ORIGINAL ARTICLE

Prevalence of plant parasitic nematodes vary with crop cultivars/clones

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ABSTRACT

The prevalence of plant parasitic nematodes on four horticultural crops *viz*. rose, Hypericum, snap bean and enset was assessed in 2016. Random sampling was used to collect soil samples using auger. Nematodes were recovered from 100 ml soil per sample using a modified Baermann tray technique, identified and enumerated from 1 ml nematode suspension. All the four crop types were found infested with root knot nematode at varying population among cultivars/clones. *M. hapla* infecting roses occurred on citron cultivar at significantly higher (P> 0.05) population density of 1481 J2 while the lowest population density was on Olympia (668 J2) per 100 ml soil. Hypericum cultivars were found infected by *M. javanica* at highest density of 5020 J2 100 ml^{-1} soils on Calypso. On the other hand, snap bean were found infected by in addition to *M. incognita* that occurred most on Faraday (6485 J2 100 ml^{-1} soil), by *Helicotylenchus, Pratylenchus, Rotylenchulus, Scutellonema* and *Tylenchorhynchus*. Greater densities of *Pratylenchus* and *Rotylenchulus* were supported by this cultivar. Compared to the other crops, enset generally supported lower densities of *Meloidogyne* spp. of 490 and 385 J2 on clone Wagu and Astera, respectively. *Hoplolaimus, Mesocriconema, Pratylenchus, Radopholus, Scutellonema, Trichodorus, Trophurus* and *Tylenchorhynchus* were also common depending on enset clone. Based on the distribution and population density, *Meloidogyne* spp. were most important nematodes on all the crops. Depending on the cultivar, heavy to light root galling was observed on rose, snap bean and hypercum. Snap bean cultivars were most attacked and plants showed yellowish foliage and patchy stand in the field. Differences in nematode population density for all the nematode genera found among cultivars/clones might provide an opportunity to explore future potentials for resistance or tolerance characteristics that can be used to combat their damage on yield.

Key words: Floriculture, Horticulture, Hypericum, Roses, *Meloidogyne*, Population density

INTRODUCTION

Horticulture is one of the sectors that received attention in Ethiopia. Large to medium-scale production that comprise of cut-flowers, vegetables, fruits and herbs are being grown by both local and foreign owned farms. The foreign exchange earned from this sector is considered to be about 250 million USD indicating its significance to the country (Anonymous, 2014). The export volume of major flowers such as Roses and Hypericum is of paramount importance for the countries' foreign exchange (Willem, 2010). Similarly Snap beans (*Phaseolus vulgaris* L.) are among the most widely grown vegetable crops for both local and export market. Moreover, Enset is one of the highly valuable horticultural crops indigenous to Ethiopia which is one of the main food and income sources especially for the resource poor farmers in the south and south-western Ethiopia.

However, recently a challenge particularly due to crop pests is becoming serious problem leading to a decline in yield and quality horticultural crops. Though they are overlooked by most growers, plant-parasitic nematodes are among the pests threatening the sustainability of the world agricultural production causing a significant global yield losses of about USD 80 billion (Piedrahita *et al.,* 2012; Singh *et al.,* 2015), through their direct damage in particular to the production of susceptible crop varieties (Koenning *et al.,* 1999). Various groups of plant parasitic nematodes are widely distributed in Ethiopia and are problems of vegetables (Bogale *et al.,* 2004; Wondirad and Mekete, 2002). Root knot nematodes cause serious damage on the intensive monoculture production system of the floriculture industry (Meressa *et al.,* 2014). Nematode infections result in symptoms that appear on both root and aerial part of the affected plant. However, even a characteristic and clearly visible damage symptom in the root tissue may not appear characteristic in the aerial part and could also mimic deficiency of nutrition. Hence, close examination of the soil and plant tissues helps to establish the relationship between the symptom of the damage and the nematodes. Depending on the infecting nematode type, symptoms may include stunting, wilting, reduced root system, root galling, root lesion, proliferation of secondary roots and subsequently yield reduction (Bačić*et al*., 2006; Pokharel, 2009; Machado *et al.,* 2012; Anita and Selvaraj, 2011). However, symptoms become more pronounced when infection occurs during the first few weeks after planting which also may depend on the level of nematode population density (Norshie *et al.,* 2011; Teklu *et al.,* 2016).

