic interactions between SCMV and MCMV, and results in chlorotic mottling and leaf bleaching, necrosis, and severe stunting that often lead to plant death. In contrast, single infections by SCMV or MCMV lead to mild mottling or mosaic and reduction of apical growth. Disease damage is greater in maize plants

infected in the early growth stages and if water availability is low and temperatures are high. Under these conditions, leaves starts to yellow and die from the margins, while the husks covering the cob desicates, before the plant dies prematurely. Any grains that may have formed in the husk discolor and become infected with fungi, rendering them useless for food or feed (Samita, 2018).

2.2.2 Host-range for MLN viruses

Research has shown that maize is the principal host of MLN which affects members of Poaceae family (Mudde *et al.,* 2019). Maize chlorotic mottle virus has not been isolated from dicotyledons, but has been established across a diverse experimental host-range which comprises of about 19 species of grass (Cabanas *et al.,* 2013). Some of MCMV plant hosts besides maize are; sorghum, finger millet, Napier grass, sugarcane, kikuyu grass (*P. clandestinium*).

2.3 Maize chlorotic mottle virus

The first reported incidence of maize infected with MCMV was in Peru then later spread to Brazil, Argentina, Mexico, USA, Thailand, and China with two geographically based and genetically different strains (Lapierre and Signoret, 2004; Xie *et al*., 2011). Initial isolation of MCMV which belongs to *Tombusviridae* family and *Machlomovirus* genus was from Peru samples in 1971 (Nault *et al.,* 1979). The first report in Kenya was in 2011 (Adams *et al.,* 2014; Wangai *et al.,* 2012). On avarage, MCMV yield losses are between 10-15% as reported in floury and sweet maize varieties in Peru (Liu *et al.,* 2015). Maize chlorotic mottle virus typical symptoms comprise of mosaic and chlorotic mottling which gets to a severe level depending on maize varieties, crop stage and farm management at the time of infection (CIMMYT Maize Program, 2004). With fast spread across

farms, regions and the synagy with Potyviruses; SCMV, MDMV, and WSMV, and the lack of resistant commercialized maize varieties, MCMV becomes the significant virus in crop production as a significant food crop within sub-Saharan region (Braidwood *et al.,* 2018). The virus structure is made up of a single-stranded positive sense RNA genome which is enclosed in a 30nm icosahedral virion (Cabanas *et al.,* 2013; Stuart *et al.,* 2004).

2.3.1. Transmission of MCMV

Insect vectors, which include maize thrips (*Frankliniella williamsi*), leaf beetles (*Oulema melanopa*) and rootworms (*Diabrotica undecimpunctata, D. lonicornis* and *D. virgifera*), are important in the transimission of MCMV. Furthermore, mechanical transmission of MCMV, particularly during farming operations has also been reported (Mahuku *et al.,* 2015; Wangai *et al.,* 2012). The virus is easily transmitted in laboratory and greenhouse experiments mechanically. Mechanical inoculation of MCMV has been found to successfully infect *Digitaria sanguinalis, Hordeum* spp., *Bromus* spp., *Eragrostis trichodes, Setaria* spp., *Panicum* spp., *Triticum aestivum* and *Sorghum* spp., (Gordon *et al.,* 1984). Kansas type 1 infected *Zea parviglumis* and *Zea luxurians* (Mahuku *et al.,* 2015). Currently, there has been extensive MCMV research on its transmission and stability, especially during farm resting periods. Maize chlorotic mottle virus was a major problem for temperate seed production in Hawaii in the 1990s although MCMV transmission through seed has been found to be insignificant; up to 0.33 % (Jensen *et al.,* 1991).

2.3.2 Maize chlorotic mottle disease symptoms

A variety of symptoms in maize are caused by MCMV, depending on maize varieties, stage of crop at infection, and ecological conditions, including leaf chlorosis, shortened male flowers with reduced spikes, malformed, shortened internodes, and poorly filled ears (Gordon *et al.*, 1984). Disease expression range in severity from mild chlorosis to severe stunted growth, necrosis, and premature plant death (Niblett and Claflin, 1978; Uyemoto *et al*., 1981). Co-infection with MCMV and a potyvirus in maize results in a variety of symptoms that are typical of viral diseases, including chlorotic mottling of leaves, which usually starts at the leaf base, spreading upwards to the tips of the leaves, and leaf margin necrosis, progressing through the mid-rib leading to ultimate leaf death (Niblett and Claflin, 1978; Uyemoto *et al*., 1981). When necrosis occurs in the leaves of a whorl prior to expansion, then 'dead heart' occurs. Severely infected plants produce small cobs that contain poor or even lack grain, or entire crop death before tasseling (Deressa and Demissie, 2017).

2.3.3. Impact of MCMVon crop yield

Maize chlorotic mottle virus, regardless of the abscence or presence of other viruses, affects maize yields. In Peru, average losses of 10 and 15% as a result of MCMV were reported in sweet corn and floury varieties (Liu *et al.,* 2015). Whereas, a reduction in maize yield loss of up to 59% was observed in experimental plots of inoculated maize plants (Castillo and Hebert, 1974). In the US, MLN (MCMV and portyviruses) caused yield losses estimated at between 50 and 90% depending on maize varieties (Uyemoto *et al*., 1980).

2.4. Distribution and transmission of SCMV

Sugarcane mosaic virus (SCMV) is found across the globe, occurring in America, Europe, Africa, Australia, and Asia as single or multiple SCMV strains (Shukla and Ward, 1989). In Kenya, SCMV was detected in maize samples collected from 28 districts (Louie and Darrah, 1980). Incidences varied from district to district with Nyanza at 15.2%, Rift Valley at 15.8% and western counties at 19.6% (Louie, 1980). Artificial inoculation studies in East Africa showed losses of up to 50% (Louie and Darrah, 1980), and there is evidence of alternative hosts, including sugarcane, johnsons grass, sorghum, oats, millet, and sudan grass.

Sugarcane mosaic virus is principally vectored by *Aphis gossypii* and *Myzus persicae* (Mahuku *et al*., 2015; Wangai *et al*., 2012). Mechanical transmission may also be through infected stalk cuttings and represents a minor transmission route which has only been recorded from glasshouse and laboratory studies (da-Silva *et al*., 2015).

The relative importance of seed cane as a source of disease was demonstrated in the 1990s from the incidence of mosaic in adjacent fields cultivated with variety CP72-2086 that had been established from different sources (Cerruti, 2006). Sugarcane mosaic virus incidence in one field was 95%, compared with 22% in the adjacent field. The two seed cane sources of CP72-2086 had been cultivated in close proximity for 15 to 20 years, indicating that transmission by aphids had not been rapid (Cerruti, 2006).

2.5. Management of Maize lethal necrotic disease

Prevention is the best strategy in MLN management, where seed inspection and seed farm screening are key. In Kenya, tests have been performed at the Kenya Plant Health Inspectorate

Services (KEPHIS) to test for MCMV in all seed for export and import, including breeders and research maize fields. Domestic regulations prevent transfer of maize and maize products from infected regions to clean regions. Communication of public information on MLN disease awareness and management is through press releases, posters, field days, public events, brochures, sensitization workshops, and radio programmes.

The recommended management approach for MLN disease is integrated pest management that includes cultural methods like crop rotation, skipping of season, cultivation of resistant varieties, and crop diversification, and chemical control through use of seed treatment and insecticides. Utilization of resistant or tolerant maize varieties is potentially an effective mode of MLN management, reducing related yield losses (Nelson *et al.,* 2011).

2.6 Impact of Maize lethal necrosis on maize yields

In high MLN diesease endemic areas in Kenya, farmers experience very high crop loss where affected plants are mostly barren as a result of small ears that contain few or no seeds (Wangai *et al.,* 2012). Given the affected areas constitute the major source of maize production, and losses may reach up to 100%, MLN becomes a significant factor to food production in Kenya and its impact may resonate throughout the entire economic maize chain.

2.7 Plant parasitic nematodes infecting maize

Maize as a very significant food crop within sub-Saharan Africa faces critical nematodes infestations leading to high levels of losses which when combined with other biotic and abiotic factors may lead to 100% loss. About 120 species of plant parasitic nematodes infect maize across

the world, however, due to environmental difference, only 3-7 species can be isolated from a field at a given time. Despite the high number of plant parasitic nematodes affecting maize production, a few are known to be of pathological significance to production (Norton, 1983). *Pratylenchus* spp. and *Meloidogyne* spp. (RKNs) are the main nematodes which cause a significant loss in maize production (Coyne *et al*., 2018). Several other plant parasitic nematode species may also be present even though high chances are lesion nematodes across most farming systems (Mc-Donald *et al*., 2017). Most maize farms within the sub-Saharan region experience poor growth and yields which is attributed to Lesion nematodes. Lesion nematodes infestation lead to necrotic lesions, reduced root mass which finally leads to poor root system thus affected uptake of minerals and water. Most identified *Pratylenchus Spp.* include; *Pratylenchus zeae* and *Pratylenchus brachyurus* being most common, *Pratylenchus sefaensis*, *Pratylenchus hexincisus, Pratylenchus delattrei, Pratylenchus penetrans*, and *Pratylenchus scribneri* have also been documented. Maize is referred to as a poor host of RKNs, due to absence of symptoms associated with typical galling Meloidogyne spp. Maize infestation with either single or both *M. incognita* and *M. javanica* may lead to high loss in yields of ≥50% (Odeyemi *et al*., 2011; Riekert and Henshaw, 1998). There are other parasitic nematodes which infest maize; *Ditylenchus, Helicotylenchus, Longidorus, Criconematidae*, *Hemicycliophora, Telotylenchus, Meloidogyne, Rotylenchus, Scutellonema, Quinisulcius, Tylenchorhynchus, Xiphinema* spp.*,* and *Hoplolaimus pararobustus* (Groote *et al*., 2016; Schuurmans Stekhoven and Teunissen, 1938). These plant parasitic nematodes have been documented to have an economic impact to maize production at different levels in various regions. *Rotylenchulus* plant-parasitic nematode genus is of key concern having been identified to infest maize in earlier years, its impact and pathogenicity on maize production remain unknown (Donald

et al., 2017; Van den Berg *et al*., 2017). However, due to the difficulty involved in identification of genera or species of plant-parasitic nematode through morphological methods or morphometrical approaches, eggs within plant roots have been identified using molecular techniques for both *Rotylenchulus* and *Meloidogyne* which led to confirmation and identification of *Rotylenchulus* (Bekker *et al*., 2016).

