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Plant Parasitic Nematodes Associated With Coffee in Kenya and Factors Influencing their Occurrence, Abundance and Diversity

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Abstract

Frequent detection of galls on coffee roots has raised concerns of nematodes in coffee production systems in Kenya. This study aimed at determining the occurrence of nematodes associated with coffee in Kenya and the role of crop management, cultivars, soil properties and agro ecological zones on the abundance and frequency of nematodes. A survey was conducted in the prime coffee growing areas in 10 counties namely; Machakos, Makueni, Kiambu, Embu, Kirinyanga, Nyeri, Meru, Kisii, Nandi and Trans-Nzoia. Nematodes were extracted using a combination of centrifugal floatation and Modified Baermann techniques and identified to genera level. Nutrient analysis was carried out using the Double Mehlich method. Results showed that nematodes belonging to 30 genera were recovered from coffee agro-ecosystems. Plant parasitic nematodes were the most prevalent with 64% frequency (19 genera) of occurrence followed by bacterial feeders at 24%. The genus *Tylenchulus*, *Meloidogyne* and *Pratylenchus* were the most dominant across all the coffee growing areas. Coffee farms in the coffee-tea zones (Upper Midland 1) harbored the highest numbers of plant parasitic nematodes, followed by Upper Midland 2 and least in the marginal coffee growing zones (Upper Midland 3). Well managed farms had less plant parasitic nematodes compared to neglected farms. K and P significantly contributed to the variation in the nematode community composition. This study demonstrated the prevalence of plant parasitic nematodes, factors that influence their abundance and distribution and justifies need for further management of nematodes in coffee production.
Key words: Abundance, agro-ecologial zones, diversity, nematode genera, nutrients

Introduction

Coffee (*Coffea arabica* L.) is among the leading commodity crops for many developing countries, contributing over US$ 10-11 billion annually (Alpizar *et al.,* 2007). Commercial coffee production is mainly on large plantations/ coffee estates (accounting for 40% of total output) and small-holder farms averaging less than 0.5 ha. Although the occurrence of nematodes in coffee production systems in Kenya has been observed in the past, they have often been ignored or misdiagnosed due to their microscopic size and symptoms confounded by either malnutrition and root infections by other pests (Castillo *et al.,* 2009). Plant parasitic nematodes are considered to be major pathogens of coffee worldwide, causing yield losses estimated at 15%, approximated at 20 million 60-kilogram bags in 2007-2008 (AEO, 2007).

Soil texture has been shown to play a vital role in nematode activities with reports indicating that sandy soils are most favourable (Bertrand *et al.,* 2001). In addition, poor agronomic practices have led to depletion of organic matter thus aggravating damage caused by *M. exigua* on coffee (Etienne *et al.,* 2000). Nematodes account for much of the losses in coffee with transplanted seedlings less than five years old drying up while mature coffee suffers leaf drop/defoliation and yields decline (Luc *et al.,* 2005). Nematode problem in coffee growing areas is amplified due to a complex in the pathogenesis, where at least two genera of fungi are present, (*Fusarium* spp. and *Rhizoctonia solani*) which are pathogenic to coffee mainly during early nursery stages (Campos *et al.,* 1990). The endoparasitic nematodes, *Meloidogyne* and *Pratylenchus* predispose the plants to fungal infection besides causing physiological alterations in the tissues. The silent nature of nematodes and non specific disease above-ground symptoms at early stages of disease progress are perhaps the main reasons for the little attention given to nematodes in coffee (Khan, 2008).

Inadequate knowledge, poor diagnosis of nematode problems and lack of data on nematodes associated with coffee are major challenges to management of nematodes (Wintgens, 2009). Plant nutrition on the other hand, strengthens the plant response capacity enabling it to withstand effect of pathogens, in particular, Phosphorus (P) is essential for development of extensive root systems for nutrient uptake and Nitrogen (N) for vigorous growth

thus enabling plants to withstand damage by soil borne pathogens (Jones, 2003; Castillo *et al.*, 2009). This study was conducted with the aim of evaluating the occurrence and distribution of nematodes in the main coffee growing areas of Kenya.

