CLIMATE SMART CLOVE PRODUCTION FOR SUSTAINABLE LIVELIHOODS: INTERVENTION FOR ROOT KNOT NEMATODE CONTROL

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Cloves is one of the important spices produced by small holder farmers in East Usambara Mountain (EUM). Previously, the farmers in EUM reported wilting and drying of crops to be associated with biotic and abiotic factors. Plant parasitic nematodes (PPN) particularly root knot nematode has been reported to cause yield reduction in cloves and are considered of highly economic importance. In the same area, climate smart agricultural practices (CSA) had been introduced to enhance use of soil micro-organism such as arbuscular mycorrhizal Fungi (AMF) to combat RKN. However the influence of CSA in the association of RKN and AMF remains largely unknown. The aim was to evaluate the association between indigenous AMF and plant parasitic nematode under CSA practices. The first chapter of this dissertation contains general introduction, the second chapter contains literature review describing cloves, PPN, AMF and Climate smart agricultural practices, the third, fourth and fifth chapters comprise manuscripts in the form of publishable papers while the sixth chapter covers general conclusion and recommendation.

Chapter 3 reports on survey conducted in EUM to assess perception and awareness of root knot nematode (RKN) among the farmers. The survey revealed that majority (89.1%) of the farmers were not aware of RKN. Most of them practiced mixed cropping and reported that they faced other constraints such insect pests and diseases that might have contribute to wilting of their clove crop. In Chapter 4 abundance of AMF and plant parasitic nematodes (PPN) in the clove fields was evaluated in CSA and NCSA. The study revealed existence of mycorrhizal colonization in clove roots (86 to 100%) and that *Rotylenchulus* spp. and *Glomus* spp. were the most abundant genera. There was no significant difference between PPN and AMF abundance under different farming practices. However, there was positive association between AMF and PPN in both farming practices. The studies showed that there was no significant association between soil properties and arbuscular mycorrhizal fungi but there was positive association between nitrogen and PPN abundance. There was no significant relationship between AMF and PPN abundance in clove fields. In Chapter 5, Pathogenicity of RKN in clove roots was studied. The findings showed that no observable galling symptoms were seen in 300 clove plants in EUM. Thus cloves were not affected by RKN. In screen house experiments clove seedling that were inoculated with second stage juvenile (J2) nematode showed no galling symptoms and reproduction factor was less than 1 indicating that clove plants were not hosts of root knot nematode. Thus RKN was not the cause of the wilting of clove plants reported in the study area. Further research should focus on other causes leading to the problem.

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DECLARATION

I Suzan Donald Machera, declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This treasurable work is dedicated to my dearly loved parents *Donald Mohono Machera* and *Rose Willigis Ndomba* and my siblings *Regina Machera* and *Clara Machera* for their tireless encouragement and support during the course of my studies.

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LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
CSA	Climate Smart Agriculture
EC	Electron conductivity
EUM	East Usambara Mountain
FAO	Food and Agriculture organization
K	Potassium
NCSA	Non climate smart agriculture
OC	Organic carbon
P	Available phosphorous
ppm	Parts per million
PPN	Plant parasitic nematode
RKN	Root knot nematode
spp.	Species
SPSS	Statistical Package for Social Sciences program
SUA	Sokoine University of Agriculture
TN	Total nitrogen

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Clove (*Syzygium aromaticum* (L) Merr and Perry) is among the most ancient and popular spice crops in the world (Maharani *et al.*, 2019; Rajalekshmy and Manimekali, 2020). It is cultivated for its dried unopened flower buds that are used for food flavouring, food preservation and medicinal purpose (Purbopuspito and Rees, 2002; Riptanti *et al.,* 2019). Clove is among the major sources of employment and income for farming households wherever the crop is grown (ITC, 2014).

Clove tree is a native to the Moluccas Island also known as the Spice island in East Indonesia. The tree belongs to the family Myrtaceae. It is a perennial tropical plant that grows to a height ranging between 10 – 20 m and thrive better in coastal environment ranging from sea level up to an altitude of 1000 meters above sea level with average temperature of 15ºC-30ºC (Rajalekshmy and Manimekali, 2020). Deep loamy soil with high humus content and good drainage is best suited for clove cultivation. It also grows well in cooler climate with well distributed annual rainfall of 1500-3000 mm (Miraji, 2013) a condition ideal for flowering. The productive life of cloves is usually 12 to 100 years, with the first harvest obtained in the sixth year of planting. Clove is widely cultivated in different parts of the world such as India, Indonesia, Madagascar, Malaysia, Sri Lanka and Tanzania particularly in Zanzibar (Riptanti *et al.,* 2019). In Tanzania, there are approximately 9 087 ha under clove with an annual production of 9 011 tonnes per year. This makes Tanzania the third largest clove producer after Indonesia which has an annual production of 123 773 tonnes, followed by Madagascar with 22 000 tonnes

(Pratama and Darwanto, 2019). However, in terms of clove productivity Tanzania is leading with 1 - 1.2 tons /ha/year compared with Indonesia 0.2 - 0.3 tons/ha/year (Pratama *et al*., 2020). Zanzibar contribute 98.3% of the total production and Tanzania mainland (East Usambara Mountain and Morogoro) contributing about 1.7 % (NBS, 2017). In East Usambara Mountain (EUM), 1440 ha are under clove cultivation with an annual production of 500 tonnes (Seguna, 2017).

Clove production in EUM is characterized by low productivity partly due insect pest and diseases, lack of superior genotypes and climate change variability (Seguna, 2017). According to Maerere and Van, (2014), clove production in Tanzania is still under traditional crop production system which is characterized by poor agronomic practices, poor soil management practices, drought stress and outbreak of pests and diseases which contribute to yield decline. To increase clove production new innovations such climate smart agriculture and adoption of Good Agricultural Practices (GAP) are needed to improve living condition of the farmer (Pratama *et al*., 2020). Climate smart agricultural practices which refers to an integrated approach to achieve food security while mitigating climate change have been introduced in East Usambara Mountains. These practices include soil conservation method, agro forestry and residue management which are reported to enhance mycorrhizal association with plants and increase plant resistance to diseases (Hernandez *et al.,* 2019).

1.2 Problem statement and justification

Plant parasitic nematodes (PPNs) such as root knot nematodes (RKN) (*Meloidogyne* spp) have been reported to be associated with clove plants (Seguna, 2017). Root knot nematodes are known to cause various damages in spices like black pepper, cardamom and turmeric (Pervez, 2018). The estimated global damage caused by RKN for all crops worldwide is $US80 billion per year (Gnamkoulamba *et al.*, 2018). The damage caused by RKN on plants create infection pathway for other pathogens that contribute to delayed crop maturity, reduced yields and quality of crop product (Gnamkoulamba *et al.*, 2018). Control of RKN is difficult on perennial crops because most of them spend their time in the soil or underground plant roots. Hence applying nematicides to the immediate surrounding targeting RKN is difficult (Ibrahim *et al.,* 2016). Several measures such as soil sterilization and crop rotation have been proposed to control RKN although with limited success (Gine, 2016). However, the use of biological control organisms such as AMF has shown great potential to manage plant parasitic nematodes including RKN problems. Apart from protection against plant parasitic nematodes, arbuscular mycorrhizal fungi (AMF) also improve nutrients uptake and reduce disease outbreak.

The use of AMF is relevant based on the fact that perennial crops such as cloves naturally establish mutualistic symbiosis with mycorrhizal fungi found in the soil (Mathimaran *et al.,* 2007). Resulting from such plant-fungi relationship, AMF induce physiological changes that enhance plant resistance to diseases by priming host defences, reduce soil borne pathogens and improve nutritional status of the plant (Miozzi *et al.,* 2019: Alban *et al.,* 2013). Singh *et al.* (2019) reported that increased root growth due mycorrhizal colonization increase plant tolerance towards nematode infection. However, AMF abundance, and activity vary greatly among sites. This variation can be attributed to differences in soil fertility and management practices (Emery *et al.,* 2017). Herrejon *et al.* (2019) reported that climate smart agriculture (CSA) practices can enhance mycorrhizal association with plants and increase plant resistance to diseases. However, very little is known about effectiveness of AMF as part of CSA in reducing nematode attack in cloves in East Usambara Mountains. Therefore, this study aims at increasing clove productivity by supressing plant parasitic nematodes through application of different management practices that would enhance mycorrhizal colonization in East Usambara Mountains.

1.3 Objective

1.3.1 Overall objective

Increasing clove productivity by supressing plant parasitic nematodes through the use of CSA farming system that will enhance mycorrhizal colonization in East Usambara Mountains.

1.3.2 Specific Objectives

The specific objectives are to:

i. To assess farmers' knowledge and management of root knot nematode in clove fields in East Usambara Mountains.
ii. To determine the relationship between plants parasitic nematodes and arbuscular mycorrhizal fungi in CSA and non-CSA clove fields in East Usambara Mountains.
iii. To determine the occurrence and pathogenicity test of root knot nematode on cloves in East Usambara Mountains.

References

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Economic importance of cloves

Clove (*Syzygium aromaticum)* is a strategic commodity that is cultivated for its aromatic dried flower buds and used as a spice all over the world (Baietto, 2014). It has significantly contributed to income security to many people in rural areas. It is applied in different industries such as pharmaceutical industry for medicine production and cosmetic industry for perfumery purposes It is used for culinary purposes, to add flavor in food, drinks and act as food preservatives,. Plant extracts obtained from cloves have been reported to combat insect pests and diseases as bio pesticides (Milind and Deepa, 2011; Riptanti *et al*., 2019).

2.1.1 Ecological requirement of clove

Clove is an evergreen tropical perennial plant that grows to a height of 20m. It requires warm and humid climate, with an annual rainfall of 2500-3000 mm. It can be grown from sea level to 1000 meters above sea level, where soils have good drainage since the crop can't withstand water logging (Miraji, 2013).

2.1.2 Clove production constraints

Clove production in Africa has been declining in the last two decades (Matin and Dabek, 1988; Martin, 1991). In Tanzania, this decline has been attributed to pests and diseases and poor agronomic practices (Nutman and Robert, 1971; Martin and Dabek, 1988; Maerere and Van, 2014; Maharani *et al*., 2019; Suprihanti *et al.*, 2020). Poor soil fertility, poor crop management and post-harvest losses have also been reported to affect clove production. Other constraints in clove production include lack of adequate information on clove production (Riptanti *et al*., 2019), clove price fluctuation and climate change (Suprihanti *et al*., 2020). Among the pests that affect cloves are plant parasitic nematodes, *Hindola* spp causing the Sumatra diseases, dieback and leaf canker caused by *Cryphonectria cubensis* and leafspots caused by C*ylindrocladium quinqueseptatum* (Waller and Sitepu, 1975). Suprihanti *et al*. (2020) reported that replanting can be used to manage clove diseases in plantations. Other practices include weeding and pesticide application (Sujianto *et al*., 2020).

2.2 Plant parasitic nematodes

Plant parasitic nematodes are obligate parasites of plants that have wide range of species and cause major damage in many important crops worldwide (Schouteden *et al*., 2015). They are capable of parasitizing thousands of plants, resulting in reduced crop yield. These parasites show a wide range of life styles, they have hollow, retractable, needlelike mouth spear called the stylet that penetrate through the cells injecting some secretions in the plant cells during feeding (Rasmann *et al.*, 2012. Plant parasitic nematodes are of two groups based on their feeding behavior, namely endo-parasites which feed in host tissue and ecto-parasites which do not enter the host tissue. The PPN can be migratory ecto-parasitic which feed externally through the root walls thus establish a short relationship with their host. This nematode i.e., *Xiphinema* spp. are capable of transmitting plant viruses. Migratory endo- parasites do cause damage to the host plant by entering the host and migrate from cell to cell, such nematodes include *Pratylenchus* spp. and *Radopholus* spp. There are sedentary endo-parasitic such as root knot nematode and cyst nematode which have the ability to invade the root and feed from the cells and transform the feeding site into specialized structures called giant cells to support nematode development and reproduction (Mbega and Nzogela, 2012). Some nematodes are semi-endoparasites i.e., they have ability to migrate and penetrate within the host plant in order to feed but later on induce a feeding site e.g., *Rotylenchulus reniformis*. Secondly PPN can be grouped according to plant parts they infest. Some of the plant parts and associated PPN include: foliar nematode e.g., *Aphelenchoides* spp, stem nematode e.g., *Ditylenchus* spp. and root nematode such as *Meloidogyne* spp. and *Rotylenchus* spp. Which later on causes perforation of leaves, leaf and stem necrosis, malformation of roots and growth reduction leading to stunted growth.

2.2.1 Root knot nematodes

Root knot nematodes (RKN) are obligate sedentary plant parasitic nematodes. There are about 150 species of *Meloidogyne*, affecting more than 2000 plant species including grass, fruits, weeds and vegetable worldwide (Makhubu *et al*., 2021). Root knot nematodes have six developmental stages including egg, first stage juvenile (J1), second stage juvenile (J2), third stage juvenile (J3), fourth stage juvenile (J4) and adult stage. The lifecycle starts when the first stage juvenile (J1)   undergoes the first moult within the eggs and erupts as J2. The J2 is the most infective stage can be found in the roots or soil and do not feed until they find the suitable host (Nzogela *et al*., 2020). To complete their lifecycle the second stage juveniles, invade the root elongation region and migrate through roots then to the vascular cylinder, upon successful parasitism they form permanent feeding sites called giant cells (Nguyen *et al*., 2018). The giant cells later prohibit absorption and transport of water and nutrients to the plants. As a result of feeding the second-stage will moult into third-stage, fourth stage and adult stage. J3 and J4 do not feed since they lack the functional stylet and the adult females will remain sedentary for the rest of their life and produce hundreds of eggs enclosed in a gelatinous matrix (Makhubu *et al*., 2021).

