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ABUNDANCE, DIVERSITY AND DISTRIBUTION OF SOIL NEMATODES IN KANGAITA AND WERU TEA CATCHMENTS OF KIRINYAGA AND THARAKA NITHI COUNTIES, KENYA

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ABSTRACT

Nematodes are the most abundant animals on earth and play essential roles in ecosystem functioning hence their abundance and diversity affect soil health. Nematodes have been reported in tea fields in some parts of Kenya and previous studies indicate that they may be a cause for the decline of tea population in some tea fields in Kenya. Nematodes of *Moloidogyne spp* have also been reported to be responsible for death of tea plants in nursery conditions. A survey was carried out in Weru and Kangaita tea factories catchment areas in Tharaka Nithi and Kirinyaga counties respectively. The survey aimed to determine the abundance and diversity of nematodes in small holder tea farms. Kangaita represented the high elevation site while Weru represented the low elevation site. Soil samples were collected from smallholder tea farms from which nematodes were extracted, identified based on their morphological characteristics, and classified according to their feeding habits then quantified using standard protocols. Nematodes from 23 *genera* were recovered in the two study sites representing all the five feeding groups: plant feeders, fungal feeders, bacterial feeders, omnivores, and predatory nematodes. Of the 23 genera, 11 were plant feeders, 6 bacterial feeders, 3 fungal feeders 2 omnivores and 1 predatory nematode. Kangaita, being a high elevation site reported higher population density in most *genera* reported than Weru which is a low elevation site. This is a departure from most studies that have reported higher nematode population densities in low altitudes. This can be attributed to differences in climatic and soil conditions in the two study sites in the same season. Kangaita was cooler with deep, well-ventilated, and loose soils while Weru was hotter with mostly compacted, shallow, and poorer soils in the tea farms. There is need for further research on the effect of elevation and farming practices on the distribution, abundance, and diversity of nematodes in tea fields.

Key words: Nematodes, abundance, soil health, tea fields, elevation, diversity, feeding group

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INTRODUCTION

Tea is an evergreen shrub from the genus Camellia. Among the camellia species, tea is the most grown and cultivated and most important commercially. Tea is processed to produce a stimulant brew. The brew from tea is classified as a major beverage worldwide, only second to water because of its health benefits [1]. In Kenya, tea is a major foreign exchange earner and supports approximately over 560,000 small holder farmers directly, large plantation farmers and others in the value chain [2]. Kenya is the leading tea exporter and among the leading tea producers in the world after China and India. Kirinyaga and Tharaka-Nithi counties are some of the major tea growing counties in Kenya in addition to Meru, Kericho, Bomet, Kisii, Nyamira, Embu among others [3]. Tea grown and processed in Kenya accesses mainly the export market while a small percentage is consumed locally [3, 4]. Tea plants are widely adaptable to different agro-ecological zones with variations in climate and physical features that affect growth, yield, and quality [5]. Tea is largely a mono crop that loses diversity. As such, after long seasons of tea cultivation such soils cannot support biodiversity. Farmers engage in various cultural practices in a bid to increase the productivity and profits of their tea fields. These practices include inorganic fertilizer (NPK 26.5.5) application, animal manure application and neglect of the farms with neither weeding, plucking nor fertilizer application. These practices affect the soil microorganisms which in turn affect nutrient availability and their eventual uptake by the plant and the productivity of the tea plant [6, 7, 8] and abundance of nematodes [9]. Nematodes are the most abundant animals on earth and the dominant component of soil [10,11]. Nematodes of the *genera Aphelenchus spp*., and *Pratylenchus spp*., have been reported to be widespread in the tea fields of Ngere tea factory catchments soils [12].

Nematodes have been reported to reduce the leaf area and stem girth of tea plants and cause tiny galls, for example the Root-knot nematodes, *Meloidogyne incognita* [13]. Meloidogyne javanica, M. incognita, have also been reported to affect tea in the nursery while *M. brevicaua* infests mature tea plants [13]. Infested tea bushes are stunted, chlorotic leaves though they may not die instantly. Plant parasitic nematodes on the other hand have been reported to lead to the decline in tea yields, and eventual death of tea bushes after pruning especially in high elevation areas [14]. The abundance and diversity of nematodes in soil is affected by the chemical, bio-physical and hydrological characteristics of soil [15] and elevation gradient [16]. There is limited information on nematode associated with tea and their distribution. Therefore, this study focused on the identification and diversity of nematodes in tea farms belonging to small holders within Weru and Kangaita catchment areas in Tharaka Nithi and Kirinyaga counties, respectively.

