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Occurrence, diversity and relative abundance of plant-parasitic nematodes: A survey of selected crop fields in Akure, Southwestern Nigeria

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Abstract

Information is scanty on the occurrence and population dynamics of Plant-Parasitic Nematodes (PPNs) in most cultivated fields in Nigeria. To this end, a study was designed to provide this information. Soil samples were collected randomly from crop fields (maize, rice, sugarcane, plantain, cassava, cowpea, cocoa and fallow land). The soil collection was with a core sampler at a distance of 10-15 cm from the plant root zone and at an approximate depth of 15-30 cm. The Baermann's method was adopted to extract nematodes from a 100 g representative soil sample. Extracted nematodes were identified to the genus level. All data collected were subjected to statistical analysis and mean separation. Results showed that a significantly highest nematode population, 791.66, was obtained from the fallow land, while the cocoa field was next, 580.00. The rice field had significantly lowest population of 125.00. In all, 17 genera, in 12 families, were identified and *Meloidogyne* spp. was the most frequently isolated and most abundant, *Tylenchulus* spp. was the least. Furthermore, the plantain field had the most diverse genera, 10.20, while the rice field had the least, 2.00. PPNs occur and are widely distributed in the study area.

Keywords: *Plant-Parasitic Nematodes (PPNs), Relative abundance, Crop fields, Fallow land, Meloidogyne spp*

1. Introduction

Plant-Parasitic Nematodes (PPNs) constitute a major threat to sustainable crop production and food security globally. Annual crop loss from nematode infestation has been estimated to be about 12.3%, with a monetary value of $157 bn (Singh *et al*., 2015). In Africa, PPNs pose a significant threat to crop production, due to the extensive damage and yield losses they cause to a wide range of crops (Bridge *et al*., 2005). Most staple crops in the continent are known to be susceptible to at least one or more of these parasites. Rice (*Oryza* sativa.), maize (*Zea mays*), and cassava (*Manihot esculenta*) occupy an important place in the diet of most Africans. Unfortunately, nematode infestation and associated yield losses have been reported for all of these crops.

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Gnamkoulamba *et al*. (2018) isolated 12 genera of PPNs, *Meloidogyne* and *Helicotylenchus* being the most abundant, in rice fields in Togo. In South Africa, Mc Donald *et al.* (2017) reported on several genera of nematodes that infested maize roots. *Meloidogyne javanica* and *M. incognita* (root-knot nematodes) were the most abundant. A total of 16 genera of PPNs were isolated from soil under cassava cultivation in Cote d'Ivoire. Six of the genera were also found to infect the roots directly. *Gracilis* and *Meloidogyne* were the most frequently encountered in the two cassava production areas surveyed (Regis *et al*., 2020). Nematode infestation can cause physical distortion or alteration in the genetic make-up of the host plant. Their direct and indirect damage activities result in delayed crop maturity, high production costs, crop failure, yield and income loss (Sikora and Fernández, 2005; and Onkendi *et al*., 2014). Additionally, attacks by nematodes can predispose the host pants to infection by pathogenic organisms (De Waele and Elsen, 2007).

Most farmers in rural Africa have little knowledge of the existence of these parasites in their fields. Consequently, the first step towards the development of effective and lasting management strategies is the provision of information on their presence and relative abundance in crop fields. The aim of this study is to provide this information, while the objectives were to: (i.) evaluate soil from cultivated fields and fallow lands in Akure, Ondo State, for the presence of PPNs; and (ii.) determine the most frequently occurring genera and their relative abundance.

2. Materials and methods

2.1. Study location

The laboratory study was conducted at the Federal University of Technology, Akure (FUTA), while samples were collected from Akure south local government. Akure is located at an altitude of 332 m above sea level, and latitude 7°07'N to 7°37'N and longitude 5°06'E to 5°38'E in the rain forest agroecology of South-Western Nigeria. The nematode isolation and identification process were carried out in the pathology laboratory of the Department of Crop, Soil, and Pest Management, FUTA.