Nematodes infest any part of maize plant, including the stem, cob, leaf, seed pods, seed, and flower. Plant parasitic nematodes bears a long stylet which pierces the root to allow feeding on the inner root tissue, while *Helicotylenchus* spp. have a short stout stylet adapted to feeding on superficial parts of the root system (Bekker *et al*., 2016).

Most plant nematode species parasitize the exterior or interior of plant roots, and during this mechanical process, roots also sustain chemical damage through the release of substances that hinder absorption of water and nutrients and also disrupt translocation vessels. Common symptoms of nematode infestation include distortion, enlargement or nodulation of the roots; reduction in root mass; increased root fibrosity; and, shortened root systems. Injurious nematode infestation leads to additional opportunistic infection by other nematodes, and fungal and bacterial infection that increase the risk of plant mortality (Bekker *et al*., 2016).

2.7.4 Life cycle of plant parasitic nematodes

Nematode life-cycle comprises six distinct stages: egg, 4 juvenile stages, and adult stage (Sally *et al.*, 2010). Duration varies among the stages, and with species, biotic and abiotic factors, like temperature, moisture, and host plant. Most species undergo shortened life cycles and undergo several stages within a season in tropical climates that may lead to rapid increases in population,

including from single pathogenic or two dioceous nematodes (Coyne *et al*., 2018). Nematodes are able to withstand environmental stress conditions, such as extreme high and temperatures, and drought, although survival varies with duration of the stressor. Most species of nematode may survive long periods of time in the egg stage encapsulated in cysts (*Heterodera* spp.), and as second *(Anguina* spp.) and forth (*Ditylenchus* spp.) stage juveniles.

2.7 Lesion nematodes

Plant lesion nematodes (*Pratylenchus* spp.) threaten crop production, especially crops in the Poaceae. Across plant parasitic nematodes, there are over 120 species known to infest maize across the globe causing significant reductions in maize yield (Tylka, 2007). *Pratylenchus* spp. occur sporadically, usually in high numbers. Aboveground symptoms of *Pratylenchus* spp. infestation varies with type of nematodes and their population, and environmental conditions; root damage consists of small lesions, destruction of the epidermis, lack of root hairs (Handoo, 1998).

2.8 Management of *Pratylenchus*

Effective *Pratylenchus* nematode management is dependent on its detection and estimation of population density. General management strategies target the reduction *Pratylenchus* soil populations to a non-pathological level which include quarantine, reducing of initial nematode population, inhibition of reproduction, and minimized crop damage. Quarantine involves preventing the introduction of nematodes to a clean area from an infested area using regulatory instruments. Cultural control methods include crop rotation, harrowing and ploughing during hot and dry periods, use of organic manure, flooding and use of resistant varieties (Prasad *et al.,* 1983).

Effective physical methods of *Pratylenchus* control include heat (solar) treatment of soil, hot water treatment of planting material, and use of 15% water soluble hydrogenated fish oil. While chemical control methods, such as soil fumigants and nematicides, may be effective, key drawbacks include prohibitive costs and negative effects on naturally occurring soil micro-flora and fauna. In contrast, biological control relies on increasing the abundance of natural enemies in soil, such as predatory nematodes and other arthropods, fungi, protozoa, and viruses. Effective bio-controls include fungi that form adhesive spores (conidia) or zoospores, such as endospore-forming actinomycetes parasites of stationary stages, facultative and obligate parasites infesting nematodes and soil organic matter (Waweru *et al.,* 2013).

2.9 Plant parasitic nematodes and plant viruses interactions

The interactive association between viruses and plant parasitic nematodes is documented to happen in two ways; specific interactions and virus transmission between certain ecto-parasitic species of nematode and plant viruses transmited by these nematodes while other interactions are mostly the overal effects of viruses and nematodes within host plant. Initial proof of plant virus transmission by parasitic nematode was documented after having a successful Grapevine fanleaf virus (GFLV) of *Nepovirus* genus transition by *Xiphinema* nematodes (Belval *et al.,* 2019; Hewitt *et al.,* 1958). This work initiated a rigorous scouting for nematode vectors for various soil-borne viruses which led to study across; taxonomy, ecology and biology of nematode vectors and viruses (Lamberti, 1975). Nematodes feed on plants infected by viruses, take in virus particles but from an estimate of 2600 nematodes species only a few (30 species) are known to be virus vectors (Archidona-Yuste *et al*., 2016). Identified nematode-virus vectors are of the *Longidoridae* and *Trichodoridae*

families of the *Dorylaimida* order which are surface parasites infesting roots of perennial and annual plants. Studies have also proven that plant parasitic nematodes affect the transimission of plant viruses. Plant parasitic nematodes indirect interactions include and not limited to changes in the host plant metabolism which have effects on nematodes population. Within tobacco crops, both *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* nematodes were hampered by Tobacco mosaic virus while *D. dipsaci* nematodes were enhanced by *A. ritzemabosi* but supressed by Tomato blackring virus (Mashkoor, 1989; Weischer, 1975).

CHAPTER THREE

INTERACTION OF PLANT PARASITIC NEMATODES AND VIRUSES CAUSING MAIZE LETHAL NECROSIS DISEASE IN SELECTED MAIZE FIELDS IN KENYA

3.1 Abstract

Maize is classified as the third largest food crop across the globe after rice and wheat, and is known to be the main crop utilized as staple food in Kenya. The emergence of Maize lethal necrosis (MLN) disease, which is a result of coinfection of maize by Maize chlorotic mottle virus (MCMV) in combination with Sugarcane mosaic virus (SCMV), has become a major threat to food security and livelihoods in Kenya. Viruses involved in MLN disease development are mainly spread by insect vectors, but the contribution of plant parasitic nematodes on disease development is yet to be established. This study was done to determine the effect of nematodes' interaction with the viruses on MLN disease severity and development in maize fields in different agro-ecological zones in Kenya. The study sites were selected based on available data on distribution of MLN disease and included Narok, Bomet, Nakuru and Nyeri counties. Farms were selected at random and the prevalence and severity of MLN, MCMV and SCMV in symptomatic maize plants was recorded. The study involved collection of leaf and soil samples, and subsequent analysis for presence of viruses and plant parasitic nematodes, respectively. Enzyme Linked immuno-sorbent assay (ELISA) was used to detect the viruses while nematode extraction was done using a modified Baermann funnel technique. Variance analysis was used to measure significant defferences ($P< 0.05$) in disease incidence and severity, and nematode populations. Narok and Bomet counties recorded the highest incidences and severity for MLN disease, and highest plant parasitic nematodes infestation. Nyeri County had lowest disease severity with the lowest nematodes infestation. Correlation coefficients (-1.0 and 1.0), being the statistical measure of relationship

between plant parasitic nematodes infestation and MLN viruses, were calculated. Correlation coefficient (r=+0.471 to r=-0.121) indicated insignificant positive and negative relationships between severity of MLN, MCMV and SCMV in presence of plant parasitic nematodes *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp. infestation across Narok, Bomet, Nakuru and Nyeri counties. This study shows that plant parasitic nematodes had no significant role in development of MLN disease in the field. However, there is need for further work under controlled conditions to validate these observations.

3.2 Introduction

Maize (*Zea mays* L.) is an important crop used as food, feed and for industrial raw materials globally (Klopfenstein *et al.,* 2013). Due to maize being an important crop for 1.2 billion people living in Latin America and sub-Saharan Africa and an important crop for animal feeds, its global production ranks third after wheat (Wheeler and Reynolds, 2013). Southern and Eastern Africa are Africa's top maize consuming regions per capita at 77 kg and 27 kg per year, respectively (Shiferaw *et al.,* 2011). In Kenya, food security is determined by maize production of which 70% annual production is by small scale farmers (Ali-Olubandwa *et al.,* 2011).

Low maize productivity is attributed to both biotic and abiotic stresses. The biotic stresses, which include insect pests, parasitic nematodes, bacterial, viral and fungal infections, lead to reduction in maize yields (Urassa, 2015). Some of the main abiotic factors are prolonged droughts, poor soils, poor agronomic practices (Chumo, 2013). The main disease constrains affecting maize production are viruses such as maize streak and maize lethal necrosis, fungal diseases such as smut and fusarium, and bacterial diseases such as blight and wilt (Jeffers, 2004). Stem borers, locusts,

and fall armyworm are some of the main insect pests that threaten maize production (Smith, 2015). In combination, biotic and abiotic constrains to crop yields have led to food insecurity concerns.

