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Research Article

Abiotic Factor Influence to Density and Distribution of Plant-Parasitic Nematodes on Citrus in Kenya

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ABSTRACT

Plant-parasitic nematodes (PPNs) pose threat to agricultural production and food security globally. They cause major crop losses by damaging the roots and causing plant diseases. Various PPNs species require adequate attention since they damage and limit production in citrus. Their taxonomy is very important for management strategies. A two-year survey in 2018 and 2019 was conducted in all citrus-growing regions in Kenya to assess the abundance, distribution, and diversity of plant-parasitic nematodes from the soil rhizosphere. Nematode population in 200cc of soil and 5g of roots was determined by extraction using modified Baermann's technique and identification by morphological features. The findings indicated that four genera were encountered in different citrus growing county regions in Kenya. The PPNs species identified were *Meloidogyne incognita, Tylenchulus semipenetrans, Helicotylenchus dihystera and Pratylenchus brachyurus.* The most predominant species in all the surveyed localities was *Tylenchulus semipenetrans.* Factor regression analysis results showed that modest rainfall amounts favored high-density counts of PPNs on citrus root where soil types of Rackers in Baringo and Luvisols, Ferralsols, and Cambisols in Machakos County were dominant. Species of Tylenchulus, Meloidogyne, and Helicotylechus were most abundant in Kitale, Taita-Taveta, Kilifi, and Kwale. The results presented here show how possible the population density of PPNs in varied soil types, rainfall amounts, and prevailing temperature would be influenced by abiotic factors therein. Hence appropriate approaches to manage the PPNs species damaging citrus roots are important.

Keywords: Citrus; Nematodes; Abundance; Plant-parasitic; Food security; Species.

INTRODUCTION

Plant-parasitic nematodes (PPNs) are recognized as of significance threat to agricultural production systems with widespread occurrence worldwide [1,2]. They infest all species of higher plants, where they feed on all various parts of the plants but they attack mostly the root system [3]. Apart from causing crop losses annually they also damage home gardens and lawn ornaments [4]. Plant-Parasitic nematodes can also bring about plant diseases which reduce the economic production every year leading in massive economic losses due to complex management practices [5,6]. Citrus global damage by PPNs is approximately $US 80 billion per year [7]. 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 presents an opportunity for bacteria colonization [8]. The abundance and distribution of plant-parasitic nematodes are influenced by soil texture, crop cycle, soil moisture and agricultural practices (9, 10). 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. The economically important nematode

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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* and spiral nematode, *Helicotylenchus spp* [11,3,12]. The citrus nematode, *T. semipenetrans*, is one of the most widely spread and highly specialized parasites among plant-feeding nematodes causing economic damage on citrus trees [3,13]. 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 [14]. Affected trees show the following symptoms: small yellow leaves, defoliation, die-back of twigs, small fruits and overall reduced tree vigor. 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 [15]. Soil salinity is known to increase the population density of citrus nematode [16]. Apart from causing damage to crops, this nematode species predisposes the citrus tree to many disease causing-microorganisms [17,18]. Root-knot nematode, *Meloidogyne spp*, is ranked the most economically damaging PPN and a threat to a wide range of crops worldwide, especially in tropical regions [1]. There are about 90 species of *Meloidogyne spp* of economic importance [19] with high range of geographical distribution capable of infecting many species of higher plants [20,21]. 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. 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 [22]. Plant-parasitic nematodes studies in Sub Saharan Africa are negligible in scope or none existent in some countries [23,24,6]. In Kenya, for instance, there is limited information on plant-parasitic nematode species found on citrus rhizosphere. Correct nematode identification for new or potentially harmful species requires extensive knowledge of nematode taxonomy. The objective of the present study was to explore species diversity of PPNs on citrus in different region of production. 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, 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 the each farm. A composite sample of 200cc of soil sample and root samples of 5g were placed inside a labeled and sealed in plastic bag to prevent drying out. The bag was labeled 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.

Nematode extraction and identification

The extraction of plant parasitic nematodes from the soil and root samples was done using modified Baermann's funnel technique 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 200 g of soil were put on double ply paper towel (serviette), supported by a course meshed plastic standing on a plate below it (Figure 1). Water was carefully added inside from the edge of the plate until the soil was wet. The set 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 20 ml sample volume portion. A fixed volume of 2 ml 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 (Figure 2).

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

Figure 2: Laboratory nematode sample soil processing and microscopic identification (KALRO Kabete 2018-19).

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.

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 means 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.) to determine correlation levels at 5%.

RESULTS

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 genus species found infesting citrus in the production counties. Baringo led other counties with highest Tylenchulus genus species at 135 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.

