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RESEARCH ARTICLE

Nematology

Prevalence and abundance of plant-parasitic nematodes associated with corn (*Zea mays* L.) in Anuradhapura, Sri Lanka

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Abstract: The prevalence and abundance of plant-parasitic nematodes (PPNs) associated with corn (*Zea mays*; Poaceae) in the Anuradhapura district of Sri Lanka are poorly understood. This study investigated the occurrence and population densities of major PPN genera associated with corn. Over 92% of the corn fields were positive for PPNs in all the sampled fields. Major PPN genera identified were *Pratylenchus* spp. (71.4%), *Helicotylenchus* spp. (28.6%), *Meloidogyne* spp. (21.4%), *Criconemella* spp. (21.4%), and *Hoplolaimus* spp. (35.7%). The mean population density of *Pratylenchus* spp. was 2020 nematodes kg^{-1} of soil, in the Anuradhapura corn fields. During the cropping season from November (2021) to February (2022), all PPN genera except *Meloidogyne* spp. were observed. *Pratylenchus* spp. were detected at levels below 1000 nematodes kg^{-1} of soil at the seedling stage, except in Kelenikawewa where the initial population was 1865 nematodes kg^{-1}. At the time of harvest, *Pratylenchus* spp. increased by 2 to 10 folds. These findings suggest a potential impact of *Pratylenchus* spp. on corn yield in Anuradhapura, highlighting the need for further research to assess damage levels and the overall effect of PPNs on corn production in Sri Lanka.

Keywords: Anuradhapura, nematode diversity, plant-parasitic nematodes, *Pratylenchus*, Sri Lanka, *Zea mays* (maize)

INTRODUCTION

Corn (*Zea mays* L.) is a key cereal crop grown after rice *(Oryza sativa* L.) in Sri Lanka (Malaviarachchi *et al*., 2007). Corn production in Sri Lanka has recently increased (DOA, 2020) due to the rising demand for poultry feed (Malaviarachchi *et al*., 2007). Corn-based food items are popcorn, boiled corn cobs, and locally produced ready-mix cereals 'Thriposha' and 'Samaposha'. Consequently, the local production of corn has increased from 150 to 360 tons between 2000 and 2020 (Esham *et al*., 2005; Esham, 2014; DOA, 2020). Corn is grown in almost 80,000 ha in Sri Lanka in climatically dry and intermediate zones with rainfed cultivation during the *Maha* season (DOA, 2020) and the Anuradhapura district alone contributes 30% of the country's extent (Hamangoda & Pushpakumari, 2019; Vidanapathirana *et al*., 2022). However, this cropping system has lower productivity compared to irrigated or protected agriculture, primarily due to erratic weather, biotic stresses, and poor adoption of improved agronomic practices (Esham, 2014).

In Sri Lanka, open-pollinated corn varieties are in use for cultivation including *Bhadra*, *Ruwan*, *Aruna*, and *Muthu* (Vidanapathirana *et al*., 2022). Despite this, hybrid varieties are more popular among farmers (Malaviarachchi *et al*., 2007; Esham, 2014; Vidanapathirana *et al*., 2022). The Department of Agriculture in Sri Lanka released the first local hybrid corn variety, *Sampath*, in 2004. Subsequently, some private companies introduced imported hybrid varieties, such as *Jet* and *Pacific* (Esham, 2014). In 2019, four

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more hybrid varieties were introduced by the Department of Agriculture. However, the *Jet* and *Pacific* varieties remain the most cultivated hybrids in the Anuradhapura district due to their high yield potential (6 tons ha^{-1}) and export demand (Vidanapathirana *et al*., 2022). In 2020, average corn yields in Sri Lanka were 4 tons ha^{-1}, which is below the maximum yield potential (Vidanapathirana *et al*., 2022). This could be due to a variety of factors including biotic factors such as pests and diseases and abiotic stresses such as drought, and soil edaphic characters (Malaviarachchi *et al*., 2007; Esham, 2014). Among biotic factors, the association of PPN with corn is highly influential on its yield (Sikandar *et al*., 2021; Khanal & Land, 2023).

Plant-parasitic nematodes are known to infect almost all cultivated crops and are responsible for 14-20% of global annual yield losses (Jung & Wyss, 1999; Mesa-Valle *et al*., 2020). These losses are estimated to be over 358 billion USD (Abd-Elgawad & Askary, 2015; Khanal & Land, 2023). A variety of nematodes are known to cause damage to corn, including genera such as *Anguina* spp., *Criconemella* spp., *Ditylenchus* spp., *Helicotylenchus* spp., *Hirschmanniella* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus* spp., *Rotylenchus* spp., *Trichodorus* spp., *Tylenchulus* spp., and *Xiphinema* spp. (Nicol *et al*., 2011). Among these, *Meloidogyne* spp. and *Pratylenchus* spp. are known to cause the most damage and potential economic losses in corn (Sikora *et al*., 2005).