The nematodes in the genus *Meloidogyne*, *Rotylenchulus*, *Xiphinema* and *Pratylenchus* are the most prevailing pests of Rose cultivation in Saudi Arabia, where all of the cultivars grown susceptible particularly to *Meloidogyne incognita* (Nour El-Deen *et al.,* 2015). Pizetta et al. (2010) evaluated nine Rose cultivars for *M. hapla* and found all to be susceptible to the nematode. However, Wang et al. (2004) noted that response of Rose cultivars depend on the geographic isolates of *M. hapla* and hence host suitability of rose rootstock range from resistant to susceptible. Due to this single nematode species, a production loss of up to 40% was reported on Roses grown on hydroponics in The Netherlands (García and Amsing, 2007). Rose production in

the warm tropics is being threatened by *M. hapla* (Meressa *et al.,* 2015). For example, collapse of some Rose greenhouses have been noted most possibly due to infestation by root-knot nematodes (Meressa *et al.,* 2014) indicating that the future of cut-rose businesses under the presence of high nematode pressure could pose a question of sustainability.

Meloidogyne spp. and *Pratylenchus* spp. have been implicated to affect the production of *Hypericum* (Tacconi and Menichetti, 1988; Hussey and Janssen, 2001). Cultivars of Snap bean are also known to be affected by different types of plant parasitic nematodes including *Meloidogyne* spp. causing yield losses especially in areas with warm temperatures (Pedrosa*et al.* 2000).The occurrence of various plant parasitic nematode taxa associated with Enset (*Enset ventricosum*) has been reported (Swart *et al.,* 2000; Addis *et al.,* 2004). Literature reports indicate that in addition to the direct damage to Enset, species of *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne* (root-knot nematodes) and *Pratylenchus* (lesion nematodes) have been implicated to predispose Enset to *Xanthomonas* wilt caused by *Xanthomonas campestris* pv. *Musacearum* (Blomme *et al*., 2013).

Management of plant parasitic nematodes is very difficult and relies primarily on preventive methods to avoid the establishment of the nematode in a nematode free soil, since there is no single measure to completely eradicate nematodes once infestation has occurred. An integrated approach using cultural practices and biological control are being used worldwide to effectively control nematode damage (Sadat *et al.,* 2007). The majority of large scale farms depend on synthetic nematicides that have negative features on environment and human health (Johnson and McClanahan, 1974; Kihara*et al.,* 2015; Salguero *et al.,* 2016). The withdrawal of commonly used nematicides from market or restricted use of these chemicals has hence led to the search for alternative management options such as cultural practices and resistance (Westerdahl, 2011). The use of resistant cultivars is one of the best methods for nematode management and reduction of associated economic losses (Ferreira *et al.,* 2012). Therefore, the development of cultivars/clones resistant to nematode attack is one of the strategies easily applicable in the management of various plant parasitic nematodes in the production of different crops. In this regard, knowledge on the presence of nematode resistance characters in crops being grown would be important for the development of resistant cultivars. In the present study, the variation in the prevalence of plant parasitic nematodes between different cultivars/clones of Rose, *Hypericum*, Snap bean and Enset was established.

MATERIALS AND METHODS

Sampling sites

The survey was conducted in four localities in December 2016 (Fig 1). Purposive and random sampling was used to select the sampling localities. Except for Enset clones that were previously collected from different part of the southern region and established at the experimental and demonstration field (Eladale) of Jimma University, the other three crops were grown for commercial purpose. Hence, the soil samples for Roses and Snap bean were collected from Ziway and Qoqa, respectively, in the south and south eastern of Addis Ababa

soil samples of Hypericum were collected from Derba, North of Addis Ababa. The three commercial farms are generally located at a fairly closer distance to Addis Ababa. Moreover, additional site information was collected (Table 1).

