Research Article

Influence of Soil Types on Citrus Nematode Species Diversity and Abundance on Varied Ecological Conditions in Kenya

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Abstract | Plant-parasitic nematodes (PPNs) cause major crop losses by damaging plant roots and causing reduced absorption of soil nutrient elements. A two-year survey in 2018 and 2019 was conducted in most citrus growing regions in Kenya to assess the abundance, distribution and diversity of plant parasitic nematodes from different soil rhizosphere. Nematode population in 200cc of soil and 5g of roots were collected for PPNs extraction by using modified Baermann's technique and identification by morphological features. The findings indicated that four major genera were abundant in different citrus growing ecological zones in Kenya. The identified species were *Meloidogyne incognita*, *Tylenchulus semipenetrans*, *Helicotylenchus dihystera* and, *Pratylenchus brachyurus*. The most abundant species in all the surveyed localities was *T. semipenetrans*. Factor regression analysis results showed that modest rainfall amounts favoured high density populations of PPNs on citrus roots where soil types of Rackers in Baringo and Luvisols, Ferralsols and Cambisols in Machakos County were dominant. Tylenchulus, Meloidogyne and Helicotylenchus species were most abundant in Kitale, Taita-Taveta, Kilifi and Kwale. The results presented here show how possible population density of PPNs in varied soil class types, rainfall amounts and prevailing temperature would be influenced by abiotic factors therein.

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Introduction

Plant-parasitic nematodes (PPNs) are recognized as of significance threat to agricultural production systems with widespread occurrence worldwide (Jones *et al*., 2013; Van den Hoogen *et al*., 2019). They infest all species of higher plants, where they feed on all various parts of the plants but they attack mostly the root system (Bakr *et al*., 2011; Anwar *et al*., 1991). Apart from causing crop losses annually PPNs also damage home gardens and lawn ornaments (Chitwood, 2003). Various PPNs can also bring about plant diseases which reduce the economic production every year leading to massive economic losses due to complex management practices (Al-Rehiayani and Fouly, 2005; Onkendi *et al*., 2014). Huge loss of citrus yield due to PPNs continue to be reported worldwide leading to subsequent income loss where management strategies are minimal (Nicol *et al*., 2011; Onkendi *et al*., 2014). The wounds created by their damage to the host plant becomes the entry points for plant pathogens like fungi and bacteria and the damaged tissue presentsan opportunity for bacteria colonization (Chirchir *et al*., 2008; Kago *et al*., 2013).

The abundance and distribution of plant-parasitic nematodes are influenced by soil texture, crop cycle, soil moisture and agricultural practices (Patel *et al*., 2009; Zalpuri *et al*., 2013). Populations of nematodes are concentrated on the upper soil layers in affected citrus orchards, where they are spread by agricultural implements, irrigation or rainwater, movement of plant material and soil (Abd-Elgawad, 2020). The economically important nematode species that infest citrus include, the citrus nematode, *Tylenchulus semipenetrans* (Cobb), the root-knot nematodes of *Meloidogyne species* (spp.), root-lesion nematodes, *Pratylenchus* spp., the burrowing nematode *Radopholus similis* (Thome) and spiral nematode, *Helicotylenchus* spp. (CABI, 2005; Bakr *et al*., 2011; Karuri *et al*., 2017).

The citrus nematode, *T. semipenetrans*, is one of the most widely spread and highly specialized parasites among plant-feeding nematodes and which causes major economic damage on citrus trees (Bakr *et al*., 2011; Olabiyi *et al*., 2016). This species is found in every citrus growing region in the world and it is known to cause a 30-50% reduction of citrus production and vegetative growth (Safdar *et al*., 2010). Infestation level depends on the density of the nematode population, the age and vigor of the tree as well as the decay and susceptibility of the rootstock (Hajihassani *et al*., 2018). Soil salinity is known to increase the population density of citrus nematode (Bakr *et al*., 2011). Apart from causing damage to crops, this nematode species predisposes the citrus tree to many disease causing-microorganisms (Wardle *et al*., 2004; De Waele and Elsen, 2007).