In Sri Lanka, there is limited information available regarding the association of PPN with corn. A survey from 1991 to 1995 in 16 districts of Sri Lanka indicated PPN associations with different crop species (Ekanayake & Toida, 1997). Ekanayake & Toida (1997) identified six nematode species associated with corn: *Criconemella ornata, Helicotylenchus dihystera, Hoplolaimus seinhorsti, Pratylenchus zeae, P. brachyurus,* and *Xiphenema elongatum,* although nematode population abundance and distribution were not investigated. In this context, the objective of this study was to identify the prevalence and abundance of PPNs associated with corn fields in the Anuradhapura district, Sri Lanka.

MATERIALS AND METHODS

Preliminary sampling

Preliminary sampling was undertaken in February 2021 across 14 corn fields located in five locations, Horowupotana, Kahatagasdigiliya, Kelenikawewa, Ranorawa, and Elayapaththuwa, situated within the Anuradhapura district (8°.3114" N, 80°.4037" E), Sri Lanka. A complete cropping history of the sampled fields was recorded. The sampling sites were divided into 1 ha blocks. From selected blocks, 25 soil core samples were taken in a zigzag pattern across the field as described by Marais *et al*. (2017). Samples were taken in the corn rhizosphere to a depth of 30 cm using a 4 cm diameter soil corer (Eijkelkamp, Japan). To minimize cross-contamination, tools were cleaned between each sampling using a metal brush and tissue paper. About a kilogram of soil from subsamples (25 cores ha^{-1}) was thoroughly mixed to make a composite sample before being transferred to labelled plastic bags. Subsequently, the bags were sealed, and stored in darkness in cooler boxes (Marina Cooler Box) and the soil samples were taken to the laboratory and placed in a refrigerator at 4 °C until they were processed.

Intensive secondary sampling

From the 14 corn fields, four fields in Horowupotana (8°33'01.4"N, 80°45'44.0"E), Kahatagasdigiliya (8°28'13.3"N, 80°44'27.7"E), Kelenikawewa (8°20'29.8"N 80°41'37.6"E), and Elayapaththuwa (8°23'20.6"N 80°18'26.0"E) with high PPN abundance were selected for intensive sampling. Soil samples were collected when the seedlings were 2-3 wks old (2021 November) and at harvesting (14-15 wks after planting in 2022 February), during the *Maha* cropping season in 2021/2022. Each field was divided into four equal quadrants. A composite sample from a quadrant was collected in a zigzag pattern, giving a total of four composite samples per field. At each sampling point in a quadrant, 12 soil cores (500 g soil) were collected randomly using a soil sampler within a 4 m radius from the centre point of the sample. Apart from this modified sampling pattern, other procedures and techniques were identical to the methods mentioned previously.

Nematode extraction: Sieving-centrifugal-sugar flotation method for soil sample

The sieving-centrifugal-sugar flotation method (Jenkins, 1964; Marais *et al*., 2017) was used to extract nematodes from the soil samples to determine nematode density. Briefly, 100 g of soil from the composite sample was placed in a 5 L container and thoroughly mixed with 1 L tap water. After manually mixing and stirring, the mixture was allowed to settle for 30 s before transferring to nested aperture sieves 150-μm on top of 38-μm aperture sieves (W.S. Tyler, USA). This process was repeated three times for each sample to recover most nematodes in the soil. The deposit from the bottom sieve (38-μm aperture) was transferred into 50 mL centrifuge tubes and centrifuged at 568 g rotational centrifugal force (RCF) for 5 min. The supernatant was removed carefully, and the pellet was thoroughly mixed with sucrose solution (624 g of sugar /L tap water) and spun again in the centrifuge (Tomy, Japan) for one min at 568 g RCF. After centrifugation, the supernatant was passed through a 38-µm-aperture sieve and carefully washed using water dispensed from a wash bottle to collect the extracted nematodes into a 100 mL sterile specimen bottle with a lid. Samples were kept at 4 °C until they were morphologically identified (Kularathna *et al*., 2019). Using an inverted compound light microscope (Nikon, Japan) at ×40 magnification, the nematodes were identified at the genus level based on morphological descriptions (Fortuner, 1988; Mai *et al*., 1996).

Statistical analysis

Nematode populations from the survey were characterized using standardized ecological nematology indices (Boag, 1993). Nematode prevalence was calculated using the equation 1.

$$\textbf{Prevalence (\%)} = (nN/nS) \times 100 \quad ...(1)$$

where the nN is the number of samples containing nematodes taxon or genus, and nS is the total number of samples collected.

The proportion of the sample comprising PPNs was derived by equation 2.

$$\textbf{Percentage of PPN} = (PPN/Nematodes) \times 100 \quad ...(2)$$

where PPN is the total number of PPNs recovered, and Nematodes is the total number of nematodes (PPN and all non-PPN) recovered.