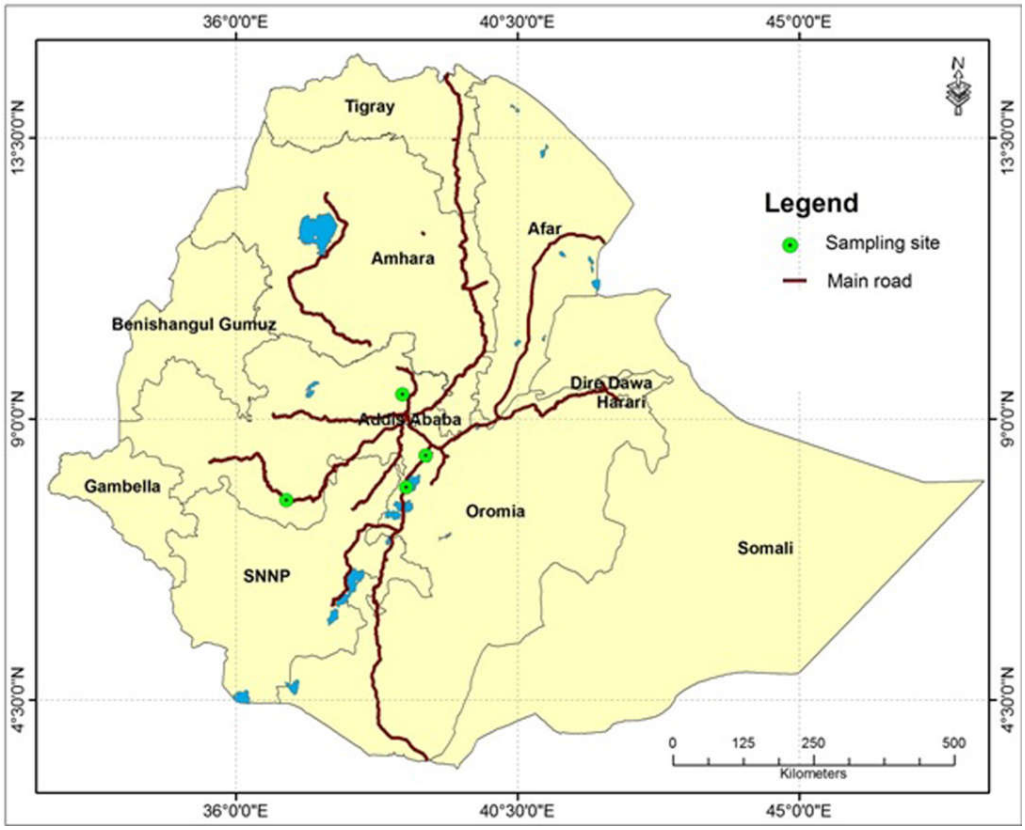

Figure 1. Map of the four study sites

Table 1. Location specific information and surveyed crops of the study sites

District	Altitude (m a.s.l)	Number of cultivars/clones	Common name	Botanical name
Ziway	1648	5	Rose	*Rosa hybrida L.*
Qoqa	1606	4	Snap bean	*Phaseolus vulgaris L.*
Derba	2430	5	St John's wort	*Hypericum spp.*
Jimma	1820	6	Enset	*Ensete ventricosum*

Soil sampling

Four crop species, namely, Rose (*Rosa hybrida*), Hypericum (*Hypericum spp*), Snap bean (*Phaseolus vulgaris*) and Enset (*Enset ventricosum*) were included in the survey. For each crop species' cultivars/clones, 20 soil cores of 2 cm in diameter were taken from a depth of 20-25 cm around the plant root using sampling tube to make a bulk of about 1.2 l soil per sample. Soil samples for each cultivars/clones were in triplicate. After thorough hand mixing a sub-sample of 200 ml soil was collected in a 0.5 l labelled plastic bag and transported with due care to the laboratory and stored in a refrigerator at 5^{o}C until nematode extraction was performed.

Nematode extraction and quantification

Nematodes were extracted from aliquots of 100 ml soil per sample using a modified Baermann technique (Hooper et al. 2005). After 48 hours, the suspension was sieved and nematodes were collected on a 20 µm aperture stainless steel sieve and transferred into plastic cups and stored in a refrigerator at 5^{o}C until counted. From each samples of Snap bean and Enset, a sub-sample of 1 ml suspension was taken and fixed in TAF fixative (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) for identification to genus level. Samples from *Hypericum* and Rose did contain only root knot nematodes; hence, fixation was not necessary. Identified genera were then enumerated from a 1 ml capacity counting dish using a Micros compound microscope. *Meloidogyne* species were identified based on molecular analysis at Julius Kuhn Institut (Braunschweig, Germany) based on the method described by Janssen et al. (2017).