2.2.2 Symptoms of nematode infection

Plants affected with nematode can results in above ground and below ground symptoms. Above ground symptoms include stunted growth, wilting, chlorosis, yellowing of leaves, yield loss, and severe infection on older plants causes the plant to wilt suddenly and die early. However, these signs are similar to those of nutrient deficiencies and drought. Below ground symptoms include root galling; which is the main symptom of *Meloidogyne* spp. infection. RKN infection is easy to identify because of the swelling in root. However, the induced formed galls in the root delays nutrient and water uptake and increase plant root susceptibility to other infections such as bacterial and fungal diseases (Ralmi *et al*., 2016).

2.2.3 Factors that influence occurrence of plant parasitic nematodes

The occurrence and populations of plant parasitic nematodes are influenced by host susceptibility and environmental factors, including agro-climatic situation, soil characteristics and agricultural practices (Fleming *et al.,* 2016; Mondal *et al*., 2019). Soil environmental variables such as soil texture, soil pH, organic matter, cation exchange affect nematode population. Upadhaya *et al*. (2019) reported that soil texture and agricultural practices affected prevalence, abundance and pathogenicity of nematode. Furthermore, nematode population is generally higher in warmer areas than cooler areas hence high damage due to their favorable conditions for their multiplication and survival (De Waele and Elsen, 2007).

2.2.4 Economic importance of plant parasitic nematodes

Plant-parasitic nematodes pose a significant threat to crop production in Africa due to the extensive damage they cause to a wide range of agricultural crops including spice. However, the economic impact of these pathogens has been significantly underestimated because most farmers in developing countries are unaware of the problem (Kagoda *et al*., 2010). Plant parasitic nematodes are diverse microscopic soil pathogens that causes unspecific symptoms, that can be confused with abiotic stress symptoms such as drought, nutrient deficiency or micro biome diseases and can also facilitate replication and spread of other pathogens such as fungi, bacteria, and viruses, through mechanical damage (Jones *et al.*, 2013; Onkendi *et al.*, 2014; Mokrini *et al*., 2019). Due to limited information on the impact of PPN. There are no effective management strategies being implemented which makes PPNs a significant threat in farms across the continent

2.2.5 Management of plant- parasitic nematodes

The main goal of controlling various PPN in the soil is to protect the plants from attack so as to achieve maximum crop yield at the end of the growing season. Pest management practices can be categorized into chemical, cultural or biological. These can be practiced singly or in combination to achieve the desired results.

2.2.5.1 Chemical control method

In many developing countries, the control of PPN has been achieved through the use of nematicides. They can be applied as pre-plant nematicides, fumigants or contact nematicides. These synthetic chemical compounds contain active ingredients such as methyl bromide which have adverse effects on environment, human and animals. They are expensive and not available for small scale farmers. Moreover, continuous use of nematicides may lead to some resistance in PPN species (Onkendi *et al*., 2014).

2.2.5.2 Cultural control methods

There are several kinds of cultural practices which involve the use of healthy seeds and transplants, field sanitation, crop rotation, intercropping, tolerant varieties, burning diseased plants, fallowing and mulching (Onkendi *et al*., 2014). Plant parasitic nematodes can easily be spread by human activities, such as transfer of plant debris and infested soils so it is highly advised to clean agricultural machinery and tools after use to avoid transfer of nematodes to other fields. Field sanitation is one of the most important factors in the control and prevention of PPN in the fields, weeds removal has been reported to control various crop pests since weeds can serve as an alternative host of a pest e.g. amaranth weed has been reported to be a good host of *Meloidogyne* spp. (Noling and Gilreath, 2002). The use of resistant cultivars is a valuable component in the management of root-knot nematode. However, not all resistant cultivar can be tolerant to RKN (Briar *et al*., 2016).

2.2.5.3 Physical control method

Heat-based methods such as steaming and solarization of the soil have been reported to kill micro-organisms found in the soil before planting. Steaming involves killing the microorganism in the heated layers of the soil. It kills the pests and beneficial organisms. The method is applicable in small areas. Another approach is solarization that involves the use of transparent plastic films to trap solar radiation and convert it into heat energy in the soil. The effectiveness of this method depends on temperature and duration of its application. Thus it must be practiced during the periods with solar radiation to achieve maximum soil temperature (Katan, 2000). Ozores-Hampton *et al*. (2004) reported solarization to be the promising method to control nematodes. However, this practice is not efficient enough because nematodes can be re-introduced from planting material, equipment's and irrigation water, also some nematode eggs may be resistant to heat thus making this technique not economically viable for a farmer. MacGuidwin, (1993) reported soil flooding as physical control of nematodes. Prolonged soil flooding reduces nematode population by cutting down oxygen supply for respiration and increasing concentration of naturally occurring substance such organic acids which are toxic to nematodes.

2.2.5.4 Biological control methods

Biological methods involve the use of living organisms whether alive or in inactive form to control plant parasitic nematodes**.** Natural enemies are promising for the control of plant-parasitic nematodes. Several nematode antagonists have been reported, including fungi and bacteria that parasitize and feed on nematodes. Fungi and bacteria can be classified on their nematophagous and antagonistic characteristics. Some fungi are endoparasites, trappers, toxin producers and egg parasites thus they are called nematophagous fungi. Parasitic fungi such as *Paecilomyces lilacinus* have been reported to reduce root galling caused by *M. incognita* and *M. javanica* in tomato crops and eggplant by parasitizing the nematode eggs (Goswami *et al*., 2006). Fungi such as *Aspergillus* spp. have lethal effect on nematodes. For example, a strain of *A. viridie* was reported to reduce egg hatching in tomato (Goswami and Mittal, 2004). Beneficial fungi such as arbuscular mycorrhizal fungi (AMF) have been reported to reduce the number of soybean cyst (*Heterodera glycines*) in soybean (Pawlowski and Hartman, 2020). Tchabi *et al*. (2018) also observed significant decrease of *Meloidogyne* spp. infection in *Solanum macrocarpon* colonized by AMF.

2.3. Arbuscular mycorrrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts, capable of colonizing more than 80% of plant species (Prates *et al*., 2019). They are capable of protecting their colonized host plants from abiotic and biotic stress such as plant-parasitic nematodes (Schouteden *et al*., 2015). The AM fungi are classified in the Phylum Glomeromycota with different genera classified on the basis of spore such as *Glomus, Paraglomus, Sclerocytis*, *Acualospora, Entrophospora, Gigaspora,* and *Diversispora*. The mutualistic association involves the fungus taking energy for its production and growth from the plant and in return AMF facilitates mineral uptake from the soil especially phosphorous which is a limiting nutrient to plants (Melo *et al*., 2017). Arbuscular mycorrhizal associations are characterized by structures called arbuscules, hyphae and vesicles in roots, spores and hyphae in the rhizosphere (Ingleby *et al*., 2007). AMF develop intra- radical mycelium (IRM) and extra-radical mycelium (ERM), where by the hyphae of IRM colonize within the roots, develops vesicles and arbuscules structures that are responsible for most of the exchanges between the fungi and the plant. The ERM forms external network of hyphae within the soil (Muller *et al*., 2017; Giovannini *et al*., 2020; Mahmoundi *et al*., 2020)

Arbuscular mycorrhizal fungi cannot be cultured in the laboratory, it can only be cultured in the presence of their host plant (Lee *et al*., 2008; Borriello *et al*., 2012).. Arbuscular mycorrhizal fungi are not host-specific (Ingleby *et al*., 2007). Many AMF may reproduce only vegetatively without producing spores (Lee *et al*., 2008). The diversity of AMF potentially influences plant fitness, community structure, and biodiversity and ecosystem variability. However, the population, diversity and distribution of AMF vary in performance depending on plant species, environment conditions and different management practices such as tillage, crop rotation and cropping system (Jefwa *et al.*, 2009) which can all have positive or negative response in their development.

2.3.1 Role of Arbuscular mycorrhizal fungi

The AMF offer many benefits to the host plants and the agricultural ecosystem, such as enhancing plant growth, increasing soil stability, and improving agricultural productivity and crop health (Zhu *et al*., 2020). Arbuscular mycorrhizal fungi play an important role in plant growth especially under nutrient poor conditions. Arbuscular mycorrhizal fungi can protect the plant against biotic e.g. pests and abiotic stress e.g. drought. The AMF hyphae form an extensive network in soil which can spread into the soil volume and greatly increase the surface area for uptake of immobile nutrients and access more nutrient resources (Singh *et al.,* 2019). Arbuscular mycorrhizal fungi increases crop productivity especially in acidic soils through uptake of phosphorous. Arbuscular mycorrhizal fungi increase host resistance and protection from native plant parasitic nematode like *Heterodera*, *Meloidogyne*, *Pratylenchus* or *Radopholus* (Reid and Emery, 2016; Singh *et al.*, 2019)**.** AMF also contribute to edaphic stability because they promote the soil aggregation via the mycelial network and glomalin production (Silva *et al*., 2010; Fernandes *et al*., 2016). Furthermore, AMF diversity contributes to the coexistence, productivity and maintenance of plant diversity under different environmental conditions. AMF are considered as natural growth regulators and are used as bio-inoculants (Begum *et al*., 2019). Therefore, stimulating indigenous AMF growth is essential in maintaining sustainable agriculture and increasing plant productivity (Hontoria *et al*., 2019).

2.4 Arbuscular mycorrhizal fungi and nematode interaction

Interactions between AMF and nematodes have been reported in numerous studies (Ferreira *et al*., 2018; Wani *et al*., 2017; Herrejon *et al*., 2019). The nature of these interactions can vary significantly, with opposing responses of nematodes to AM fungi. Various studies have shown that AMF enhances host resistance against PPN (Elsen *et al*., 2008). However, nematode response may be negative, neutral and positive to mycorrhizal host plants (Pinochet *et al*., 1996; Ferreira *et al*., 2018).

Arbuscular mycorrhizal fungi and PPN colonize the same area in roots of the host plant (Hol and Gook, 2005). Interaction between AMF and plant parasitic nematodes depends on several factors such as host plant, nematode and AMF species, which may either induce host tolerance or increase host tolerance (Hol and Cook, 2005). Moreover, AMF colonization can reduce development of sedentary PPN and the increase number of migratory parasitic nematodes (Hol and Cook, 2005). In a split-root experimental set-up, Elsen *et al*. (2008) observed a significant decrease in *Radopholus similis* infection in banana roots colonized by AMF. There was suppression of nematode after inoculation of native AMF on coffee plants (Alban *et al*., 2013). Pinochet *et al*. (1996) reported that mycorrhizal colonization reduced population of migratory PPN in perennial crops. Singh *et al.* (2019) reported that AM fungi suppressed the population of plant-parasitic burrowing nematode *Radopholus similis* in the roots of various banana genotypes. Castillo *et al*. (2006) reported significant decrease of *Meloidogyne* spp. and root gall severity after inoculation of different *Glomus* spp. in olive seedlings. Another study shows that twenty strains of indigenous AMF and commercial AMF suppressed root knot nematode in tomato in pots and in the field (Affokpon *et al.*, 2011). This indicates that AMF has a great potential in managing plant parasitic nematodes.

2.5 Climate smart agriculture

Climate-smart agriculture (CSA) as defined by FAO refers to agriculture practices that increases crop productivity sustainably, reduce greenhouse gases and increases achievement of national food security (Nyasimi *et al.*, 2017; Kurgat *et al*., 2020). However, with high variability of climate change, food security is reduced due to dynamic rainfall pattern, floods and drought which affect agriculture production at different level (Makate, 2019; Mugabe, 2020). Thus, adoption of CSA practices are promoted worldwide so as to reduce carbon emission while increasing food security. The different climate smart agriculture practice include: water and soil conservation, which involves practices such as agroforestry, trenches, ridges, soil bunds and planting pits; Efficient use of fertilizer through micro-dosing; intercropping which improves soil fertility and controls pest and diseases. Other practices include crop residue application such as compost and mulching; soil nutrient restoration such as crop rotation, bush fallow, and minimum tillage; improved seed varieties and integrated pest management (Mehra *et al*., 2018; Amadu *et al*., 2020)

2.6 Climate smart agriculture and arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi is of significant importance in agro-ecosystem and can be used in adoption of climate change by increasing plant yield and provide resistance against abiotic and abiotic stress (Di Salvo *et al*., 2020). However, some agricultural practices such as heavy tillage, phosphorous fertilization and chemical fertilization reduce mycorrhizal potential in the ecosystem (Boddington and Dodd, 2000; Jansa *et al*., 2006; Field et al., 2020). Thus climate smart practices are reported to enhance mycorrhizal colonization (Saso-Hernandez et al., 2019).

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Reference

CHAPTER THREE

3.0 FARMERS' KNOWLEDGE AND MANAGEMENT OF ROOT KNOT NEMATODE IN CLOVE (*Syzygium aromaticum* (L.) Merry and Perry) CULTIVATION AREAS IN EAST USAMBARA MOUNTAIN

3.1 Abstract

Root knot nematodes (*Meloidogyne* spp.) are one of the most serious pests of spices in Tanzania. The objective of this study was to assess farmers' knowledge of root knot nematode and their management practices in cloves. A survey was conducted in two wards (Zirai and Misalai) in Amani nature reserve located in Muheza district, Tanga region in December 2019. Data was collected through face to face interviews using semi–structured questionnaires and analyzed using descriptive statistics. Majority (89.9%) of farmers were not aware of root knot nematode. Only 10.1% of farmers were aware of root knot nematode. Majority (97.1%) of farmers did not apply any control measures. In addition to root knot nematode, farmers also reported different clove production constraints in their fields where 52.5% of the respondents cited insect pests affecting clove production while diseases were reported by 50.7% of the respondents. The main associated symptoms with the reported diseases were twig die-back (74.6%), wilting (69.6%) and (31.9%) stunted growth.