Genus-specific prevalence data were recorded as the presence (1) or absence (0) of each genus at each survey site and the total number of genera was the number of different genera detected at a single survey site.

Simpson's diversity index (1-D), was calculated by equation 3:

$$\text{Simpson's diversity index } (1\text{-}D) = 1-[\textstyle\sum n(n-1)/N(N-1)] \quad ...(3)$$

where n is the total number of nematodes of a specific genus, and N is the total number of nematodes across all genera in the sample.

Evenness (E) expresses how evenly individuals in a community are distributed over the different genera and was calculated by equation 4:

$$\text{Evenness } (E) = 1-[\textstyle\sum n(n-1)/N(N-1)]/s \quad ...(4)$$

where s is the total number of different genera (Fleming *et al*., 2016). Simpson's biodiversity index (1-D) and evenness (E) were calculated only for the PPN diversity. Higher values (ranging from 0-1) of the Simpson biodiversity index (1-D) indicate higher nematode diversity; if it was zero there was a single nematode genus in the population.

Nematode abundance data were analyzed using a one-way ANOVA and a post hoc Bonferroni test at 95% confidence intervals was performed (GenStat statistical software ver. 20). The data was checked for normality and then transformed using the square root method before statistical analysis.

RESULTS AND DISCUSSION

Prevalence of plant parasitic nematodes in the corn fields of Anuradhapura, Sri Lanka - Preliminary samples

The prevalence of PPN was 92.9% and was found in 13 out of 14 corn fields (Table 1). Among the recovered PPN, the root-lesion nematode (RLN) *Pratylenchus* spp., had a prevalence of 71.4% occurring in 10 out of 14 fields sampled. *Pratylenchus* spp. were the most prevalent PPN detected, compared to other PPN genera such as the spiral nematode (*Helicotylenchus* spp.) (28.6%), root-knot nematode (*Meloidogyne* spp.) (21.4%), ring nematode (*Criconemella* spp.) (21.4%) and lance nematode (*Hoplolaimus* spp.) (35.7%). All these genera, except *Meloidogyne* spp., were previously reported in corn fields in Sri Lanka (Ekanayake & Toida 1997). The mean population of *Pratylenchus* spp. was 2020 nematodes kg^{-1} of soil recovered from the preliminary sampling. The mean population of *Helicotylenchus* spp. (spiral nematode) had a mean abundance of 963 nematodes kg^{-1} of soil. The abundance of *Meloidogyne* spp. J2 (root-knot nematode) and *Criconemella* spp. (ring nematode) was observed to be 54 nematodes per kg of soil and 411 nematodes per kg of soil, respectively.

The prevalence percentages and diversity indices of PPN identified in 14 corn fields are presented in Table 2. The percentage of PPNs ranged from 0 to 81% of the total nematode population. Field 8 in Kelenikawewa had the highest PPN percentage of 81% followed by Field 6

(Kahatagasdigiliya) at 72%, Field 4 (Horowupotana) at 72%, Field 2 (Horowupotana) at 55% of PPN population of total nematodes. No PPN (0%) was detected in Field 10 in Ranorawa. Simpson's biodiversity index (1-D) ranged from 0 to 0.49 with Evenness from 0 to 0.25. Field 14 (Elayapaththuwa) was the richest in biodiversity among the 14 fields that were sampled as (1-D) was 0.49 with an Evenness of 0.25. Comparatively, the same field recorded an exceptionally low percentage of PPN (0.83%).

Table 1: Prevalence and abundance of plant-parasitic nematodes in the corn fields of Anuradhapura, Sri Lanka : preliminary sampling

Nematode (taxa/genus/ common name)	Prevalence [a]	Prevalence % [b]	Abundance kg^{-1} of soil			
			Mean[c]	Median [d]	Maximum	Damage level*
PPN (All genera)	13	92.9	1862	1142	8500	-
PPN Genera						
Pratylenchus (lesion)	10	71.4	2020	1038	7517	1000
Helicotylenchus (spiral)	4	28.6	963	54	3708	4000
Meloidogyne J2 (root-knot)	3	21.4	54	50	100	200
Criconemella (ring)	3	21.4	411	200	900	6000
Hoplolaimus (lance)	5	35.7	113	33	400	1500

[a] Number of positive samples/total sample count
[b] The % of positive samples/total sample count
[c] Total number of nematodes/numbers of positive samples
[d] Median for nematode abundance in positive samples
* Damage level as summarised (Olthof, 1987; Evans *et al*., 1993; Thompson *et al*., 2010; Niblack, 2014; Fleming *et al*., 2016)

Table 2: Abundance and diversity indexes of plant-parasitic nematodes in the corn fields of Anuradhapura district, Sri Lanka : Preliminary sampling