Root-knot nematode, *Meloidogyne* spp., is ranked one of the economically damaging on vegetables and annual crops worldwide, especially in tropical regions (Bélair and Simard, 2008). Effect of the root-lesion nematode (Tylenchulus semipenetrans) (Jones *et al*., 2013). There are about 90 species of *Meloidogyne* spp. of economic importance (Lamovšek *et al*., 2013) with high range of geographical distribution and capable of infecting many species of higher plants (Adegbite, 2011; Favery *et al*., 2016). They form a complex feeding site which results in the formation of galls on injured plant tissues, leading to stunted growth and impaired nutrient flow as a result of difficulties in the water and nutrient flow (Khan and Hassan, 2010). Root-lesion nematodes *Pratylenchus* species are also known to damage citrus by producing necrotic lesions on the surface of the roots which become entry points of disease-causing pathogens (Sayed *et al*., 2014).

Plant-parasitic nematodes studies in Sub Saharan Africa on citrus are negligible in scope or none existent in some countries (Sikora *et al*., 1987; Agbenin, 2011; Onkendi *et al*., 2014). In Kenya, for instance, there is limited information on plant-parasitic nematode species found on citrus rhizosphere and soil types harboring high populations and species diversity. The present work was aimed at identification of PPNs associated with citrus and analysis of the rhizosphere in different agro-ecological zones in Kenya in consideration to soil type and class, annual temperature and rainfall amount.

Materials and Methods

Sample sites of citrus nematodes

The present work was aimed at assessing infestation levels of plant-parasitic nematodes (PPNs) on citrus crop in Kenya. Plant-parasitic nematode samples were collected for two years (2018 and 2019) from citrus orchards within small-scale and large-scale farms during the wet and dry seasons in seven counties namely, Baringo, Trans-Nzoia, Machakos, Makueni, Taita-Taveta, Kwale and Kilifi. These farms were distributed within the ecological zones of high altitude (Lower Highland Zone 2 - LH2) regions of Rift valley, low midlands (Low Midland 4 - LM4) and coastal lowlands (Coastal Lowland 3 -CL3).

In each sampled farm, soil samples were collected from the citrus tree base, inside the canopy soil cover, at a depth of 15-30 cm including a part of plant roots using a soil auger and a garden trowel. A zigzag sampling pattern was carried out from one end of each farm. A composite sample of 200cc of soil sample and root samples of 5g were placed inside a labelled aerated bag and sealed in plastic bag to prevent drying out. The bag was labelled with the date collected, county, village, and the farmer's name. All samples were kept in a cool box to prevent the nematodes from dying and transported to KALRO Kabete Laboratory for extraction and identification of PPNs. Sampling was done during wet and dry seasons. Temperature and rainfall data were obtained from nearest meteorological station for each county.

Extraction and identification of specimens

The extraction of plant parasitic nematodes from the soil and root samples was done using modified Baermann's funnel technique (Hooper *et al*., 2005) where sieves with mesh sizes; 150, 38 and 25 were used. Briefly, the roots were washed to remove the soil particles and examined for galling and root-knot infection. A sample of 200g of soil were put on double ply paper towel (serviette), supported by a course meshed plastic standing on a plate below it (Figures 1 and 2). Water was carefully added inside from the edge of the plate until the soil was wet. The sieved solution was left undisturbed for 24 hours to facilitate swimming of the nematodes from soil to water after which the soil was removed, discarded and the nematodes suspension were collected (Figure 2). The suspensions collected were sieved through; 150, 38 and 25 micrometer apertures, to obtain 20ml sample volume portion. A fixed volume of 2ml nematode suspension was drawn using a micropipette and placed onto a counting dish under a stereoscopic microscope. The counting was carried out and scored then repeated three times and average was recorded. Morphological examination under microscope was carried out to determine species/ genus identification following pictorial keys (Siddiqi, 2000).

Correlation of abiotic conditions

Site soil type was identified as major factor to PPNs abundance in relation to chemical and physical conditions of the rhizosphere. This would lead to interpretation of the PPNs preference and abundance on citrus among the sample sites. Similarly, wet and dry field conditions were assumed to influence population density of PPNs thus mean area rainfall amounts were used to correlate to density population and nematode species of the sample sites. In addition, the site mean temperature was also used in the correlation to determine species and population density of PPNs occurrence and abundance.