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MATERIALS AND METHOD

The study was carried out in Kangaita and Weru tea factory catchment small holder farms within Kirinyaga and Tharaka-Nithi counties, Kenya, respectively. Each factory catchment was zoned into three based on elevation (high LH0, medium LH1, and low UM1) as described by Jaetzold *et al.* [17]. Three farms were randomly selected in each of the three zones in each factory catchment within Kirinyaga and Tharaka Nithi counties. Kangaita tea catchment is in high elevation with most of the farms lying above 2,000 meters above sea level while Weru, in Tharaka-Nithi County is in low elevation of about 1,400 meters above sea level.

Random soil sampling was done in each farm under study. Five soil sub samples of approximately 200g each were collected in each farm from the surface to a depth of 45 cm using a soil auger. Nine farms were sampled per county making total sampled farms 18. The soil sub samples were mixed thoroughly to come up with a composite sample of 500g. The samples were transported to the laboratory in a cool box at 15^{o}C.

Centrifugal floatation technique as described by Jenkins [18] was employed to extract the nematodes. Initially, 200g soil sample was dissolved in 5 liters of water in a bucket and stirred to make homogenate slurry. The stirring was necessary to release the nematodes from the soil. The slurry was then passed through sieves of fine apertures; 250µm, 150 µm and 38 µm sieves. The solution collected from the 38 µm aperture sieve was backwashed and loaded into 50 ml falcon tubes. The solution in the tubes was centrifuged at 1700 rpm for 7 minutes. The supernatant obtained from the first spin was discarded and the pellet was topped up with sugar solution to balance at the 30ml mark. The sugar solution was prepared by dissolving 454g of sugar in 1 liter of water. This formed a sugar solution at 1.18 s.g. The contents of the falcon tubes topped up with the sugar solution underwent a second spin at 1700 rpm for 3 minutes. The supernatant formed was then passed over a 38µm sieve. The contents were then backwashed to make a 3 ml nematode suspension. This suspension was placed in tubes and the nematodes were fixed using formalin at 75^{o}C. Morphological features were used to identify the nematodes to genus level. This was done by observation through a compound microscope. The nematode numbers per *genera* were determined by counting. The nematode *genera* were assigned to trophic groups as described by Yeates *et al.* [19]

These nematodes were grouped into the five main trophic levels namely, herbivores/plant feeders, bacteriophores, fungivores, omnivores and predatory nematodes. The nematodes were assigned to the colonizer-persister value (C-P Value) based on a scale of 1-5 [20].

Where:
C-P 1s - are colonizers characterized by short generation time.
C-P 5 s -are persisters characterized by long generation time.

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The population densities of different nematode species in the samples were calculated using equations 1 and 2 below [21]

$$\mathrm{FR} = \frac{\text{Number of times individual nematodes occurred}}{\text{sample size}} \times 100 \quad (1)$$

FR-Frequency rating

$$\mathrm{N} = \frac{\text{Number of individuals of a species in a sample}}{\text{total of all individuals in a sample}} \times 100 \quad (2)$$

N-Nematode population

Statistical analysis

Statistical analysis was done using XLSTAT software.

RESULTS AND DISCUSSION

Nematodes from 20 families and 23 *genera* were recovered in the two study sites: Kangaita and Weru (Table1). The nematodes were grouped into the five main trophic levels namely Herbivores/Plant feeders (PF), Bacteriophores (BF), Fungivores FF), Omnivores (OM), and Predatory (PR) nematodes. Out of the 23 recovered nematode genera, eleven of them were plant parasitic nematodes (Plant Feeders). The plant parasitic nematodes identified were *Criconemella Spp., Filenchus Spp., Helicotylenchus Spp., Hemicyclophora Spp., Heterodera Spp., Longidorus Spp., Meloidogyne Spp., Pratylenchus Spp., Rotylenchus Spp., Tricodorus Spp.,* and *Tylenchus Spp*

Six *genera* of bacterial feeders were identified and these included *Alaimus, Cephalobus, Cervidellus, Eucephalobus, Prismatolaimus,* and *Wilsonema*. Three Fungal feeding nematode *genera* were identified including *Aphelenchus, Ditylenchus* and *Leptonchus*. Two *genera* of Omnivorous nematodes identified were Dorylaimus and Prodorylaimus. Only one genus of predatory nematodes was identified, and this was *Mononchus*.

