Relationship between Plant Parasitic Nematodes, Arbuscular Mycorrhizal Fungi and Soil Characteristics on Clove (*Syzygium aromaticum* (l.) Merr and Perr) Agroecosystem in East Usambara Mountains-Tanzania

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Abstract

Native communities of arbuscular mycorrhizal fungi (AMF) and plant parasitic nematodes (PPN) were examined in fields previously under climate-smart agriculture (CSA) and non-climate smart (NCSA) of the East Usambara Mountains. The field were differing in soil properties and agricultural practices. Soil samples were taken from 10 sites in each of the 30 fields. AMF spores and PPN were isolated using wet- sieving method and the Baermann tray method, respectively. The isolated fungal spores and PPN were morphologically identified, classified and quantified. A total of 10 AMF and 27 PPN genus were recorded. The CSA and NCSA fields had 51% and 56% of genus Glomus, respectively. About 73.2% and 72% of genus Rotylenchulus were recovered in CSA and NCSA, 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 suort that CSA practices can facilitate mycorrhizal colonization.. Our results showed that both agriculture practices didnot influence AMF and PPN abundance in the soil.

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

Introduction

The clove tree, (*Syzygium aromaticum* (L.) Merr and Perr), originating from Indonesia has been used globally for its spice and aromatic properties. In Tanzania, cloves trees are grown in East Usambara Mountain characterized by a 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 provide the 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. Also, it serves as a source of income to smallholder farmers and a source of foreign exchange money for the country. Despite its economic importance, clove production is still very low with only 360 kg/ha of clove buds produced compared to its potential production of 600 kg/ha (Mardiningsih *et al.,* 2020).

Clove production tends to decline due to poor agronomic practices, climate change and susceptibility to pests and diseases (Maerere, 2014; Suprihanti, 2020). Among these constraints, plant parasitic nematodes (PPN) have been reported to be one of the main factors limiting clove production (Seguna, 2017). These parasites are capable of damaging roots and tubers (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.,* 2018) 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.,* 2016).

Estimated crop yield loss due to PPN annually is US$100 billion worldwide because these soil-borne pathogens are very difficult to control (Benjlil *et al.,* 2019; Mateille *et al.,* 2020). Several strategies have been developed to control phytonematodes. These include the use of nematicides, crop rotation and the use of resistant varieties (Mwesige *et al.,* 2016; 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 a detrimental effect on public and environmental health (Olaifa and Adenkule, 2016) but is also too expensive for farmers to afford. In the eye 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 much safer, sustainable and eco-friendly solution for the management of PPN. AMF form an obligate mutualistic symbiotic association with various plant species, which often improve uptake of plant nutrients, improve plant growth and protect the plant from pathogens (Singh *et al.,* 2019; Wolfe *et al.,* 2020; Zhu *et al.,* 2020). Application of AMF to a 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). Sayed and Kesba (2005) reported that AMF suppressed PPN in grapes. Affokpon *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 is beneficial, however, they both share plant roots as a source of space and food (Majic *et al.,* 2008; Hol and Cook, 2015). But their populations and interaction vary among plant species and sites, this variation 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.,* 2018; Adeyemi *et al.,* 2019; Hontoria *et al.,* 2019; Summuna *et al.,* 2019). The 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 influence the mutualistic association between AMF and PPN because they can as well affect the populations of PPN and AMFs (Upadhaya *et al.,* 2018).