Materials and methods

To assess the distribution of plant parasitic nematodes, a survey was undertaken in the three coffee agro-ecological zones, namely: coffee-tea zone which is the Upper Midlands 1(UM1); main coffee zone which is the Upper Midlands 2 (UM2) and the marginal coffee zone which is the Upper Midlands 3 (UM3). Anthropogenic factors were visually evaluated by ranking the crop management levels in coffee farms into high, medium, low and neglected farms. Alongside this, soil properties were determined at each sampling point. The survey was carried out during the short rain seasons of 2008 and 2009 and a checklist was used to collect the farm profile data namely farmer's name, factory where they process their coffee, date of tree establishment, gender, cultivar, agro ecological zone, altitude, farm size, crop management level and whether intercropped or not during the survey. Above-ground symptoms of pathogen infestation were also recorded during sampling. Counties sampled were ten namely; Machakos, Makueni, Kiambu, Embu, Kirinyanga, Nyeri, Meru, Kisii, Nandi and Trans-Nzoia. Two hundred composite soil samples (20 samples per district) were collected in duplicates using an auger and buckets at a depth of 5-15cm where the feeder roots of coffee are predominant.

The unit of sampling was the individual coffee farm and the available sampling units or sampling frame was the coffee growing areas. Stratified sampling which entailed drawing a random-type sample separately from each stratum was applied to obtain composite samples. A minimum of six sampling points, per farm laid out along a zigzag pattern were done. Soil collected was composited, put in a bag and kept in a cool box awaiting transit to the laboratory.

Observable symptoms of nematode infestation were recorded during the sampling. In the laboratory, nematodes were extracted using a combination of centrifugal floatation and the Modified Baermann Techniques (MBT) as described by Hooper *et al.* (2005). The nematodes were fixed, mounted on slides and identified to genus level. Nutrient status of the soil samples collected from the field was determined at Coffee Research Station's Chemistry laboratory using the Double Mehlich method (Tisdale, *et al.*, 1997).

The extracted nematodes were killed using solutions of 5-10% formalin plus 2% glycerol under fume chamber to avoid toxic fumes. $CaCO_3$ powder was added to the stock solution to neutralize the free formic acid that cause darkening and granulation of tissues. Identification was based on differences in morphological characters using an identification key for plant nematodes genera. The important morphological features were readily seen in freshly killed/fixed specimens while the numbers were determined by using a 1ml counting slide with suitable grid alongside a tally counter with the aid of stereoscopic microscope with magnifications of 10x and 40x. For verification, nematodes were mounted on thin microscopic Cobbs slides using 19mm diameter round cover slips to allow examination from either side and identified to genera level.

Nematode and soil parameters data collected was log transformed (Log(x+1)) and subjected to analysis of variance (ANOVA) using Genstat computer software package (Lawes Agricultural Trust Rothamsted Experimental Station 2006, version 9). Means, when significantly different, were separated using the Fisher' protected LSD test at 5% probability level. Nematode community structure was initially analyzed using the unconstrained Principal Component Analysis (PCA). The relationship between soil environmental and nematode species relationship was then analyzed using Redundancy Analysis (RDA), the constrained multivariate linear response method (Te Braakand and Verdonschot, 1995). All nematode genera were then assigned to trophic guilds according to Bongers and Bongers (1998). The environmental dataset was checked for exceptional observations that could be outliers.

Results

Nematodes belonging to thirty (30) genera were found to be associated with coffee (Table 1). Among these, 19 genera are known to contain plant parasitic nematodes. Nematodes classified as plant parasitic dominated the trophic guild accounting for 64% followed by bacteria feeding nematodes (24%). Among the plant parasitic

group, nematodes belonging to the genera *Tylenchulus*, *Meloidogyne* spp. and *Pratylenchus* spp. were the most dominant across all the coffee growing areas investigated.