Figure 1: *Sample assemblage of nematodes from citrus regions of Kenya (2018-2019).*

Figure 2: *Sample soil processing and microscopic identification (KALRO Kabete 2018-19).*

Data analysis

As most data sets were in number counts transformation by x+0.5 was carried out before analysis for significance difference. The transformed numbers of plant parasitic nematodes were subjected to Analysis of Variance (ANOVA) on General Linear Model, to compare significance of the number of nematodes population density per plant sample on citrus crop and mean values were separated by Fishers' Least Significance Difference at 5% level. To get the correlation effect the factor (rainfall amount, mean temperature) values were regressed with dependent variable (PPNs counts) of significance value using SAS Linear Model Procedure (LM Proc., SAS Version 9) to determine correlation levels at 5%.

Results and Discussion

Site nematode species abundance

A total of ninety-eight (98) samples were collected from citrus orchards in different locations bearing 712 specimens. Altogether, four genera of plant parasitic nematodes were identified from the sites. Figure 3 indicate the nematode genera found infesting citrus in the production counties. Baringo led other counties with highest *Tylenchulus* genus species at 135 counts while second abundant genus was *Melodogyne* at 56 cumulative from the citrus samples. The least occurring nematode genus species was *Pratelynchus* at 6 from Taita-Taveta and observed to be only present in Baringo at 2 counts from the samples. The fourth genus was *Helicotylenchus* (spp.) highest at 19 in Baringo and absent in Kilifi and Kwale.

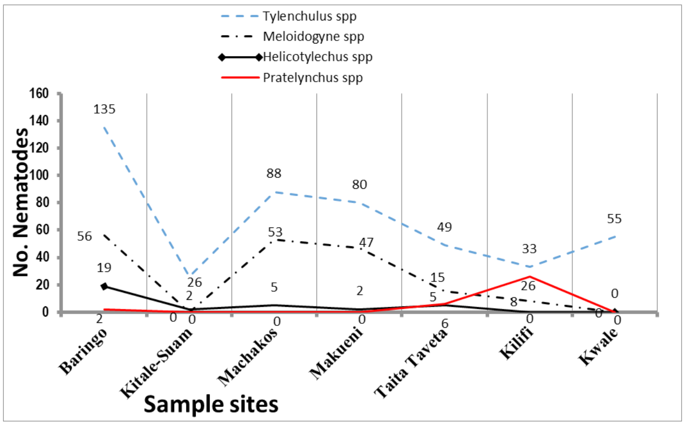

Figure 3: *Nematode genus species abundance in the sample sites in Kenya citrus growing counties (2018-19).*

Species significance at sites

Tylenchulus semipenetrans species was found in all the sampled citrus orchards in seven counties (Table 1). *Meloidogyne incognita* species was recorded in six counties only, with none recorded in Kilifi County. *Helicotylenchus dihystera* species was not identified in Kwale and Kilifi while *Pratylenchus brachyurus* species was not scored from Makueni, Machakos and Kitale sample sites. Highest nematode species appeared to occur during the wet period with few counts during the dry season. Baringo sample sites yielded the highest composition of various species of the four genera. In ascending order PPNs mean counts were 1.3, 18.8, 56.0, and 134.6 for *P. brachyurus*, *H. dihystera*, *M. incognita* and *T. semipenetrans* respectively in Baringo. This presented 30% of the total identified specimens. Other areas accounted for the remainder as follows: Kitale-Suam 4%, Machakos 21%, Makueni 18%, Taita-Taveta 10%, Kilifi 9% and Kwale 8%. In most county samples *T. semipenetrans* species led as the most significant ($p < 0.05$) abundant species in Kwale, Kilifi, Machakos, Kitale (Suam) and Baringo. Species abundance showed *T. semipenetrans* leading in Baringo followed by Kitale (Suam) and Machakos. Similarly, *H.dihystera* led in abundance in Baringo at 18.8 and with lower counts in other county sample areas. *Pratelynchus brachyurus* was not scored from Makueni, Machakos and Kitale sample sites. During the dry season only *T. semipenetrans* was scored in Baringo, Makueni and Kilifi.