From the survey, it was observed that the nematodes in the genus Meloidogyne and Rotylenchus are well distributed in the Kangaita and Weru, respectively (Tables 2 and 3). This is consistent with research carried earlier in India which concluded that root knot nematodes which are plant parasitic nematodes; *Meloidogyne javanica, Meloidogyne incognita* and *Meloidogyne brevican* are widely distributed in tea farms [22] Five trophic groups of nematodes were recovered in the study areas. These are herbivores, bacteriovores, fungivores, omnivores and predators. This is in line with previous research findings which have indicated that the five trophic levels are represented in almost every soil sample [12, 23].

Nematodes of Aphelenchus and Tylenchus spp. were widespread in the tea fields of both Kangaita and Weru, respectively. These findings agree with studies conducted previously in tea fields [12, 24]. The study also revealed that more nematode species are found in tea farms than those observed earlier in other studies. The current study revealed that 23 *genera* of nematodes in tea farms while previous studies [24] revealed

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16 *genera* of nematodes. This variation could be due to increased use of manure on tea fields or variations in climatic conditions.

In Kangaita, the most frequently occurring nematodes were Aphelenchus, Cephalobus, Criconemella, Eucephalobus, Filenchus, Helicotylencus, Leptonchus, and tylenchus at 100% frequency rating with a population of 1373, 1073, 887, 600, 320, 467, and 767 nematodes per 200g of soil respectively (Table 2). Meloidogyne had the highest population of 1527/200g of soil followed by Aphelenchus (1373/200g of soil) and Cephalobus (1073/200g). Cervidellus had the least population of 13/200g of soil. Fungal feeders were also frequently occurring in the tea fields. Nematodes of *Aphelenchus spp*., a fungal feeder, were widespread in the tea fields of both Kangaita and Weru with a frequency rating of 100% in both study areas. These findings agree with studies previously conducted in tea fields [12,24]. [12] noted that large numbers of fungal feeders like *Aphelenchus spp*, could be an indication of decomposition of substrates with high C: N ratios by fungi and colonization of the tea root zones with large number of fungi. Similar findings were also reported by Feng et al [24] and Koenning *et al.* [26].

In Weru, the most frequently occurring nematodes were aphelenchus, cephalobus, eucephalobus, meloidogyne, mononcus and tylenchus at 100% frequency rating with a population of 444, 774, 762, 822, 186 and 1338 nematodes/200g of soil respectively (Table 3). The least frequently occurring nematodes in Weru were hemicyclophora, heterodera, longidorus, prodorylaimus and Trichodorus at 33.3% frequency rating and population of 162, 174, 36 and 48 nematodes/200g of soil respectively. Rotylenchus had the highest population/200g of soil at 2628 with a frequency rating of 66.7% followed by Tylenchus and Meloidogyne in that order. Cervidellus had the least population of 18/200g of soil with a frequency rating of 44%.

Kangaita recorded higher nematode abundance in most *genera* recovered than in Weru. This is a departure from most studies that have reported higher nematode population densities in low altitudes [16]. This can be attributed to differences in climatic and soil conditions or crop diversity [27] in the two study sites in the same season. Kangaita was cooler with deep, well ventilated, and loose soils while Weru was hotter with mostly compacted, shallow, and poorer soils in the tea farms. This information is important considering the limited land resources and the need to cultivate tea in lowlands.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

The study demonstrated that the two sites, Kangaita and Weru catchments, harbors soil nematodes. A total of 23 *genera* were recovered in both catchments. The fields had a high occurrence of both plant parasitic nematodes and fungal feeders. More studies need to be done to determine the distribution of nematodes in different elevations within the same catchment area. There is also need for further research on the effect of elevation and farming practices on the distribution, abundance, and diversity of nematodes in tea fields.