Many research works on AMF - nematode interaction has focused on specific groups of nematodes. Only limited information is available on the main drivers of AMF population dynamics as influenced by different agriculture practices (Herrejon *et al.,* 2019). Few studies have examined the AMF-PPN interactions (Pinochet *et al.,* 1996; 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 studies have been reported such interactions with perennial crops including clove. The objectives of this study were to firstly establish the association between the 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

Material and methods

Location of the study area

The study was conducted in Zirai and Misalai wards within the East Usambara Mountains (EUM) in the Muheza district, Tanga region. Both wards are located at longitude 38°32′ and 38°48E, latitude 4°4′and 5°13′S and 600m-1, 300m above sea level. The study area is characterized by soft and steep slopes, humid tropical zone, with an average annual rainfall of 1 918 mm which is bimodal and a mean annual temperature of 20.6°C. The fields in the study area were comprised of perennial spice crop plantations grown as mixed cropping systems with cloves as the main crop. Cloves

were intercropped with other spices such as cardamom, cinnamon and black pepper, and cloves intercropped with food crops such as root crops e.g. yams and cassava; cereals e.g. maize; and legumes e.g. beans.

Soil Sampling

The wards were selected purposively based on long term cloves growing and had previously practiced CSA practices. In each ward, 15 clove fields were purposively sampled. Soil sampling was done during the wet season on August 2019. Samples were collected during the clove reproductive phase (bud formation). The total sample size was thirty fields sampled purposively based on used climate-smart agricultural practices such as soil conversation methods including terraces, agroforestry and mulching, characteristics symptoms of plant parasitic nematode attack such as dwarf and drying of clove trees. At each field, one composite sample was made from 10 sub-samples that were randomly collected within a distance of 3m apart in a zigzag pattern. Two kilograms of rhizosphere soil and 500gram of roots were collected at a depth of 40cm using a soil auger. Plant roots were carefully collected in order to access the fine active roots where mycorrhizal colonization occurred. The samples were packed in plastic zip-lock plastic bag, transported to the laboratory, and kept at 40C before direct evaluation of plant parasitic nematodes, arbuscular mycorrhizal fungi and soil analysis.

Extraction and identification of plant parasitic nematode

The PPN in the soil and roots were extracted by using the Baermann funnel 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 1ml of glycerol, 10ml of formalin (40% formaldehyde) and 89 ml of distilled 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.

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 a method in Song *et al.,* (2019). To disperse the soil aggregates and release AMF spores, a 50g sample of air-dried soil 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 a petri plate and placed under the dissecting microscope. Spores were picked via a micropipette glass and transferred to a microscopic slide. The spores were counted on a plate after 40X magnification using the stereomicroscope. The spore samples were later mounted on slides with PVLG (polyvinyl alcohol in lacto glycerol) and PVLG + Melzer's reagent (1:1 v/v). The AMF spore samples were identified by a microscope up to the genus level. The identification of AMF genera was made through morphological structures of spores, such as colour, 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 the taxonomic key (Perez and Shenck, 1990). Also, the spores were identified by comparison with the aid of the site content of the international culture collection of arbuscular mycorrhizal fungi (INVAM) guideline http://invam.wvu.edu/the-fungi/classification.

Isolation of AMF from clove roots

Clove roots were separated, rinsed in tap water and cut into 1 cm pieces. Three grams of fine roots were cut into 0.5–1.0 cm pieces immersed in 10% KOH at 100°C for 1hour. Later the roots were washed in distilled water and stained using 0.05% trypan blue, 8% acetic acid and 92% distilled water for 30minutes. Mycorrhizal colonization was assessed using the root intersection method by Trouvetal *et*

al., (1986). The estimate of the proportion of infected roots was measured after 40X magnification 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 performed 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 coverslip. Each fragment was carefully checked throughout its length, at magnifications of 100X and 400X. The presence of colonization in a root segment was recorded if only hyphae, arbuscule or vesicles were found. Total root colonization was calculated using the following formula: % Colonization = Total number of positive segments / Total number of segments studied x 100.

Soil laboratory analysis

Soil physiochemical 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 following soil characteristics were determined: Soil pH, total nitrogen (N), organic carbon (OC) and available phosphorus (P). Total N, organic carbon, available P and soil pH were analyzed following standard methods for tropical soils (Anderson and Ingram, 1993). Phosphorus was extracted using 0.5M $NaHCO_3$+0.01M ethylenediaminetetraacetic acid (EDTA) (pH 8.5, modified Olsen) using a 1:10 soil/solution ratio 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). The total nitrogen was measured using the Kjeldahl nitrogen method (Kjeldahl, 1883). Soil pH was measured in an a queous suspension (1:2.5 w: v). The physical properties regarding the proportions of sand, silt and clay were determined by the pipette method (Claessen *et al.,* 1997).