Table 1: Mean population densities of nematodes associated with coffee in Kenya

Genera	Rank Abundance	Abundance J2counts/200cc soil	Trophic group
Tylenchulus	1	166.6	Plant parasitic
Meloidogyne	2	161.4	Plant parasitic
Tylenchorhynchus	3	159.5	Plant parasitic
Pratylenchus	4	158.0	Plant parasitic
Tylenchus	5	156.4	Plant parasitic
Rotylenchus	6	147.7	Plant parasitic
Hemicyclophora	7	138.5	Plant parasitic
Ucephalobus	8	138.5	Bacterial feeder
Acrobeles	9	138.3	Bacterial feeder
Mononchus	10	136.7	Predator
Rhabditis	11	135.8	Bacterial feeder
Hoplolaimus	12	134.0	Plant parasitic
Aphelenchus	13	132.9	Fungal feeder
Chromadora	14	132.6	Bacterial feeder
Scutellonema	15	130.1	Plant parasitic
Prodorylaimus	16	127.8	Predator
Helicotylenchus	17	125.0	Plant parasitic
Hemicriconema	18	115.8	Plant parasitic
Iotonchus	19	114.5	Predator
Xiphinema	20	113.9	Plant parasitic
Criconema	21	109.5	Plant parasitic
Cephalobus	22	105.7	Bacterial feeder
Aphelenchoides	23	103.5	Plant parasitic
Plectus	24	101.4	Bacterial feeder
Bunonema	25	93.0	Plant parasitic
Trichodorus	26	90.8	Plant parasitic
Paratrichodorus	27	90.4	Plant parasitic
Radopholus	28	89.8	Plant parasitic
Longidorus	29	87.3	Plant parasitic
Alaimus	30	61.1	Bacterial feeder

The abundance of plant parasitic nematodes associated with coffee was variable ($P \leq 0.05$) among the three coffee growing zones (Figure 1). Amongst the regions, coffee farms in the coffee-tea zones (UM1) harbored the highest numbers of plant parasitic nematodes, followed by main coffee zones (UM2) and least in the marginal coffee growing zones (UM3).