Data analysis

Population density per cultivars/clones was expressed in nematode numbers per 100 ml of soil. Total mean population density (PD) of each nematode genus of each cultivar/clone, frequency of occurrence (FO) and prominence value (PV) of each genus were calculated. The frequency of occurrence of each genus was determined based on Norton (1978). Prominence value was calculated when necessary as $PV= PD*(FO^{-1/2})*10^{-1}$ (De Waele *et al*., 1998). Nematode counts were first log transformed (ln x+1) prior to analysis (Karuri, *et al.,* 2016) to fit the assumptions of ANOVA, but means of actual figures collected are reported. Nematode data per crop species was subjected to analysis of variance (ANOVA) using STATISTICA 7 (StatSoft, Inc., 2004). Tukey's HSD (Honest Significant Difference) test was used to compare the means at the 0.05 level.

RESULTS

The root knot nematodes (*Meloidogyne* spp.) were found in all the soil samples collected from the rhizosphere of the respective crop types (Fig 2-5). In all the four crops, the mean population density varied between cultivars or clones ($P< 0.05$). Moreover, the infecting *Meloidogyne* species were different.

Rose (*Rosa hybrida L.*)

Meloidogyne hapla was the species identified associated with Rose cut-flowers grown under greenhouse (Fig.2). The yield reduction and tendency of increased frequency of old crop replacement experienced by the farm was also noticed. The highest nematode population recorded in 100 ml soil was on

the cultivar citron (1481 ± 40J2), which had minor symptoms of wilting on the first two layers of its petals. Population density of *M. hapla* was not statistically significant (P > 0.05) among Dreamland, Moon-Walk and Opus cultivars. On the other hand, the lowest population recorded was from cultivar Olympia (668 ± 62 J2), which showed intense leaf discoloration compared to the other cultivars.

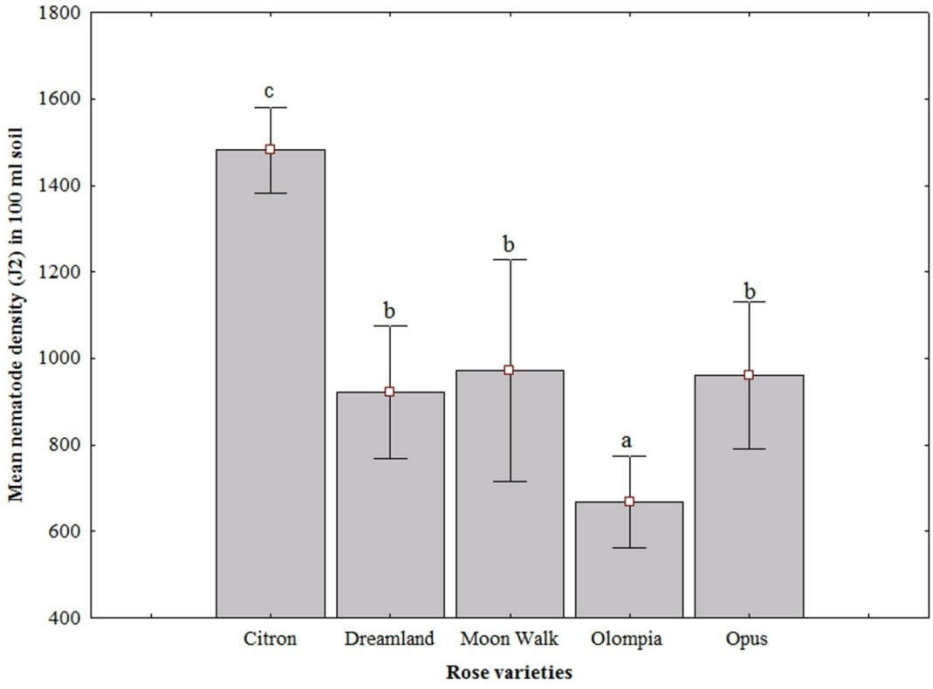

Figure 2. *Meloidogyne hapla* population density in 100 ml soil on different Rose cultivars

Hypericum (*Hypericum spp.*)

All cultivars of Hypericum were infected by *M. javanica* (Fig3). However, the infection level significantly differed (*P*<0.05) among cultivars. The highest mean population density recorded with 5020 ± 141 J2 in 100 ml soil was on the cultivar Calypso. This cultivar had visible damage symptoms including leaf discoloration, stunted and patchy growth stand and higher number of small root galls with abnormal root architecture (Fig 4). The cultivar Rio was the second heavily infested, with a mean nematode density of 1493 ± 491 J2 that had similar several patchy spots and stunted growth. Bamboo and Diablo cultivars had no visible above ground damage symptoms, although the mean nematode density recovered was still high, 467± 50 and 413 ± 144J2, respectively. RioB supported a 120 ± 20 J2 in a 100 ml soil with better growth performance than the other cultivars grown in the greenhouse.