Fields	Corn variety	PPN % [a]	Most abundant PPN	Simpson biodiversity (1-D)	E (Evenness)
1	Pacific 984	31.6	*Pratylenchus*	0.00	0.00
2	Pacific 984	54.8	*Pratylenchus*	0.04	0.02
3	Pacific 999	45.9	*Pratylenchus*	0.00	0.00
4	Pacific 984	71.7	*Pratylenchus*	0.15	0.05
5	Pacific 999	26.7	*Pratylenchus*	0.00	0.00
6	Pacific 999	72.1	*Pratylenchus*	0.10	0.03
7	Pacific 999	42.6	*Pratylenchus*	0.34	0.11
8	Pacific 999	81.3	*Pratylenchus*	0.21	0.05
9	Pacific 984	44.2	*Helicotylenchus*	0.03	0.02
10	Pacific 999	0		NA	NA
11	Pacific 999	40.9	*Pratylenchus*	0.00	0.00
12	Pacific 984	5.3	*Hoplolaimus*	0.00	0.00
13	Jet	14.7	*Pratylenchus*	0.08	0.04
14	Jet	0.8	*Hoplolaimus*	0.49	0.25

[a] Plant-parasitic nematodes (PPN) % = number of PPN/total nematodes in a field

Abundance of plant-parasitic nematodes in the Anuradhapura corn fields, Sri Lanka - Preliminary sampling

The 14 sampled fields in five locations within the Anuradhapura district, Sri Lanka differed significantly (p<0.001) in total nematode number, PPN, RLN, and spiral nematode abundance (Table 3). The Kelenikawewa area was highly populated with PPN of 4804 nematodes kg^{-1} and RLN 4213 nematodes kg^{-1}. In the Kelenikawewa area, there was a low abundance of other PPN, which included *Meloidogyne*, *Criconemella*, and *Hoplolaimus* nematodes, with a combined total of 592 nematodes kg^{-1} and *Helicotylenchus* not detected. RLN abundance in Kahatagasdigiliya and Horowupotana was 1717 nematodes kg^{-1} and 1350 nematodes kg^{-1}, respectively. Of the five areas, Ranorawa (186 nematodes kg^{-1}) was the only area with RLN abundance below 500 nematodes kg^{-1} of soil. *Helicotylenchus* was the next abundant PPN with numbers varying depending on the sampling area (Table 3). The abundance of *Helicotylenchus* differed significantly (p<0.001) between the areas, with relatively low levels detected in Horawupotana (27 nematodes kg^{-1}) and Kahatagasdigiliya (17 nematodes kg^{-1}) while comparatively, the Ranorawa area had nearly 45 times greater numbers with 1236 nematodes kg^{-1}. *Helicotylenchus* spp. was not detected in the Kelenikawewa and Elyapaththuwa areas.