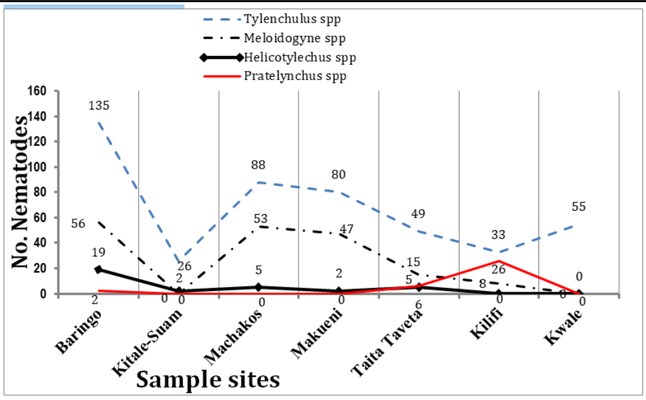

Figure 3: Nematode genus species abundance in the sample sites in Kenya citrus growing counties (2018-19).

Genus 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. semipenetran* 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. semipenetran* species led as the most significant ($p < 0.05$) abundant species in Kwale, Kilifi, Machakos, Kitale (*Suam*) and Baringo. Species abundance showed *T . semipenetran* leading in followed by Kitale (Suam) Baringo 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. semipenetran* was scored in Baringo, Makueni and Kilifi.

Wet season/ Site	*Tylenchulus sp*	*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	0.02
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 BA	1.3 ± 1.0 C^B	0 C^B	10.1	0.01
Taita Taveta	48.8 ± 23.6 A^C	15.0 ± 13.2 B^C	5.0 ± 5.0 BB	6.3 ± 3.2 BB	7	0.022
Kilifi	32.5 ± 12.4 A^C	7.5 ± 2.2 B^C	0 BB	26.3 ± 12.5 B^B	6.4	0.264
Kwale	54.3 ± 12.6 A^C	0 B^C	0 BB	0 B^B	85.1	< 0.001
F	26.6	11.5	6.3	10.5		
P	< 0.001	0.002	0.03	0.04		
Dry Season						
Baringo	5.0 ± 2.0 A^A	0 B^A	0 B^B	0 B^B	12	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	0.006
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	0.005
Kwale	0 B^A	0 A^A	0 A^A	0 A^A	~	
F	11.8	-	-	-		
P	0.002	-	-	-		

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

Nematode counts marked within county sites with similar capital letters denote no significant ($p > 0.05$, F6, 47) difference while similar superscript capital letters across species show no significant difference of population density among species at 5% level.

Site abiotic factor significance

The Rackers soil type in Baringo identified with high fertility and adequate drainage led to significantly ($p < 0.05$, F6, 47) high numbers of PPNs species and abundance as indicated in Table 2 where the four genera led in high density count peaks compared to other sites. The *Luvisols*, *Ferralsosl* and *Cambisols* in *Machakos* County bore second highest numbers of species of genera *Tylenchulus sp*, *Meloidogyne sp* and *Helicotylechus sp* significantly ($p < 0.05$) leading 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*. 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 and Greysols) or sandy to coarse-textured (Arenosols and Planosols).

Genera	**Baringo**	**Kitale-Suam**	**Kwale**	**Machakos**	**Makueni**	**Taita Taveta**	**Kilifi**
Tylenchulus	+	+	+	+	+	+	+
Meloidogyne	+	+	+	+	+	+	-
Helicotylechus	+	+	-	+	+	+	-
Pratylenchus	+	-	+	-	-	+	+
Soil type/ descriptions	Lithosols: shallow soils; dry out fast, while Regosols are poorly developed,and Rackers: very deep, well drained soils	Acrisols: old acidic clay soils; while Gleysols are usually water logged with low organic content; Ferralsols: highly weathered soils; Vertisols: heavy clay soils	Cambisols: red-brownish young weakly differentiated soil strata; as Verticals / Luvisols: clay forms. Arenosols: sandy-textured soils with non/low clay	Luvisols: subsurface clay; Cambisols: red-brownish, and Acrisols: acidic clay and Ferralsols: highly weathered soils of leached soils at top surface	Nitisols: red to red-brownish clay; Andosols : developed from volcanic ash; Planosols: coarse-textured surface Gleysols: water logged, low organic content	Gleysols: waterlogged; Luvisols: clay rich, and Ferralsols: highly weathered soils of leached soils at top surface	Luvisols: heavy with clay at subsurface and more humus at the top; Arenosols: low clay content and high sand soils in the tropics.
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± 1.8	5.7 ± 0.8
p	1.00E-04	0.012	0.199	0	0.22	0.06	0.037
t (temperature)	-0.67	-0.8	1.69	4.88	0.38	-0.8	0.98
t (rainfall))	9.01	4.2	0	0.23	1.88	1.54	2.64
r	0.92	0.85	0.44	0.88	0.41	0.66	0.71

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

The regression results showed that high counts of PPNs were positively correlated to modest rainfall amounts with r-values being significantly high at 0.88 and 0.92 for the sites of Machakos and Baringo. The temperature regimes at the sites did not show definite effect to PPNs count densities of the samples taken.