2.2. Sample collection

Soil samples were obtained from cultivated fields of different crop types (maize, rice, sugarcane plantain, cassava, cowpea and cocoa) and one fallow land. The sampling method employed was the random sampling pattern described by Coyne *et al.* (2007). Three (3) farms, replicates, were visited for each crop and three sub-samples were collected from each farm, to make a total of nine composite samples for each crop field (treatment) and 72 for all treatments. Soil samples were collected on each site, using a soil core sampler, at a distance of 10-15 cm from the plant root zone and at an approximate depth of 15-30 cm. The samples were collected from land areas measuring 5 m × 5 m (25 m^2) in each location. The weight of soil for each sample was 1 kg. They were sealed in polythene bags, protected from the sun to prevent moisture loss, labeled and transported to the laboratory in ice packs. Each composite sample was mixed thoroughly and passed over a 10 mm diameter mesh sieve to remove stones and debris.

2.3. Extraction of nematodes from the soil samples

The Baermann-funnel method of nematode extraction, as described by Cesarz *et al*. (2019), was adopted with slight modifications. The funnel was replaced with perforated plastic of 8 cm diameter and 6 cm depth, while the filter was two-layered serviette papers. One hundred (100) g of soil, from each composite sample, was transferred to the serviette papers within the perforated plastic and suspended in a shallow container having 100 ml of water. The extraction period was 48 h, after which nematode suspension was obtained from each treatment. Centrifugation was done and the supernatant was decanted, leaving 10 ml of nematode suspension in labeled test tubes.

2.4. Killing, fixing and staining of extracted nematodes

This was done using the hot fixative method as described by Ryss (2017). Extracted nematodes in test tubes were fixed using formalin solution (37% formaldehyde). The heating temperature was 70°C in a water bath. Two (2) ml of the heated formalin solution was added to 10 ml of extracted nematodes in each treatment, following the procedure described by Van Bezooijen (2006). The staining of fixed nematodes was with 0.2 ml lactophenol.

2.5. Microscopy, counting and identification of isolated nematodes

Extracted nematodes were viewed, at X250 magnification, using an AmScope® microscope equipped with a 5.0-megapixel camera and connected to a laptop computer. One (1) ml of the heat-fixed and stained nematode was obtained, with a pipette, from the suspension of each treatment, and transferred into a calibrated hollow slide. The identification of isolated nematodes was based on guides from standard texts (Coyne *et al*., 2007) and pictorial keys (Eisenback, 2002; and Mekete *et al*., 2012). Counting and identification were done in triplicate for each crop field and the fallow land.

2.6. Data collection, experimental design and statistical analysis

Data were collected on the following;

i. The population of nematodes in all the treatments evaluated

   The total number of nematodes in the 1 ml representative aliquot of each replicate was counted. The values obtained were extrapolated, using simple mathematical relationships, to obtain the total population of nematodes in 100 g of the soil sample from which the extraction was done.

ii. Distribution and types of nematodes in all treatments.

   The nematodes isolated from each sample were identified, up to the genus level, and recorded. The criteria for identification were; length of the body, width and orientation of the body and shape of the tail and head. The type of stylet and reproductive structures were other very important factors that were taken into consideration.

iii. The relative abundance of nematode genera (100 g of soil sample) in each treatment evaluated

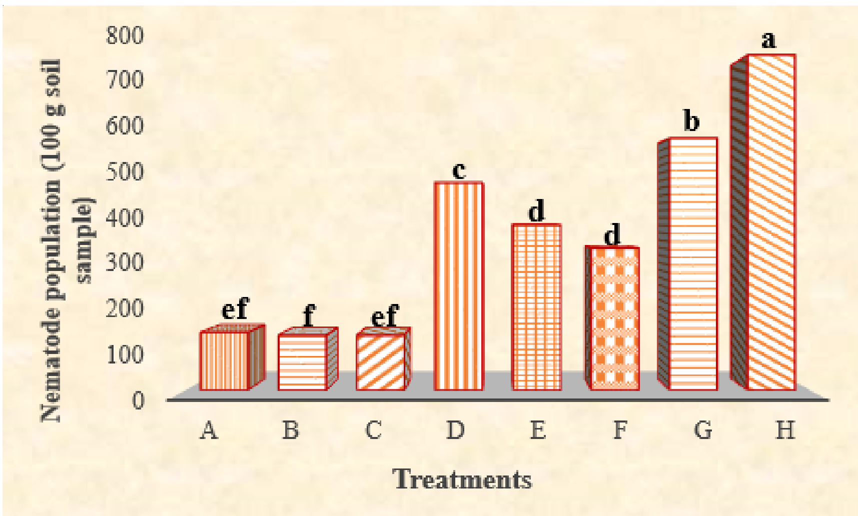
   The number of genera and the population of each genus isolated from each treatment were counted and compared to determine their relative abundance.