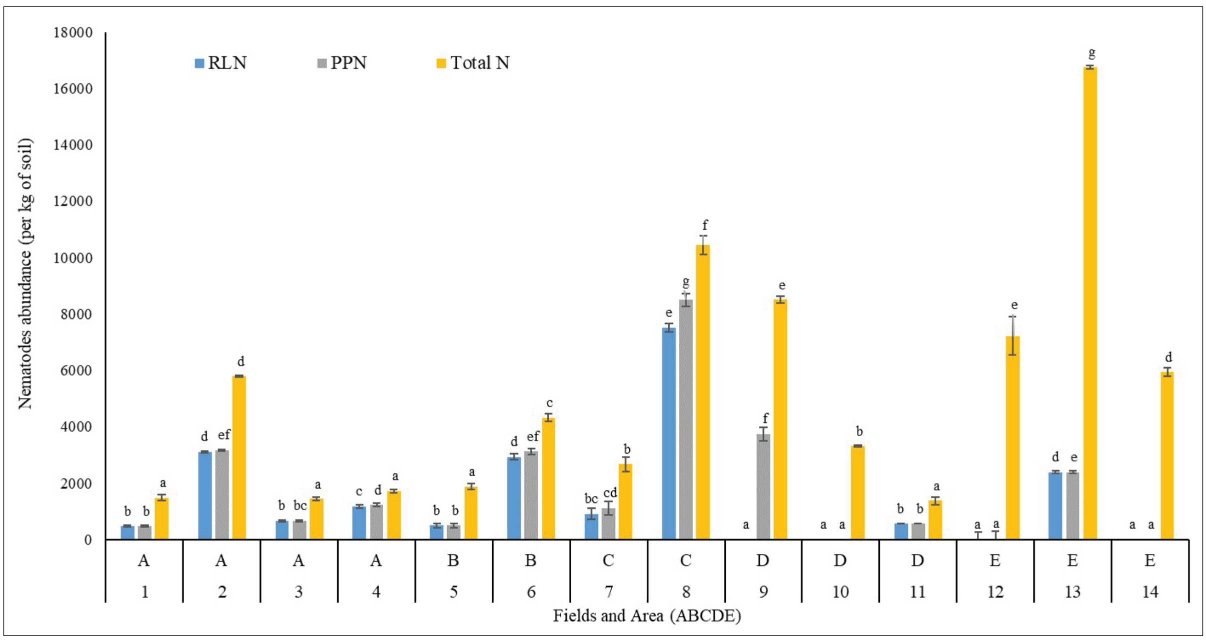

Figure 1: Abundance of plant-parasitic nematodes in the corn fields of Anuradhapura district, Sri Lanka-Preliminary sampling
The same letters on the coloured bars, representing RLN (blue), PPN (grey), and Total (yellow) nematodes in different areas and fields, are not significantly different according to the Bonferroni test at a 95% confidence level (Error bars with SEM).
Area: A-Horowupotana, B- Kahatagasdigiliya, C- Kelenikawewa, D- Ranorawa and E- Elayapaththuwa, 1-14 sampled fields numbers
PPN plant-parasitic nematodes, RLN root-lesion nematodes, and Total N- total nematodes (PPN and non-PPN)

Table 3: Abundance of plant-parasitic nematodes recorded in the sampled area of Anuradhapura district, Sri Lanka- Preliminary sampling.

Area	Mean abundance of nematodes kg^{-1} of soil (± SEM)				
	Pratylenchus spp.	*Helicotylenchus* spp.	PPN	Other PPN	Total
Horowupotana	1350 ± 219.1^{b}	27.1 ± 6.7^{a}	1377.1 ± 223.8^{a}	0 ± 0^{a}	2602.1 ± 384.9^{a}
Kahatagasdigiliya	1716.7 ± 372.3^{bc}	16.7 ± 9.4^{a}	1808.3 ± 398.8^{ab}	75 ± 24.2^{b}	3095.8 ± 377.1^{ab}
Kelenikawewa	4212.5 ± 1004^{c}	0 ± 0^{a}	4804.2 ± 1123.2^{b}	591.7 ± 122.9^{c}	6558.3 ± 1187.8^{bc}
Ranorawa	186.1 ± 66.2^{a}	1236.1 ± 429.8^{b}	1433.3 ± 407.3^{a}	11.1 ± 7.6^{a}	4391.7 ± 734.6^{ab}
Elayapaththuwa	794.4 ± 284.9^{a}	0 ± 0^{a}	801.1 ± 283.8^{a}	7.2 ± 2.2^{ab}	9973.9 ± 1192.3^{c}

Nematode abundance in 14 corn fields sampled in the Anuradhapura district, Sri Lanka is illustrated in Figure 1. Total nematode abundance between the fields was significantly different ($p<0.001$) with levels varying from 16,767 to 1,367 nematodes kg^{-1} of soil. There were five fields with lower levels of total nematode abundance per kg of soil compared to the other 9 corn fields. Nematode abundance did not differ significantly ($p>0.05$) between the following five fields with lower levels: Horowupotana Fields 1 (1475 nematodes kg^{-1}), 3 (1433 kg^{-1}) and 4 (1717 nematodes kg^{-1}), Field 5 in Kahatagasdigiliya (1875 nematodes kg^{-1}), and Field 11 in Ranorawa (1367 nematodes kg^{-1}). Higher total nematode abundance was recorded (from highest to lowest) in Field 13 from Elayapaththuwa (16,767 nematodes kg^{-1}), Fields 8 and 9 in Kelenikawewa (10442 nematodes kg^{-1}), (8500 nematodes kg^{-1}), Field 12 in Elayapaththuwa (7220 nematodes kg^{-1}), Field 2 Horowupotana (5783 nematodes kg^{-1}), and Field 6 Kahatagasdigiliya (4317 nematodes kg^{-1}). The PPN abundance differed significantly ($p<0.001$) between fields. In this survey, *Pratylenchus* spp. (RLN) was recorded as the predominating nematode in the sampled fields and its abundance differed significantly ($p<0.001$) with the highest (7517 RLN nematodes kg^{-1} of soil) in Field 8 from Kelenikawewa.