It is estimated that the MLN outbreak in Kenya in 2011 caused more than 80% yield losses (Mahabaleswara, 2016; Wangai *et al*., 2012). The outbreak, caused by MCMV in combination with SCMV, spread fast in most of the maize growing regions including the Rift Valley, the main maize producing area (Wangai *et al.,* 2012). The disease has since spread further in Eastern African countries including Uganda, Tanzania, Rwanda, and Democratic Republic of Congo (Adams *et al.,* 2014; Lukanda *et al.,* 2014; DeGroote *et al*., 2016). A lot of research on MLN epidemiology has been done but there remains a big gap on understanding the effects of other pathogens on development of the disease.

Transmission of viruses causing MLN (MCMV and SCMV) is mainly by vectors; MCMV transmittion by maize thrips (*Frankliniella williamsi*), maize-root worms (*Diabrotica undecimpunctata*, *D. virgifera* and *D. lonicornis*) and leaf beetles (*Oulema melanopa*) while SCMV vectors are aphids (*Aphis gossypii* and *Myzus persicae*) (Mahuku *et al.,* 2015; Wangai *et al.,* 2012). Transmission of MCMV has also been documented from infected or contaminated seed, infested soil, plant debris, farm implements and machinery (Mahuku *et al.,* 2015).

Plant parasitic nematodes brings about great losses to many plants leading to large production and economic losses (Barker and Koenning, 1998). Pathogenicity by nematodes vary from region to region and across species (Jagdale *et al.,* 2013). Maize production decreased in Kenya in 2005 and went up to 50% due to phytonematodes (Ogolla, 2005). In Kenya, root lesion nematodes (*Pratylenchus spp.*) occur sporadically in maize, but usually in high numbers and is therefore a pest of economic significance (Kimenju *et al.,* 1998). Increased adverse effects of *Meloidogyne*

spp. in maize production has been observed (Adegbite *et al.*, 2005; Adegbite, 2011). In most maize farms, apart from *Pratylenchus* spp. and *Meloidogyne* spp., the other plant parasitic nematodes affecting maize production include *Helicotylenchus* and *Tylenchus* spp (Coyne *et al*., 2018). This study was carried out to determine the effect of plant parasitic nematodes' interaction with viruses causing MLN on disease development and severity in the field. The findings will lead to recommendations for MLN management.

3.3 Materials and methods

3.3.1 Survey regions

Maize lethal necrosis disease hotspot counties within Kenya's maize producing regions were selected for this study. Counties surveyed were Narok, Bomet, Nakuru and Nyeri. These are regions where maize production is also constrained by plant parasitic nematodes (Kimenju *et al.*, 1998). Selected farms were only those under continuous maize production over four seasons (2 years). A random simple sampling technique was deployed to get one hundred and twenty-nine (129) samples from twelve (12) maize farms. Since affected farmers by Maize lethal necrosis disease were unknown, snowball sampling technique was used to identify affected farms. Once the first farm was identified, it became easier to locate the next and the subsequent affected farms. The affected farmers were the ones to direct where to go next as they knew affected farmers in the community (Makone *et al*., 2014). Scores for MLN disease severity were recorded using a scale of 1–5 as described by Gowda *et al*. (2015), where; 1 = no symptom; 2 = <10% of plant leaf surfaces showing symptoms; 3 = 10–30% plant leaf surfaces showing symptoms; 4 = 31–50% of plant leaf surfaces showing symptoms; and 5 = >51% of plant leaf surfaces showing symptoms.

Maize lethal necrosis disease for the entire farm was also scored using the above scale. The score for each farm was the average of 30 scores of individual plants made while walking zig-zag through the farm and recording each score then getting farm average score (Manandhar, *et al*., 1988). Twelve samples were sampled per farm. Leaves were sampled for virus assays while roots and soil from same sampled plants were obtained for plant parasitic nematodes extraction. The whole root system was scooped out using a disinfected clean spade to avoid cross contamination. Leaf samples were labeled in correspondence to soil samples.

3.3.2 Plant parasitic nematodes extraction

A modified Baermann technique was used for nematode extraction (Coyne *et al*., 2018)**.** Roots were removed from soil samples and extracted in a different dish. Using a sieve, debri and stones were removed from the soil samples and lumps were broken by hand. Each soil sample was mixed thoroughly and 100 ml weighed for nematode extraction. Roots were chopped to about 1cm length and set for nematodes extraction. Tissue paper was placed in the extraction sieve on a plastic plate taking care to cover sieve base with the towel. Soil at 100 ml was added onto the towel in the sieve. Water was gently added to the extraction plates and kept undisturbed, in darkness, for 48 hours. After 48 hours, the remaining water was drained from extraction unit and root/soil discarded. Nematodes were extracted from the filtrate through a series of varied aperture sieves (down–top): 38, 90, 150 and 250, 150, 90 then 38 μm to get rid of debris and trap nematodes on the final sieve. *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp. and *Meloidogyne* spp. nematode species were identified under dissecting microscope at 440× magnification (Ciancio *et al.,* 2000; Mekete *et al.,* 2012).

3.3.3 Virus assays

Leaf samples collected from the field were assayed for presence of viruses at the Kenya Agricultural and Livestock Research Organization, Biotechnology Centre molecular laboratory. Leaves were screened for SCMV using Indirect Elisa while Double Antibody Sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) was used to test for the presence of MCMV (Gowda *et al.,* 2015). For MCMV, IgG antibody was added to DAS ELISA buffer (carbonate buffer) at a ratio of 1:1000 (capture antibody). This was used to coat the plate by dispensing 100 µl of the buffer to each test well. To avoid wastage and reduce experimental error, fresh capture antibody was prepared for each test. The plates were kept at 4°C overnight within moistened incubator and washed three times with phosphate buffered saline (PBS, ×1) + Tween 20 (0.05%) after incubation period. Leaves were homogenized in sample buffer and 100µl was loaded on antibody pre-coated plates. After incubation and washing, antigen–enzyme conjugate was prepared to a working concentration of 1:5000 using conjugate buffer (PBST-PVP-BSA), before adding 100 µl of the antigen–enzyme conjugate to each test well. The plates were incubated for one hour at 37°C and then washed 4 Xs with PBS-T to remove the unbound antibody–enzyme conjugate. Phosphate substrate was prepared by dissolving pNPP tablets in substrate buffer (1 mg/ml) and 100 µl of the buffer added to every test well. The experiment was incubated within room temperature for one hour after which absorbance was read using ELISA reader (Elx 808) at wavelength of 405 nm. Positive samples were those with absorbance value greater than three times the negative control absorbance divided by two.

Samples for SCMV screening were homogenized in 1× carbonate coating buffer and loaded into ELISA Nunc plate, then set at 37°C in moist chamber for one hour. Plates were rinsed as described above. About 5% non-fat dry milk (NDM), 200 µl, prepared in Tris–HCl was used as a blocker and incubated for one hour at room temperature. Blocker was dispensed off and 100 µl of SCMV IgG antibody pre-prepared in PBST-PVP-BSA-5% NDM in a ratio of 1:1000 was added, and the plates incubated at 37°C for one hour. After PBST wash, anti-Rabbit-IgG-AP prepared in PBST-PVP-BSA-5% NDM in the ratio of 1:10000 was used. After incubation, 100 µl of substrate was added and reaction read on Elx 808 reader at 405nm.

3.3.4 Data analysis

The relationship between nematode populations and MLN viruses was determined after results were analyzed by correlation analysis using Genstat software (15th Edition service pack 1, SP1 VSN International Ltd, UK- 2012). Disease symptom scores and test results were entered on Excel spreadsheets and run on Genstat. Means of *Pratylenchus* spp., *Meloidogyge* spp., *Helicotylenchus* spp. and *Tylenchus* spp. nematodes populations across MLN viruses were obtained through comparing treatments using Fisher's protected least significance difference test ($P \leq 0.05$).

3.4 Results

3.4.1 Disease incidence, severity and nematode populations in the field

Sampled maize farms had MLN infection with varied symptoms including mild mosaic and mottling, mild chlorosis to severe chlorosis and necrosis. In some farms, MLN severity index was very high in which most plants had severe chlorotic mottling to dead heart symptoms, premature

husks' drying and poor or no grain filling (Figure 3.1). Plants had chlorotic mottling, leaf necrosis and early drying of leaf sheath and dead hearts symptoms. All the four counties had a significant difference in MLN disease severity. On average, counties were rated depending on MLN severity score at the scale of 1 to 5; Nakuru had MLN disease average score of 2, Nyeri-3, Bomet-4 and Narok-4 (Table 3.1). Nakuru County had the lowest MLN severity score while Narok and Bomet had the highest MLN score.

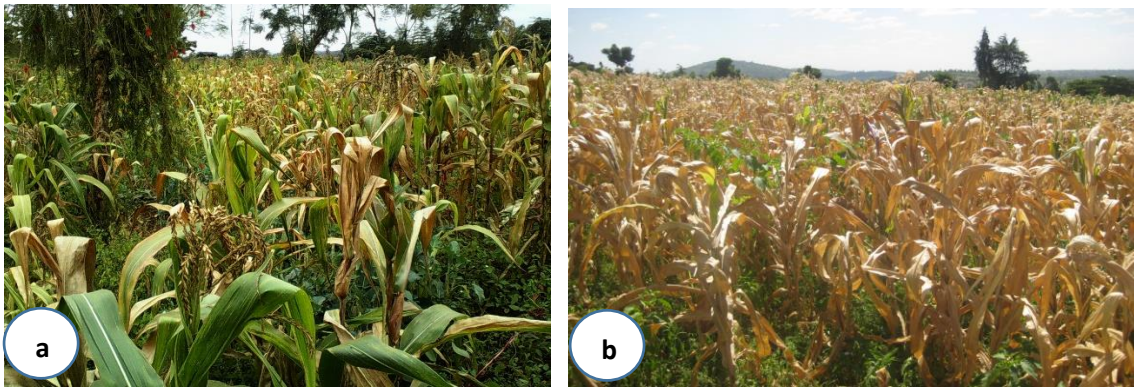

Figure 3.1 Maize fields showing symptomatic farm infected with maize lethal necrosis disease; a). shows mid-leaf chlorosis b). shows severe leaf chlorosis and necrosis being symptoms of MLN.