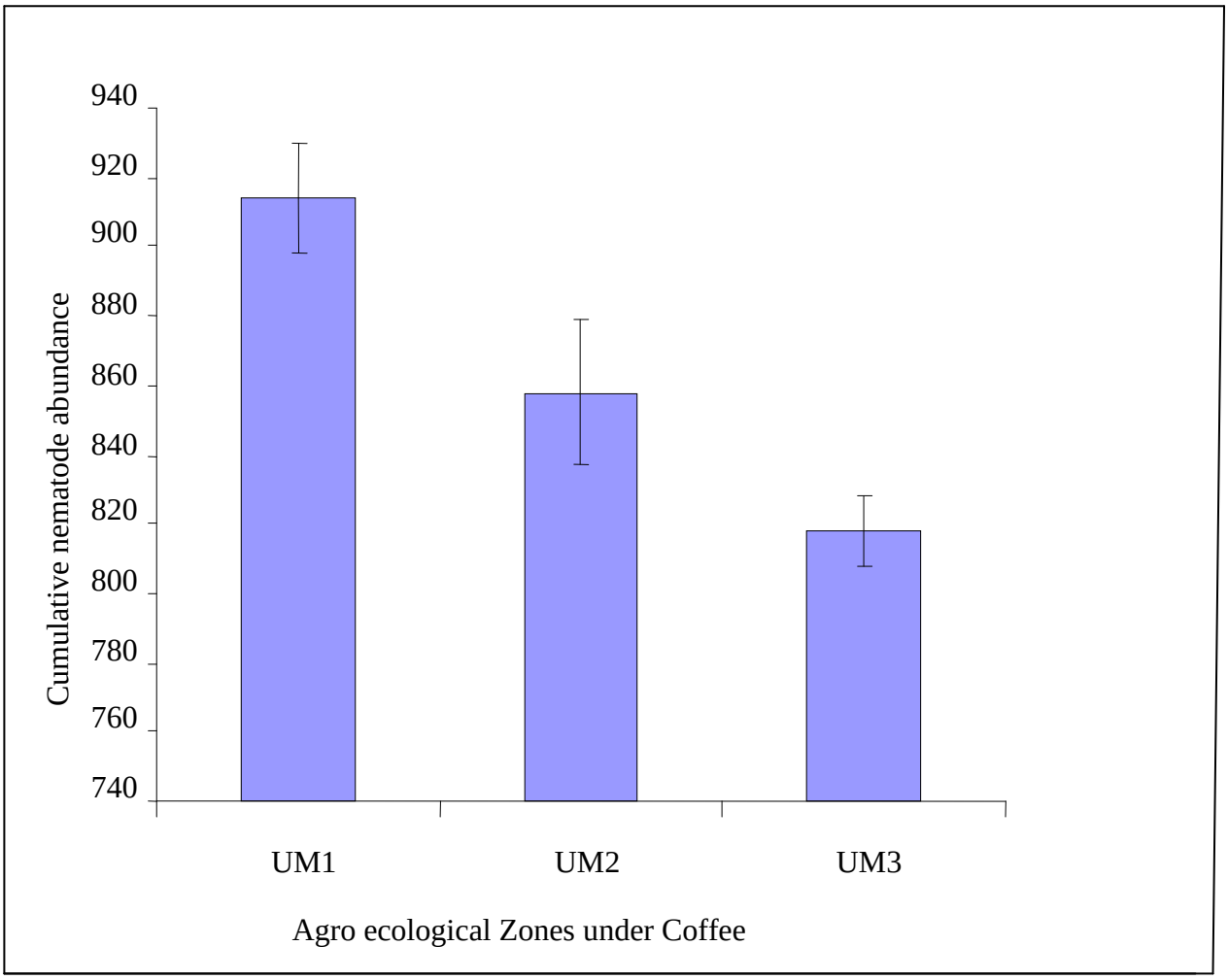

Figure 1: Cumulative abundance of nematodes in the three of agro ecological zon**es**

KEY: UM1 -Upper Midland zone 1 UM2 -Upper Midland zone 2
UM3 -Upper Midland zone 3

The lowest ranked nematode genus was *Alaimus* with an abundance of 61.1 juvenile counts/200cc soil. The results indicated that some of the nematode genera have higher abundance than others implying that genera are not evenly distributed across the sampling sites. The detection of nematode species increased with increase in the number of sampling sites (Fig. 2). As the curve indicates, nearly all possible nematode species were recovered in the 90th sample where the species accumulation curve starts to level out. Similarly, collecting at least 50% of the samples would have yielded more than 90% of the nematode species.