Statistical analysis

The frequency of occurrence (FO), relative abundance (RA) and spore density (SD) equations (1, 2 and 3) were used to estimate the structure of the AMF community. These parameters were calculated by using the following formula: SD = number of spores in 300g air-dried soil, RA = (spore number of genus/ total spore number) x 100%, FO = (number of samples in which the genus was observed / total samples) x 100%. Where we determined the dominant AMF genus according to relative abundance (RA>3%) and frequency of occurrence (FO > 40%).

$$Spore\ density = \frac{Number\ of\ spores\ of\ genus}{Weight\ of\ air\ driedsoil} \quad (1)$$

$$Relative\ abundance = \frac{Number\ of\ spores\ of\ genus}{Total\ number\ of\ spores} \times 100 \quad (2)$$

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

Following the formula in equations 4, 5 and 6, the population density, abundance, frequency of plant parasitic nematode genera were also assessed. The frequency and abundance of each nematode genus were assessed based on the limits established by (Fortuner and Merny, 1973). The frequency was calculated by dividing the number of positive samples in which the nematode was observed by a total number of nematodes and expressed as a percentage. Abundance indices were calculated as the logarithm of the average observed on nematode population density in the farms in which the genus was found (log 10 x +1) and a nematode was regarded as abundant if abundance value ≥ 2.3 (=200 individuals/L soil). A nematode was regarded as frequent in soil or roots when it was observed in at least 30% of the samples.

Population density (PD)

$$PD = \frac{Number\ of\ nematodes\ genus\ per\ sample}{300g\ of\ soil\ sample} \times 100 \quad (4)$$

Frequency of occurrence (FO)

$$FO = \frac{Number\ of\ fields\ positive\ for\ genus}{Total\ number\ of\ sampled\ fields} \times 100 \quad (5)$$

Abundance index (AI)

$$AI = \frac{Number\ of\ nematodes\ per\ gram\ of\ soil}{number\ of\ samples\ in\ which\ the\ nematodes\ was\ found} \quad (6)$$

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 agricultural practices, soil parameters, PPN and AMF abundance.

Results

Occurrence of PPN in clove fields of EUM

Twenty-seven genera of PPN were identified in all the sampled fields of EUM (Table 1, Fig. 1). In CSA cloves fields, the prevalent nematodes were *Rotylenchulus* (100%), *Helicotylenchus* (93%) and *Meloidogyne* (83%) while NCSA fields, *Helicotylenchus* (100%), *Rotylenchulus* (93.3%) and *Meloidogyne* (86%). In CSA fields, *Rotylenchulus* (1029/300 g soil) had the highest

Table 1: Population density, prevalence and average abundance of plant parasitic nematode from soil (300g) sampled from clove fields in East Usambara Mountain