Keywords: Cloves, production constraints, root knot nematodes, smallholder farmers

3.2 Introduction

Cloves (*Syzygium aromaticum* (L.) Merr and Perry) is a tropical perennial spice crop, belonging to the family Myrtaceae. It has been cultivated in different areas in Tanzania including such as Morogoro, Tanga and Zanzibar (Baietto, 2014). It is acrop of high economic value used in food flavouring, preservative and for medicinal purpose (Devasahayam *et al.,* 2020; Mardiningsih *et al*., 2020; Rajalekshmy and Manimekali, 2020). Tanzania is the third producer of cloves after Madagsacar and Indonesia with a production of 9 011, 22 000 and 123 773 tonnes respectively (FAO, 2017). Although Indonesia is the first clove grower with 330 000 ha under cultivation, its productivity is very low with 0.3 tonnes per hectare per year where the average annual yield in Tanzania is 1.2 tonnes per hectare per year. In spite of this, Tanzania is still facing decline in clove production (Pratama and Darwanto, 2019). Low yield in cloves has been attributed to insect pests and diseases, use of improper agricultural practices, drought, soil infertility, and lack of knowledge on good agricultural practices (Martin and Dabek, 1988; Baietto, 2014; Das *et al.,* 2018; Riptanti *et al*., 2019).

Among these constraints, plant parasitic nematodes (PPNs) have been reported to be one of the devastating pests in the world causing 29.5% yield loss worth $ 173 billion globally (Kumar *et al.,* 2020). Recently, it has been reported that PPNs such as root knot nematodes (*Meloidogyne* spp) are the most harmful PPNs in Africa with a wide host range (Eche *et al*., 2015) leaving no crop variety safe (Ajay, 2019). The PPNs have been reported to infest and cause severe damage to other spices such as cardamom, ginger, black pepper (Eapen *et al.,* 2005; Ravindra *et al*., 2014; Pervez, 2018; Siddiqui *et al.,* 2019; Maris *et al.,* 2020). Plant parasitic nematodes are difficult for farmers to detect because symptoms caused by PPNs such as shoot dieback in perennials, stunted growth, wilting and death of host plant can be attributed to other pathogens (Ajayi, 2019) including abiotic stresses. Presence of PPNs cause wounding of crop roots which increases the chance of infection by other soil pathogens (Talavera *et al.,* 2012; Ireri *et al.,* 2018). Mwesige *et al*. (2016) reported that presence of *Meloidogyne* spp in Africa will decrease agricultural productivity and will be worsened by the fact that farmers have limited information on PPN.

Farmers' knowledge, perceptions and management of the pest is crucial in crop production of any crop (Schreinemachers *et al.,* 2015; Hashim *et al.,* 2018 and Prabhu *et al.,* 2018). Development of good agriculture practices depends on information about farmers' knowledge and management of pests and disease; lack of this information may result into high persistence of insect pest and poor adoption of management practices (Schreinemachers *et al.,* 2015). Farmer’s knowledge and control practices for pests have been stated elsewhere in different cropping system and related pests such as cotton pests, vegetable pests and disease (Mendesil *et al*., 2016). However, despite cloves economic importance, such pertinent information is not available for root knot nematodes in the crop. Therefore, the objective of this study was to determine farmers’ awareness on root knot nematodes in cloves in East Usambara.

3.3 Materials and Methods

3.3.1 Description of study area

This study was conducted in August 2019 in Amani Nature Reserve in East Usambara in two wards of Zirai and Misalai, Muheza district, Tanga region in Tanzania. Muheza district is located at 5º00′00″S and 38º 55′00 ″E, with an altitude of 400–1300 masl and annual rainfall of about 2 000 mm. These areas experience bimodal rainfall pattern with the short rains falling in October to December and the long rains in February to May each year. Purposive sampling was used to select the wards based on their long history and popularity on clove production.

3.3.2 Selection of respondents

Interviewed farmers were picked using multistage random sampling procedures. The sample size (n= number of farmers interviewed) was determined using the formula suggested by Wonnacott and Wonnacott, (1990).

$$N = \frac{Z^2 P(1-P)}{Q^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

Where: -N = required sample size, Z = confidence level at 95% (standard value of 1.96), p = estimated proportion of an attribute (average per cent of clove farmers in a population of spice farmers in the villages), estimated at 90%, Q = margin of error at 5% (standard value of 0.05).

$N = Z2p(1 - p) / Q2 = (1.96)^2(0.9)(1 - 0.9)/(0.05)^2 = 138.297 \approx 138$……………..…. (ii)

Accordingly, one hundred thirty-eight (138) farmers in two wards were interviewed, 69 farmers were picked per ward.

3.3.3 Data collection

Data were collected through face-to-face interview. A semi structured questionnaire was prepared based on different factors related to farmers’ characteristics in clove production,

production constraints and control practices. To assess farmers' perception and awareness of root knot nematode, respondents were shown a series of coloured photograph of different crops (black pepper, maize, coffee and tomato) having root knot nematode symptoms (Schreinemachers *et al.,* 2015). The data collected included farmers demographic characteristics (e.g., gender, age and level of education), farm characteristics (source of clove seedlings, farm practices), clove production constraints, farmers knowledge and perception of symptoms and management of root knot nematode disease.

3.3.4 Data analysis

The IBM SPSS Statistics version 20 was used to generate descriptive statistics in terms of frequencies and percentages for the variables. Factors determining farmers' awareness on root knot nematode were assessed using the binary logistic regression model.

3.4 Results

3.4.1 Social economic characteristics of the farmers

Table 3.1 shows the demographic characteristics of respondents involved in the study. Of the 138 farmers interviewed. Majority of the farmers were males (72.5%) and of various age. Most of the farmers had formal education (77.8%).

Table 3.1: Socio demographic characteristics of respondents in East Usambara Mountain

Characteristics	Categories	Percent (%) N=138
Sex of respondents	Male	72.5%
	Female	27.5%

Age of respondents (years)	Below 25	0.7
	26 to 35	20.3
	36 to 45	19.5
	46 to 55	13.8
	above 56	45.7
Education level	No formal education	23.2
	Primary education	56.5
	Secondary education	13.8
	College education	6.5

3.4.2 Respondents' farm characteristics

Farmers' responses on farm characteristics are summarized in Table 3:2. Most (81.2%) of the farmers owned 1 to 3ha of land. Majority (65.2%) of the farmers had been in clove production for 11 to 20 years. Clove seedlings planted in EUM were mainly obtained from neighbor's farm (67.4%). All farmers practiced mixed cropping. Based on field observations cloves were intercropped with more than one crop, without any systematic arrangement or specific spacing.

Table 3.2: Farmers experience and farms characteristics of the interviewed respondents in East Usambara Mountain

Characteristics	Categories	Percent (%) (n=138)
Farm size (ha)	< 1	13.8
	1 to 3	81.2
	3.1 to 5.1	2.9
	> 5.1	2.2
Experience	1 to 10	20.2
	11 to 20	65.2
	21 to 30	8.7
	31 to 40	2.9
	above 41	2.9
Source of clove seedling	Farmers group	5.8
	Neighbors	67.4
	Own farm	26.8
Cloves cropping system	Mixed cropping	100.0
Crops grown in the mixed cropping system	Other spices	55.1
	Cereals	66.7
	Legumes	52.2
	Root crops	60.9
	Coffee	2.9
	Banana	10.9
	Sugarcane	15.9

3.4.3 Farmers clove production constraints and control measures

Symptoms such as wilting, stunted growth and die-back (Figure 3.1), insect pests and diseases were reported by farmers as the main clove production constraint in East Usambara Mountains. Majority (52.2%) of farmers reported insect pests as the main constraints limiting clove production, followed by plant diseases (50.7%). The major clove symptoms as reported by the farmers were twig die-back (74.6%), wilting (69.6%) and stunted growth (31.9%). The appearance of the healthy (Figure 3.2A), wilting of clove trees (Figure 3.2B) and leaves shedding due to wilting (Figure 3.2C) was observed during survey. Different control measures were practiced by farmers to combat production constraints. Of this, 82.6% did not apply any control measures, 14.5% applied crude oil and 2.9% uproot diseased plant.

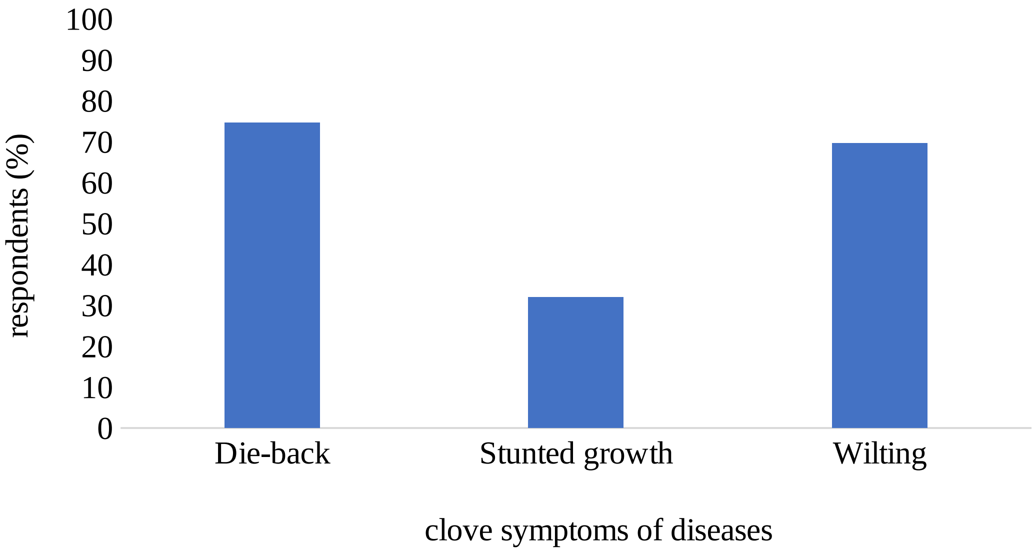


Figure 3.1: Farmers' response on clove production constraints in EUM

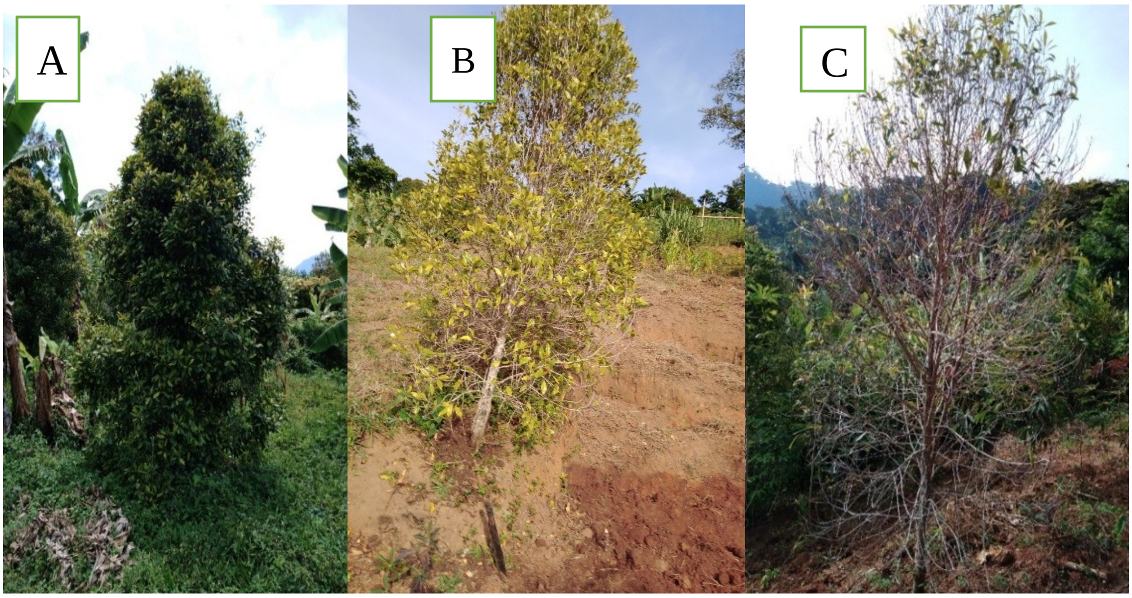


Figure 3.2: Appearances of the clove tree from healthy to wilting. From right-left (A); healthy clove tree (B); wilting clove tree and (C) leaves shedded due to wilting.

3.4.5 Farmers awareness of root knot nematode

Response from the questionnaire survey in relation to farmer's knowledge and perception about RKN show that 89.9% of the respondents were completely unaware of RKN while only 10.1% had knowledge of the pest. Of the 10.1% farmers who were aware of RKN, 64.3% had no formal education while 35.7% had primary education; 64.3% were males and 35.7% females. Results also revealed that 85.7% of farmer with 11 to 20 years of experience were aware of root knot nematode. 97.1% of farmers did not apply any control measure. Only 2.9% of farmers reported to practice uprooting to control RKN.

3.4.6 Factors determining farmers' knowledge on root knot nematode

Binary Logit model was used for determining farmers' awareness of RKN. Among the social economic variables, only source of seedlings obtained from neighbors' had a significant effect on the awareness of RKN (*P* = 0.028). Indicating that farmers who obtained seedlings from their neighbors had high probability of being aware of RKN.

Table 3:3 Logit results on factors determining farmers' knowledge on root knot nematode

Variables	Categories	Estimate	S. E-	Wald	Sig
Education	College education	5.6281	152.7	0.0014	0.9706
	No formal education	-1.2594	320.8	0	0.9969
	Primary education	-5.5851	152.7	0.0013	0.9708
	Secondary education	-4.3518	152.7	0.0008	0.9773
seedling source	Farmers group	-0.8455	0.6254	1.8279	0.1764

Neighbors	0.9739	0.4451	4.7864	0.0287
Ownfarm	.-0.3983	0.5207	0.5851	0.4443

Key: Mean significant P < 0.05, S.E- standard error

3.5 Discussion

The results of the survey indicated that clove production in East Usambara Mountain is a male dominated business. Similar results were reported by Doss *et al*. (2013) that men are more likely to own land than women. With regard to farmers educational background data show that the majority of farmers had primary education and some no formal education at all. This is in agreement with Piras *et al*. (2018) who reported that majority of farmers engaged in farming have relatively low levels of education and many have not completed even a primary school education. However, trained, experienced and knowledgeable farmers have been cited as a must, so as to improve crop production (Crosby *et al*., 2000; Benjamin *et al.*, 2012). The educated farmers can easily and promptly adopt new innovative ideas that can bring significant changes in their farming system (Madisa *et al*., 2010).