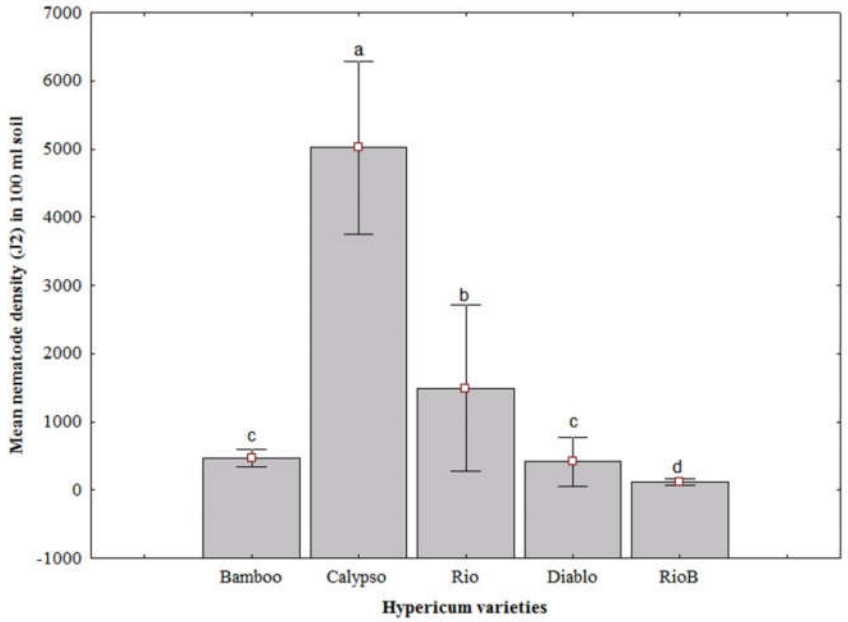

Figure 3. *Meloidogyne javanica* population density in 100 ml soil on different cultivars of *Hypericum*

Figure 4. Root damage symptom caused by *Meloidogyne javanica* on hypericum cultivar Calypso

Snap bean (*Phaseolus vulgaris*)

Cultivars of Snap bean significantly varied in the mean population density of *M. incognita* recorded (Fig. 5). "Faraday" was the most affected cultivar in which its roots had to be extremely galled (Fig 6). The nematode density recovered in

100 ml was such high (6485 ±922 J2), that this cultivar moreover had typical wavy crop stand in the field and plants were extremely short branched with abnormal leaves. The mean nematode density found on Bobby bean was 4130 ± 155 J2. However, its root only had slight galling though patchy growth was typical in the field (Fig. 7). The other two cultivars (PeaA4 and PeaB5) were less affected by this nematode with hardly visible symptoms on both above and below ground parts.

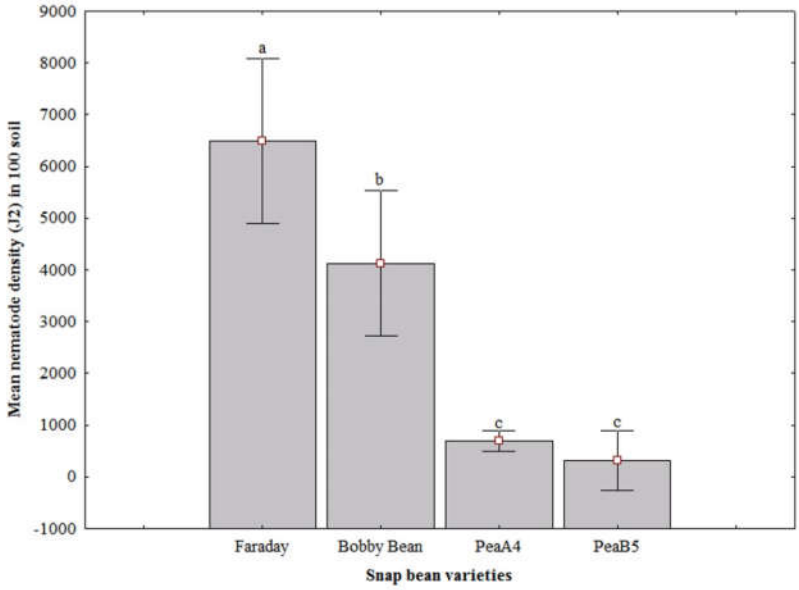

Figure 5. *Meloidogyne incognita* population density in 100 ml soil on different cultivars of Snap bean