Most of the collected symptomatic leaf samples from the field tested positive for MCMV and SCMV, while others tested positive of either of the two viruses causing MLN. Means of common plant parasitic nematodes; *Pratylenchus* spp., *Tylenchus* spp., *Helicotylenchus* spp. and *Meloidogyne* spp. across MLN causing viruses; MCMV and SCMV, respectively, were obtained. Plant parasitic nematodes was obtained across MLN causing viruses, nematodes population across plants infested with *Pratylenchus* spp., *Tylenchus* spp., *Helicotylenchus* spp. and *Meloidogyne* spp. were recorded. Results also indicated disease severity variation across regions. Sampled farms within Bomet and Narok had MLN average score of 4, Nyeri scored 3 while Nakuru scored 2

Table 3.1 Sampled counties GPS coordinates, farms and level of disease severity across sampled farms within the four counties

County	Sub county	Latitude	longitude	Altitude	Farms	Farm Score	Avarage county scores
Narok	Narok North	0o53’40.83” S	35o53’21.48” E	2301 m	Farm 1	3	4
Narok	Narok West	0o58’10.30” S	35o27’50.03” E	1905 m	Farm 2	5	
Bomet	Bomet C.	0o45’33.22” S	35o20’43.30” E	2015 m	Farm 3	4	4
Bomet	Bomet C.	0o43’17.15” S	35o20’29.09” E	2095 m	Farm 4	4	
Bomet	Konoin	0o38’38.40” S	35o17’15.62” E	1984 m	Farm 5	4	
Nakuru	Molo	0o14’39.29” S	35o46’50.69” E	2270 m	Farm 6	2	2
Nakuru	Molo	0o17’13.22” S	35o49’54.17” E	2310 m	Farm 7	2	
Nakuru	Njoro	0o20’38.85” S	35o56’43.22” E	2175 m	Farm 8	2	
Nakuru	Bahati	0o8’27.84” S	36o9’12.00” E	2093 m	Farm 9	3	
Nyeri	Kieni	0o21’1.81” S	37o2’1.27” E	1792 m	Farm 10	3	3
Nyeri	Mathira W.	0o20’49.62” S	37o6’0.44” E	1935 m	Farm 11	3	
Nyeri	Mathira W.	0o20’27.35” S	37o5’26.01” E	1923 m	Farm 12	3	

3.4.2 Association of MLN, MCMV, SCMV and plant parasitic nematodes

Correlation analysis of data from surveyed regions indicated that there was positive but insignificant (r=+0.173) relationship between numbers of plants infected with MLN and *Pratylenchus* nematodes infestation (Table 3.2). A similar relationship was also recorded between plants infected with MLN and *Helicotylenchus* spp. An increase in MLN infection across maize plants population was not associated directly associated with increase in *Helicotylenchus* spp. nematode population. With the case of *Tylenchus* spp., correlation coefficient indicated an insignificant relationship with MLN (r=+0.019) as well as that of MLN and *Meloidogyne* spp (r=+0.005).

A positive insignificant relationship between MCMV and *Pratylenchus* spp. (r=+0.169) across the sampled counties was observed (Table 3.3). Number of plants infected with MCMV is directly propotional to *Pratylenchus* nematode populations. There was insignificant positive correlation

between MCMV and *Helicotylenchus* spp. (r=+0.396). Results indicated that *Tylenchus* spp. and *Meloidogyne* spp. nematodes infestation has no significant impact to MCMV severity even if the relationship was positive for *Tylenchus* spp. and a negative relationship for *Meloidogyne* spp. on MCMV+(r=+0.026, r=-0.052).

Table 3.2 Description of correlations (n=12) with significance at 0.05 and 0.01 levels (2-tailed) for MLN and *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp.

Nematode species	MLN	*Pratylenchus*	*Helicotylenchus*	*Tylenchus*
Pratylenchus	0.173	1		
Helicotylenchus	0.471	.656*	1	
Tylenchus	0.019	.781**	0.554	1
Meloidogyne	0.005	.718**	0.272	.754**

*. Correlation significance at the 0.05 level (2-tailed).

**. Correlation significance at the 0.01 level (2-tailed).

Table 3. 3 Description of correlations matrix (n=12) with significance at 0.01 and 0.05 levels (2-tailed) for MCMV and *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp.,and *Meloidogyne* spp.

	MCMV	*Pratylenchus*	*Helicotylenchus*	*Tylenchus*
Pratylenchus	0.169	1		
Helicotylenchus	0.396	.876**	1	
Tylenchus	0.026	.827**	.701*	1
Meloidogyne	-0.052	.800**	.669*	.794**

**. Correlation significance at the 0.01 level (2-tailed).

*. Correlation significance at the 0.05 level (2-tailed).

With SCMV correlation analysis, plant parasitic nematodes analysis was to establish the nature of relationship and whether it is significant. Table 3.4 indicates insignificant positive relationship between SCMV and *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp. and *Meloidogyne* spp. plant parasitic nematodes (r=+0.125, +0.049, +0.018, +0.172). The relationship indicates

number of plants infested by SCMV is not significantly associated with high number of plant parasitic nematodes population.

Table 3. 4 Description of correlation matrix (n=12) with significance at 0.01 and 0.05 levels (2-tailed) for SCMV and *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp.

Nematode species	SCMV	*Pratylenchus*	*Helicotylenchus*	*Tylenchus*
Pratylenchus	0.125	1		
Helicotylenchus	0.018	.817**	1	
Tylenchus	0.049	.863**	.630*	1
Meloidogyne	0.172	.781**	0.484	.876**

**. Correlation significance at the 0.01 level (2-tailed).

*. Correlation significance at the 0.05 level (2-tailed).

Table 3.5 indicate that there is positive relationship between plants not infected with MLN viruses with *Pratylenchus* and MLN viruses with *Helicotylenchus* (r=+0.174, +0.009) although the relationship is insignificant. Number of plants free of MLN viruses had insignificant level of infestation with plant parasitic nematodes; *Pratylenchus* and *Helicotylenchus*. Correlation of virus free plants and *Tylenchus*/*Meloidogyne* indicated an insignificant negative relationship (r = -0.183, -0.121).

Table 3. 5 Description of correlation matrix (n=12) with significance at 0.01 and 0.05 levels (2-tailed) for plants infected with *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp.

	None	*Pratylenchus*	*Helicotylenchus*	*Tylenchus*
Pratylenchus	0.174	1		
Helicotylenchus	0.009	-0.036	1	
Tylenchus	-0.183	-0.099	.740**	1
Meloidogyne	-0.121	-0.035	0.236	-0.037

**. Correlation significance at the 0.01 level (2-tailed).

3.5 Discussion

The study was conducted to establish the effect of interaction of nematodes that parasitize maize with viruses causing MLN (MCMV and SCMV) on disease severity and development within Kenyan maize growing agro-ecological regions. The study found out that in all surveyed regions, there was MLN disease as well as plant parasitic nematodes. It was also found that, plant parasitic nematodes with the highest prevalence on maize in the surveyed region were; *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp. *Pratylenchus* spp. was the most predominant in all sampled regions. Disease symptoms recorded on the field survey which included leaf necrosis, chlorotic mottling, yellow streaks and premature death of leaf sheath are similar to those reported by previous field studies as well as greenhouse trials (Adams *et al*., 2014; Mahuku *et al*., 2015). Maize crop in all sampled farms were at the same growth stage across study agro-ecological regions. Susceptability of maize plants to MLN disease is the same at all plant stages of crop development however, early crop infection leads to high yield loss (Beyene *et al*., 2016).

Tests for MLN disease symptomatic leaf samples from all the four counties scored positive for MCMV and SCMV. This is an indication that MLN in the surveyed region is caused by MCMV and SCMV. It also confirms previous reports on Kenyan cause of MLN being caused by the two viruses (Wangai et al., 2012). Most sampled farms scored 3 on a scale of 1-5 in relation to MCMV, SCMV and compined infection. In reference to sampled counties, results indicate presence of MLN within sampled regions. Presence of both MLN causing viruses and plant parasitic nematodes led to further research on nematodes population across MLN viruses.

Analysis of collected samples showed that MLN was a result of co-infection of maize by MCMV and SCMV. Studies have also indicated both MCMV and SCMV viruses being soilborne (Bond and Pirone, 1970; Phillips *et al*., 1982; Mahuku *et al*., 2015). Soil transmission of MCMV has been recorded being significantly high than that of SCMV (Hilker *et al*., 2017). Despite MCMV transmission rate from soil, there was no significant impact of plant parasitic nematodes to MLN severity across the sampled regions and across MLN viruses infected plants. Interactions between nematodes that are parasitic to plants in relation to other pathogens have been investigated with an indication of high disease severity in co-infections. *Pratylenchus* spp. increased fusarium wilt severity in potatoes with a significant increase in root infestation by *F. oxysporum spp.* (Castillo *et al.,* 1998). Due to formation of root lesions and injury by *Pratylenchus* spp. and their movement, transmission and tissue to tissue infection by bacterial and fungal pathogens increased.