The trend in age distribution shows that majority of farmers are below 56 years. Similar observation were reported by Sessay *et al*. (2013), that the increase in number of young farmers will not only spur crop production but also actively improve and modernize agricultural production. Increase in number of young farmers is also expected to enhance and facilitate in increasing sustainable crop production and easily assist the adoption of new innovations (Benjamin *et al.*, 2012).

The survey results revealed that farmers in EUM had very little knowledge of RKN. Awareness of RKN is crucial in the development of new management strategies and for farmers to accept new innovations to control the pest. The low level of knowledge of RKN could be due to farmers' inability to detect damage symptoms caused by the pest since they occur below-ground before aerial symptoms become visible (Ireri *et al*., 2018). Different studies reported similar results: 71% of okra farmers (Danso and Kwoseh, 2016), 90% farmers (Eche *et al*., 2018) and 81.5% maize farmers (Kagoda *et al*., 2010) were not aware of RKN or their damage effect or potential. This shows that majority of farmers in different areas are not aware of RKN. Farmers who obtained seedling source from neighbors had greater influence on awareness of RKN. This is in as disagreement with Obopile *et al*. (2008), who reported among the variables age had significant effect on awareness of different pests and diseases.

In this study clove production is affected by presence of insect pests and diseases. Their associated symptoms include; stunted growth, wilting and twig dieback is adversely clove production. Safni *et al.* (2018) and Waller and Sitepu. (1975) reported the same devastating symptoms in clove trees in Indonesia. They further reported that these symptoms were associated with low temperature and high altitude. Conversely, however, Kagoda *et al.* (2010) attributed wilting and stunted growth to nematode infection.

Most farmers in the study area practicing mixed cropping, so as to increase farm productivity and minimize risk of crop failure as reported by Mendesil *et al*. (2016). This result is similar to Benjamin *et al*. (2012), who reported mixed cropping as traditional practice done by most farmers that highly deters multiplication of pests.

The farmers also practiced uprooting of diseases plant; however, they damp the plant in the same field; this indicates they are not aware of the good agricultural practices. Similar results were observed by Tafesse *et al*. (2018) who reported majority of potato farmers practiced roguing in controlling bacterial wilt, ditched the diseased plant in the same field whereby this facilitate spread of more pathogen.

3.6 Conclusion and Recommendation

The results indicated that farmers had low knowledge of root knot nematode. The majority of clove growers took no measures in the control of insect pests and diseases. To improve clove yields, there is a need provide knowledge of good agricultural practices to extension officers and local farmers and carry out more research in cloves to fill the gap of identifying different pests and diseases affecting the crop.

References

CHAPTER FOUR

4.0 ASSOCIATION BETWEEN PLANT PARASITIC NEMATODE AND ARBUSCULAR MYCORRHIZAL FUNGI ON CLIMATE SMART CLOVE (*Syzgium aromaticum*) FIELDS IN EAST USAMBARA MOUNTAINS TANZANIA

4.1 Abstract

Native communities of arbuscular mycorrhizal fungi (AMF) and plant parasitic nematode (PPN) were examined in fields previously under climate smart agriculture (CSA) and non-climate smart (NCSA) of the East Usambara Mountains. The field had different agricultural practices. Soil samples were taken from 30 fields and from each field 10 soil and clove root samples were collected to make a compost sample. Arbuscular mycorrhizal fungi spores and PPN were isolated using wet-sieving method and Baermann tray method respectively. The isolated fungal spores and PPN were morphologically identified, classified and quantified. A total of 10 AMF and 22 PPN genera were recorded. There were no statistical difference between AMF and PPN in both agricultural practices. In both CSA and NCSA clove fields, fine roots were colonized 80% to 100% respectively. No association was found between AMF and PPN, a significant correlation between PPN and AMF abundance with agricultural practices was observed (p=0.001). No significant difference was found between AMF (p=0.8) and PPN communities (p=0.6) with agriculture practices. Correlating AMF and PPN with soil properties showed no association and no significant difference except for PPN with total nitrogen (p=0.03). Whatever the causes of no significant difference between the treatments, the results showed that CSA and NCSA practices both facilitate mycorrhizal colonization.

Keywords: Arbuscular mycorrhizal fungi, climate smart agriculture, cloves, plant parasitic nematode

Introduction

The clove tree (*Syzygium aromaticum* (L.) Merr and Perr), originated from the Indonesia and has been used globally for its spice and aromatic properties. In Tanzania, cloves trees are grown in East Usambara Mountain characterized warm and humid climate (Baietto, 2014). It is cultivated for its unopened flower buds (Laban *et al.,* 2020; Suprihanti, 2020). The flower buds are used as spices and provides raw material for cigarette production. It is also used in the production of essential oils which is needed by a variety of industries such as pharmaceuticals, food and drinks. Moreover, it serves as source of income to small holder farmers and a source of foreign exchange for the country. Inspite of its economic importance, clove yield is still very low with only 360 kg/ha of clove buds produced compared to its potential production 600 kg/ha (Mardiningsih *et al*., 2020).

Clove production worldwide tend to decline due to poor agronomic practices, climate change and susceptibility to pests and diseases (Maerere and Van, 2014; Suprihanti, 2020). Among these constraints, plant parasitic nematodes (PPN) have been reported to be among factors limiting clove production (Seguna, 2017, personal communication). These parasites are capable of damaging roots (Schouteden *et al*., 2015; Myint *et al.*, 2017; Vieira and Gleason, 2019). Nematode attack can lead to plants infection by other pathogens (Ye *et al.,* 2015; Gnamkoulamba *et al.,* 2018) such as plant viruses (Schouteden *et al.,* 2015) and fungi (Upadhaya *et al.*, 2019) which later lead to delayed crop maturity and yield reduction (Onkendi *et al.,* 2014; Gnamkoulamba *et al.*, 2018) and eventually death of the plant (Mwesige *et al*., 2018).

Estimated crop yield loss due to PPN annually is US$100 billion worldwide (Benjlil *et al.*, 2019; Mateille *et al*., 2020). Several strategies have been developed to control phytonematode including the use of nematicides, crop rotation and use of resistant varieties (Mwesige *et al*., 2018; Chitambo *et al.*, 2019; Mateille *et al.,* 2020; Nzogela *et al*., 2020). However, these practices have not been effective enough because some resistant crop varieties are reported to increase PPN (Chitambo *et al.*, 2019). Some component crops in crop rotation are known to hosts a wide range of PPN (Nzogela *et al*., 2020). The use of synthetic pesticides has detrimental effect on public and environment health (Olaifa and Adenkule, 2016) but also too expensive for farmers to afford. In the context of integrated Pest Management (IPM), the use of biocontrol agents like arbuscular mycorrhizal fungi (AMF) is proposed. The use of AMF is known to be a much safe, sustainable and eco-friendly solution for the management of PPN. AMF form an obligate mutualistic association with various plant species, which often improve uptake of plant nutrients, improve plant growth and protect plant from pathogens (Singh *et al.,* 2019; Wolfe *et al.*, 2020; Zhu *et al.,* 2020). Application of AMF to large number of crop species has proved to lower the number of PPN (Pinochet *et al*., 1996; Hol and Cook, 2005; Elsen *et al*., 2008). Affokpin *et al*. (2011) reported that native AMF suppressed nematode populations in the vegetable crops.

In general, the PPN are detrimental to plant health while AMF are beneficial, however they both share plant roots as a source of space and food (Majic *et al*., 2008; Hol and Cook, 2015). The populations and interaction of PPNs vary among plant species and sites, is attributed to differences in soil fertility and management practices (Jefwa *et al.*, 2009; Dobo *et al*., 2016; Fleming *et al.,* 2016; Herrejon *et al*., 2019; Modal *et al*., 2018;

Upadhaya *et al.*,2019; Adeyemi *et al.*,2019; Hontoria *et al.*, 2019; Summuna *et al.*,2019). Abundance of these microbial communities are influenced by soil characteristics like soil pH, soil type and organic matter (Upadhaya *et al.*, 2018; Mokrini *et al*., 2019). Other studies indicate that farming practices can influence mycorrhizal association with plants (Herrejon *et al.*, 2019). The practices that influence the mutualistic association between AMF as nd PPN because they can as well affect the populations of PPN and AMFs (Upadhaya *et al.*, 2019).

Many researches on AMF - nematode interaction have focused on only specific group of nematode, forgetting the influence of agriculture practices in promoting AMF population dynamics (Herrejon *et al*., 2019). Few studies have examined the AMF-PPN interactions (Elsen *et al.*, 2008; Herrejon *et al*.,2019; Hontoria et al., 2019) .A review by Pinochet *et al.* (1996) indicates that numerous articles have addressed the interaction of AMF and PPN in diverse crops but only a few have reported such interactions in perennial crops including clove. The objectives of this study were to firstly establish the association between population of native PPN and AMFs and secondly establish the influence of climate smart, non-climate smart agricultural practices and soil properties with AMF and PPN soil communities.

4.3 Material and methods

4.3.1 Description of the study area

The study was conducted in Zirai and Misalai wards within the East Usambara Mountain (EUM) in Muheza district, Tanga region. Both wards are located at longitudes 38°32′and 38°48E, latitude 4°4′and 5°13′S and 600 - 1,300 m above sea level. The study area is characterized by steep slopes, humid tropical zone, with average annual rainfall of 1 918 mm which is bimodal and a mean annual temperature of 20.6 ^{o}C. The fields in the study area were comprised of perennial spice crop plantations grown in mixed cropping system with cloves as the main crop. Cloves were intercropped with other spices (cardamom, cinnamon and black pepper), root and tubers (yams and cassava), cereals (e.g. maize) and legumes (e.g. beans).

4.3.2 Site selection and soil sampling

The wards were selected purposively based on long history of growing cloves and had previously practised CSA practices. In each ward, 15 clove fields were purposively sampled. The total sample size for the two wards was 30 fields which where purposively sampled: In Misalai ward 7 CSA fields 8 NCSA while in Zirai ward 8 CSA and 7 NCSA fields were sampled making a total of 15 fields that adapted CSA practices and the other 15 fields had not adopted CSA practices .CSA practices included soil conversation methods including terraces, agroforestry, “fanya juu fanya chini,” ridges, soil bands and mulching. Also, fields were sampled based on characteristics symptoms of plant parasitic nematode attack such as dwarf and drying of clove trees.

Soil and root samples were collected during the clove reproductive phase (bud formation) in the wet season (August 2019). At each field, one composite soil and clove root sample was made from 10 sub-samples that were randomly collected within a distance of 3 m apart in a zigzag pattern. Two kilograms of rhizosphere soil and 500 gram of roots were collected at a depth of 0- 40 cm using soil auger. Plant roots were carefully collected in order to access the fine active roots where mycorrhizal colonization occur. The samples were packed in plastic zip lock bags, transported to the laboratory, and kept at 4^{o}C before direct evaluation of plant parasitic nematodes, arbuscular mycorrhizal fungi and soil analyses.

4.3.3 Extraction and identification of plant parasitic nematode

The, PPN in 300 g of soil were extracted by using Baermann funnel method as described by Coyne *et al*. (2007). Before extraction, the roots were washed in running water, cut into small pieces and blended. Nematodes were killed and fixed in 1 mL of glycerol, 10 mL of formalin and 89 mL of distilled water and permanent slides were mounted in glycerol. The PPN of each sample were identified to genus level based on morphological characteristics including body shape, stylet type, stylet length, mouth type, lip region, pharyngeal overlap, vulva position and tail shape (Mekete *et al.*, 2012). Nematodes were categorized by genera and enumerated under a stereomicroscope at 100× magnification.

4.3.4 Isolation and identification of Arbuscular mycorrhizal fungi

Soil samples were air-dried before AMF spores were isolated and enumerated. AMF spore isolation was performed using the method by Song *et al*. (2019). To disperse the soil aggregates and release AMF spores, a 50 g soil sample of air-dried was placed in a 2 L glass beaker filled with tap water to form a suspension. The suspension was agitated with a glass rod. The soil mixture was left for one hour and poured onto nested sieves with 500, 300, 180 and 53 μm openings. The residue collected in the smallest sieve were washed, transferred into petri plates and placed under dissecting microscope. Spores were picked via a micropipette glass and transferred to microscopic slide. The spores were counted on a plate after 40X magnification using a stereomicroscope. The spore samples were later mounted on slides with polyvinyl alcohol in lacto glycerol (PVLG) and PVLG

+ Melzer's reagent (1:1 v/v). The AMF spore samples were identified using a microscope up to genus level. The identification of AMF genera was made through morphological structures of spores, such as color, size, characteristics of the spore wall (thickness and adornments), reaction to Melzer and spore bearing hyphae and compared with descriptions of fungal genus according to taxonomic key (Shenck and Perez, 1990). In addition, International Culture Collection of Arbuscular Mycorrhizal Fungi guideline http://invam.wvu.edu/the-fungi/classification was referred to for comparison.

4.3.5 Isolation of AMF from clove roots

Clove roots were separated from soil, rinsed in tap water and cut into 1 cm pieces. Three grams of fine roots were cut into 0.5–1.0 cm pieces which were immersed in 10% KOH at 100°C for 1 hour. Later the roots were washed in distilled water and stained using 0.05% trypan blue, 8% acetic acid and 92% distilled water for 30 minutes. Mycorrhizal colonization was assessed using the root intersection method by Trouvetal *et al.* (1986). Estimation of the proportion of infected roots was done using a dissecting microscope (40X) according to Trouvetal *et al*. (1986). Five replicates of 10 roots per slide were assessed for the presence or absence of AMF structures (arbuscules, vesicles, and hyphae) using a light microscope. The percentage of root colonization was detected by the observation of fifty root fragments of 1 cm, randomly selected to quantify mycorrhizal in each sample. These fragments were arranged in parallel groups of 10 to 15 in a drop of glycerinated water between slide and cover slip. Each fragment was carefully checked throughout at magnifications of 100X and 400X. The presence of colonization in a root segment was recorded only if hyphae, arbuscules or vesicles were found.