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.

DISCUSSION

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 plant-parasitic nematodes recognized of scientific and economic significance to various crops [1]. A total of 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. 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. 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 [25].

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. Of note was the fact that Baringo sample sites yielded the highest composition of various species of the four genera. It was apparent that PPNs counts occurred significantly in counts at 1.3, 18.8, 56.0, and 134.6 for *P. brachyurus, H. dihystera, M. incognita and T. semipenetran* respectively in Baringo. As shown from the results Baringo presented 30% of the total identified specimens as other areas accounted for the remainder with Kitale-Suam 4%, Machakos 21%, Makueni 18%, Taita-Taveta 10%, Kilifi 9% and Kwale 8%. The significance of this was that certain counties were likely to continue losing high fruit yield due to PPNs presence and specifically these were Baringo, Machakos, Makueni and Taita-Taveta, as PPNs abundance showed. Of immediate interest to research workers would be the factors leading to the high population density of PPNs on citrus fruits in Baringo, Machakos, Makueni and Taita-Taveta. The common soil type in Baringo was Rackers in most citrus farms which literature by other workers report high fertility and adequate drainage leading to significantly high numbers of PPNs species and abundance where the four genera led in high density count peaks as found on fertile soils [25,26]. The Luvisols, Ferralsosl and Cambisols in Machakos County bore second highest numbers of species of genera *Tylenchulus sp*, *Meloidogyne sp* and *Helicotylechus sp* significantly leading counties of Kitale, Taita-Taveta, Kilifi and Kwale. A review of the properties of these soil groups show that Luvisols are rich of sub-surface fertile clay while Vertisols bear high content of expanding clay resulting to enhanced nematode diversity in the presence of host plant [25,27]. On the other hand Ferrasols poor fertility condition could have led to little diversity of nematode in the mentioned areas of Kitale-Suam [12,29].

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 [25]. 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 and Greysols) or sandy to coarse-textured (Arenosols and Planosols). The said nematode species absence here delimits preference of specific soil rhizosphere and nematode species abundance.

The regression results showed that high counts of PPNs were positively correlated to modest rainfall amounts with r-values being significantly high at 0.88 and 0.92 for the sites of Machakos and Baringo respectively. The temperature regimes at the sites did not show definite effect to PPNs count densities of the samples taken. 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 [10,28], and may be caused by several factors such as differences in soil types, soil moisture, climatic factors, irrigation system and other agricultural practices. The occurrence and mean abundance of nematode genera varied with seasons such that during rainy season there were more plant parasitic nematodes than during dry season. In this study more nematodes were recorded in different agro ecological zones during rainy/wet seasons, as compared to dry season. This is in agreement with other studies where the number of plant parasitic nematodes increased significantly with increased to soil water content [29,30]. 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 [31]. 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 [31]. Another important environmental factor that has been reported to influence populations of plant parasitic nematodes is temperature [27,32]. Increased temperature is reported to increase the development and reproduction of PPNs [33]. The abundance of citrus nematodes in Machakos, Makueni, Baringo (Marigat) and Kilifi was significantly higher than in Kitale-Suam. This was probably due to the higher mean temperatures in those counties. This is in agreement with similar work on 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 [31]. Soil substrate and its texture have been shown to affect populations of PPNs, mainly because they influence their migration and reproduction [18,34,39], 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 [35]. The present study results showed variation in occurrence and distribution of nematodes due to the soil texture, similar to reports by earlier workers [36,37]. The soil texture in Baringo (Marigat), Makueni and Machakos are mostly friable loam soils, which harbor high PPNs due to good aeration in the soil and fertile nature unlike the soils in Kitale and Taita-Taveta. The nematodes of genera Pratylenchus, Tylenchulus and Meloidogyne are more predominant in sandy soils worldwide [33,38] 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 [34,39].

CONCLUSION

The present study has presented PPN species found attacking citrus in Kenya in the varied agro ecological zones diversity relating to different soil types, rainfall amounts and temperature regimes and the occurrence of nematode diversity and abundance in such conditions. Further future studies would reveal what nematode species were causing most yields limiting scenarios in each region of the country comparable to worldwide status.

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