Site abiotic factor significance

The Rackers/ Luvisols soil types in Baringo identified with high fertility and adequate drainage led to significantly ($p < 0.05$) high numbers of PPNs species and abundance at 21.5 ± 3.8as indicated in Table 2 where the four genera led in high populations compared to other sites. The Luvisolsand Cambisols in Machakos County bore second highest numbers of PPNsat14.6 ± 4.6for species of genera *Tylenchulus*, *Meloidogyne* and *Helicotylechus* significantly ($p< 0.05$) leading the counties of Kitale, Taita-Taveta, Kilifi and Kwale. Further, it was observable that genus species of *Helicotylechus* was absent in Kwale where the major soil type was the sandy Arenosols known for low fertility. Similarly, both *Meloidogyne* and *Helicotylechus* genera were absent in Kilifi site which was abundant with same Arenosol soil type. Overall genus *Pratelynchus* was absent in the county sites of Kitale-Suam, Machakos and Makueni where soils were acidic type (Acrisols, Luvisols, and Ferrasols) or sandy to coarse-textured (Arenosols and Planosols). The regression results showed that high populations of PPNs were significantly ($p < 0.05$, $t = 9.01$, $r = 0.92$) positively correlated to rainfall (675±225mm) amounts in Baringo. Similarly, rainfall amounts (850 ± 250mm and1,000 ± 200) significantly ($p < 0.05$, $t = 8.23$, $r = 0.88$and $p < 0.05$, $t = 7.88$, $r = 0.81$) led to higher PPNs population for Machakos and Makueni, respectively.

The results of the present study have shown the diversity of some plant-parasitic nematodes associated with citrus in different agro-ecological zones in Kenya of seven counties of Baringo, Trans-Nzoia, Machakos, Makueni, Taita-Taveta, Kwale and Kilifi. The four genera recorded were *Tylenchulus*, *Meloidogyne*, *Helicotylechus* and *Pratylenchus* and are among the top ten (10) plant-parasitic nematodes recognized of being of economic significance to various crops (Jones *et al*., 2013).

Table 1: *Site nematode genera occurrence on citrus crop in Kenya during wet and dry seasons of 2018-2019.*

Wet season/ Site	*Tylenchulus* spp.	*Meloidogyne* spp.	*Helicotylechus* spp.	*Pratelynchus* spp.	F	*P*
Baringo	134.6 ± 17.4 A^{A}	56.3 ± 32.0 B^{A}	18.8 ± 11.6 B^{A}	1.3 ± 1.0 B^{A}	21.6	0.013
Kitale-Suam	26.3 ± 6.9 A^{C}	0 B^{C}	1.3 ± 1.0 B^{B}	0 B^{B}	43.2	0.020
Machakos	87.5 ± 27.7 A^{B}	52.5 ± 15.6 B^{A}	5.0 ± 2.0 C^{B}	0 C^{B}	30.6	0.001
Makueni	80.0 ± 25.9 A^{B}	46.3 ± 25.5 B^{A}	1.3 ± 1.0 C^{B}	0 C^{B}	10.1	0.010
Taita Taveta	48.8 ± 23.6 **A^{C}**	15.0 ± 13.2 B^{C}	5.0 ± 5.0 B^{B}	6.3 ± 3.2 B^{B}	7.0	0.022
Kilifi	32.5 ± 12.4 A^{C}	7.5 ± 2.2 B^{C}	0 B^{B}	26.3 ± 12.5 B^{B}	6.4	0.264
Kwale	54.3 ± 12.6 A^{C}	0 B^{C}	0 B^{B}	0 B^{B}	85.1	< 0.001
F	26.6	11.5	6.3	10.5		
P	< 0.001	0.002	0.030	0.040		
Dry season						
Baringo	5.0 ± 2.0 A^{A}	0 B^{A}	0 B^{B}	0 B^{B}	12.0	0.006
Kitale-Suam	0 B^{A}	0 A^{A}	0 A^{A}	0A^{A}	-	-
Machakos	0 B^{A}	0 A^{A}	0 A^{A}	0 A^{A}	-	-
Makueni	5.0 ± 2.0 A^{A}	0 A^{B}	0 A^{B}	0 A^{B}	12.0	0.006
Taita Taveta	0 B^{A}	0 A^{A}	0 A^{A}	0 A^{A}	-	-
Kilifi	5.0 ± 1.0 A^{A}	0 A^{B}	0 A^{B}	0 A^{B}	12.0	0.005
Kwale	0 B^{A}	0 A^{A}	0 A^{A}	0 A^{A}	-	-
F	11.8	-	-	-		
P	0.002	-	-	-		

Nematode counts marked within county sites with similar capital letters denote no significant (p > 0.05, $F_{6, 47}$) difference while similar superscript capital letters across species show no significant difference of population density among species at 5% level.