4.3.6 Soil laboratory analysis

Soil physio-chemical analyses were carried out in the Soil Analysis laboratory of the Sokoine University of Agriculture in Morogoro. The soil samples were air dried and sieved through a 2 mm sieve. The soil was analyzed for Soil pH, total nitrogen (N), organic carbon (OC) and available phosphorus (P). Total N, organic carbon, available P and soil pH was analyzed following the standard methods for tropical soils (Anderson and Ingram, 1993). Phosphorus was extracted using 0.5M NaHCO3+0.01M ethylene diaminetetraacetic acid (EDTA) using a 1:10 soil/solution and considered to indicate “available” phosphorus (Olsen *et al.*, 1954). Organic carbon content was determined with the oxidation method of Walkley and Black (Nelson and Sommers, 1996). Total nitrogen was measured using Kjeldahl nitrogen method (Kjeldahl, 1883) while soil pH was measured in aqueous suspension (1:2.5 w: v). Physical properties regarding the proportions of sand, silt and clay were determined by the pipette method (Claessen *et al.*, 1997).

4.3.7 Statistical analysis

The frequency of occurrence (FO), relative abundance (RA) and spore density (SD) were used to estimate structure of AMF community. These parameters were calculated by the following formulas:

$$Spore\ density = \frac{Number\ of\ spores\ of\ genus}{Weight\ of\ air\ dried\ soil} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..$$

(1)

$$Relative\ abundance = \frac{Number\ of\ spores\ of\ genus}{Total\ number\ of\ spores}\ X\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

(2)

$$Frequency\ of\ occurrence = \frac{Number\ of\ samples\ per\ genus\ observed}{Total\ number\ of\ samples} X\ 100$$ ………………… (3)

$$Colonization\ percentage = \frac{total\ number\ of\ positive\ segments}{Total\ number\ of\ segments\ studied} X\ 100$$ …………………….. (4)

Population density, abundance, frequency of plant parasitic nematode genera were also assessed. Frequency and abundance of each nematode genus were assessed based on the limits established by Fortuner and Merny. (1973). Nematode genera was regarded as abundant if abundance value was ≥ 2.3 (=200 individuals/L soil) and frequent in soil when it was observed in at least 30% of the samples.

Population density (PD)¿ $$\frac{Number\ of\ nematode\ genus\ per\ sample}{300\ g\ of\ soil\ sample}$$ ………………………….. (5)

Frequency of occurrence (FO) ¿ $$\frac{Number\ of\ fields\ positve\ for\ genus}{Total\ number\ of\ sampled\ fields} x\ 100$$ …………………. (6)

Abundance index (AI) ¿ $$\log \frac{Number\ of\ nematodes\ per\ gram\ of\ soil}{number\ of\ samples \in which\ the\ nematode\ was\ found}$$ ………… (7)

Data were log transformed to meet normality, then t-test was used to compare significant effect of PPN and AMF abundance on CSA and NCSA practices. Pearson correlation analysis was used to examine the relationship between CSA and NCSA, soil parameters on PPN and AMF abundance.

4.4 Results

4.4.1 Occurrence of PPN

Twenty-seven genera of PPN were morphologically identified in all the sampled fields of EUM (Table 4.1, Figure 4.3). In CSA clove fields, the most prevalent nematodes were *Rotylenchulus* (100%), *Helicotylenchus* (93%) and *Meloidogyne* (83%). In NCSA fields, *Helicotylenchus* (100%), *Rotylenchulus* (93.3%) and *Meloidogyne* (86%) were the most prevalent. In CSA fields, *Rotylenchulus* (1029/300 g soil) had the highest population density followed by *Helicotylenchus* (147/300 g soil), while the rest of the genera had lower population densities ranging from 1 to 48/300 g soil. However, there was no significance difference between abundance of PPN and agricultural practices ($t=0.0012$, $p=0.8$).

Table 4.1: Population density, frequency of occurrence and abundance index of plant parasitic nematode from soil (300 g) sampled from clove fields in East Usambara Mountain

Climate smart agriculture practices				Non climate smart agriculture practices		
Nematode genera	PD	FO%	AI	AI		FO%
Rotylenchulus	1029	100.0	1.84	1022	1.87	93.3
Radopholus	11	40.0	0.45	11	0.57	26.7
Helicotylenchus	147	93.3	1.06	145	1.03	100.0
Tylenchus	18	60.0	0.48	16	0.80	20.0
Tetylenchus	4	13.3	0.48	3	0.60	6.7
Tyleptus	0	0.0	0.00	1	0.30	6.7
Hoplolaimus	8	26.7	0.48	44	1.08	26.7
Scutellenoma	17	20.0	0.82	10	0.54	26.7
Pratylenchus	49	53.3	0.85	15	0.50	46.7
Ditylenchus	27	53.3	0.64	34	0.77	46.7
Seinura	6	26.7	0.40	23	1.10	13.3
Xiphinema	15	26.7	0.68	3	0.30	20.0
Merlinius	1	6.7	0.30	4	0.48	13.3
Melodoigyne	51	80.0	0.72	3	0.09	86.7
Oionchus	1	6.7	0.30	42	1.63	6.7
Tylenchorhynchus	8	26.7	0.48	1	0.07	40.0
Aphelenchoides	0	0.0	0.00	7	0.90	6.7
Actinolamiane	1	6.7	0.00	1	0.30	6.7
Criconema	1	13.3	0.18	1	0.30	0.0
Dolichodorous	3	6.7	0.60	1	0.30	6.7
Psilenchus	1	6.7	0.30	1	0.30	0.0
Hirshmaniella	1	6.7	0.30	0	0.00	0.0
Caloosia	1	6.7	0.30	1	0.00	6.7
Criconemella	1	0.0	0.00	0	0.00	0.0
Paratylenchus	1	6.7	0.30	1	0.00	0.0
Trichodorous	1	6.7	0.30	0	0.00	0.0
Tyencholaimalles	2	0.0	0.48	2	0.48	6.7

Key: PD-population density. AI-abundance index. FO-frequency of occurrence

The frequency and abundance of plant parasitic nematodes in climate smart fields (CSA) and non-climate smart fields (NCSA) with population density above 5/300g soil are presented in Figure 4.1 and 4.2. *Rotylenchulus* was the most prevalent genera in CSA fields with 100 % frequencies of occurrences, followed by *Helicotylenchus* (93%) and

Meloidogyne (80%) but with low (2.3) abundance indices. In NCSA fields, *Helicotylenchus* (100%), *Rotylenchulus* (93%) and *Meloidogyne* (87%) were highly prevalent with frequency of occurrence 80%. However, all nematode genera identified had low abundance indices (<2.3). According to the dominance diagrams (Figures. 4.1 and 4.2) which combine abundance (A) and frequency (F), 4 0% of the genera were observed widely spread to be over the prospected location (F≥30%).

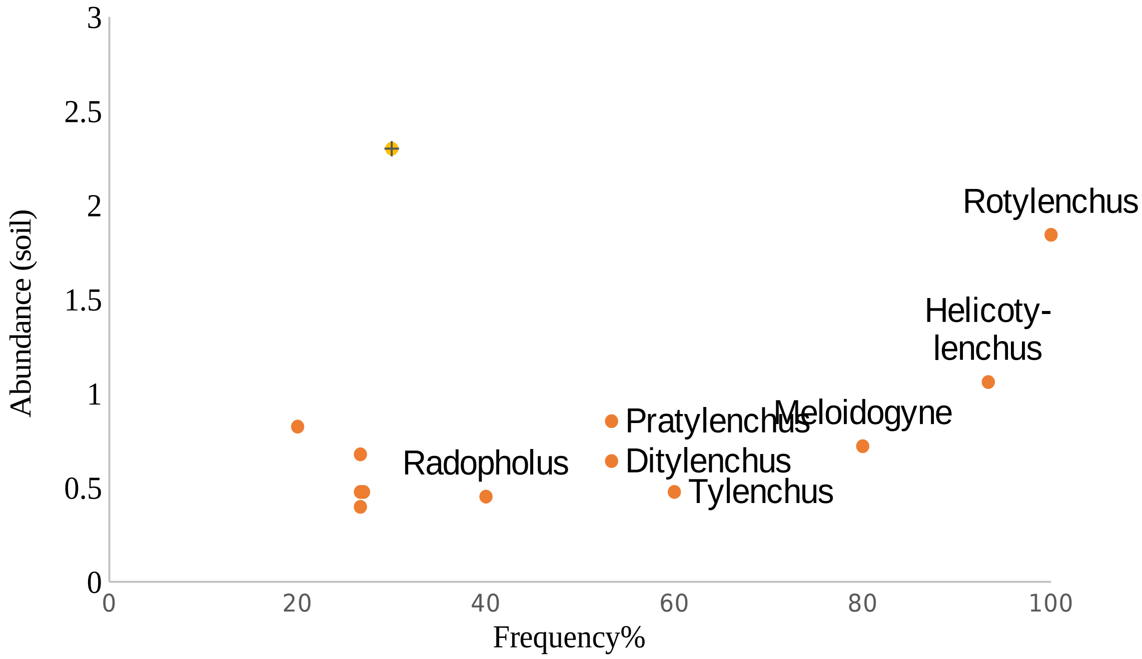


Figure 4.1: Frequency and abundance of plant-parasitic nematode genera associated with climate smart fields. Straight vertical lines represent nematode frequency limit (F, 30%) and straight horizontal lines represent the abundance threshold in soil (AI, 2.3).

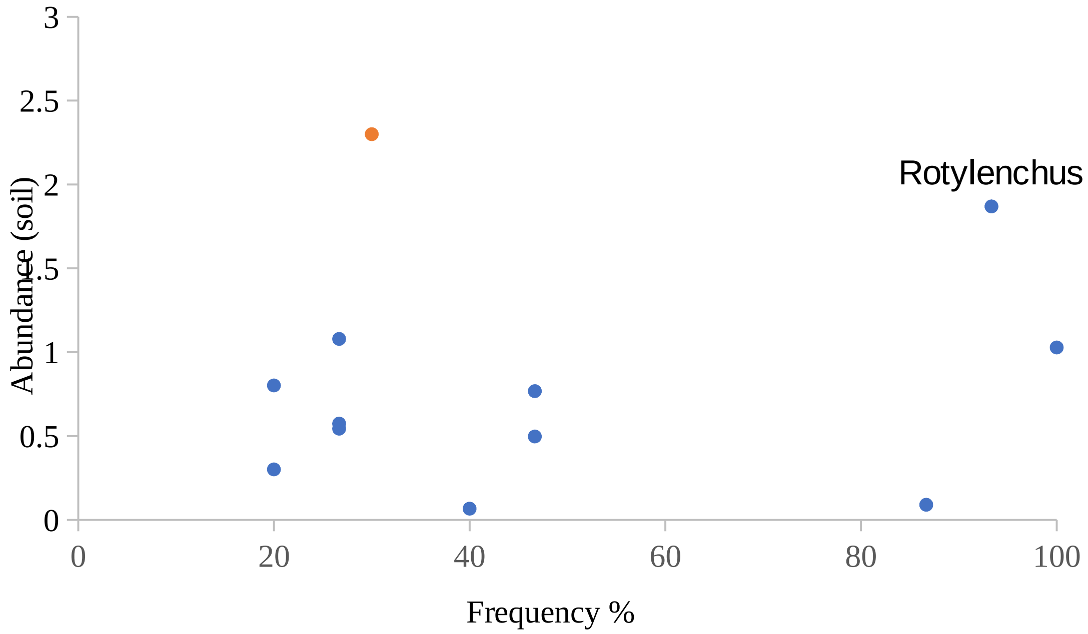
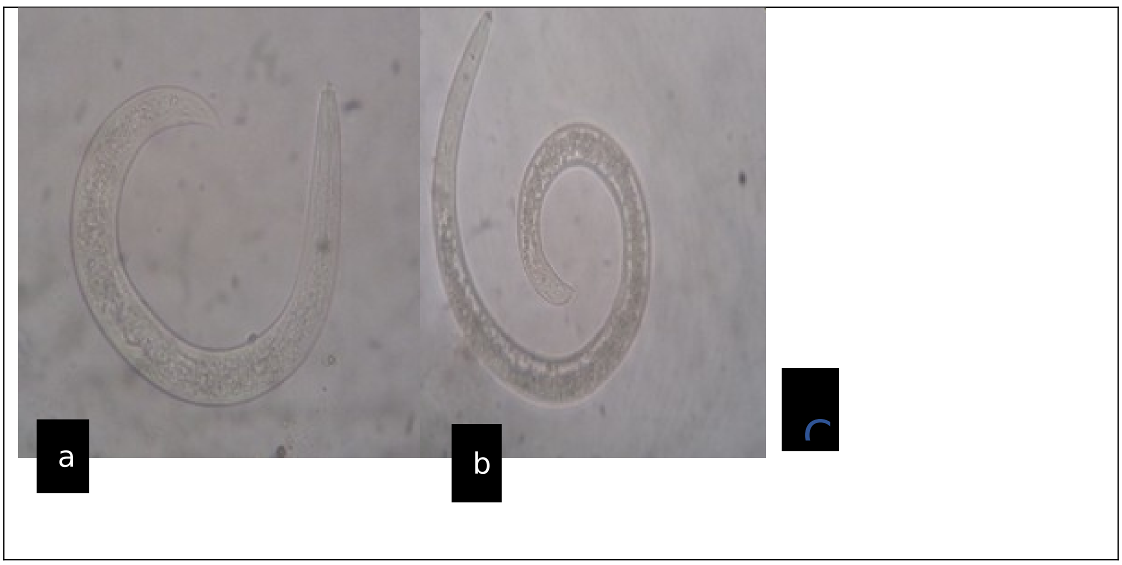


Figure 4.2: Frequency and abundance of plant-parasitic nematode genera associated with non-climate smart fields (NCSA). Straight vertical lines represent nematode frequency limit (F, 30%) and straight horizontal lines represent the abundance threshold in soil (AI, 2.3).