	CSA			NCSA		
Nematode genera	**PD**	**FO%**	**AI**	**PD**	**AI**	**FO%**
Rotylenchulus	1029	100.0	1.84	1022.0	1.87	93.3
Radopholus	11	40.0	0.45	11.0	0.57	26.7
Helicotylenchus	147	93.3	1.06	145.0	1.03	100.0
Tylenchus	18	60.0	0.48	16.0	0.80	20.0
Tetylenchus	4	13.3	0.48	3.0	0.60	6.7
Tyleptus	0	0.0	0.00	1.0	0.30	6.7
Hoplolaimus	8	26.7	0.48	44.0	1.08	26.7
Scutellenoma	17	20.0	0.82	10.0	0.54	26.7
Pratylenchus	49	53.3	0.85	15.0	0.50	46.7
Ditylenchus	27	53.3	0.64	34.0	0.77	46.7
Seinura	6	26.7	0.40	23.0	1.10	13.3
Xiphinema	15	26.7	0.68	3.0	0.30	20.0
Merlinius	1	6.7	0.30	4.0	0.48	13.3
Melodoigyne	51	80.0	0.72	3.0	0.09	86.7
Oionchus	1	6.7	0.30	42.0	1.63	6.7
Tylenchorhynchus	8	26.7	0.48	1.0	0.07	40.0
Aphelenchoides	0	0.0	0.00	7.0	0.90	6.7
Actinolamiane	1	6.7	0.30	1.0	0.30	6.7
Criconema	1	6.7	0.1	1.0	0.30	6.7
Dolichodorous	3	6.7	0.60	1.0	0.30	6.7
Psilenchus	1	6.7	0.30	1.0	0.30	6.7
Hirshmaniella	1	6.7	0.30	0.0	0.00	0.0
Caloosia	1	6.7	0.30	1.0	0.30	6.7
Criconemella	1	6.7	0.30	0.0	0.00	0.0
Paratylenchus	1	6.7	0.30	1.0	0.30	6.7
Trichodorous	1	6.7	0.30	0.0	0.00	0.0
Tyencholaimalles	2	6.7	0.30	2.0	0.30	6.7

Key: *PD-population density. AI-abundance index. FO-frequency of occurrence. CSA = Climate smart agricuture, NCSA = Non climate smart agriculture*