The diversity of PPN varied across the sampled fields, with different nematode genera exhibiting varying prevalence percentages. The abundance of PPNs varied among the sampled fields. Kelenikawewa exhibited the highest abundance of PPN, particularly *Pratylenchus* spp. (4213 nematodes kg^{-1} of soil) compared to other areas, potentially indicating its severe infestation. This observation is consistent with findings from previous studies indicating high populations of *Pratylenchus* spp. recorded in various crops, including corn (Schmitt & Barker, 1981; Grabau & Chen, 2016; Simon *et al*., 2018). Comparatively, the incidence and abundance of the genus *Pratylenchus* spp. in the Anuradhapura corn fields were considerably higher than those found in corn and cereal crops in other parts of the world (Thompson *et al*., 2010; Tylka *et al*., 2011; Fleming *et al*., 2016; Simon *et al*., 2018). This could be influenced by many factors such as host susceptibility (Batista da Silva, 2013; Fleming *et al*., 2016), soil and ecological conditions in the respective area (Kable & Mai, 1968; Castillo *et al*., 1996; Kandji *et al*., 2003; Govaerts *et al*., 2007; Fleming *et al*., 2016; Karuri *et al*., 2017), and crop rotation and management practices (Wang & McSorley, 2005; Thompson *et al*., 2010; Simon *et al*., 2018; Tylka *et al*., 2019).

Prevalence of the plant-parasitic nematodes associated with selected corn fields in the Anuradhapura district, Sri Lanka - Intensive sampling.

The prevalence of PPNs and their abundance at the seedling (November 2021) and harvesting stages of corn (February 2022) varied between the two samples (Table 4). The total nematode population increased from sampling in November 2021 to February 2022, with higher numbers observed at the time of harvesting (8404 nematodes kg^{-1}) compared to the seedling stage (1689 nematodes kg^{-1}). The prevalence of *Pratylenchus* spp. at the seedling stage (SS) of corn was 81%, with 13

Table 4: Prevalence and abundance of plant-parasitic nematodes in selected corn fields in the Anuradhapura district, Sri Lanka- Intensive sampling.

Nematode (taxa/genus/ common name)	Prevalence [a]		Abundance kg^{-1} of soil					
			Mean [b]		Median [c]		Maximum	
	SS	HS	SS	HS	SS	HS	SS	HS
PPN (All genera)	16 (100)	16 (100)	854.16	5545.30	825.01	4983.33	1433.34	8416.68
PPN Genera								
Pratylenchus (lesion)	13 (81)	16 (100)	727.08	4338.54	641.68	3893.75	1270.84	6675.00
Helicotylenchus (spiral)	5 (31)	15 (93.75)	81.95	873.95	50.00	754.18	116.66	2429.16
Criconemella (ring)	9 (56)	14 (87.5)	113.55	245.84	106.26	262.51	145.84	366.66
Hoplolaimus (lance)	6 (38)	14 (87.5)	100.00	187.33	104.16	98.95	104.16	504.18

SS- Sampling at the seedling stage of corn (November 2021)
HS- Sampling at the harvesting stage of corn (February 2022)
[a]No. of positive samples/total sample count and the values in parenthesis are in percentages
[b]Total no. of nematodes/no. of positive samples
[c]Median is for nematode abundance in positive samples

out of 16 composite samples from four fields showing its presence. However, at the harvesting stage (HS), the prevalence increased to 100%, as observed in all 16 composite samples collected from the same four fields sampled. *Helicotylenchus* spp. prevalence at SS and HS were 31% and 94%, respectively. *Criconemella* spp. was 56% and 87.5% and *Hoplolaimus* spp. 38% and 87.5% at the SS and HS, respectively. The mean abundance of the nematodes increased from the SS to the HS of corn irrespective of genera. *Pratylenchus* was the most abundant PPN genus in all the fields at both sampling times. However, sampling at HS yielded an abundance of *Helicotylenchus* (874 nematodes kg^{-1}). *Criconemella* (113 and 246 nematodes kg^{-1}) and *Hoplolaimus* (100 and 187 nematodes kg^{-1}) were observed in all the fields sampled. The genus *Meloidogyne* spp. (J_2 stage) was not observed in any fields sampled.

The abundance and reproduction factor of plant -parasitic nematodes associated with corn at the seedling and harvesting stage in the Anuradhapura district, Sri Lanka - Intensive sampling

Nematode abundance in the four selected corn fields were sampled in November 2021, and February 2022; abundance was greater in HS compared to SS (Table 5). The mean abundance of the total nematodes was significantly different (p<0.001) among the fields at both times of sampling. The HS yielded a higher abundance compared to the SS. The mean of the total nematode population at the SS (pi) and HS (pf) were at Kelenikawewa (2996, 19075 nematodes kg^{-1}), followed by Horowupotana (2258, 7321 nematodes kg^{-1}), Elyapaththuwa (796, 3415 nematodes kg^{-1}) and then Kahatagasdigiliya (450, 2890 nematodes kg^{-1}), respectively. The abundance of the PPN and *Pratylenchus* was also significantly different (p<0.001) in the four fields sampled. Plant-parasitic nematodes abundance at the SS in all the fields were lower than 1000 nematodes kg^{-1} of soil except in Kelenikawewa (2100 nematodes kg^{-1}). However, at the HS, the PPN abundance of all the fields was recorded above 2000 PPN kg^{-1}. The highest PPN abundance was recorded at Kelenikawewa (11017 nematodes kg^{-1}), followed by Horowupotana (6017 nematodes kg^{-1}), Elyapaththuwa (2535 nematodes kg^{-1}), and Kahatagasdigiliya (2223 nematodes kg^{-1}).