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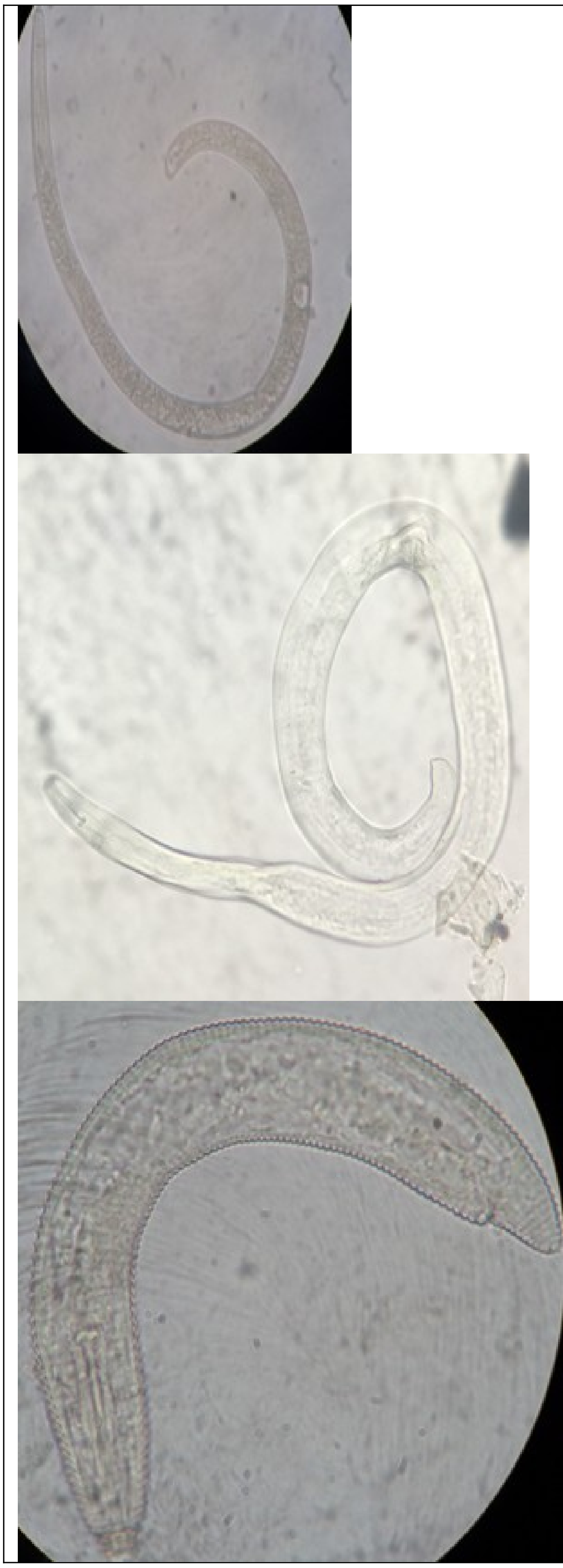


Figure 4.3: Morphology appearance of the plant parasitic nematode genera recovered from clove growing agroecosystem in East Usambara Mountain (a). Xiphinema spp, (b). Helicotylenchus spp, (c). Scutellonema spp (d). Rotylenchulus spp and (e). Criconema spp

4.4.2 Occurrence of AMF

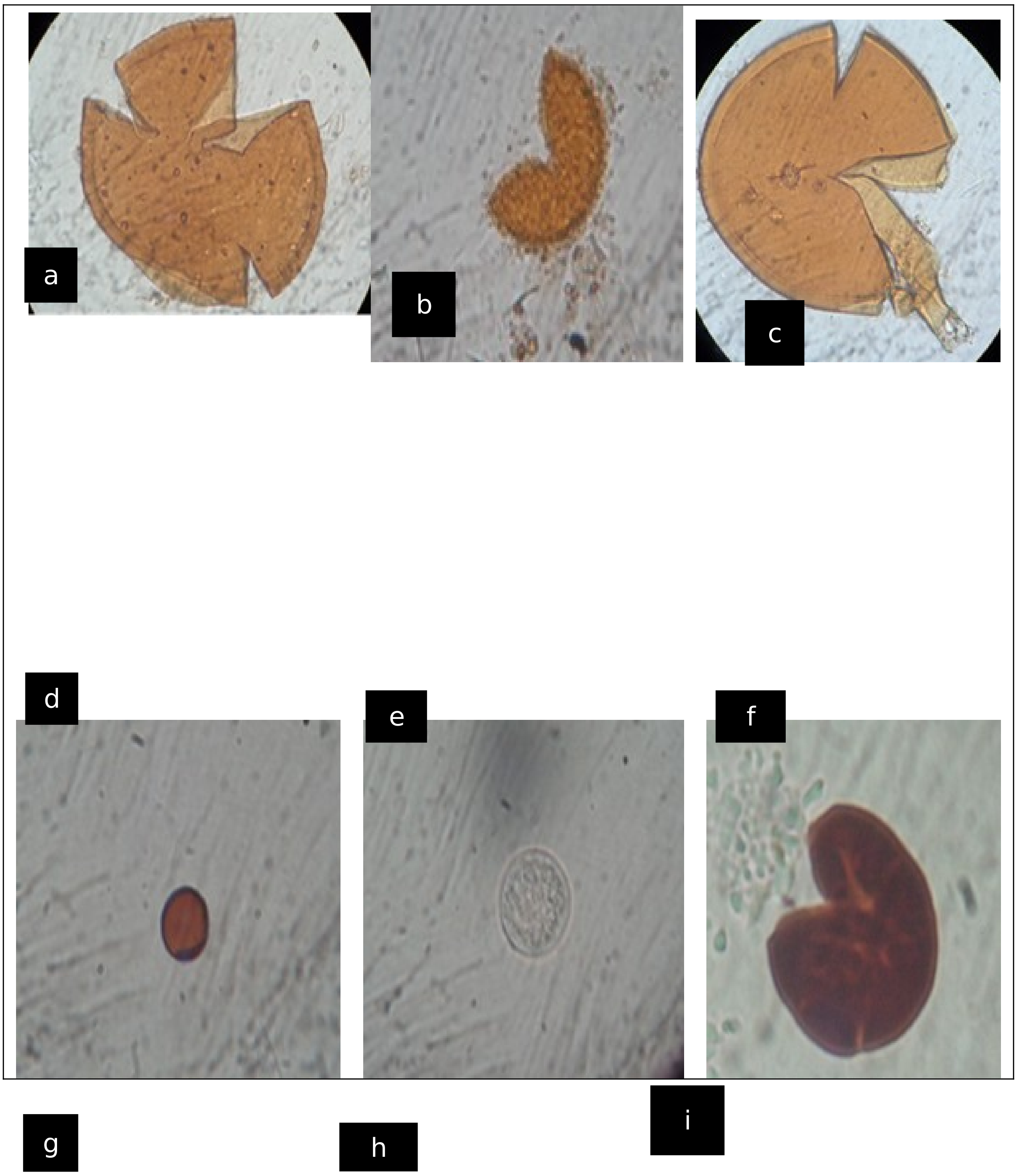
A total of 11600 spores in CSA and 9747 spores in NCSA were isolated from the soil sample from the rhizosphere. Out of these spore specimens 10 genera were identified (Table 4.2, Figure 4.4). However, there was no significant difference between AMF abundance and agricultural practices (t= 0.528, p= 0.6).

Table 4.2: Spore density (SD), relative abundance (RA), isolation frequency (IF) of AMF from soil (300g) sampled from clove fields in East Usambara Mountains

Genera	Climate smart agriculture practices			Non climate smart agricultural practices		
	SD	RA (%)	IF (%)	SD	RA (%)	IF (%)
Glomus	5988	51.62	100	5469	56.11	100
Gigaspora	1194	10.29	100	1191	12.22	100
Acualospora	2828	24.38	100	2235	22.93	100
Scutellospora	990	8.53	53	570	5.85	53
Racocetra	120	1.03	20	54	0.55	20
Redeckra	42	0.36	20	36	0.37	13
Sclerocytis	192	1.66	33	108	1.11	33
Dentiscuta	54	0.47	27	24	0.25	7

Claroideoglomus	138	1.19	53	30	0.31	13
Cetraspora	54	0.47	40	30	0.31	13
Total	11600	100.00		9747	100.00	

Dominant AMF genus was determined according to relative abundance (RA > 3%) and isolation frequency (IF > 40%) according to Dandan and Zhiwei (2007).



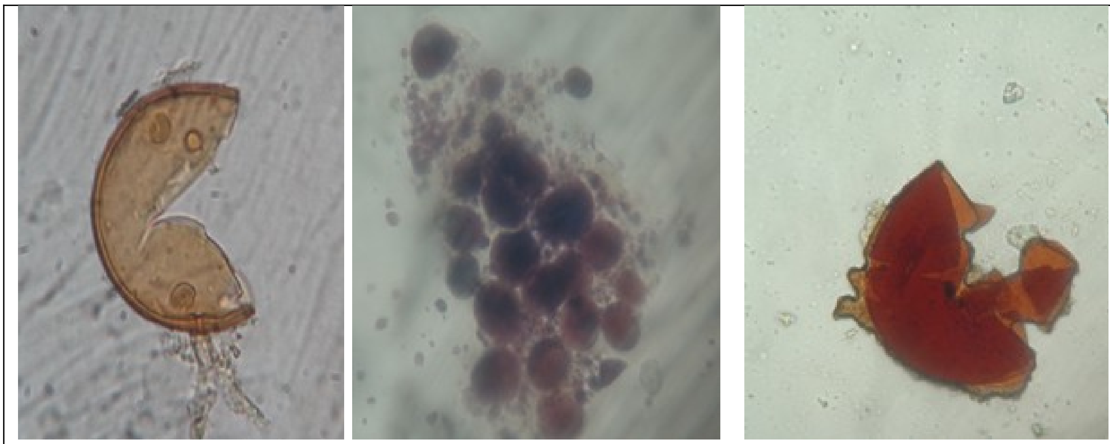


Figure 4.4: Morphological appearance of AMF spores isolated from rhizosphere soil of clove fields in East Usambara Mountain a -c. Acualospora spp., d-g. Glomus spp. h. Gigaspora spp.

4.4.3 Mycorrhizal colonization

Generally, there was no significant difference between mycorrhizal colonization in both CSA and NCSA ($p = 0.0914$).The mean mycorrhizal colonization in clove roots was 98.1% in CSA and 97.3% in NCSA (Fig 4.5; Fig 4.6).

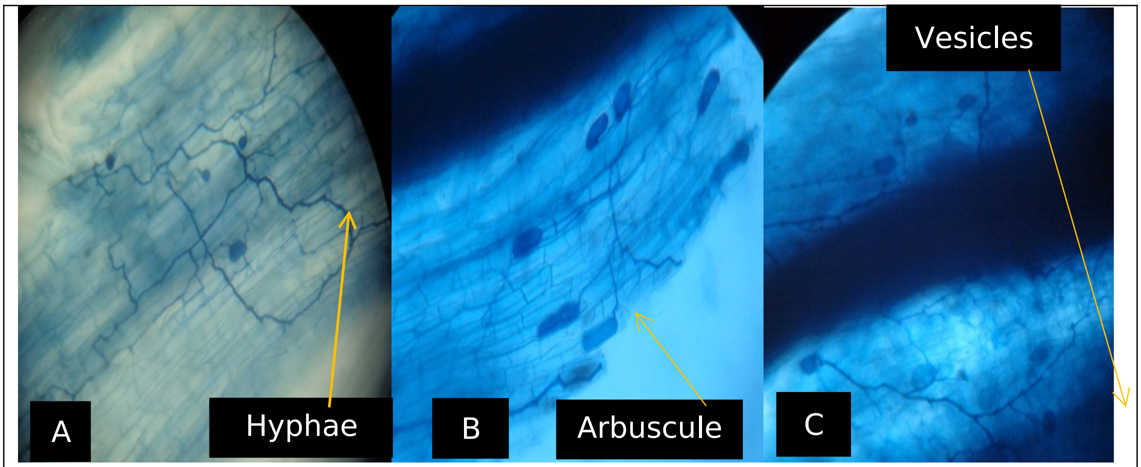


Figure 4.5: Examples of AMF structures used as indicator of clove root colonization from clove fields

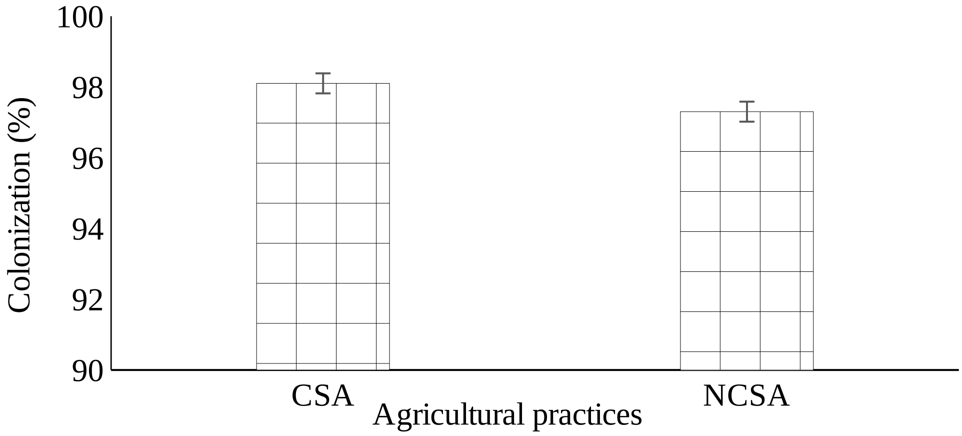


Figure 4.6: Graph showing mycorrhizal colonization (presence of hyphae, vesicles and vesicles) in both CSA and NCSA fields.