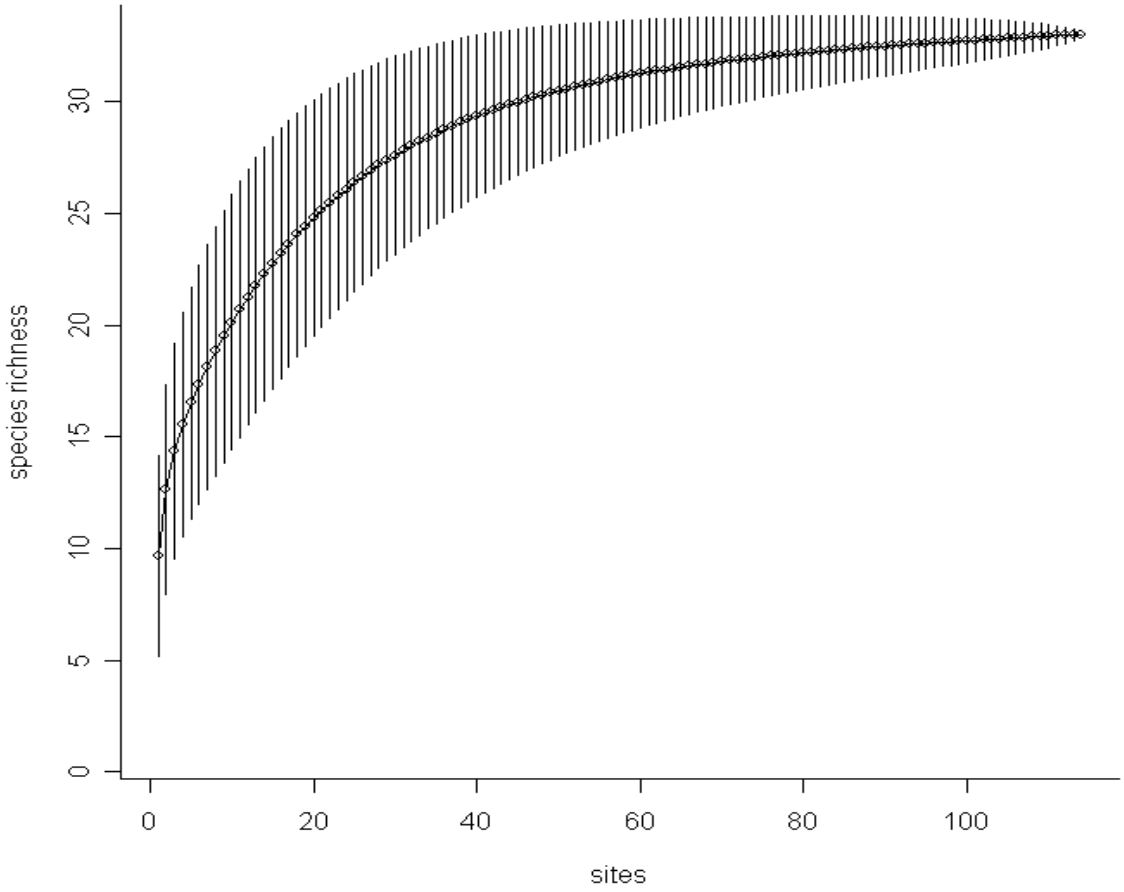

Figure 2: Species accumulation curve for nematodes in various districts of Kenya

The level of crop husbandry was found to significantly impact on the abundance of nematodes (Figure 3). Farms that were ranked to be highly managed recorded the least number of nematodes while those ranked as neglected had the highest number of plant parasitic nematodes. Among the soil chemical parameters analyzed, significant differences were observed in pH and K in the sub-soils (Table 2).

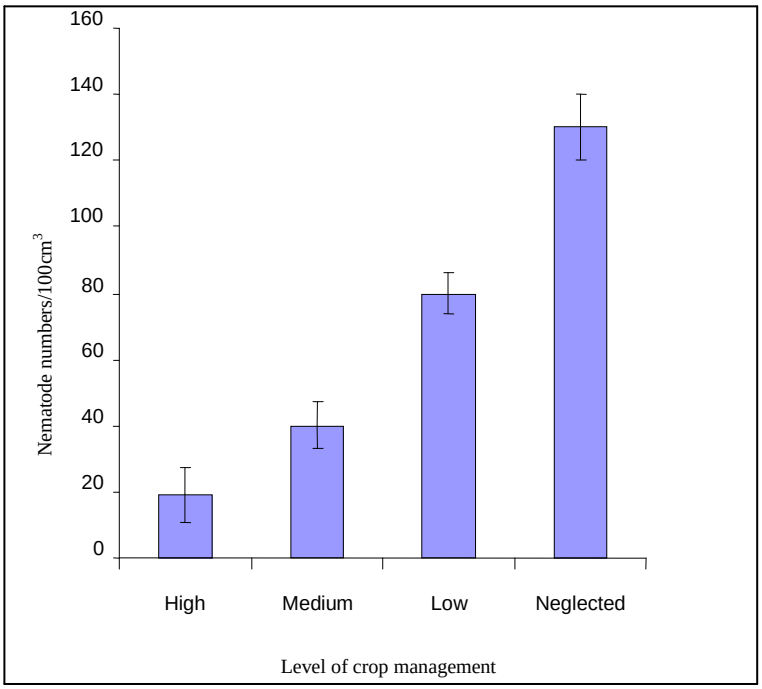

Figure 3**:** Influence of management level on the abundance of nematodes in coffee.

Table 2: Average physico-chemical properties of soils from coffee growing regions in Kenya

Ecological zone	K (me%)		P (ppm)		pH
	Top soil	Sub Soil	Top soil	Sub soil	
UM1	0.92	0.62	63.64	38.11	4.4
UM2	0.93	0.61	77.61	47.82	4.9
UM3	1.13	0.87	83.86	47.30	4.9
P (0.05)	0.16	0.02	0.62	0.73	<0.001

The combined soil pH had narrow range of 4.4-4.9 being slightly acidic to strongly acidic and was significantly variable ($P<0.05$) among the agro ecological zones. The soil pH in the coffee-tea zone (UM1) was most acidic compared to the main coffee zone (UM2) and the marginal coffee zones (UM3). Unlike topsoil, Potassium (K) levels in the subsoil were significantly variable. In top and subsoil, K levels were lowest in UM1 gently increasing to peak in marginal coffee zone (UM3). The K levels observed in this study were well within the prescribed range of 0.4 – 2 Me. The phosphorus (P) levels observed in this study though not influenced by agro ecological zonations, were within the optimal range of 20-100 ppm.