In all the fields, the *Pratylenchus* population was as high as the PPN abundance in the respective fields. The mean abundance of *Pratylenchus* at the SS Kelenikawewa field was 1863 nematodes kg^{-1} and Horowupotana field 500 nematodes kg^{-1} but in the fields of Kahatagasdigiliya (204.2 nematodes kg^{-1}) and Elayapaththuwa (88 nematodes kg^{-1}) RLN abundance was below 500 kg^{-1} of soil. At the HS, the *Pratylenchus* abundance in all four fields were above 1000 nematodes kg^{-1}. The highest *Pratylenchus* abundance was recorded with a 5-fold increase compared to the SS at the Kelenikawewa field (9863 nematodes kg^{-1}). Comparing the Kelenikawewa field with other fields the mean population of *Pratylenchus* was lower but above 1000 nematodes kg^{-1} of soil as follows: Horowupotana 3925 nematodes kg^{-1}, 7.9 fold increased), Elayapaththuwa 2000 nematodes kg^{-1}, 22.9 fold increased, and Kahatagasdigiliya 1567 nematodes kg^{-1}, 7.7 fold increased. These findings indicated that when corn matures the abundance of *Pratylenchus* also increases. All types of nematodes, except for spiral nematodes, were recorded in high abundance in the Kelenikawewa field, and the next most prevalent was Horowupotana. A high *Helicotylenchus* population was recorded in the Horowupotana field (1767 nematodes kg^{-1}) at the HS compared to other fields.

Intensive sampling in subsequent seasons was carried out on fields with the highest *Pratylenchus* abundance, considering the potential severity of its infestation in these specific areas. Similarly, McDonald & Nicol (2005) and McDonald *et al*. (2017) reported that *Pratylenchus* spp. is sporadic when their numbers are substantially higher with high prevalence in corn fields. Analysis of PPN abundance at different stages of corn growth revealed an increase in population from the SS to the HS. The studies conducted by Simon *et al*. (2018); Chowdhury *et al*. (2020); Han *et al*. (2021), & Thapa *et al*. (2023) indicated that sampling at different corn growth stages could alter the nematode abundance. However, Thapa *et al*. (2023) observed higher PPN abundance in corn fields at the HS than in the SS, as we also discovered in this study. In contrast, Simon *et al*. (2018) stated that the SS would be better for recovering most of the PPNs in terms of diversity. However, this study indicates the diversity and abundance were higher at the HS than at SS in corn fields in Sri Lanka.

The reproductive factor (Rf) of *Pratylenchus* was calculated (Rf=pf/pi) using the mean initial population (pi) at the SS and the mean final population (pf) at the HS of corn. The *Pratylenchus* multiplications were Kelenikawewa 5.3, Horowupotana 7.9, Kahatagasdigiliya 7.7, and Elayapaththuwa 22.9. However, the abundance of *Pratylenchus* in the Kelenikawewa field (9863 abundance kg^{-1}) was 2.5 times higher than in the Horowupotana (3925 nematodes kg^{-1}) and 5 times higher than the other two fields.

The population of PPN and *Pratylenchus* spp. at Elayapaththuwa and Kahatagasdigiliya locations had lower levels of nematodes in February (2022) compared to the previous year (February 2021). PPN populations in the Kelenikawewa and Horowupotana field had increased (Figure 2). The results indicated that there was a population increase of 31.2% (2021 February- 7517 nematodes kg^{-1} and in 2022 February- 9863 nematodes kg^{-1}) of *Pratylenchus* spp. at the Kelenikawewa field compared to the previous year. The mean population of *Pratylenchus* spp. in both seasons (February 2021 and February 2022) showed a 26.3% increase in Horowupotana (3108 and 3925 nematodes kg^{-1}). However, the population of *Pratylenchu*s spp. decreased by 19.2% and 46.6% in Elayapaththuwa (2383 and 2000 nematodes kg^{-1}) and Kahatagasdigiliya (2933 and 1567 nematodes kg^{-1}) fields, respectively (Figure 2). These population changes could be influenced by soil characteristics such as texture, structure, pH, moisture content, and nutrient contents, which are known to be major contributors to the change in population density and dissemination of nematodes (Kandji *et al*., 2003; Fleming *et al*., 2016). Also, climatic factors such as rainfall, and temperature have impacts on the population densities of PPNs (Govaerts *et al*., 2007; Karuri *et al*., 2017). Thus, further studies need to be conducted to determine the relationship of soil and environmental characteristics on nematode populations.