The study indicates that parasitic nematodes affecting maize production namely *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Meloidogyne* spp. infestation does not affect MLN disease severity in maize in the field. However, there is need for further work under regulated conditions to evaluate the effect of individual nematode interactions with MLN causing viruses.

CHAPTER FOUR

DEVELOPMENT OF MAIZE LETHAL NECROSIS DISEASE IN PLANTS INFECTED WITH LESION NEMATODES (*PRATYLENCHUS* SPP.)

4.1 Abstract

In 2011, Maize lethal necrosis (MLN) disease outbreak was reported in East Africa. Consequently, MLN has since become a big threat to food security in the region. The disease is caused by a synergistic interaction between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). However, more research is needed to establish the role of other plant pathogens associated with the cropn needs to be conducted. A study was conducted in the greenhouse to determine the effect of lesion nematodes (*Pratylenchus* spp.) on MLN disease development in maize varieties H614D and Emph 1101. Variety H614D is known to be susceptible to both MLN and *Pratylenchus* spp. whereas maize variety Emph 1101 is susceptible to MLN but resistant to *Pratylenchus* nematodes. The two maize varieties were subjected to three distinct treatments: single inoculation with MCMV and SCMV; combined MCMV + SCMV inoculation; the third treatment was the addition of *Pratylenchus* nematodes to the previous two treatments. Over a period of two months, disease severity and incidence were recorded weekly. Plant growth parameters which included plant height and weight, and root length and weight were evaluated at the end of the two-month experiment. The plants were then uprooted and root length and weight, lesion index and nematode counts recorded. Severity of MLN disease was higher in H614D than in Emph 1101. Disease severity was higher in plants inoculated with both MLN viruses and *Pratylenchus* in H614D. Measurements of area under disease progress curve (AUDPC) in plants inoculated with MLN-causing viruses and *Pratylenchus* was higher in H614D and was not

significant in Emph 1101 compared to plants inoculated with MLN-causing viruses alone. *Pratylenchus* nematodes in combination with MLN-causing viruses had a significant effect on plant growth parameters in variety H614D than in variety Emph 1101. This study concludes that *Pratylenchus* infestation contributes to MLN disease development in maize genotypes that are susceptible to both the viruses and the nematodes.

4.2 Introduction

Maize (*Zea mays*) is a globally important food, feed, and industrial crop and is ranked third worldwide after wheat and rice, and is consumed by over 1.2 billion people living in Latin America and sub-Saharan Africa (Wheeler and Reynolds, 2013). Maize accounts for 40 to 50% of Eastern and Southern Africa dietary calorie and protein requirements (Cairns *et al.,* 2013). In Kenya, small scale farmers accounts for 75% of maize production while 20% of total production is sold (Nagarajan *et al.,* 2019; Nyoro *et al.,* 2004). Several biotic and abiotic constraints affects maize production. Insect pests, parasitic nematodes, bacterial, viral and fungal diseases are among the biotic factors that constrains maize production. On the other hand, abiotic factors include prolonged drought, poor soils, poor agronomic practices and variety selection (Ong'amo *et al.,* 2013). Production constraints have led to a decline in maize production leading to food insecurity (Lesk *et al.,* 2016). Maize lethal necrosis (MLN) disease is among the new constrains affecting maize production (Wangai *et al.,* 2012). The MLN outbreak in Kenya in 2011 was caused by MCMV and SCMV (Wangai *et al.,* 2012; Adams *et al.,* 2013), spreading rapidly to other parts of the country (Wangai *et al.,* 2012; Mahabaleswara, 2016). Since then, other countries of East Africa, including Rwanda, the Republic of Congo (Adams *et al.,* 2014; Lukanda *et al.,* 2014),

Uganda, Tanzania and Ethiopia (Mahuku *et al.,* 2015) have been affected. Reports from previous research indicate that MCMV is spread by maize thrips (*Frankliniella williamsi*), maize root worms (*Diabrotica undecimpunctata, D. virgifera* and *D. lonicornis*) and leaf beetles (*Oulema melanopa*), while mechanical disease transmission may occur during farm operations (Mahuku *et al.,* 2015). Vectors for SCMV are aphids (*Aphis gossypii* and *Myzus persicae*) as indicated in Wangai *et al.* (2012). There is a gap in the understanding of the effect of other pathogens on MLN disease development. Infestation of maize with plant parasitic nematodes increases the production costs and economic losses with an estimated 10.2% maize yield loss in East and Southern Africa (Barker and Koenning, 1998; Talwana *et al.,* 2016). Root lesion nematodes (*Pratylenchus* spp.) significantly affect maize production in Kenya (Kimenju *et al.,* 1998; Namu *et al.,* 2018). Although their occurrence in maize is sporadic, their high abundance has led to their being categorized as economically significant in maize production (Nicol *et al.,* 2011). However, more studies regarding the association of *Pratylenchus* spp. on MLN disease development needs to be conducted. The current study aimed at determining the effect of *Pratylenchus* spp. in the development of MLN disease.

4.3 Materials and methods

4.3.1 Experimental design

A greenhouse experiment was set up to determine the effect of *Pratylenchus* spp. nematodes on development of MLN disease using a completely randomized design (CRD) with five replications. The treatments consisted of plants inoculated with SCMV and MCMV (the viruses that cause MLN) and *Pratylenchus* spp. nematodes.

4.3.2 Multiplication of *Pratylenchus* nematodes inoculum

Initial soil and root samples from maize suspected of infestation with *Pratylenchus* nematodes were collected from farmers' fields as described in section 3.3.1. Sampling was done on two-month-old maize plants showing general nematode infestation symptoms of stunting, yellowing of leaves and dark root lesions, all pointing to Pratylenchus symptoms (Davis and Macguidwin, 2000).

Extraction of nematodes from root and soil samples was done using a modified Baermann funnel technique as described in Section 3.3.2, where debris were removed from soil using a coarse sieve; roots were macerated before extraction (Coyne *et al*., 2007). After sample preparation, 100 g of thoroughly mixed soil was placed on top of tissue paper in an extraction plastic sieve/basket on a plastic plate; the base of the sieve was covered by tissue. A standard volume of water was added to the dish to moisten the soil or root tissue, without flooding, ensuring there was sufficient water to avoid drying of the sample during the 48-hour incubation period in darkness. Nematodes were extracted using decreasing aperture sieves (250, 150, 90 and 38 μm). Pratylenchus nematodes were collected in 25-ml beakers by backwashing the sieve with clean, nematode-free water. Identification and counting was performed using a dissecting microscope (440× magnification) in a counting dish.

4.3.3 Preparation and multiplication of *Pratylenchus* nematode cultures

Extracted nematodes were re-suspended in 10 ml of sterile water in a measuring cylinder, to which 6 mg of streptomycin sulfate (6000 ppm) was added for surface sterilization. The nematodes were allowed to settle for about one hour, excess water reduced using micro-Pasteur pipette. This

process was repeated several times. Three nematode multiplication methods (two *in-vitro* and one *in-vivo*) were used for *Pratylenchus* spp. multiplication as follows. Carrot disc method as described by Coyne *et al*., 2007, vermiculite method on root mass (Kagoda *et al.* 2010) and Sterile soil method (Santana-Gomes *et al.*, 2018).

The carrot disc method was performed in a tissue culture laboratory (Coyne *et al*., 2007). Fresh carrots that were proven free from nematodes by microscopy were cleaned, rinsed with distilled water and surface-sterilized using 70% ethanol and flame. They were peeled and cut into discs of 0.5 cm thick and 4 cm in diameter. Each disc was set in a 5 cm diameter glass petri dish and inoculated with surface-sterilized *Pratylenchus* ($25/ml^{-1}$) (Coyne *et al.*, 2007). Petri dishes were sealed using parafilm and put under incubation (28°C) for four weeks. Harvesting was done every month for a period of four months, stored at 4°C and pooled for experimental inoculation.

Multiplication of *Pratylenchus* in vermiculite was performed on surface-sterilized pre-germinated maize seeds as described by Kagoda *et al.* (2010). Surface sterilization of maize seeds was performed using 95% ethanol followed by 20% sodium hypochlorite. The seeds were then rinsed in sterile distilled water. Seeds were planted on sterilized vermiculite and incubated in a sterile moist incubator at 28°C. At germination, a suspension of 250 surface-sterilized *Pratylenchus* nematodes was added to sterilized carrot discs followed by incubation at 28°C in a sterilized moist incubator. Seedlings of 13 cm height were cut at the base of the stem, foliar material discarded and the root system placed in vermiculite and incubated for one month prior to maceration of the *Pratylenchus* infected roots for extraction using the Baermann technique.

Sterile soil was also used for *Pratylenchus* multiplication on maize (Santana-Gomes *et al.*, 2018). Upon germination, potted maize seedlings were inoculated with *Pratylenchus* nematodes. Plants

were watered as required and *Pratylenchus* nematodes harvested at tasseling stage.

4.3.4 Assessing the development of Maize lethal necrosis disease in lesion nematode infected plants

Complete randomized experimental design was used to study the development of MLN in *Pratylenchus* spp. infected plants. Each treatment consisted of four plants and was replicated five times. The four plants in each replicate were inoculated with different combinations of MLN-causing viruses and *Pratylenchus* spp. nematodes in a disinfected glasshouse (Table 4.1). Sterile soil was potted (top diameter: 9 cm; base diameter: 6 cm; height: 8 cm), in which four maize seeds were planted.