The experimental design adopted was a Completely Randomized Design (CRD) and all treatments were replicated thrice. Data collected were subjected to Analysis of Variance (ANOVA) where applicable, using Minitab software (version 17) and the means were separated using Tukey's test at a 5% level of probability.

3. Results

3.1. Total population of all isolated nematode genera (100 g of soil sample) in all the treatments evaluated

Results from the study showed that PPNs were present in all the treatments evaluated, but most of them differed significantly in terms of the total population isolated (Figure 1). The fallow land had the highest



Note: Key: A = Maize, B = Rice, C = Sugarcane, D = Plantain, E = Cassava, F = Cowpea, G = Cocoa, and H = Fallow land

Figure 1: Total population of isolated nematodes in each treatment

nematode population, 791.66, among the treatments. This value was statistically significant (Figure 1). The cocoa field was next, 580.00 nematodes were isolated from it. This value was 26.73% less than the fallow land but was significantly higher than all the other treatments.

Table 1: Nematode genera isolated across all treatments (cultivated field and grassland)

Nematode genera	Common name	Family
Anguina spp.	Seed gall nematodes	Anguinidae
Aphelenchoides spp.	Leaf and bud nematodes	Aphelenchoididae
Bursaphelenchus spp	Pine wilt nematodes	Parasitaphelenchidae
Gracilacus spp.	Pin nematodes	Tylenchulidae
Helicotylenchus spp.	Spiral nematodes	Hoploliamidae
Hemicycliophora spp.	Sheath nematodes	Criconematidae
Heterodera spp.	Cyst nematodes	Heteroderidae
Hoplolaimus spp.	Lance nematodes	Hoploliamidae
Meloidogyne spp.	Root-knot nematodes	Heteroderidae
Paratylenchus spp.	Pin nematodes	Tylenchulidae
Pratylenchus spp.	Lesion nematodes	Pratylenchidae
Radopholus spp.	Burrow nematode	Pratylenchidae
Rotylenchulus spp.	Reniform nematode	Hoploliamidae
Scutellonema spp.	Yam nematode	Hoploliamidae
Tylenchorhynchus spp.	Stunt nematode	Belonolaimidae
Tylenchulus spp.	Citrus root nematode	Tylenchulidae
Xiphinema spp.	Dagger nematode	Longidoridae

A significantly lowest nematode population of 125.00 was obtained from the rice field. The maize and sugarcane fields also had low nematode populations and the values were statistically similar (149.00 and 200.00 respectively). The cowpea and cassava fields had 321.00 and 376.00 respectively and were statistically similar (Figure 1).

All isolated nematodes were identified as belonging to 17 genera and 12 families (Tables 1 and 2). The list included; *Aphelencoides* spp. (Rice leaf nematode), *Heterodera* spp. (Cyst nematode), *Meloidogyne* spp. (Root-knot nematode) and *Radopholus* spp. (Burrow nematode) amongst others. The distribution pattern of these genera showed that all treatments had at last one genus (Table 2.). The plantain field had the highest diversity of nematodes, 10.20 genera (Figure 2). This value was, however, not significantly different from the maize and cocoa fields that both had 10.00 genera. The sugarcane, cassava, cowpea and fallow land all had 9.00 genera, while the rice field had the least diversity of nematodes. Only 2.00 genera were isolated from it. (Figure 2).