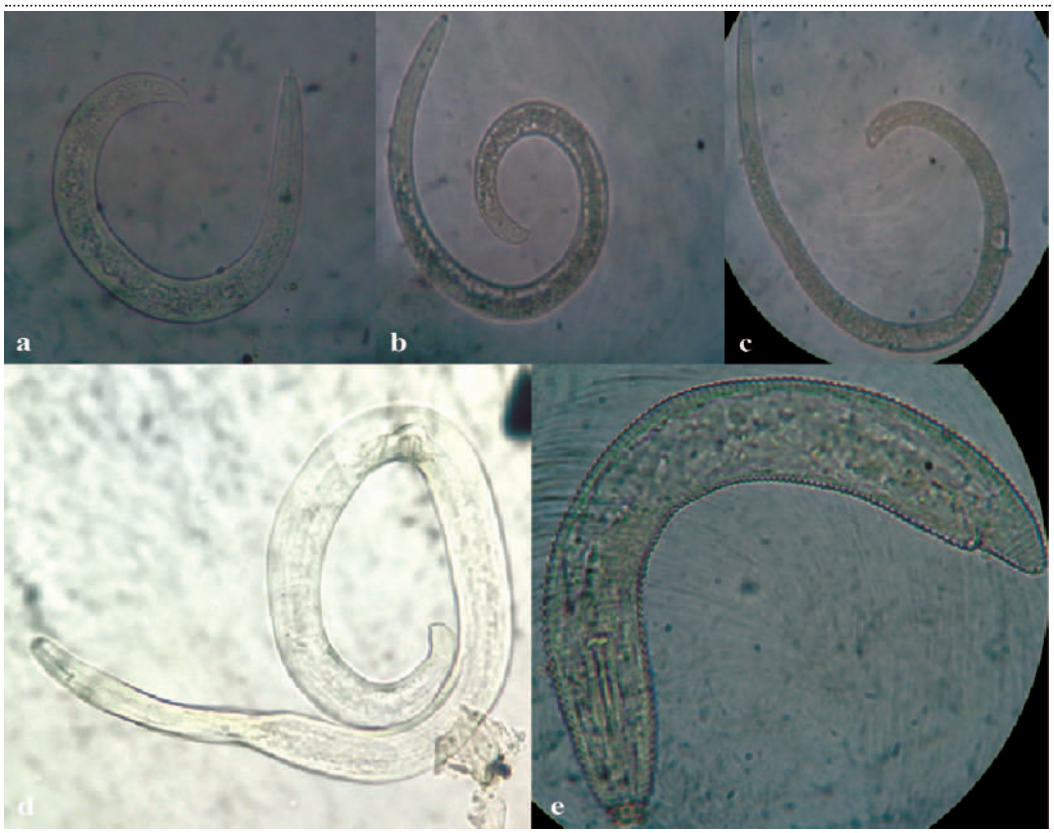

Figure 1: Shows some of the morphology identification of plant parasitic nematode genera recorved from the survey from clove growing agroecosystem in East Usambara Mountains (x40) a. *Xiphinema* spp, b. *Helicotylenchus* spp, c. *Scutellonema* spp d. *Rotylenchulus* spp and e. *Criconema* spp

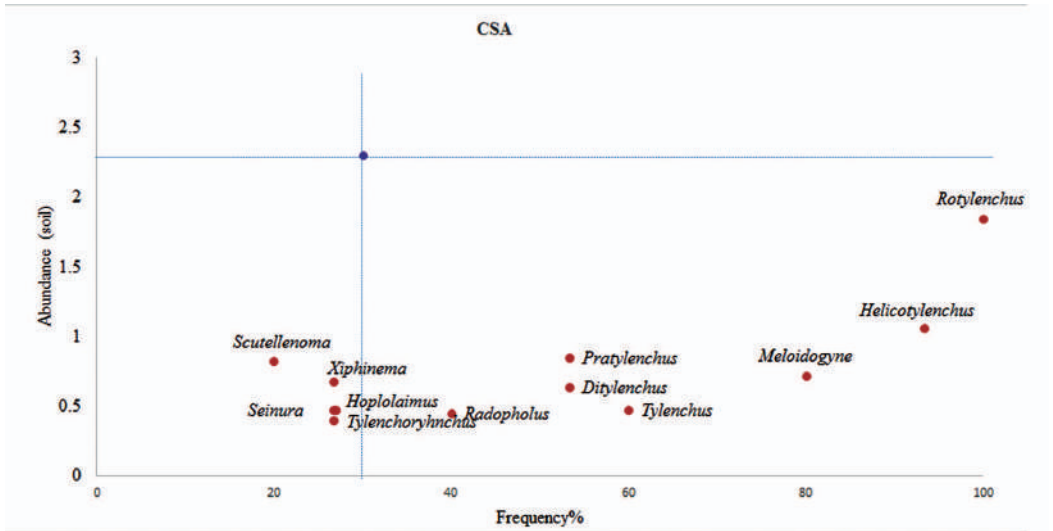

Figure 2: Frequency and abundance of plant-parasitic nematode genera associated with climate-smart fields. Dotted vertical lines represent nematode frequency limit (F, 30%) and dotted horizontal lines represent the abundance threshold in soil (AI, 2.3) according to Fortuner and Merny (1973)