Table 4.1 List of treatments (viruses and their combinations) used to evaluate the effect *Pratylenchus* nematodes to MLN development on maize varieties H614D and Emph 1101

Maize variety	Treatment description
H614D	Non-infected
	MCMV
	SCMV
	MCMV+SCMV
	MCMV+*Pratylenchus*
	SCMV+*Pratylenchus*
	MCMV+SCMV+*Pratylenchus*
	Pratylenchus infected
Emph 1101	Non-infected
	MCMV
	SCMV
	MCMV+SCMV
	MCMV+*Pratylenchus*
	SCMV+*Pratylenchus*
	MCMV+SCMV+*Pratylenchus*
	Pratylenchus infected

MCMV = Maize chlorotic mottle virus, SCMV = Sugarcane mosaic virus, *Pratylenchus* = Lesion nematodes.

4.3.5 Inoculation of experimental plants with MLN viruses and lesion nematodes

Maize chlorotic mottle virus and SCMV inocula were obtained from cultures maintained in the greenhouse in individual young plants inoculated with the viruses. The virus-infected plants were kept in separate greenhouses to avoid cross-contamination. Potassium phosphate inoculation buffer, which was prepared at 0.1 M concentration, for mono-basic (potassium di-hydrogen) and di-basic (di-potassium hydrogen) phosphate, was used in an inoculum sample buffer ratio of 1:10 (v/v). Maize leaves from symptomatic plants that were confirmed positive for only one virus were harvested and chopped into small pieces using scissors. The chopped leaf material was mixed with the buffer and blended to a fine homogenous mix (two separate household blenders; Redberry RB103 were used at medium speed for 5 minutes per spin for SCMV and MCMV) that was sieved using cheese cloth. For MLN disease, positive materials of MCMV: SCMV (1:8 v/v) were mixed and to which carborundum metallic dust was added at the ration of 1:1000. Artificial inoculation of MLN, MCMV and SCMV was performed on two-week-old maize seedlings in the morning for high-rate virus translocation (Balogun, 2008; Hull, 2009). Treatments consisting of MCMV and SCMV were carried out in separate greenhouses to avoid cross-contamination of MCMV and SCMV. Sub-cultured *Pratylenchus* nematodes were used as inoculum for the maize plants. The two-week-old maize seedlings already inoculated with the test viruses were also inoculated with 1000 *Pratylenchus* spp. suspension per plant, in a hole made next to each seedling (9 mm diameter and 1 cm deep). The experiment was performed using MLN-susceptible maize variety (H614D) and MLN susceptible but *Pratylenchus* spp. resistant maize variety (Emph 1101) (Kagoda *et al.*, 2010).

4.3.6 MLN disease severity assessment

Disease severity was assessed every week for a period of 8 weeks using a scale of 1 to 5 as by Gowda *et al.* (2015) described as described in Section 3.3.1 where 1 indicates no symptoms; 2 = <10% of leaf surface is symptomatic; 3 = 11-30% plant leaf surface is symptomatic; 4 = 31-50% of plant leaf surface is symptomatic; and 5 indicates that >50% of plant leaf surface is symptomatic.

4.3.7 Detection of MLN disease-causing viruses

Maize leaves were sampled and screened for MLN disease-causing viruses using double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) technique for MCMV and indirect ELISA for SCMV as described by Gowda *et al*. (2015) and explained in Section 3.3.3. For the detection of MCMV, immunoglobulin G (IgG) primary antibody in phosphate buffer was used in a 1:1000 ratio coated on Nunc MaxiSorp™ plates and incubated at +4°C overnight in a moist chamber for antibody adherence, and then washed three times at 2 minutes intervals using phosphate buffered saline with Tween 20 (PBST). Leaf samples homogenized in sample buffer were loaded as duplicates on antibody pre-coated plates and incubated at 37°C in a moist chamber for one hour before being washed as described above. Then conjugated secondary antibody in PBST-PVP-BSA (Polyvinylpyrrolidone, Bovine Serum Albumin) buffer was used at a concentration of 1:5000, and subsequently incubated at 37°C for one hour. Plates were washed and phosphate substrate pNPP (p-nitrophenyl phosphate 1 mg ml^{-1}) was added to the test well. Plates were incubated for one hour at room temperature and absorbance readings were obtained from an ELISA reader (BioTek Company Elx 808/Winooski, Vermont, U.S.A.) at a wavelength

of 405 nm. Positive samples were determined by using an ELISA cutoff point calculated as twice the average of the negative control.

Samples for SCMV detection were homogenized in carbonate coating buffer and loaded onto ELISA plates (Nunc MaxiSorp™ flat-bottom; Thermo Fisher Scientific). After one hour incubation at 37°C, in a moist chamber, ELISA plates were rinsed with PBST (three times) and blocked using 5% non-fat dry milk powder (Cheng *et al.,* 2019). The SCMV IgG antibody used in the study was prepared in PBST-PVP-BSA-5% NDM in a ratio of 1:1000, and then conjugate antibody (anti-Rabbit-IgG-AP) was prepared in 5% NDM PBST-PVP-BSA buffer – at a ratio of 1:10000. Substrate pNPP was added to the plates and readings were recorded after incubation for 45 minutes on an ELISA reader (BioTek Company Elx 808/Winooski, Vermont, U.S.A.) at a wavelength of 405 nm.

4.3.8 Estimation of lesion nematode populations

Pratylenchus nematodes were extracted from inoculated maize plants before flowering stage (Taba *et al*., 2008). The maize plants were uprooted with their root system intact. The roots were then cut off from stems, washed to remove soil and weighed. Same leaf sampled plants were uprooted followed by collection of their respective soil samples in a container using a spade, thoroughly mixed to uniformity and a 500 g sample collected. Nematodes were extracted and counted as described above.

4.3.9 Data analysis

Treatment differences were tested using analysis of variance and Fishers protected least significant difference at $P = 0.05$. Disease symptom expression scores were used to calculate area under disease progression curve (Jeger *et al*., 2001). Analyses were conducted using Genstat (Payne *et al*., 1996).

4.4 Results

Multiplication of *Pratylenchus* nematodes varied across the three methods; soil, vermiculite and carrot discs. After 3 months, *Pratylenchus* population on soil, vermiculite and carrot discs was 4211, 5344 and 1533, respectively. *Pratylenchus* population was significantly lower on carrot disks media than vermiculite and sterile soil. While nematode population in soil and vermiculite increased, populations in carrot reduced as compared to the initial population which was introduced at inoculation time. The carrot disk method was also prone to high rates of fungal contamination before establishing healthy cultures.

Symptoms of MLN disease increased with time on MLN-susceptible maize variety 'H614D' and varied among treatments in season one. Plants inoculated with MLN-causing viruses and *Pratylenchus* spp. had significant chlorosis and necrosis symptoms compared to those inoculated with MLN-causing viruses alone (Fig. 4.1). Disease severity was higher in plants subjected to a combined treatment of virus and nematode than in those plants inoculated with a combined treatment of MCMV and SCMV only. High levels of chlorosis, necrosis and in some cases dead hearts, resulting to a severity score of 5 within eight weeks after inoculation. There was a

significant disease severity in plants inoculated with MLN-causing viruses and those inoculated with MCMV+*Pratylenchus* spp. and MLN+ *Pratylenchus* spp. than in those without *Pratylenchus* spp.

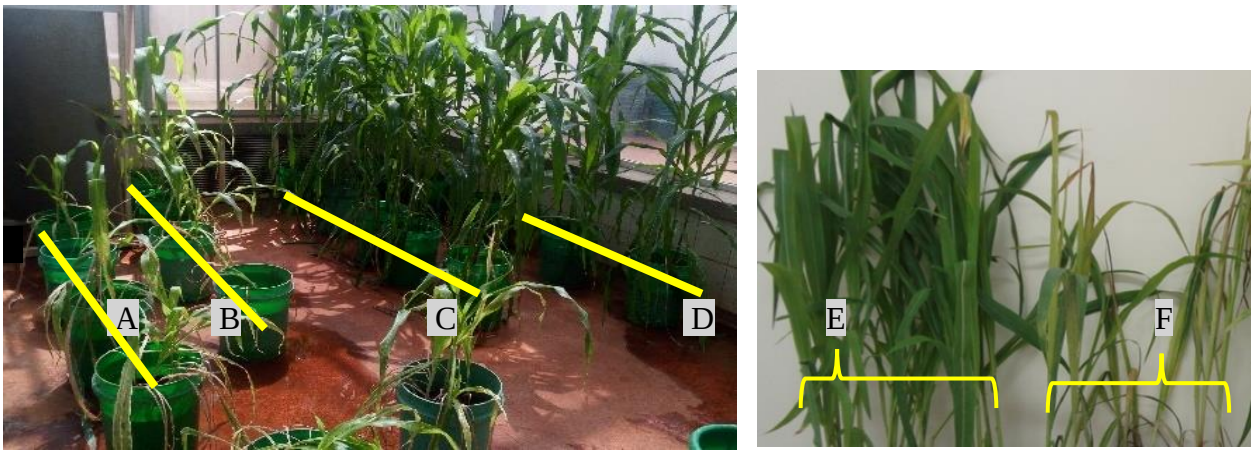

Figure 4. 1 Stunted growth symptom expression in plants inoculated with; (row A) Maize lethal necrosis (MLN) disease-causing viruses MCMV+SCMV, (row B) MLN+Pratylenchus, (row C) SCMV, (row D) SCMV+Pratylenchus and (E) Healthy plants, (F) MLN positive plants

Effects of *Pratylenchus* on disease progression with time in season one were similar to those in season two, with the presence of *Pratylenchus* spp. in plants inoculated with MCMV and MCMV+SCMV (MLN) viruses significantly increasing the disease severity over time. The disease progression in SCMV and SCMV+*Pratylenchus* treatment stagnated after 3 weeks while there was no significant difference between disease developments on plants infected with SCMV+ *Pratylenchus* spp. and SCMV without *Pratylenchus* spp. (Table 4.2).