3.2. Relative abundance of isolated nematode genera in the treatments evaluated

The isolated nematode genera and their population differed across the eight treatments evaluated. In the maize field, *Meloidogyne* spp. (Figure 3e) was the most abundant. The total number isolated was 26.70 (Table 4). This value was significantly the highest. It was followed by *Rotylenchus* spp. (Figure 3f). Only 20.00 were isolated. On a general note, nematode infestation was low in the maize field. Only two nematode genera were isolated from the rice field, *Scutellonema* spp. (Figure 3g) and *Meloidogyne* spp. having 116.67 isolates. The

Table 2: Occurrence/Distribution of nematodes within the cultivated fields

Isolated nematode	Cultivated fields							
	A	B	C	D	E	F	G	H
Anguina spp	†	–	†	–	–	–	–	–
Aphelenchoides spp	–	–	–	†	†	–	–	–
Bursaphelemnchus spp	–	–	†	†	–	–	†	†
Gracilacus spp	–	–	–	†	†	–	–	†
Helicotylenchus spp	†	–	†	†	†	†	†	†
Hemicycliophora spp	–	–	–	–	–	†	†	–
Heterodera spp	†	–	–	†	†	†	†	†
Hoplolaimus spp	†	–	†	–	–	†	–	–
Meloidogyne spp	†	†	†	†	†	†	†	†
Paratylenchus spp	†	–	–	†	–	–	†	–
Pratylenchus spp	–	–	†	†	†	†	†	†
Radopholus spp	–	–	†	†	–	†	–	–
Rotylenchus spp	†	–	†	–	†	†	†	†
Scutellonema spp	–	†	†	†	†	–	†	–
Tylenchoryhchus spp	†	–	–	–	†	†	†	†
Tylenchulus spp	†	–	–	–	–	–	–	–
Xiphinema spp	†	–	–	–	–	–	–	†

Note: Keys: † indicates nematodes presence indicates nematodes absence; Key: A = Maize, B = Rice, C = Sugarcane, D = Plantain, E = Cassava, F = Cowpea, G = Cocoa, and H = Fallow land.

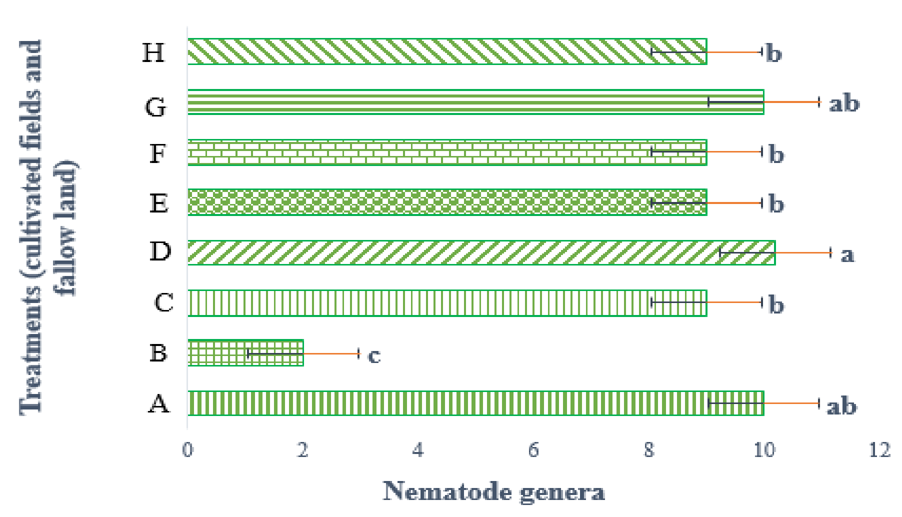


Figure 2: The total number of isolated genera of nematodes in each treatment evaluated