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Table 1: Family, genera, C-P value, and trophic groups of nematodes recovered at Kangaita and Weru

Family	Genera	C-P Value	Trophic Group
Alaimidae	Alaimus	4	BF
Aphelenchidae	Aphelenchus	2	FF
Cephalobidae	Cephalobus	2	BF
Cephalobidae	Cervidellus	2	BF
Criconematidae	Criconemella	3	PF
Anguinidae	Ditylenchus	2	FF
Dorylaimidae	Dorylaimus	4	OM
Cephalobidae	Eucephalobus	2	BF
Tylenchidae	Filenchus	2	PF
Hoplolaimidae	Helicotylenchus	3	PF
Hemicyclophoridae	Hemicyclophora	3	PF
Heteroderidae	Heterodera	2	PF
Leptonchidae	Leptonchus	4	FF
Longidoridae	Longidorus	5	PF
Meloidogynidae	Meloidogyne	3	PF
Monochidae	Mononchus	4	PR
Pratylenchidae	Pratylenchus	3	PF
Prismatolaimidae	Prismatolaimus	3	BF
Bunonematidae	Prodorylaimus	5	OM
Hoplolaimidae	Rotylenchus	3	PF
Trichodoridae	Trichodorus	4	PF
Tylenchidae	Tylenchus	2	PF
Plectidae	Wilsonema	2	BF

C-P: Colonizer-persister scale 1-5 where cp 1 are colonizers characterized by short generation time and cp 5 are persisters characterized by long generation time (Bongers, 1990). PF – plant feeders, BF – bacterial feeders, FF – fungal feeders, PR – predatory, OM - omnivores

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Table 2: Nematode population and frequency of occurrence in Kangaita

Genera	C-P Value	Population/ 200g of soil	Frequency of occurrence	*Frequency rating (%)	**Nematode population (%)
Alaimus	4	360	8.0	88.89	3.82
Aphelenchus	2	1373	9.0	100.00	14.59
Cephalobus	2	1073	9.0	100.00	11.40
Cervidellus	2	13	2.0	22.22	0.14
Criconemella	3	387	9.0	100.00	4.11
Ditylenchus	2	33	3.0	33.33	0.35
Dorylaimus	4	60	3.0	33.33	0.64
Eucephalobus	2	887	9.0	100.00	9.42
Filenchus	2	600	9.0	100.00	6.37
Helicotylenchus	3	320	9.0	100.00	3.40
Hemicyclophora	3	53	3.0	33.33	0.57
Heterodera	2	40	3.0	33.33	0.42
Leptonchus	4	467	9.0	100.00	4.96
Longidorus	5	87	6.0	66.67	0.92
Meloidogyne	3	1527	8.0	88.89	16.22
Mononchus	4	133	7.0	77.78	1.42
Pratylenchus	3	33	3.0	33.33	0.35
Prismatolaimus	3	367	6.0	66.67	3.89
Prodorylaimus	5	67	5.0	55.56	0.71
Rotylenchus	3	87	3.0	33.33	0.92
Trichodorus	4	53	3.0	33.33	0.57
Tylenchus	2	767	9.0	100.00	8.14
Wilsonema	2	627	6.0	66.67	6.66

* n/N × 100 (n = number of times individual nematodes occurred and N = Sample size (9). ** In/TN × 100 (In = Individual nematode in all the samples and TN = Total Population of all the nematodes extracted in all the samples)

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Table 3: Nematode population and frequency of occurrence in Weru

Genera	C-P Value	Population/ 200g of soil	Frequency of occurrence	*Frequency rating (%)	**Nematode population (%)
Alaimus	4	167	6	66.70	1.75
Aphelenchus	2	444	9	100.00	4.69
Cephalobus	2	774	9	100.00	8.12
Cervidellus	2	18	4	44.00	0.21
Criconemella	3	372	8	88.90	3.92
Ditylenchus	2	78	6	67.00	0.84
Dorylaimus	4	24	4	44.00	0.28
Eucephalobus	2	762	9	100.00	7.98
Filenchus	2	576	8	88.90	6.02
Helicotylenchus	3	306	8	88.90	3.22
Hemicyclophora	3	162	3	33.30	1.68
Heterodera	2	174	3	33.30	1.82
Leptonchus	4	138	6	66.70	1.47
Longidorus	5	36	3	33.00	0.35
Meloidogyne	3	822	9	100.00	8.61
Mononchus	4	186	9	100.00	1.96
Pratylenchus	3	198	6	66.70	2.10
Prismatolaimus	3	162	6	66.70	1.68
Prodorylaimus	5	36	3	33.00	0.35
Rotylenchus	3	2628	6	66.70	27.57
Trichodorus	4	48	3	33.00	0.49
Tylenchus	2	1338	9	100.00	14.06
Wilsonema	2	78	6	67.00	0.84

* n/N × 100 (n = number of times individual nematodes occurred and N = Sample size (9). ** In/TN × 100 (In = Individual nematode in all the samples and TN = Total Population of all the nematodes extracted in all the samples)

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