Multivariate analysis using the Monte-Carlo permutation test showed that among the three variables, only agro-ecological zonation and management levels significantly correlated with nematode community composition structure (Table 3). Most plant parasitic nematodes including *Meloidogyne*, *Helicotylenchus*, *Trichodorus*, *Longidorus* were better represented in the low and neglected coffee farms. Conversely, non-parasitic nematodes

among them, *Cephalobus*, *Mononchus*, *Rhabditis*, and *Acrobeles* were highly associated with increased levels of management in the coffee farms.

According to the Monte-Carlo permutation test for significance of variables, only four of the eight environmental variables namely Na, K Mn and P added sequentially significantly contributed to the variation on the nematode community composition (Table 3). The selected soil chemical parameters had variable (P<0.01) effect on various nematode genera. Nematodes belonging to the genera *Bunonema* and *Radopholus* were associated with increased Mn.

Table 3. Significance of environmental variables on nematode community composition

Environmental variable	Pr(>F)
Agro Ecological zone	<0.01 ***
Coffee variety	0.27
Level of management	0.01 **
pH	0.18
Na	<0.01***
K	0.04**
Ca	0.2
Mg	0.55
Mn	<0.01***
P	0.02**
Ca+Mg)(K)	0.87

***Highly significant ** Significant

Based on 100 permutations under direct model with terms added sequentially (first to last)

Discussion

Nineteen genera of plant parasitic nematodes were found to be associated with coffee in Kenya. Among the plant parasitic group, nematodes belonging to the genera *Tylenchulus* spp., *Meloidogyne*, and *Pratylenchus* spp were the most prominent plant parasitic genera across all the coffee growing areas investigated. The survey findings agree with other studies conducted on nematodes attacking coffee elsewhere in major coffee producing countries. These nematodes include very damaging species that cause great losses to the coffee growers and the local economy of developing countries (Campos *et al.*, 1990; Nguyen *et al.*, 2009). The most important nematodes reported in coffee shrubs are nematodes in the genus *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, and *Pratylenchus (*Luc *et al., 2005*, Bressan, 2008, Castillo *et al.*, 2009, De'Souza and Bressan 2008; Nguyen *et al.*, 2009). Others like *Tylenchulus*, *Tylenchorhynchus*, Tylenchus and *Hemicyclophora* spp. are among the most abundant nematodes reported in coffee but they are not very destructive (Campos *et al.*, 1990; Castillo *et al.*, 2009).

Farms that were highly managed recorded the least number of nematodes while those ranked as neglected had the highest number of plant parasitic nematodes. Generally, highly managed farms were dominated by bacteria feeders, whereas, in the neglected farms, plant parasitic nematodes or herbivores, were more dominant. Highly managed farms had a history of regular use of manure and weeding. In this study, the pH of the soil samples ranged from 4.4-4.9. This agrees with findings by De'Souza & Bressan (2008) and Nguyen *et al.*, (2009).

Factors found to influence nematode abundance and distribution in the survey concur with the documented factors that account for variation in disparities by zones in nematode abundance such as rainfall, soil properties,

cultivar, temperature and other soil conditions (Kandji *et al.,* 2001; Ogol and Abredit (2001); Campos and Villain, 2005). Nematode population density and distribution in the soil has been found to be influenced by many factors such as initial population density, plant species, crop management practices, soil structure and environmental conditions (De'Souza and Bressan 2008). Studies have further shown that areas where climate is characterized by prolonged and severe hot dry season may result to reduction of plant parasitic nematodes (Hussey, 1996) consistent with findings in UM3. It has also been shown that nematodes are abundant in well drained soils but wet with adequate oxygen levels as they are suitable for coffee growing than dry or flooded soils (Huang and Cares, 1995).

In this study, the pH was well within the range tolerable by the plant nematodes and reports state that most agro ecosystems tend to fall within a range of 4-8 (Nyasani *et al.,* 2008) thus coffee zones are suitable habitat for nematode reproduction. The effects of pH on nematode behavior have been examined in several species (Gaugler and Bilgrami, 2004) and the range of 4.5-6.0 was found to be quite tolerable by entomopathogenic nematodes (EPNs) (Nyasani *et al*., 2008). Other findings indicate that extreme pH is detrimental to soil nematodes (Thomas *et al.,* 2005**;** Campos and Villain, 2005; Nyasani, *et al.,* 2008).