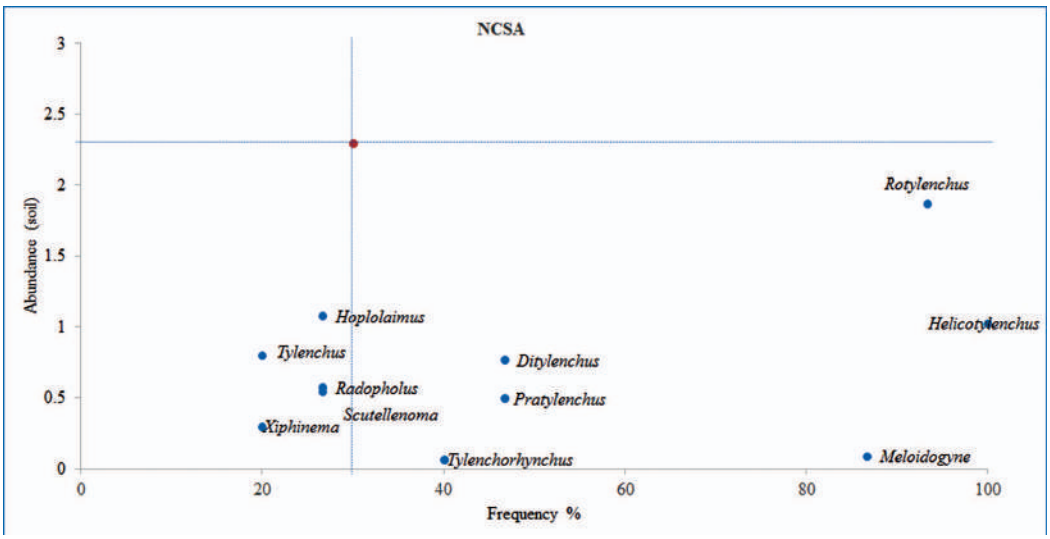

Figure 3: Frequency and abundance of plant-parasitic nematode genera associated with non-climate smart fields (NCSA). Dotted vertical lines represent nematode frequency limit (F, 30%) and dotted horizontal lines represent the abundance threshold in soil (AI, 2.3) according to Fortuner and Merny (1973)

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 (Table 1). In NCSA fields *Rotylenchus* had the highest population density (1022 /300 g soil), followed by *Helicotylenchus* (145/300 g soil). The population density of *Meloidogyne* was lower in NCSA (30/300 g soil) than in CSA fields (51/300 g soil). The genera with the highest mean abundance in both CSA (73.2%) and NCSA (72.7%) was *Rotylenchulus* (Table 1). However, there was no significance difference between abundance of PPN and agricultural practices (t=0.0012, p=0.8).

The frequency and abundance of plant parasitic nematodes in climate-smart fields (CSA) and non-climate smart fields (NCSA) with a population density above 5/300g soil are presented in dominance diagram in Figures 2 and 3. All nematode genera identified had low abundance indices (<2.3) and 40% of the genera were widely spread (F>30%).

Occurrence of AMF in the clove fields in EUM

A total of 11600 fungal spores in CSA and 9747 fungal spores in NCSA were isolated from the soil rhizosphere. Out of these spore specimens, 10 genera were identified (Table 2, Fig. 3). Expressing relative abundance and isolation frequency in brackets. In CSA, *Glomus* (51.6, 100%), *Acaulospora* (24.38, 100%) *Gigaspora* (10.29, 100%), *Scutellospora* (8.53, 53%) and in NCSA Glomus (56.1, 100%), *Gigaspora* (12.22, 100%) and *Acaulospora* (22.93, 100%), *Scutellospora* (5.85, 53%) were the dominant genera (Table 2). However, there was no significant differenct between AMF abundance and agricultural practices (t=0.528, p=0.6)

Mycorrhizal colonization

Generally, there was no significant difference between mycorhhizal 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, Fig 5).

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. Soil collected from all 15 sites had moderately high pH values in NCSA field, ranging from 4.97 (field 2) to 6.41 (field 4) compared to CSA field where pH was generally low with the majority field having

Table 2: Spore density (SD), relative abundance (RA) and isolation frequency (IF) of AMF from soil (300g) sampled from clove fields in East Usambara Mountains

Genera	CSA			NCSA		
	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). CSA = Climate smart agricuture, NCSA = Non climate smart agriculture