Table 2: *Soil type influence to nematode genus mean abundance from 10 farms at the different citrus production sites (in 2018-2019).*

Genera	Citrus growing counties in Kenya and indicative presence/ absence of nematode genus species with varied soil types						
	Baringo	**Kitale-Suam**	**Kwale**	**Machakos**	**Makueni**	**Taita Taveta**	**Kilifi**
Tylenchulus	+	+	+	+	+	+	+
Meloidogyne	+	+	+	+	+	+	-
Helicotylechus	+	+	-	+	+	+	-
Pratylenchus	+	-	+	-	-	+	+
Major soil type	**Rackers, Lithosols**	**Acrisols, Ferralsols**	**Cambisols; Arenosols**	**Luvisols, Ferralsols**	**Nitisols, Planosols**	**Gleysols,**	**Luvisols, Arenosols**
Rainfall (mm)	675 ± 225	1,300 ± 300	1,250 ± 250	850 ± 250	1,000 ± 200	425 ± 125	1350 ± 175
Temperature (°C)	23.2 ± 2.2	17.3 ± 2.4	27.4 ± 2.1	19.1 ± 1.5	22.3 ± 1.3	27.4 ± 1.8	29.5 ± 2.3
No. Nematodes	21.5 ± 3.8	2.8 ± 1.2	5.5 ± 0.8	14.6 ± 4.6	12.9 ± 4.6	7.5 ± 4.6	5.7 ± 0.8
p	0.0001	0.0116	0.1989	0.0025	0.2198	0.0558	0.0373
t (temperature)	-0.67	-0.80	1.69	4.88	0.38	-0.77	0.98
t (rainfall))	9.01	4.20	0.00	8.23	7.88	1.54	2.64
r	0.92	0.85	0.44	0.88	0.81	0.66	0.71

Key descriptions of soil characteristics as reported in literature. Factor regression results at p = 0.05 for temperature and rainfall influence on plant-parasitic nematodes on citrus roots in varied soil types.

Altogether, four genera of plant parasitic nematodes were identified from the sites. The major four identified species were *Tylenchulus semipenetrans* occurring in the seven counties while *Meloidogyne incognita* species was recorded in six counties and missed only in Kwale. *Helicotylenchus dihystera* species was not found in Kwale and Kilifi while *Pratylenchus brachyurus* species was not scored from Makueni, Machakos and Kitale sample sites.

Highest nematode species appeared to occur during the wet period with few counts during the dry season. This brings in the importance of optimum moisture for population growth of PPNs associated with citrus crop. Nita *et al.* (2021) showed that nematode optimum moisture requirement was over 30% and in a warm environment of 21°C. Of note in the present study was that Baringo sample sites yielded the highest composition of various species of the four genera with mean sample at 21.5 nematodes per sample where yearly temperature range was 21.0 to 25.4°C and rainfall 675±225mm in soil types of Rackers and Lithosols. Cooler regions like Machakos (17.5-20.6°C) scored nematode at 14.6 per sample and Makueni (21.0-23.6°C) at 12.9/ sample of rainfall range of 850 ± 250mm and1,000 ± 200mm, respectively. Of interest would be what contribution of soil type was leading to high abundance and diversity of nematodes in Baringo, Machakos, Makueni and Taita-Taveta citrus orchards. The common soil type in Baringo was Rackers in most citrus farms which literature by other workers report highfertility and adequate drainage leading to significantly high numbers of PPNs species and abundance where the four genera led in high population counts as found on fertile soils (Renco *et al.*, 2020; Melakeberhan *et al.*, 2021). The Luvisolsin Machakos County bore second highest numbers of species of genera Tylenchulus, Meloidogyne and Helicotylechus significantly leading in the counties of Kitale, Taita-Taveta, Kilifi and Kwale. A review of the properties of these soil groups show that Luvisols are rich with sub-surface fertile clay content of resulting to enhanced nematode diversity in the presence of host plant (Ghabour *et al.*, 2002; Melakeberhan *et al.*, 2021). On the other hand, Ferralsols poor fertility condition could have led to little diversity of nematode in the mentioned areas of Kitale-Suam (Karuri *et al.* 2017; Renco *et al.*, 2020).