This study confirmed that management practices and ecological conditions influence the presence and abundance of nematodes (Kandji *et al.,* 2001). This corroborates a study on bananas, where results showed that certain weeds act as reservoirs of plant pathogenic nematodes such as *R. similis* and *Meloidogyne* spp (Coyne, 2002). Such findings were found to be useful in devising appropriate nematode control measures prior to field establishment with nematode free planting materials especially coffee seedlings (Queneherve *et al.,* 2006). The absence of weeding and inadequate soil fertility in the neglected farms is particularly to blame for the high number of nematodes given that weeds offer alternative food sources for the pests (Kimenju, 1998; Castillo *et al.,* 2009). Past studies conducted to investigate the effects of different tillage practices on the nematode community structure showed that tillage changes the nematode composition by trophic group and life strategy (Lenz and Eisenbeis, 2000). For instance, the density of plant parasitic nematodes decreased while the density as well as dominance of bacterivorous nematodes increased in the highly managed farms (Queneherve *et al.,* 2006).

The high levels of K observed in UM3 may be due to higher rates of mineralization occasioned by the high temperatures associated with this agro ecological zone. Active application of organic and inorganic fertilizers may explain the higher levels of K in the topsoil compared to the subsoil and consequently the fewer nematodes found in UM3 since potassium has been reported to reduce nematode infestation (Carvalho *et al.,* 2008). The presence of plant pathogenic nematodes in UM3, a hot zone could also be attributed to their tolerance to environmental stress and survival through 'anhydrobiosis' when soil dries up in dry season (Kimenju, 1998). It is also important to note that overtime, coffee farming system has been changing from the predominant mono-cropping to intercropping due to changes in socio-economic factors. This is expected to affect the below ground ecosystems including nematode populations.

Conclusions and recommendations

It can be concluded that nematodes do affect coffee in Kenya and the plant parasitic nematodes found in Kenyan coffee comprise at least 19 genera, but eleven of these are beneficial nematode species associated with soil fertility. The most damaging and destructive nematodes such as *Meloidogyne* spp and *Pratylenchus* spp were among those present. The study also revealed that agro-ecological zones, soil chemical properties and management levels determine the abundance of nematodes and distribution of species. Arabica coffee cultivar, the only species grown in Kenya, is susceptible and hence did not influence the distribution of nematodes. The study showed that nematode problem exist in coffee farms in Kenya and there is need for management of the problem to curb the losses associated with the diseases caused by nematodes. In this study, it was apparent that many genera and species of nematodes occurred in association with coffee, including potentially damaging nematodes of significance to crop production in Kenya.

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References

Alpzar, E., Etienne, H and Bertrand, B. (2007). Intermediate resistance to *Meloidogyne exigua* root knot nematode in *Coffea arabica.* Crop Protection 26 (7):903-910.

African Economic Outlook (AEO), (2007). http://www.oecd.org/dataoecd/26/34/38562812.pdf

Bertrand, B., Anthony, F. and Lashermes, P. (2001). Breeding for resistance to *Meloidogyne exigua* of *Coffea arabica* by introgression of resistance genes of *C. canephora*. Plant Pathology 50: 637-643.

Campos , V.P., Sivaplan, P. and Gnanapragasam, N. C (1990). Namatode parasites of coffee, cocoa and tea. In Plant parasitic nematodes in sub tropical and tropical agriculture (Eds). Luc, M, Sikora, R.A. and Bridge, J. (Eds.). CAB International. Wallingford, Uk. 387-430.

Carvalho, M.P., Rodrigues, F.A., Siveira, P.R., Andrade, C.C., Baron, J.CP., Paye, H.S.A and Louriro, J.E. (2008). Rice resistance to brown spot mediated by Nitrogen and Potassium. Nutrition journal 158(3)160-166.

Castillo, G., Wintgens, J N. and Kimenju, J.W. (2009). Nematodes of coffee (in) Coffee: growing, Processing, Sustainable production, A Guidebook for Growers, Processors, Traders and Researchers 2nd Ed. edited by Wintgens J.N. pp 478-494. ISBN: 978.527-32286-2.

Coyne, D.L., Sahrawat K. L. and Plowright R. A. (2004). The influence of mineral fertilizer application and plant nutrition on plant-parasitic nematodes in upland and Lowland rice in Cote D'Voire and its implications in long term Agricultural Research trials. Experimental Agriculture, 40, pp 245-256.doi: 10.1017/S00144797031595.