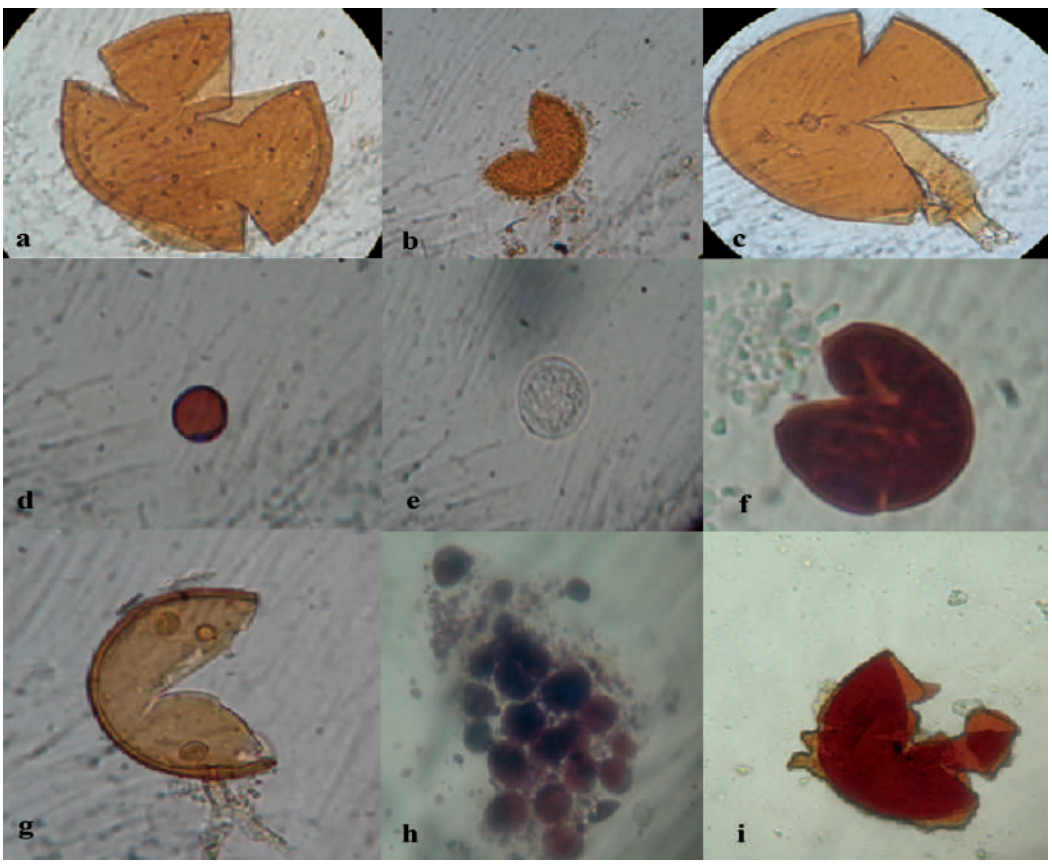

Figure 4: Shows AMF spores isolated from rhizosphere soil of clove fields in East Usambara Mountain a-c. *Acualospora* spp, d-g. *Glomus* spp h. *Gigaspora* spp

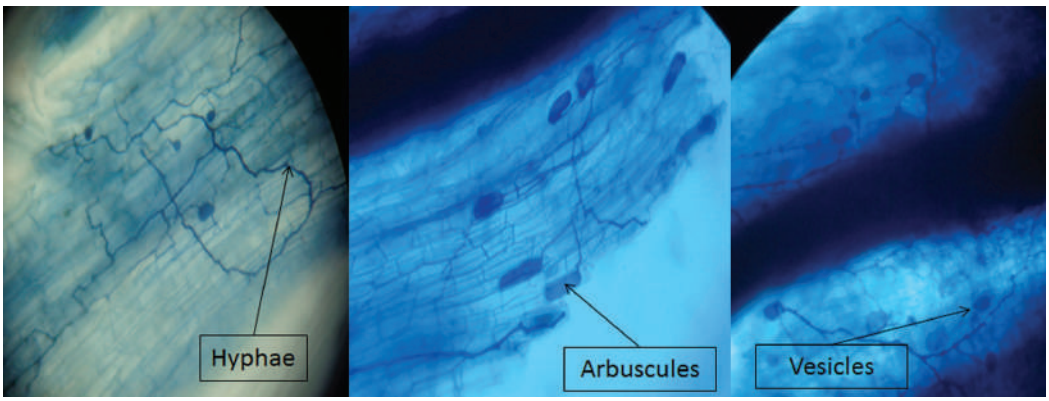

Figure 5: Show clove root segment exhibited mycorrhizal colonization identified by the presence of hyphal structures such as arbuscules, vesicles and hyphae