Figure 6. Heavily galled roots of Faraday cultivar caused by *Meloidogyne incognita* infestation

Figure 7. Patch growth of Bobby bean cultivar caused by *Meloidogyne incognita* infestation

Enset *(Enset ventricosum)*

Population density of root knot nematode significantly (P< 0.05) varied among Enset clones (Fig. 8). The highest density recorded in 100 ml soil was from Wagu and Astera, 490 and 385 J2, respectively. These two clones, however, showed no nematode damage symptoms and had better growth performance as compared to the other clones with less nematode density. There was no significant difference in nematode density among Nobo, Astera and Fia clones. However, Fia (306 J2 100 ml^{-1} soil) had poor growth performance with occasional wilting particularly on young leaves. Regardless, of the lower nematode density, Getema also appeared to be stunted similar to Fia unlike Bereda which had better growth performance of all with no observable nematode damage symptom.

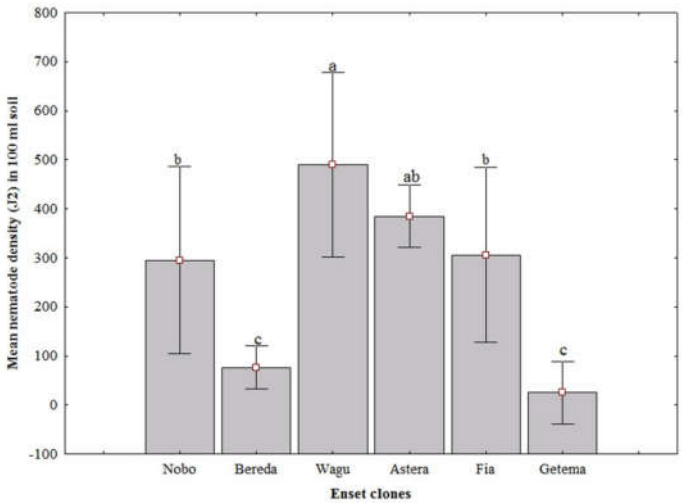

Figure 8. *Meloidogyne spp.* population density in 100 ml soil on different clones of Enset

Snap bean and Enset

Plant-parasitic nematodes other than *Meloidogyne* spp. were recorded from both Snap bean and Enset cultivars/clones (Table 2). Each cultivar differed in both the type of nematode genus and population density. A total of five nematode genera were recovered from Snap bean cultivars. Faraday hosted all the genera viz. *Helicotylenchus*, *Pratylenchus*, *Rotylenchulus*, *Scutellonema* and *Tylenchorhynchus* identified from all across the snap bean cultivars. The highest nematode density (1283.3 ± 402) was *Rotylenchulus* followed by *Pratylenchus* (617.3 ± 351). Bobby bean hosted three genera of which *Tylenchorhynchus* was found at a density of (374.3 ± 130) followed by *Scutellonema* and *Pratylenchus*. The population density of *Pratylenchus* (96.7 ± 38) from PeaA4 and *Tylenchorhynchus* (46.7 ± 12) from PeaB5 were relatively lower compared to other Snap bean cultivars. Except *Pratylenchus* (on Faraday) and *Tylenchorhynchus* (on Bobby bean) with a frequency of occurrence (67%), the rest nematodes occurred in all samples (FO= 100%) of their respective cultivars. *Rotylenchulus* appeared to be the most important on Faraday followed by *Pratylenchus* with a prominent value of 1283 and 381, respectively.