De'Souza, M. R. (2008). Plant parasitic nematodes of coffee, Springler, 313pp

Etienne, H., Anthony, F., Dussert, S., Fernandez, D., Lashermes, P. and Bertrand, B.(2002). Biotechnological applications for the improvement of coffee (*Coffea arabica* L.) In Vitro Cellular & Developmental Biology. Plant, 38(2) 129-138.

Gaugler and Bilgrami (2004). Nematode behavior. Wallingford. ISBN 0-85199-818-6. CABI Publishing. 419p

Hooper, D.J., Hallman, J. and Subbolin, S. (2005). Methods of extracting and processing of plant and soil nematodes in Plant parasitic nematodes in subtropical and Tropical Agriculture. 2nd Edition edited by Luc M., Sikora R.A & Bridge J. (2005). CAB international, Institute of Parasitology. UK ISBN 0-85198-630-7, pp 45-68.

Huang S. P. and Cares J. H. (1995). Community Composition of Plant-parasitic Nematodes in Native and Cultivated Cerrados of Central Brazil. Journal of Nematology 27(2):237-243.© The Society of Nematologists.

Hussey, R.S. (1996). Nematode pathogenesis and resistance in plants. Plant cell 8: 755-741.

Jones, J.B, (2003). Agronomic handbook: management of crops, soils and their fertility. CRC Press. ISBN 0-8493-08977-6.

Kandji, S.T., Serigne, T., Ogol, Callistus C. P. O., Albrecht, Alain (2001). Diversity of plant parasitic nematodes and their relationships with some soil physic-chemical characteristics in improved furrows in Western Kenya. Applied Soil Ecology, 18(2): 143-157.

Khan M.R. (2008). Current options for managing nematode pests of crops in India. Department of agricultural entomology, West Bengal, India. Http://www.articlebase.com/science-articles/current-options-for-managing-nematodes

Kimenju, J. K. (1998). Identification and biological control of lesion nematodes (Pratylenchus spp.) affecting maize production in Kenya. (Ph.D. Thesis). Department of Crop science, University of Nairobi. Nairobi. Kenya

Lenz R. and Eisenbeis G. (2000). Short-term effects of different tillage in a sustainable farming system on nematode community structure. Institute of Zoology, Johannes Gutenberg-University of Mainz, D-55099 Mainz, Germany. Springer Berlin / Heidelberg. Journal of Biology and Fertility of Soils 31(3-4): 237-244.

Luc M., Sikora R.A and Bridge J. (2005). Plant parasitic nematodes in subtropical and Tropical Agriculture. CAB international, Institute of Parasitology. UK ISBN 0-85198-630-7, pg 1-65, 387-430.

Nguyen, C. N., Nguyen H.C., and Moens, M. (2009). Plant parasitic nematodes assocated with coffee in Vietnam. Russian journal of Nematology, 17(1) 73-82.

Nyasani, J.O., Kimenju, J.W., Olubayo F.M., Shibairo S.I. and Mutua. G.K. (2008). Occurrence of Entomopathogenic Nematodes and their Potential in the Management of Diamondback Moth in Kale. Asian Journal of Plant Sciences. 7(3): 314-318.

Ogol, C. and Abredit, A. (2001). Diversity of plant parasitic nematodes and their relationship with some soil physic-chemical characteristics in improved furrows in western Kenya. Applied soil biology. 18(2)143-157.

Queneherve P., Cinabner C., Anwerkerken A., Topart P., Martiny B., MarieLuce S. (2006). Status of weeds as reservoirs of plant parasitic nematode in banana fields in Martinique. A recherché agronomique pour le development. Annuaire des siles du Cirad. Crop protection, 25(8) : 860-867

Thomas, S.H., Schroeder, J. and Murray, L.W.. (2005). The role of weeds in nematode management. Weed Science Society of America, 53: 923-928.

Tisdale, S.L., Nelson, W.L., Beaton, J.D. and Havlin, J.L. (1993). Soil fertility and fertilizers. Fifth edition. ISBN 0-02-42.835-3. U.S.A By Prentice-Hall, inc. upper Sandle River, New Jersey 07458. U.S.A.

Wintgens, J. N. (2009). Coffee: growing, processing, sustainable production. A Guidebook for growers, processors, traders and researchers. Second Revised Edition. Switzerland ISBN 978-3-527-32286-2.