Note: Key: A = Maize, B = Rice, C = Sugarcane, D = Plantain, E = Cassava, F = Cowpea, G = Cocoa, and H = Fallow land sugarcane field had nine isolated genera with *Meloidogyne* spp. having the significantly highest number of 50.00 (Table 4). This value was however not significantly different from 40.67 isolates of *Helicotylenchus* spp. (Table 4, Figure 3c). The plantain field had 10 isolated genera. Once again, *Meloidogyne* spp. had the significantly highest population of 230.00 isolates, making it the most abundant. *Helicotylenchus* spp. and *Pratylenchus* spp. had 70.67 and 70.33 isolates respectively. The two values were similar statistically but differed significantly from that of *Meloidogyne* spp. The cassava field had nine isolated genera, out of which *Meloidogyne* spp. had 157.67 isolates. This value was once again the highest and differed significantly from the other genera. *Helicotylenchus* spp., 76.70 isolates, and *Pratylenchus* spp., 70.00 isolates, were not different statistically but were the next most populous. 10 genera were isolated from the cowpea field. The number of *Meloidogyne* spp. was 120.00 and it was once again the most populous. *Pratylenchus* spp. had 90.00 isolates and was next to *Meloidogyne* spp. while *Heterodera* spp. (Figure 3d) had 26.67 isolates. It was the third most populous (Table 4). In a similar pattern to the cowpea field, the cocoa field had ten genera isolated from it. The population of *Meloidogyne* spp. was 300.33. It was statistically the highest in the treatment and the second most populous across all treatments. The treatment also had the highest isolate of *Heterodera* spp. (76.70) across all treatments. *Pratylenchus* spp. (70.00 isolates) and *Helicotylenchus* spp. (63.33 isolates) were third and fourth most populous respectively. The two values were similar statistically. The fallow land had the highest populations of *Meloidogyne* spp. (350.00 isolates), *Heterodera* spp. (190.33 isolates), *Pratylenchus* spp. (110.00 isolates) and *Bursaphelenchus* spp. (63.33 isolates).

On a general note, *Meloidogyne* spp. was the most commonly isolated and the most abundant with the significantly highest population in all treatments. *Helicotylenchus* spp., *Heterodera* spp. and *Pratylenchus* spp. were also widely distributed among treatments with reasonable populations. *Aguina* spp., *Aphelenchoides* spp. and *Tylenchorhynchus* spp. (Figure 3h) where some of the least encountered with very low populations. The same is true for *Tylenchulus* spp. and *Xiphinema* spp. (Table 4).