No significant difference was observed regarding disease severity on disease development in H614D between season one and season two with respect to MLN combined with *Pratylenchus*. MCMV+SCMV+*Pratylenchus*-innoculated plants significantly exhibited a higher MLN severity than than those with MLN-causing viruses alone (Table 4.2 and 4.3).

Table 4.2 Effect of presence of nematodes (*Pratylenchus* spp.) on development of MLN disease among the virus treatments in susceptible maize hybrid 'H614D' in season one.

Treatment combination	Weeks after inoculation							
	1	2	3	4	5	6	7	8
MCMV	1.35d	2.1d	3b	3.3b	3.6b	3.7c	3.7c	3.8c
MCMV/PRATY	1.75ba	2.5c	2.6c	3c	3.4c	3.9bc	4.1b	4bc
SCMV	1.5bc	2.3d	2.75c	2.5d	3d	3d	3d	3d
SCMV/PRATY	1.6cd	2.35d	2.75c	2.65d	2.95d	3d	3d	3d
MLN/PRATY	1.7cba	3a	3.65a	4.3a	4.65a	4.8a	4.85a	4.95a
MLN	1.9a	2.7b	3c	3.35b	3.7b	4.1b	4.2b	4.8b
Healthy	1e	1e	1e	1e	1e	1e	1e	1e
PRATY	1e	1e	1e	1e	1e	1e	1e	1e
CV%	27	20.8	12.9	11	10.6	8.6	9	6.7
LSD	0.25	0.28	0.20	0.18	0.19	0.16	0.17	0.13

Data are means ±SE of disease expression scores at a scale of 1-5. Means followed by different letters within a column are significantly different (Fisher's protected LSD test at P< 0.05). MLN - Maize lethal necrosis, PRATY- *Pratylenchus* spp., SCMV -*Sugarcane mosaic virus*, MCMV - *Maize chlorotic mottle virus*, PRATY -Nematodes without viruses, Healthy - Nematodes and virus free, AUDPC- Area under disease progression curve.

Table 4.3 Effect of presence of *Pratylenchus* on disease development among the virus treatments in susceptible 'H614D' in season two.

Treatment combination	Weeks after inoculation							
	1	2	3	4	5	6	7	8
MCMV	1.6d	2d	2.7d	2.9d	3.05d	3.1c	3.2d	3.2c
MCMV/PRATY	1.9c	2.9b	3.15bc	3.3c	3.7c	4.15b	4.7b	4.95a
SCMV	1.9c	2.85c	2.85d	2.85d	2.85d	2.85d	2.85d	2.85d
SCMV/PRATY	1.95c	2.9b	2.95dc	2.95d	3d	3dc	3d	3dc
MLN/PRATY	2.2ab	3.1a	3.9a	4.55b	4.95b	4.95e	5a	5a
MLN	2.05cb	2.4b	2.95dc	3.3c	3.65c	4.15b	4.35c	4.7b
Healthy	1e	1e	1e	1e	1e	1e	1e	1e
PRATY	1e	1e	1e	1e	1e	1e	1e	1e
CV%	18.3	17.7	15.8	14.7	12.9	10.9	12.8	12.1
LSD	0.20	0.26	0.25	0.25	0.23	0.21	0.25	0.24

Data are means ±SE of disease expression scores. Means followed by different letters within a column are significantly different (Fisher's protected LSD test at P< 0.05). MLN - Maize lethal necrosis, PRATY- *Pratylenchus* spp., SCMV -Sugarcane mosaic virus, MCMV - Maize chlorotic mottle virus, PRATY -Nematodes without viruses, Healthy - Nematodes and virus free, AUDPC- Area under disease progression curve.

Plants innoculated with SCMV had initial mosaic symptoms on the second week after innoculation (Fig. 1). This was observed across treatments with Emph 1101 and H614D. Results indicated that SCMV expression on both H614D and Emph 1101 had slow disease progression from symptoms onset to week 8 after inoculation. Plants infected with SCMV and SCMV+ *Pratylenchus* spp. showed no significant difference across treatments. Disease progression in plants inoculated with SCMV and *Pratylenchus* spp. was similar to that of SCMV without *Pratylenchus* spp. in both season one and two (Table 4.2 and 4.3) with the highest expression being at 30% at week 8 after inoculation. Regarding MCMV disease expression, inoculated plants showed the first chlorotic mottle symptoms with a score 2 on young leaves located above the inoculated leaves after 7 days post-inoculation; these symptoms advanced to score of 3 after 2 weeks. Maize chlorotic mottle disease started at week 2 on H614D and at week 3 for Emph 1101 variety after inoculation. MCMV disease progression significantly varied across varieties. MCMV disease expression was higher on H614D than on Emph 1101 from week 7 after inoculation. Disease progression in plants infected with MCMV+ *Pratylenchus* spp. was significantly different from that of MCMV without *Pratylenchus* on H614D (Table 4.2 and 4.3).

No significant variation was observed between MCMV and MCMV+*Pratylenchus* on Emph 1101 (Table 4.4). Maize lethal necrosis disease progression on both H614D and Emph 1101 was higher than MCMV and SCMV, MCMV and SCMV. There was no significant difference in MLN disease progression between H614D and Emph 1101: both had the same disease severity score of 5 at week 8. In H614D variety in both seasons, MLN disease progression was significantly higher on plants inoculated with MLN+*Pratylenchus* from week 4. No sigficant variation was observed in

disease progression for plants inoculated with MLN viruses alone and those inoculated with MLN viruses and *Pratylenchus* spp.

Table 4.4 Effect of *Pratylenchus* on MLN severity among the virus treatments in *Pratylenchus*-resistant maize variety Emph 1101.

Treatment	Weeks after inoculation							
	1	2	3	4	5	6	7	8
MCMV	1.7dc	2.6b	3.3a	3.4b	3.4bc	3.6b	3.6b	3.6b
MCMV/PRATY	1.9cb	2.7b	3.4a	3.5b	3.6b	3.5b	3.7b	3.7b
SCMV	1.6d	2.3c	3.0dcb	3.0c	3.0d	3.0c	3.0c	3.0c
SCMV/PRATY	1.7dc	2cd	2.9bc	3.0c	3.0d	3.0c	3.0c	3.0c
MLN/PRATY	2.0ab	3.0a	3.1b	4.0a	4.1a	4.4a	4.8a	5.0a
MLN	2.0ab	2.6b	3.0cb	4.0a	4.2a	4.3a	4.5a	4.9a
Healthy	1e	1e	1e	1e	1e	1e	1e	1e
PRATY	1e	1e	1e	1e	1e	1e	1e	1e
CV%	21.9	21	10.8	8.3	8.3	10.3	10.6	8
LSD	0.22	0.30	0.17	0.14	0.14	0.18	0.19	0.15

Data are means ±SE of disease expression scores. Means followed by different letters within a column are significantly different (Fisher's protected LSD test at $P< 0.05$). MLN - Maize lethal necrosis, PRATY- *Pratylenchus* spp., SCMV -Sugarcane mosaic virus, MCMV - Maize chlorotic mottle virus, PRATY -Nematodes without viruses, Healthy - Nematodes and virus free, AUDPC- Area under disease progression curve.

There was a significant difference across treatments on plant growth parameters in plants inoculated with MLN-causing viruses and their combination with *Pratylenchus* in the two maize varieties. All plant growth parameters measured were affected by MLN viruses, either alone or with the addition of nematodes. Plant height/weight and root length/weight of plants innoculated with viruses and *Pratylenchus* spp. significantly varied from the control group. Treatments of SCMV applied singly varied significantly from the combined SCMV+*Pratylenchus* treatment on root length, plant height and plant weight on H614D and Emph 1101. However, on Emph 1101 there was no significant difference in root weight under either treatment. For plants inoculated

with MCMV, there was a significant reduction in root length, plant height and a significant loss of plant and root weight. There was even further significant impact across all growth factors on plants inoculated with both MCMV and *Pratylenchus* in relation to controls. There was no significant effect between MCMV-inoculated plants and those inoculated with MCMV+*Pratylenchus* with respect to root length for both H614D, and plant height and plant weight for Emph 1101. A significant difference was recorded on plants inoculated with MCMV and MCMV+*Pratylenchus* on plant height and plant weight for H614D, and on root weight for both varieties. The greatest impact of MLN was observed on root length and weight, plant height and weight. Plants showed stunted growth in both maize varieties. There was no significant difference in all growth factors across plant varieties for plants inoculated with MLN and MLN+*Pratylenchus* (Table 4.6).