Apart from the root knot nematode, stated above, a total of eight plant parasitic nematode genera were recorded to be associated with Enset clones. The frequency of occurrence, mean population density and prominence values of each nematode genus for each cultivar/clone is shown (Table 2). Clone Wagu hosted five of the nematode genera (*Mesocriconema, Hoplolaimus*, *Pratylenchus*, *Tylenchorhynchus* and *Trichodorus*) while Nobo, and Fia hosted four genera each. *Trichodorus* was the most frequently encountered genus on five of the clones followed by *Radopholus* that was recorded from four clones. *Tylenchorhynchus, Trophurus* and *Mesocriconema* were detected in three clones. *Hoplolaimus* was detected from Nobo and Wagu clones, but *Pratylenchus* was only from Wagu. *Scutellonema* was only recovered from Fia. Based on abundance, *Tylenchorhynchus* was the most abundant genus on Nobo and Bereda with 720 ± 28.3 and 470 ± 39.4 nematode per 100 ml^{-1} soil, respectively, followed by *Radopholus* (460 ± 57.8) and *Trophurus* (258 ± 31) on Nobo and Getema, respectively.

Table 2. Frequency of occurrence and abundance in 100 ml^{-1} ml soil and the prominence value of major nematode genera from the soil rhizosphere of Snap bean cultivars and Enset clones.

Crop Type	Cultivars/clones	Nematode genus	PD 100 ml^{-1} soil*	FO (%)**	PV***
Snap beans	Faradays	*Helicotylenchus*	51.7 ± 28	100	50
		Pratylenchus	617.3 ± 351	67	381
		Rotylenchulus	1283.3 ± 402	100	1283
		Scutellonema	163.3 ± 21	100	160
		Tylenchorhynchus	100 ± 26	100	100
	Bobby Bean	*Tylenchorhynchus*	374.3 ± 130	67	163
		Scutellonema	170 ± 20	100	170
		Pratylenchus	133.3 ± 42	100	133
	PeaA4	*Pratylenchus*	96.7 ± 38	100	97
	PeaB5	*Tylenchorhynchus*	46.7 ± 12	100	47
Ensete	Nobo	*Mesocriconema*	25 ± 7.1	67	16
		Hoplolaimus	40 ± 14.1	67	33
		Radopholus	460 ± 57.8	67	376
		Tylenchorhynchus	720 ± 28.3	67	588
	Wagu	*Mesocriconema*	44 ± 5.7	67	33
		Hoplolaimus	61 ± 29.7	67	33
		Pratylenchus	154 ± 79.2	67	171

	Trichodorus	73.3 ± 15.7	100	73
	Tylenchorhynchus	36 ± 7.8	67	24
Astera	*Mesocriconema*	46 ± 8.5	67	33
	Trichodorus	47 ± 9.1	67	33
	Trophurus	98 ± 31	67	98
Getema	*Trophurus*	258 ± 31	67	229
	Radopholus	39 ± 12.7	67	24
	Trichodorus	63 ± 9.8	67	57
Fia	*Radopholus*	35 ± 6.4	67	24
	Scutellonema	26 ± 8.4	67	16
	Trichodorus	48 ± 11.3	67	33
	Trophurus	115 ± 35.4	100	115
Bereda	*Radopholus*	135 ± 28.6	100	135
	Trichodorus	130 ± 14.2	100	130
	Tylenchorhynchus	470 ± 39.4	67	384

*Population density (PD)= mean number of individual nematodes genus per 100 ml soil (n=3).**Frequency of occurrence (FO%): number of sites with positive detected/total number of sites sampled*100***Prominence Value (PV) = PD *$(FO)^{1/2}$ *10^{-1}.