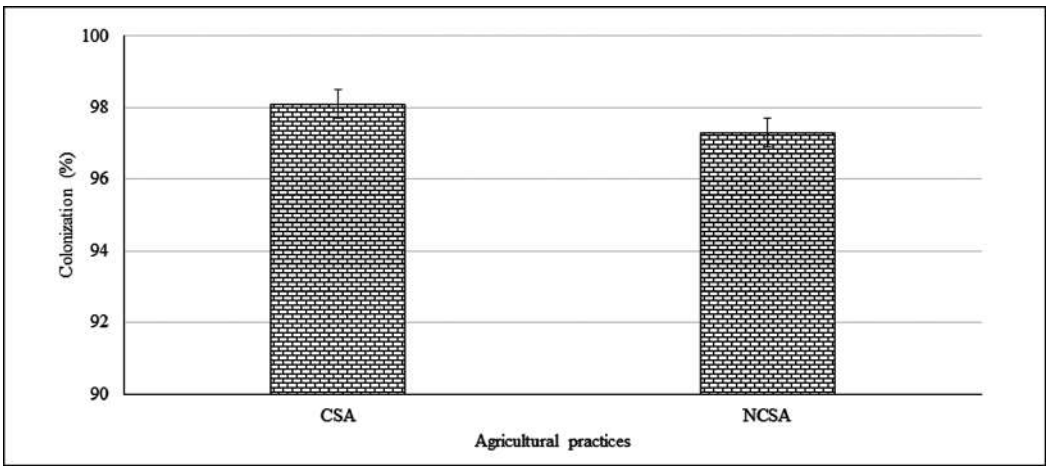

Figure 6: Graph showing percentage of mycorrhizal colonization (presence of hyphae, vesicles and arbuscules) in CSA and NCSA fields. CSA = Climate smart agricuture, NCSA = Non climate smart agriculture

pH lower than 6. The available P levels in CSA and NCSA fields were low and generally not different. Total nitrogen levels were low between the field that previously has CSA practices and those which has no CSA being practiced. Similar trends were observed for the potassium element. Organic carbon content was higher in CSA than in NCSA fields however it was very low in both fields.

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

Correlation analysis was performed among different fields in order to determine if there was a relationship between the abundance of AMF, PPN and the use of agricultural practices. There was significant (p=0.001) positive correlation (r^2=0.999) between PPN abundance and AMF abundance (r^2=0.843, p=0.001) on different agricultural practices (CSA and NCSA). In general, there was no relationship between the abundance of AMF and PPN in the soil (r^2=0.128, p=0.498). There was no significant correlation between PPN abundance and AMF abundance with soil properties. PPN vs soil pH (r^2=0.208, p=0.268), ,organic carbon (r^2=0.09, p=0.601), phosphorous (r^2=-0.02, p=0.902) and potassium (r^2=-0.03, p=0.85). AMF abundance verses soil pH (-0.08, r=0.641), nitrogen ((r^2=-0.11, p=0.538) pottassium (r^2=0.134, p=0.478) and organic carbon (r^2=0.12, p=0.495). However, significant associations were found only between nematode abundance and total nitrogen (r^2=0.393, p=0.032) only.

Table 3: Soil properties from different fields in EUM

	CSA							NCSA					
Field No.	Ph	Ec	TN	OC	P	K	Field No.	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	5.8	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	5.3	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

Discussion

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 maybe due to seasonal variation, geographical location or agricultural practices. On the basis of potential pathogenic ability,despite of there 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 along 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.

Conclusion and recommendation

This study indicated that Rotylenchulus and Glomus were the dominant genera recorded in clove fields in East Usambara Mountain. Moreover, no association was found between PPN abundance 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.

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Conflict of Interest

The author declare no conflict of interest. The funders had no role in the design of the study;in the collection, analysis or interpretation of data;in the writing of the manuscript, or in the decision to publish the results.

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