4.4.4 Soil properties of cloves in CSA and NCSA fields in East Usambara Mountain

Three classes of soil were identified in assayed clove fields, this includes clay, sandy clay and sandy clay loam. Based on the mean soil properties, NCSA practices had high values compared to CSA fields with exception of potassium was seen high in CSA. All in all, clove fields are experienced with low soil nutrient (Table 4.3).

Table 4. 3: Soil properties from different sampled fields in EUM

CSA							NCSA						
Field	pH	Ec	TN	OC	P	K	Field	pH	Ec	TN	OC	P	K
1	6.06	208	0.31	2.41	2.42	0.43	16	6.15	337	0.37	1.22	5.42	0.23
2	5.93	210	0.36	2.49	0.42	0.48	17	4.97	139.9	0.24	2.60	1.07	0.25
3	4.48	120.7	0.21	1.56	0.99	0.21	18	5.52	115.8	0.25	1.71	1.49	0.25
4	5.96	124.4	0.28	1.03	0.92	0.38	19	6.41	167.8	0.47	2.24	0.82	0.51

5	6.15	106.2	0.23	1.82	0.42	0.24	20	6.22	127.7	0.10	1.67	0.06	0.22
6	5.49	126.2	0.20	1.18	1.78	0.26	21	5.97	202	0.34	2.38	0.64	0.21
7	5.24	83.2	0.24	1.48	0.24	0.18	22	6.04	138	0.32	2.24	0.63	0.21
8	5.74	150.3	0.29	2.03	0.06	0.37	23	6.34	204	0.30	2.20	1.13	0.45
9	6.03	142.3	0.25	2.38	0.99	0.22	24	6.02	200	0.36	2.24	2.35	0.21
10	5.80	129.2	0.38	2.28	1.10	0.18	25	6.0	134	0.30	1.86	0.42	0.32
11	6.02	200	0.36	2.24	2.35	0.21	26	6.03	142.6	0.25	2.38	0.99	0.22
12	5.34	119.2	0.24	1.92	0.95	0.24	27	5.87	198.2	0.29	2.01	0.56	0.22
13	6.05	108.2	0.27	2.40	2.54	0.36	28	6.15	129.3	0.39	2.28	2.20	0.19
14	5.30	122.2	0.19	1.45	0.47	0.35	29	5.36	124.9	0.26	1.76	0.33	0.28
15	6.01	204	0.34	2.26	2.39	0.39	30	6.26	129.3	0.28	1.98	0.85	0.27
Average	5.71	143.6	0.28	1.93	1.20	0.30	Average	5.95	166.0	0.30	2.05	1.26	0.27

KEY: OC-Organic carbon, TN-Total nitrogen, EC-electron conductivity, P-Available phosphorous, K-Potassium

4.4.5 Relationship between agricultural practices, soil properties with PPN and AMF abundance

Correlation analysis was performed among different variables in order to determine if there was a relationship between abundance of AMF, PPN and use of agricultural practices. There was significant positive correlation (r^2 =0.999, p= 0.001) between PPN abundance and AMF abundance (r^2 =0.843, p=0.001) for different agricultural practices (CSA and NCSA). In general, there was no relationship between abundance of AMF and PPN in the soil. There was no significant correlation between PPN abundance, AMF abundance, AMF colonization with soil properties ($p > 0.05$). However, significant associations were found between nematode abundance and total nitrogen (r^2 = 0.393, p=0.032).

4.5 Discussion

Contrary to the previous claim that PPN is an important pest devastating clove trees in east Usambara mountains of Tanzania, this study could not establish sufficient evidence to confirm that claim. This study is reporting the occurrence of PPN and AMF in rhizosphere in clove fields in which CSA and NCSA practices were used. It is evident that PPN and AMF were present in all clove field irrespective of the used agricultural practices. Although it is known that co-occurrence of PPN and AMF in rhizosphere has positive or negative associations, this study however shows, there was no association between PPN and AMF communities suggesting that possibly there is no co-change in populations of these two communities in tree clove fields.

Twenty seven plant parasitic nematodes were identified in this study, however *Rotylenchus, Helicotylenchus, Meloidogyne, Pratylenchus Radopholus* and *Ditylenchus* were most frequent encountered and dominant. This contradicts with the work done by

Bridge (1978), who found association of up to 12 plant parasitic nematodes of cloves such as *Caloosia paradoxa, Meloidogyne incognita* and *Macroposthonia onoesis* being the most abundant .This may be due to seasonal variation, geographical location or agricultural practices. On the basis of potential pathogenic ability, despite of their abundance they are considered omnipresent pathogens (Fortuner and Merny, 1973).

The findings also revealed that *Rotylenchus* spp is the most abundant genera in cloves in EUM. And these is in agreement with other studies which reported a great abundance of *Rotylenchus* in spice crops such as turmeric, ginger (Rama and Dasgupta, 2010; Nguyen *et al.,* 2020). The *Rotylenchus* spp have the ability to feed inside the root and form association with fungal and bacteria pathogens producing disease complexes (Mondal *et al.*, 2019; Nguyen *et al*., 2020). In addition cloves roots in the field were not found affected by *Meloidogyne* spp this may be due to the fact *Meloidogyne* spp are not the main pathogen of cloves (Lau *et al*., 2018).

Among the arbuscular mycorrhiza fungi identified in cloves, *Glomus* was the most dominant genera followed by *Acualospora*, *Gigaspora* and *Scutellospora*. This finding is in agreement with the work by Choudhary *et al*. (2010) who reported the same genera was the most dominant in their study. However similar observation of *Glomus* being dominant followed by Acualospora was reported in different crops such as banana (Jefwa *et al*., 2012), tomato (Songachon *et al*., 2012) and apple (Summuna *et al*., 2019). The point that an equal number of spores of genus *Glomus* were present in both the CSA and non-CSA agricultural practices, was in agreement with the work by Dandan and Zhiwei, (2007) who reported that *Glomus* is highly dominated by small spores and widely distributed in a wide range of ecological conditions. The AMF is adapted to live in a wide range of environmental conditions and different agricultural practices, can survive in alkaline and acidic soils and also produce a quite high number of spores within a very short period of time (Oehl *et al*., 2009, Soka *et al.*, 2018; Summuna *et al*., 2019; Adeyemi *et al*., 2019).

Agricultural practices are important indicators of the abundance of PPN and AMF in soil (Depontes *et al*., 2017; Adeyemi *et al*., 2019). However, in this study both CSA and NCSA practices showed positive association but there were no significant effects of PPN and AMF abundance. This could be attributed of the fact that nematode and AMF communities take a long time to respond to changes in agriculture practices (Herrejon *et al.,* 2019). In addition, the measured soil properties showed no association with AMF abundance. Similar observations were reported by Manoharan *et al*. (2017) where no association was observed between soil properties and AMF abundance.

AMF have been shown to reduce development of root diseases caused by pathogens include plant parasitic nematodes (Hill *et al*., 2018; Wolfe *et al.*, 2020). However, in this study no association was established between AMF spore abundance and nematode abundance in the soil. Similar results were also obtained by Ferreira *et al.* (2018) who reported no association was found between AMF and nematode abundance. Also the insignificant AMF-nematode interaction observed in this study is in agreement with Herrejon *et al*. (2019) and Hol and Cook (2005) who reported the interaction between AMF and plant parasitic nematodes can be positive, negative or neutral and also depends on several factors such as host plant, agricultural practices ,AMF and plant parasitic nematode species.

4.6 Conclusion and recommendation

This study indicated that *Rotylenchul*us and *Glomus* were the dominant genera recorded in clove fields in East Usambara Mountain. No association was found between PPN and AMF abundance. CSA and NCSA agriculture practices had association on AMF abundance and plant parasitic nematodes, despite a lack of clear difference in AMF and PPN community. This could be influenced by other factors such as environmental factors, geographical location, host plant, soil microbial community. Soil properties did not influence nematode and AMF composition in the soil although there was a significant relationship between total nitrogen and plant parasitic nematodes abundance. More study is needed to generate more information on what drives these communities in clove fields and how these drivers can be influenced by climate change.

References

CHAPTER FIVE

5.0 OCCURRENCE AND PATHOGENECITY TEST OF ROOT KNOT NEMATODE (Meloidogyne spp.) IN CLOVE FIELDS IN EAST USAMBARA MOUNTAINS.

5.1 Abstract

Root knot nematode have been reported to cause wilting and drying of clove trees in East Usambara Mountain (EUM) although RKN virulence on clove trees has not been reported before. This study was carried out to evaluate the occurrence of RKN in the soil and roots of clove trees under field condition in EUM. Later, pathogenicity tests were conducted under screen house condition. After 150 days of inoculation, evaluation was done to determine gall index and reproduction factor. The results showed that no galls were observed in 300 plants under field condition. Screen house experiments showed that no galls were present on cloves seedlings and the nematode population in the soil declined as the seedlings were growing. These results suggest that clove plants grown in EUM could be none preferred hosts for root knot nematode infection

Key words: Cloves, East Usambara Mountain, *Meloidogyne* spp., reproduction factor, wilting

5.2 Introduction

Clove *(Syzygium aromaticum* (L.) Merr and Perry) is among the most popular spice crops in Eastern Usambara Mountain (EUM). It is among the major sources of employment, income and food security for farming households (ITC, 2014). Over the past 40 years, total production of cloves was declining (Baietto, 2014). In Tanzania clove production declined from 16 000 tonnes in 1970 to 1 500 to 3 000 tonnes in 2013 (Pratama and Darwanto, 2019). The decline in clove production has been caused by low productivity due to insect pests, diseases, old plants and poor agronomic practices (Sujianto *et al*., 2020; Suprihanti, 2020). Recently, a gradual decline of clove yields was reported in East Usambara Mountain and is associated with rapid wilting and death of plants where different plant pathogens such viral, fungi or nematodes were suspected to be the cause (Seguna , 2017). However, there is no scientific evidence supporting that cloves trees in EUM were susceptible host for RKN.

Meloidogyne spp (root knot nematode) are economically important plant parasitic nematode affecting broad range of hosts plants (Kolombia *et al*., 2017). The genus *Meloidogyne* is known to infect more than 5500 plant species (Akyazi *et al*., 2012). These parasites interact with other disease causing organisms to produce diseases complexes (Gnamkoulamba *et al*., 2018; Kumar et *al*., 2020). The damage caused by these nematodes may lead to reduced yields and stunted growth (Onkendi *et al*., 2014). Kumar *et al*. (2020) reported that yield loss of spices of up to 29.5 % is attributed to root knot nematodes. Root knot nematode in cloves, have received little attention and only limited number of reports are available (Bridge, 1978). Furthermore, information regarding the association of root knot nematodes with clove plants in EUM is lacking. Therefore, the objective of this study was to establish the occurrence of RKN in clove fields in EUM and confirm their pathogenicity to clove trees.

5.3 Materials and Method

5.3.1 Description of the study area

The Study was conducted in the African seed health Centre laboratories and at the screen houses Crop science department located at Sokoine University of Agriculture (SUA). The area is located at latitude of 06 º 50`S, longitude of 37 º 39`E and altitude of 526 m. above sea level.

5.3.2 Survey and collection of clove samples

A survey was conducted to establish occurrence of RKN in soil of clove fields of Zirai and Misalai wards of Muheza district in Tanga region. Thirty clove fields were purposively selected based on incidence of symptoms associated with plant parasitic nematode infestation. These include stunted growth, twig die-back and wilting/drying of clove trees. From the selected fields, composite samples of clove roots and rhizosphere soil were prepared from 10 sub-samples that were randomly collected within a distance of 3 m apart in a zigzag pattern. Two kilograms of rhizosphere soil were collected at a depth of 0- 40 cm using soil auger. The samples were packed in zip lock bags and transported to the laboratory at Sokoine University of Agriculture.

5.3.3 Pathogenicity test experiments

5.3.3.1 Preparation of potting mixture

Soil for pot experiments was sterilized to kill harmful micro-organisms. Top soil was sieved to remove foreign materials such as plant debris, plastic materials, broken pots and glasses. The soil was sterilized using a metal tray for 30 minutes at 100ºC with fire wood as the source of heat. The sterilized soil was spread on a large metal sheet after heating and left over night to cool off.

5.3.3.2 Collection and multiplication of nematode

The tomato plant was used because it is highly susceptible to RKN infection. The tomato cv. Carl J seeds were sown in sterilized soil. After three weeks, tomato seedlings were transplanted in pots containing soil that was previously infected by RKN from clove trees in EUM. The 5 L pots were maintained in the screen house for root knot nematode development and to obtain inoculum. After 5 months, second stage juvenile (J2) nematode was extracted using root incubation method as described by Coyne *et al*. (2007). Tomato plant roots were washed thoroughly under a gentle stress of water, cut into small pieces and placed in modified Baermann funnel. The level of water in the Baermann's funnel were maintained to keep the tissue paper wet and left undisturbed for 14 days. After every 48 hours, second juvenile stage nematodes suspensions were collected and sieved. Counting of nematode population was done under a dissecting microscope.

5.3.3.3 Inoculation of clove plants under screen house

One year old nematode free clove seedlings were planted singly in plastic pots containing 5 kg of sterilized soils. The seedlings were inoculated with 0, 1100, 1550 and 1650 J2s *Meloidogyne* spp. Four holes were made closely to the plant stem and nematode J2s suspension was poured into them using 10ml micropipette. Six replicates were kept for each inoculum levels including a control without any inoculation (J2). The pots were arranged in a completely randomized design and kept in the screen and watered after every two days.