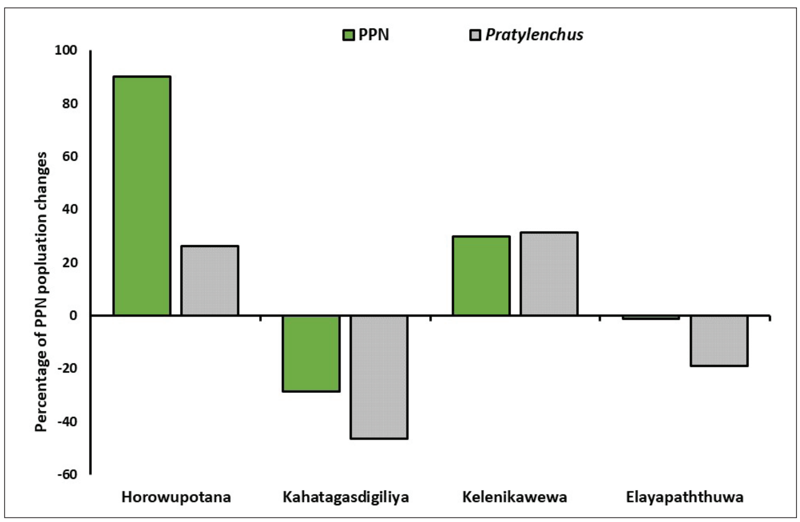

Figure 2: Changes in abundance (%) of plant-parasitic nematodes in 2022 February compared to 2021 February in four sampled fields in the Anuradhapura district. PPN-plant-parasitic nematodes, *Pratylenchus*- root-lesion nematodes

Pratylenchus spp. were the most abundant PPN genus throughout both sampling periods, indicating its persistence and potential threat to corn production in Sri Lanka. The Rf of *Pratylenchus* was substantially higher compared to other nematode genera in most fields, indicating its greater reproductive potential. In the Anuradhapura district, corn is cultivated as a rainfed crop where crops get zero irrigation and are completely reliant on rainfall. In the *Maha* season (September to March) the Anuradhapura district receives 49% (615 out of 1255 mm) of the average annual rainfall from October to December (Climate Data, 2023). There is limited information available in Sri Lanka regarding the susceptibility of corn varieties used against PPNs in the sampled areas of Anuradhapura, Sri Lanka. Furthermore, there are limitations for soil testing facilities to assess soil physiochemical properties or to evaluate the relationship of these soil factors with PPN populations in Sri Lanka. Therefore, due to the lack of information and facilities, corn farmers in the Anuradhapura district continuously cultivated corn for more than a decade without implementing any crop rotation practices, driven only by market demand. Additionally, their practice includes growing the same corn varieties for multiple seasons. As reports indicate, this type of cropping practice could lead to a severe buildup of PPNs (Arjun *et al*., 1983). Therefore, this could be the reason behind the high levels of PPNs, especially *Pratylenchus* nematodes.

This research could not compare the population levels of *Pratylenchus* nematodes, which causes economic losses to the Sri Lankan corn industry. Nevertheless, findings from Thompson *et al*. (2010), Tylka *et al*. (2011), Simon *et al*. (2018), Chowdhury *et al*. (2020), and Niblack (2014) have reported generalised threshold levels for *Pratylenchus*: up to 100 RLN kg^{-1} of soil with no significant damage to the crops, above 100 to 250 RLN kg^{-1} of soil with minor damage, and 250 to 500 RLN kg^{-1} of soil with a moderate level of damage. Therefore, their findings demonstrate that severe damage to corn could arise from an RLN population between 500 to 1000 RLN kg^{-1} of soil or any number above 1000 kg^{-1} of soil. Further, it suggests that RLN levels were above the levels mentioned by previous authors and could lead to a severe economic threat to the Sri Lankan corn industry. Further investigations are necessary to assess the true extent of the damage. Additional studies should focus on developing a threshold level specific to the corn-growing regions of Sri Lanka and evaluating the pathogenicity and reproduction of RLN in corn varieties commonly used in Sri Lanka.

CONCLUSION

This study highlights the widespread presence of PPNs in corn-producing areas of the Anuradhapura district, with *Pratylenchus* spp. emerging as the predominant genus. Variations in PPN abundance and diversity were observed, with some areas, such as Kelenikawewa, exhibiting higher infestation levels. *Pratylenchus* spp. had a higher reproductive potential than other PPN genera observed in this study. Temporal analysis revealed dynamic trends in nematode population, with some fields increasing while others declining. These fluctuating nematode dynamics emphasised the need for continuous monitoring and adaptive management strategies. Overall, the findings highlight the importance of nematode management strategies to mitigate the impact of PPNs on corn yields and ensure sustainable production in the region. Further research focusing on the primary factors driving PPN population dynamics would be essential for the development of targeted and sustainable management practices.

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