Calculations of the area under the disease-progress curve (AUDPC) showed varied effects of treatments on the two maize varieties (Table 4.6). The AUDPC for plants inoculated with MCMV+SCMV+*Pratylenchus* was significantly higher than for all other treatments. For H614D, the area was significantly different between plants inoculated with MCMV and MCMV+*Pratylenchus,* but there was no difference between plants inoculated with SCMV and SCMV+*Pratylenchus*. For Emph 1101, there was no significant difference in AUDPC between MCMV and MCMV+*Pratylenchus*, SCMV and SCMV+*Pratylenchus*. In contrast to H614D, there was no significant difference in AUDPC for Emph 1101 MCMV-inoculated plants and those inoculated with MCMV+*Pratylenchus,* SCMV/SCMV+*Pratylenchus* in relation to the controls (Table 4.5).

Table 4.5 Effects of MLN, MCMV, SCMV and *Pratylenchus* on root and above ground plant growth parameters and the Area Under Disease Progresion Curve (AUDPC) among treatments for H614D and Emph 1101 maize varieties

Treatment	Root Length (cm)		Plant Height (cm)		Root Weight (g)		Plant Weight (g)		AUDPC	
	H614D	Emph 1101	H614D	Emph 1101	H614D	Emph 1101	H614D	Emph 1101	H614D	Emph 1101
MLN	6.5d	10.5d	27.8e	24.2e	4.7e	3.3d	15.7d	15.3d	220.1b	212.8a
MLN/PRATY	4.9d	6.9d	17.1e	19.5e	1.95e	2.3d	11.5d	12.8d	240.1a	217.2a
MCMV	23.0c	21.9c	114.2c	100.2d	16.6cd	13.6bc	111.3bc	80.5c	166.2d	160.7b
MCMV/PRATY	19.0c	22.4c	72.0d	84.3d	12.9d	11.1c	95.4c	76.2c	183.8c	161.0b
SCMV	29.0b	29.2b	125.3bc	135.3bc	20.5bc	17.2ab	125.7ab	100.3c	156.3d	148.1b
SCMV/PRATY	22.6c	18.7c	106.7c	125.1c	18.6c	16.1ab	114.2bc	89.1abc	162.9d	159.6b
PRATY	31.3ab	33.5ab	143.8ab	152.8ab	24.0ab	17.9a	120.5abc	106.9bc	56.0e	56.0c
Healthy	35.5a	39.7a	163.6a	162.3a	28a	19.4a	143.7a	113.8a	56.0e	56.0c
CV	40.10	43.80	41.30	33.00	48.20	55.00	50.10	52.40	11.80	14.20
LSD	5.40	6.20	24.80	20.70	4.80	4.30	28.90	24.30	11.42	12.97

Means followed by different letters within a column are significantly different (Fisher's protected LSD test at $P< 0.05$). MLN - Maize lethal necrosis, PRATY- *Pratylenchus*, SCMV -Sugarcane mosaic virus, MCMV - Maize chlorotic mottle virus, PRATY -Nematodes without viruses, Healthy - Nematodes and virus free, AUDPC- Area under disease progression curve.

Discussion

This study aimed to determine the effect of lesion nematodes (*Pratylenchus* spp.) on MLN disease development in maize variety H614D that is susceptible to both MLN and *Pratylenchus* spp. and on variety Emph1101 that is susceptible to MLN but resistant to the nematodes. Difference in susceptability to nematodes would give a clear indication of effects of nematodes to MLN severity. Confined greenhouse study results indicated a significant impact of *Pratylenchus* spp. to MLN disease development on H614D maize variety. Under same treatments, *Pratylenchus* spp. had no impact on MLN development on Emph 1101 maize variety. Maize lethal necrosis symptoms were more significant on H614D than on Emph 1101. The study indicate that effects of *Pratylenchus* spp. on MLN disease development varies across maize varieties depending on variety level of tolerance or resistance to both/either MLN viruses and *Pratylenchus* spp.

Variety plays a significant role in disease severity with respect to MLN and plant parasitic nematodes infestation (Groote *et al.,* 2016; Puerari *et al.,* 2015). According to a study by Gowda *et al.* (2015), a high number of inbred lines and hybrids from temperate climate are susceptible to MLN but germplasm tolerance and/or resistance level is very low compaired to tropical lines. Tolerance to several potyviruses has been reported in maize germplasm which may be used to develop MLN tolerant maize varieties (Gowda *et al.,* 2015). Maize varieties play a significant role to lesion nematodes population and nematodes impact rate (Kimenju *et al.,* 1998).

Maize lethal necrosis disease progression/symptoms expression varied across the two varieties, progression being faster on H614D than on Emph 1101. Symptoms analysis indicated that SCMV expression on both H614D and Emph 1101 had a slow disease progression from symptoms commencement at week 2 to week 8 after inoculation. Infection of plants with SCMV and

Pratylenchus spp. showed significant difference across treatments. SCMV disease severity did not extend beyond 30% in both varieties despite prolonged period to 8 weeks after inoculations. This supports published research reports that single infections of SCMV is not significant in maize production (Xia *et al.,* 2016). SCMV+*Pratylenchus* disease progression was not significant compared to that of SCMV without nematodes – the highest expression being at 30% at week 8 after inoculation.

Infection of plants with MCMV+ *Pratylenchus* spp. showed significant difference compared with infection with MCMV without *Pratylenchus* on H614D, but not on Emph 1101. This is an indication that *Pratylenchus* spp. contribute significantly to disease severity in varieties susceptible to MCMV and to *Pratylenchus* spp. *Pratylenchus* also contributed significantly to MLN disease severity of MLN in H614D variety. While *Pratylenchus* nematodes are not known vectors for either MCMV (or SCMV), they may aid virus transmission from soil into the plants via injured roots, or weaken plant response to infection by the viruses in susceptible hosts. Specifically, MCMV has been reported as being stable in the soil for a long time, and as *Pratylenchus* spp. move with the roots, the nematodes may aid the mechanical transmission of the virus from one plant to another within root proximity (Mahuku *et al.,* 2015). However, this remains to be confirmed. This study demonstrated that infection with *Pratylenchus* spp. had a negative effect on the growth of H614D and Emph 1101 maize cultivars.

In this study, it was found that *Pratylenchus* spp. infestation significantly increase MLN disease development and severity in MLN and *Pratylenchus* spp. susceptible maize varieties. This knowledge can contribute to strategies for managing this disease. Managing *Pratylenchus* spp. on H614D and *Pratylenchus* spp. susceptible maize varieties will reduce MLN incidence and severity

within maize agro-ecological regions. Management of MLN in regions infested with *Pratylenchus* nematodes may also be achieved through cultivation of *Pratylenchus* spp. resistant maize varieties. However, there is a need for further studies to determine the possibility of mechanical transmission of MCMV by *Pratylenchus* spp.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discusion

There has been limited information regarding how other plant pathogens affect Maize lethal necrosis (MLN) disease development. This study, therefore, was carried out to determine the role of plant parasitic nematodes in MLN disease development. The study showed that plant parasitic nematodes may not significantly affect the development of MLN disease in the field. However, under controlled (greenhouse) environment, *Pratylenchus* spp. nematode infestation significantly increased MLN disease development and severity in susceptible maize varieties. According to a study on levels of MLN severity across regions done by Tefero and Gudero (2019), MLN severity varied from region to region in relation to altitude. In previous studies done on interactions between plant parasitic nematodes and other crop pathogens, high disease severity has been reported (Back, *et al*., 2002). Some studies have associated *Pratylenchus* spp. with the severity of *Fusarium* wilt in potatoes (Castillo *et al.,* 1998). However, according to this study, plant parasitic nematodes did not significantly affect MLN disease severity in the field. Further studies in the greenhouse indicated significant effect of *Pratylenchus* spp. on severity of MLN disease in H614D and not in Emph 1101 maize variety. Variety was found to plays a significant role in MLN disease severity with respect to viruses that cause MLN and plant parasitic nematodes within a contained experiment.

Temperate climate inbred lines and hybrids have been reported as being more susceptible to MLN with low levels of tolerance compaired to tropical lines. Infection of plants with SCMV and *Pratylenchus* spp. showed no significant difference across treatments in relation to controls.

Infection of plants with MCMV+ *Pratylenchus* spp. showed significant difference in H614D, but not in Emph 1101. This is an indication that *Pratylenchus* spp. contribute significantly to disease severity in varieties susceptible to MCMV and to *Pratylenchus* spp. *Pratylenchus* nematode infestation has significant impact on MLN disease severity in both H614D and Emph 1101. This indicates that within confined trials, parasitic nematodes do play a significant role in MLN severity of which it is not significant in the fields.

5.2 Conclusion

This study indicates a significant effect of *Pratylenchus* Spp. on development of MLN in confined greenhouse trials on sterile soils void of any other microorganisms. There was no significant impact of *Pratylenchus* Spp. on severity of MLN in the surveyed regions. However, in the controlled experiment, *Pratylenchus* spp. had a significant impact to MLN disease development. This implies that there are other interactions that regulate and manage the effect of nematodes on MLN disease development in the field. Among the two selected varieties, there was an increase in MLN severity in the presence of *Pratylenchus* Spp. on H614D than on Emph 1101 maize variety. This shows that the ability of varieties to resist the viruses and the nematodes play a critical role on MLN disease development.

5.3 Recommendation

Given the information generated in this study, the following are the recommendations:

i. Breeders should develop maize varieties that are resistant to plant parasitic nematodes to manage maize lethal necrosist disease through reduced MLN disease severity

ii. Long term field trial studies should be carried out to determine the effect of interaction between nematodes, viruses and other soil microbes in disease development.

iii. There is also need for evaluation of different *Pratylenchus* spp. for their effect on MLN development.

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