5.3.4 Data collection

Clove roots collected from 300 clove trees were observed for presence or absence of galls. Root knot nematodes infestation was assessed as number of galls per plant and it was described on the scale of 0 to 5 as proposed by Taylor and Sasser. (1978), where 0 = no galls; 1=1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls and 5 = > 100 galls.

The clove plants were uprooted from the soil after 150 days of inoculation, plants were carefully removed from their roots and excised from the aerial parts. The roots were gently washed and blotted dry for gall count and gall index determination as proposed by Taylor and Sassor (1978). In each pot, four soil cores were made and 500 g of soil sample was collected randomly around each plant. Thereafter, composite soil sample was prepared for determination of nematode population density. Nematodes were extracted from 300 g soil sub sample using the Baermann method (Coyne *et al*., 2007). The reproduction factor (RF) was calculated as described by Oostenbrink (1966) as follows:

RF = FP/IP, where PF = final nematode population and PI = initial nematode population (PI = 1100, 1550 and 1650 j2 nematodes). Plants presenting RF <1.0 were considered resistant and RF ≥ 1.0 susceptible.

5.3.5 Statistical analysis

The data obtained on number of counted nematodes were transformed using log (x+1) before analysis. The transformed data were subjected to analysis of variance (ANOVA). Where significant difference was observed, treatment means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.\

5.4 Results

5.4.1 Symptoms and nematode detection in clove fields

Clove plants in EUM exhibited leaf yellowing, severe leaf drop, twig dieback, stunted growth and wilting (Fig.5.1a). The uprooted clove plants did not show any root galling symptoms in both symptomatic and non-symptomatic plants (Figure 5.1b and c).

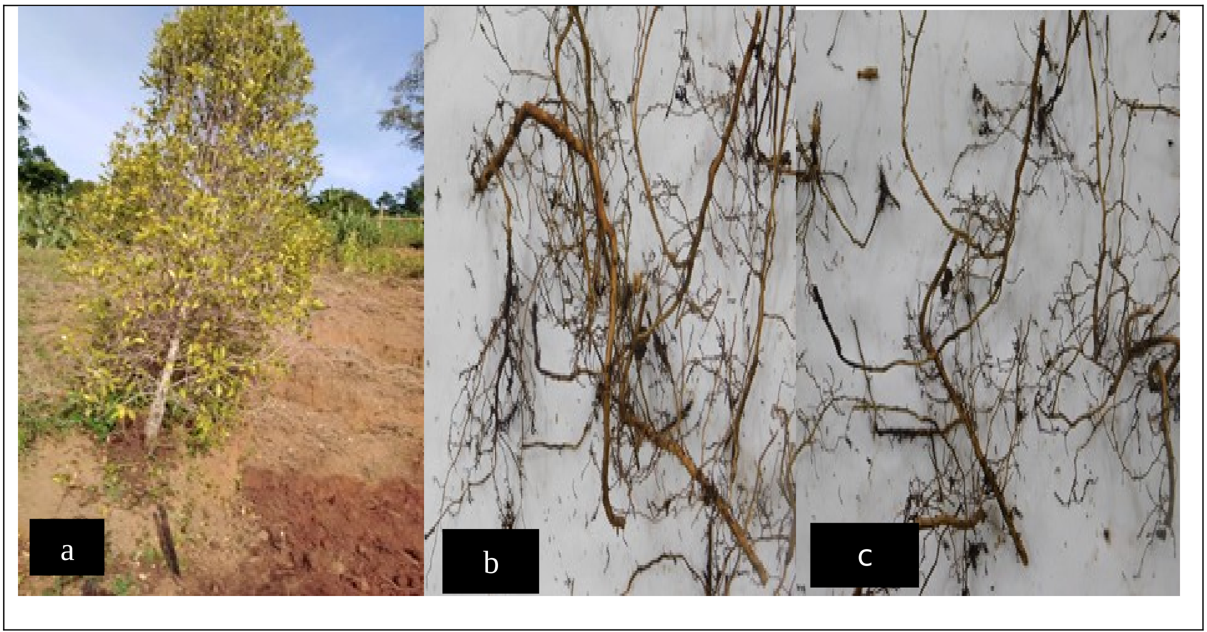


Figure 5.1: Symptoms of diseased clove trees as observed in the field (a): Stunting and yellowing of clove trees (b, c): no galling symptoms were observed in both healthy and unhealthy plant.

5.4.2 Meloidogyne spp. infection in clove roots

The results showed that tomato plant grown on soils which were collected from clove field showed root galling symptoms (Fig.5.2). Clove roots in the fields (5.2a) and clove roots inoculated with J2 nematode did not show any galling symptoms similar to those observed in the field (Fig. 5.2b). The non-inoculated plants did not present any galls in the roots. Galls index in clove plants were zero and reproduction factor was less than 1 in all treatments (Table 5.1).

Figure 5.2: Plant roots (a). Tomato root showing galling symptoms after using clove soil containing RKN, (b). Inoculated clove root showing no galling symptoms

Table 5.1: Gall index, final population, reproduction factor of Meloidogyne spp. in experiment carried out cloves at different inoculum levels

Treatment (J2/pot)	Gall index	Final population	Reproduction factor
0	0	0.00a	0.00a
1100	0	40.83b	0.03b
1550	0	57.33b	0.03b
1650	0	43.50b	0.02b
Mean		35.4	0.0233
p-value		0.014	0.010

Means followed by same letter are not significantly different $P \leq 0.05$ as per DMRT

5.5 Discussion

Farmers in EUM face problems of severe wilting and drying of the clove trees which was thought to be attributed to infestation by root knot nematodes. However, this study did not confirm that root knot nematode was the cause of the observed wilting and drying of cloves in EUM. This is because results from field survey showed no galling infection on clove field in EUM suggesting that cloves are either non host or highly resistant to RKN infection. Similar results were obtained by Bridge. (1978), who reported *Meloidogyne spp*. didn't cause any significant harm to cloves.

Reproduction factor (RF) is a good indicator of plant resistance or susceptibility as it measures nematode establishment and reproduction in the host plant. In this study, RF was less than 1 implying that cloves are RKN resistant as defined by Mukhtar *et al*, (2014). Therefore, RF obtained in the screen house experiment indicated that *Meloidogyne* spp. did not attack and establish in clove or the cloves may be resistant to such nematodes (Oosternbrink, 1966). Furthermore, the resistance against *Meloidogyne* spp. observed in cloves in this study, may be due to the host plant ability to produce root exudates that starve, reduce, or inhibit the entry of J2 nematode into clove roots (Dhandaydham *et al*., 2008; Karuri *et al*., 2017; Chitambo *et al*., 2019; Nzogela *et al*., 2020).

Plant roots infected with root knot nematodes develop galls in which severe cases gives roots knotted appearance and sometimes cause wilting and death in crops (Mwesige *et al*., 2016; Ajayi, 2019). The suspicion that wilting, stunted growth, twig dieback and rapid death of clove trees which were attributed with root knot nematode could not be verified by this study. Therefore, based on results of this study it is obvious that those symptoms in the diseased clove tree in EUM were caused by plant pathogens other than RKN (De

Waele and Elsen, 2007; Thuy *et al*., 2012). Thus, this study regarding the reproductive factor of the nematode on clove plants is the first time being reported in Tanzania, It may be used as the basis for further nematode studies on clove plants in the EUM. Also these findings pave away to investigate clove production constraints (wilting, yellowing, die-back and stunted growth)

5.6 Conclusion

This study showed that RKN is not the real cause of the wilting, stunted growth, twig dieback and rapid death of clove trees as earlier thought. Therefore the symptoms could have been caused by other biotic and abiotic factors. Thus training of farmers and extension officers on RKN is essential so as not to confuse RKN symptoms with other abiotic and biotic factors.

References

CHAPTER SIX

6.0 GENERAL CONCLUSION AND RECOMEMMENDATION

6.1 General conclusion

The study concludes that majority of farmers were not aware of root knot nematode (RKN) because its symptoms occur below ground. Plant parasitic nematode and arbuscular mycorrhizal fungi were present in both CSA and NCSA clove field however, no association was found between them. In addition, clove fields have very low nutrient thus poor soil fertility. Lastly, root knot nematodes was not the cause of wilting and drying of clove trees in EUM as earlier thoughts since no galling symptoms was observed during field survey and screen house experiment.

6.2 Recommendation

Generally, information or knowledge on plant parasitic nematode i.e. RKN should be given to farmers, researchers to avoid the confusion of the symptoms associated with RKN and that of insect pests and diseases on the crops. Different management practices should be adopted by farmers to avoid spread of this PPNs from one field to the other. For sustainable ecosystem, climate smart practices should be adopted by farmers to enhance mycorrhizal colonization which has multi-benefit in increasing crop health. There is need for more research on what drives wilting and drying of clove trees in EUM.

APPENDICES

Appendix 1: Questionnaire used for data collection among clove growers in East Usambara Mt.

1. **Basic data**

   Names of the enumerator…………………………………………………………

   Date form filled (dd/mm/yyyy) ………………………………………………

   District……………………………… Ward……………………………

   Village……………………………… Hamlet…………………………

2. **Respondent's personal data**
   a) Respondent's name………………………………
   b) Sex of respondent: FEMALE (F) MALE (M)
   c) Age of respondent 18 to 25 ( ) 26 to 35 ( ) 36 to 60 ( ) 61 to 75 ( )> 75 ( )
   d) Size of the farm
   e) Level of completed education
      i. No formal education
      ii. Primary education
      iii. Secondary education
      iv. College education

3. **Importance of cloves in the local production system**
   a) How long since you started growing cloves?
   b) Compared to other crops you are producing, to what extent does clove contribute to your income? Greater ( ) low ( ) medium ( )
   c) How much cloves (in kg) do you harvest per season?
   d) The clove you harvest, what do you use it for
      i. commercial ( )
      ii. home consumption ( )s

4. **Clove production and cultural practices**

a) **Age of clove trees in the farm**

   a. 1-10
   b. 10-20
   c. 20 -30

d. 30 and above

b) **Source of clove seedlings**

A. Government

B. Own farm

C. Neighbours

c) **What kind of production system is utilized on your farm?**

A. Mixed cropping

i. Cloves and other spices (cinnamon, black pepper, cardamom)

ii. Cloves and cereals (maize)

iii. Cloves and legumes (beans)

iv. Cloves and root crops (yams, cassava)

v. Anything else

B. Mono cropping

d) **What variety of cloves do you grow?**

e) **Tillage practices**

A. No till

B. Reduced tillage

C. Conventional tillage

f) **Soil conversation method**

A. Mulching

B. Fanya juu/fanya chini

C. Anything else

g) **Do you use any pesticide in your farm?**

A. Organic pesticide (specify)

B. Chemical pesticide (specify)

C. None

h) **Do you apply any fertilizer in your farm? YES ( ) NO ( )**

i) **If yes, what kind of fertilizer do you apply in your farm?**

A. Organic fertilizer (specify)

B. Synthetic fertilizer (specify)

C. Other- explain

5. **Clove production constraints and root knot nematode awareness**

a) What production constraints do you face in clove production?

1. Insect Pests
2. diseases
3. Wilting
4. Anything else

b) Have you ever seen these associated symptoms in your plant? A. Yes B. No

1. Twig dieback
2. Yield decline
3. Drying clove plant
4. Chlorotic leaves/ yellowing of leaves
5. Stunted growth
6. Root galls
7. Root rot
8. Anything else

c) Have you experienced wilting problem in your farm? A. Yes ( ) B. No ( )

d) In which months have you noticed wilting of the clove trees?

e) Can you estimate the ages of the trees that have been affected by the wilting?

1 – 10 ( ), 11 -20 ( ), 21 -30 ( ), 31 and above ( )

f) Have you tried any management practices to help with the wilting? Yes /no

g) What management practices have you tried on the cloves?

Weeding ( ) Mulching ( ) Trenches ( ) tillage ( ) anything else ( )

h) Are you aware of root knot nematode (minyoo fundo)? A.Yes ()B. NO ( )

i) If yes, which symptoms did you encounter and what management practice did you use to control them?

Appendix 2 Showing different surveyed clove fields intercropped with other crops

S.no	Field code	Ward	Village	Altitude	Crops intercropped
1	F20	Mgambo	Kivumo	1049	banana, maize
2	F36	Kizerui	Kizerui	931	cardamom, cinnamon
3	F37	Kwelumbizi	Maweni	796	Cardamom
4	F38	Misalai	Misalai	1043	Maize
5	F42	Kizerui	Kizerui	977	pineapple, cassava
6	F46	Misalai	Misalai	1041	Maize
7	F47	Mgambo	Kivumo	1039	Maize
8	F48	Misalai	Misalai	1025	Maize
9	F49	Misalai	Misalai	1029	maize, cassava
10	F50	Misalai	Misalai	1035	Maize
11	F51	Misalai	Misalai	1034	Maize
12	F52	Kwelumbizi	Maweni	813	banana, cassava
13	F53	Misalai	Misalai	1073	Maize
14	F54	Kizerui	Nkombola	782	yams, coffee, cardamom
15	F55	Misalai	Misalai	981	Beans
16	F56	Kizerui	Nkombola	629	cardamom, blackpepper, yams
17	F57	Kizerui	Nkombola	669	Maize
18	F58	Kizerui	Nkombola	671	cassava, maize
19	F63	Kizerui	Nkombola	677	cinnamon, jackfruit
20	F65	Kizerui	Nkombola	763	cardamom
21	F66	Misalai	Barabarani	1084	Maize
22	F70	kwelumbizi	Maweni	869	Maize
23	F72	Kizerui	Nkombola	770	maize, cassava, cardamom
24	F74	Kizerui	Nkombola	443	Palm
25	F76	Kizerui	Kizerui	783	cinnamon, pineapple
26	F77	Kizerui	Kizerui	796	cardamom
27	F78	Kizerui	Kizerui	821	cardamom
28	F79	Kizerui	Kizerui	821	cardamom
29	F81	Kizerui	Kizerui	931	sugarcane, cassava
30	F82	Kizerui	Kizerui	977	maize, cassava, banana