DISCUSSION

Cultivars/clones names used in this article are based on the information specified by the farm manager of the respective farms. In Ethiopia, almost all commercial growers grow crops directly on the soil, which exposes the plant to infections by various groups of plant parasitic nematodes. The northern root knot nematode, *Meloidogyne hapla* was first reported infecting commercial cut-rose flowers in *Ziway*, *Debrezeit*, *Sebeta* and *Holeta* (Meressa *et al.,* 2014). This species has been revealed elsewhere to be associated with severe infection of a wide range of rose cultivars (Pizetta *et al*.2010). Rose nurseries of Dutch commercial Rose cultures were often challenged by *M. hapla*, which made it difficult in Rose production under hydroponic culture (Garcia and Amsing, 2005). Application of synthetic chemicals and rotating the field with other crops was previously considered effective to reduce the nematode population present before Rose planting (Wolny, 2002). However, with the removal of most effective nematicide for reasons associated with environmental and human health; the less probability of rotation in such commercial monocroping based, these methods are unlikely applicable. Wang *et al.* (1999) evaluated Rose rootstock cultivars of *Rosa multiflora* and *R. indica* and found substantial resistant characters on two cultivars of the former rose species to *M. hapla*. Based on root gall severity, host suitability of 32 rootstocks was tested that resulted in difference in root galling ranged from gall free to heavily galled roots (Voisin *et al.,* 1996), which is not in fact a suitable indicator of resistance character. However, no Rose species resistance for the nematode is commercially available. On the other hand, the fact that resistant cultivars exist within the pool (Ohkawa and Saigusa, 1981), could give opportunities to harness the potential for searching rootstocks that could be commercialized. This could be explained by the presence of a significant variation in the nematode population among rose cultivars grown under a conditioned greenhouse in our survey. Genes that confer resistance to root knot nematodes were shown to lose their effectiveness at a soil temperature above

28^{o}C (Hu *et al.,* 2015). Ziway, the sampling area, however, possesses a mean annual temperature of about 35^{o}C that a cultivar with better growth performance under the population pressure recorded can be used for further screening by inoculating with several ranges of *M. hapla* population.

Records of *Meloidogyne* spp. association with *Hypericum* spp. are not available. In New Zealand, *M. hapla* was previously reported from these plants (Knight *et al.,* 1997). The report of Marais and Swar (2002) from South Africa showed no host-nematode interactions between *M. javanica* on *Hypericum perforatum*. . In this survey, we revealed the severity of infection based on the ability of the cultivar to support the reproduction of the nematode (Esfahani and Ahmadi, 2010). The damage threshold of this nematode to Hypericum plants is not known. However, the fact that cultivar Calypso was still giving yield at the highest nematode pressure might indicate tolerance character of this cultivar. On the other hand, cultivar RioB growing at its optimum performance while hosting lower nematode population might be ascribed to the presence of resistance character to *M. javanica*. The association of *Hypericum* cultivars with *M. javanica* is new record for Ethiopia.

Our results demonstrated that Snap bean cultivars were damaged by various plant-parasitic nematodes where in severity varied between cultivars. Planting beans when the soil temperature is less than 20^{o}C was suggested to minimize yield loss caused by root knot nematodes (Korayem *et al.,* 2015). However, for this most tropical species (*M. incognita*), the farm is situated in the Great Rift Valley where warmer soil temperature is experienced, that makes manipulation of planting date infeasible. In addition, being a monocroping based cultivation; the reproduction rate of this nematode in this area should be higher to build large population density per cropping season. The use of resistant cultivars is the best option to limit the damage thereby reduce the economic loss caused (Singh and Schwartz, 2011; Ferreira *et al.,* 2012).Yet, no consideration for resistance cultivars is available under such commercial production.

Bogale et al. (2004) found a greater difference in nematode population density among Enset clones in particular to the root lesion nematode, *Pratylenchus goodeyi*. Though this species is well known pest of the crop in Ethiopia (Peregrine and Bridge, 1992), our result revels no detection of *Pratylenchus* spp. Moreover, Mandefro and Dagne (2000) reported *M. incognita, M. javanica* and *M. ethiopica* from Enset in Ethiopia that occurred at low densities. On the other hand, difference in nematode abundance of root knot nematode may indicate the presence of inherent difference in susceptibility among each other. As resistance for nematode is one of the promising strategies to keep damage to a minimum (Speijer and De Waele, 1997), a project on collecting and screening of 85 available Enset clones to various densities of specific nematode species of *Meloidogyne* has been recently started. Moreover, a serious bacterial wilt that is common on most Enset growing areas is considered to have an interrelationship particularly with any of the endogenous parasitic nematodes.

The results obtained from this study show that the prevalence and damages implicated by plant parasitic nematodes may not be only dependant on the density of the nematode species but also

on the cultivar or clone used as a planting material. The fact that various cultivars/clones supported different population densities of the different nematode genera identified highlights that these could be plausible alternatives to tackle the damage involved by these nematodes. To this effect, large scale screening of these available cultivars/clones to a varying population density of plant parasitic nematodes is crucial. Furthermore, this finding could also provide an opportunity to further explore available potentials for management of the plant parasitic nematodes such as tolerance or resistance features.

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