Further, it was observable that genus species of Helicotylechus was absent in Kwale where the major soil type was the sandy Arenosol. It ought to be observed that the Arenosols lack quality soil organic carbon (SOC) which consists of main source of food for most soil-living microbes inclusive of nematodes (Melakeberhan *et al.*, 2021). Similarly, both *Meloidogyne* and *Helicotylechus* genera were absent in Kilifisample sites which was abundant with same Arenosol soil type. Mokrin *et al.* (2020) reported genus Helicotylenchus preference for clay soil composition while Meloidogyne most preferred presence of Manganese (Mn), Calcium (Ca), Copper (Cu) and Zinc (Zn) elements rich soils. Overall genus Pratelynchus was absent in the county sites of Kitale-Suam, Machakos and Makueni where soils were acidic type (Acrisols and Greysols) or sandy to coarse-textured (Arenosols and Planosols). The regression results showed that high counts of PPNs were positively correlated to modest rainfall amounts as in the case of Machakos and Baringo respectively. The nematodes of genera *Pratylenchus, Tylenchulus* and *Meloidogyne* are more predominant in sandy soils worldwide (Audebert *et al.*, 2000; Yavuzaslanoglu *et al.*, 2012) and were found in Kwale, Kilifi and Taita-Taveta sites which bore similar soil conditions. Acidic medium and sandy soil root base lead to low infestations of PPNs (Koenning *et al.* 1999). The results confirmed continued presence of PPNs not only on vegetables and fruit crops in Kenya but more even so on citrus crop across diverse agro-ecological regions (Melakeberhan *et al.*, 2021).

Conversely, the present study has revealed significant differences in the abundance of plant-parasitic nematode genera in different agro-ecological zones. Such variation has been reported elsewhere by other workers (Anwar *et al.*, 1991; Zalpuri, 2010), and may be caused by several factors such as differences in soil types, soil moisture, climatic factors, irrigation system and other agricultural practices. This is in agreement with other studies where the number of plant parasitic nematodes increased significantly with increased soil water content (Castillo *et al.*, 1996; Govaerts *et al.*, 2007). This was because these parasites depend on moisture for their survival and they live and migrate in soil particles surrounded by water as they invade roots and multiply. Dry soil conditions do not allow the PPNs to be metabolically active and lead to slow movement of the parasites and low colonization of citrus crop root systems as in wet conditions (Bakonyi *et al.*, 2007).

As observed in most reports PPNs pose a threat to citrus production due to the changing climate and increased rainfall patterns which result in increased population levels of nematodes (Asseng *et al.*, 2015). As confirmed in the present study optimum temperature influences populations of plant parasitic nematodes and an essential factor for these parasites to increase in numbers on host plants (Tzortzakakis and Trudgill, 2005). Increased temperature is reported to increase the development and reproduction of PPNs (Evans and Perry, 2009). The abundance of citrus nematodes in hot to warm areas of Machakos, Makueni, Baringo (Marigat) and Kilifi was significantly higher than in the cooler Kitale-Suam. This was probably due to the higher mean temperatures in those counties. This is in agreement with Asseng *et al*. (2015), who reported that pests and disease incidences becoming unpredictable due to the changing climate especially increasing temperatures which tend to accelerate reproduction and development of many plant parasitic nematodes. Likewise, soil substrate and its texture have been shown to affect populations of PPNs, mainly because they influence their migration and reproduction (Koenning *et al*., 1999; De Waele and Elsen, 2007), hence increasing or reducing their abundance and distribution. Likewise, soil pH and the nutrient content such as potassium and phosphates in the soil influence the prevalence and diversity of plant parasitic nematodes (Kandji *et al*., 2001; Olabiyi *et al*., 2016).

Conclusions and Recommendations

The present study has presented diversity and abundance of PPN species found attacking citrus in Kenya in the varied agro-ecological zones relating to different soil types, rainfall amounts and temperature regimes. The information here on genera species site occurrence will guide management options to be aligned with analysis for each nematode biology review and what other ecologists have recommended elsewhere in relation to amount of rainfall and fertility status in the citrus orchard of specific agro-ecological zone and soil type.

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Novelty Statement

Plant-parasitic nematodes' species diversity associated with citrus rhizosphere in different agro-ecological zones in Kenya.

Author's Contribution

JK, DM, PN, LI and GR contributed in field data collection and analysis while RA identified the nematode species and wrote the paper with the rest of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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