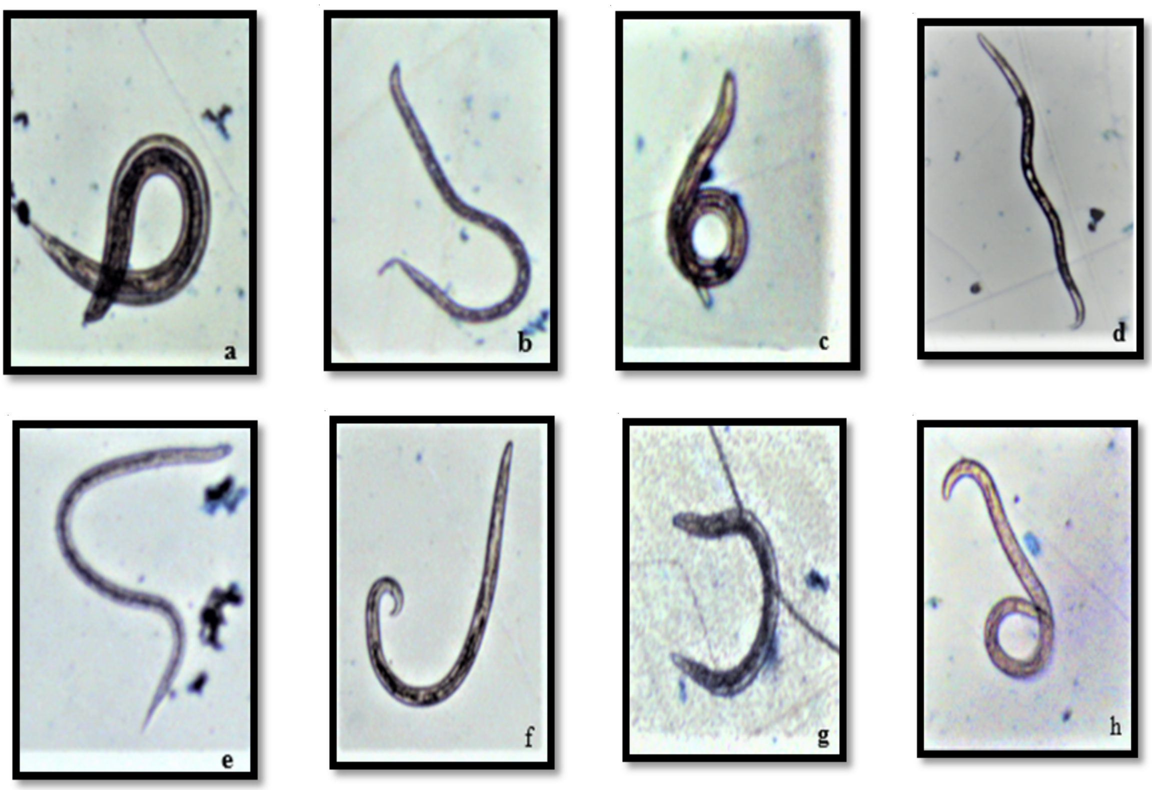


Figure 3: Microscopic image of some of the isolated nematode genera: (a) *Anguina* spp. **(b)** *Aphelenchoides* spp. **(c)** *Helicotylenchus* spp. **(d)** *Heterodera* spp. **(e)** *Meloidogyne* spp. **(f)** *Rotylenchus* spp. **(g)** *Scutelonema* spp. **(h)** *Tylenchorhynchus* spp.

Table 4: Relative abundance (100 g of soil sample) of nematode species isolated across each treatment

Isolated nematode	Treatments							
	A	B	C	D	E	F	G	H
Anguina spp.	16.70bc	0.00c	10.00e	0.00e	0.00d	0.00f	0.00f	0.00g
Aphlenchoides spp.	0.00d	0.00c	0.00f	10.00de	10.00d	0.00f	0.00f	0.00g
Bursaphelenchus spp.	0.00d	0.00c	20.00cd	13.30de	0.00d	0.00f	10.00e	63.33d
Gracilacus spp.	0.00d	0.00c	0.00f	10.00de	10.00d	0.00f	0.00f	10.00f
Helicotylenchus spp.	13.30bc	0.00c	40.67a	70.67b	76.70b	40.33c	63.33c	10.67f
Hemicycliophora spp.	0.00d	0.00c	0.00f	0.00e	0.00d	10.00ef	10.33de	0.00g
Heterodera spp.	16.00bc	0.00c	0.00f	33.30c	26.67c	26.67d	76.70b	190.33b
Hoplolaimus spp.	10.00c	0.00c	10.00e	0.00e	0.00d	10.00ef	0.00f	0.00g
Meloidogyne spp.	26.70a	116.67a	50.00a	230.00a	157.67a	120.00a	300.33a	350.67a
Paratylenchus spp.	10.67bc	0.00c	0.00f	10.00de	0.00d	0.00f	10.33de	0.00g
Pratylenchus spp.	0.00d	0.00c	30.67b	70.33b	70.00b	90.00b	70.00bc	110.00c
Radopholus spp.	0.00d	0.00c	10.00e	10.00de	0.00d	10.33e	0.00f	0.00g
Rotylenchus spp.	20.00ab	0.00c	20.67c	0.00e	10.00d	10.00ef	20.00d	10.67f
Scutellonema spp.	0.00d	10.00b	10.33de	20.67cd	10.00d	0.00f	10.00e	0.00g
Tylenchorhynchus spp.	10.00c	0.00c	0.00f	0.00e	10.00d	10.00ef	10.00e	10.67f
Tylenchulus spp.	10.00c	0.00c	0.00f	0.00e	0.00d	0.00f	0.00f	0.00g
Xiphinema spp.	10.00c	0.00c	0.00f	0.00e	000d	0.00f	000f	16.00e

Note: Key: A = Maize, B = Rice, C = Sugarcane, D = Plantain, E = Cassava, F = Cowpea, G = Cocoa, H = Fallow land

4. Discussion

Results from this study showed a widespread distribution of PPNs across all the treatments evaluated. This is a confirmation of the ubiquitous nature of these groups of organisms. Previous research work has shown that PPNs are widely distributed across all ecological zones and in different soil types (Munawar *et al*., 2018; Upadhaya *et al*., 2019; and Pulavarty *et al*., 2021). It is interesting to note that some nematodes have been reported to survive in very dry soil where conditions are hostile and inhospitable by employing anhydrobiotic survival strategies (Treonis and Wall, 2005). Seventeen genera were identified in the eight treatments evaluated. The large number of genera in such a few numbers of treatments is another testament to the highly diverse nature of PPNs. Similar findings have been reported previously. In a survey of field and vegetable crops in Jordan, Karajeh and Al-Ameiri identified 11 genera in 10 families (Karajeh and Al-Ameiri, 2010). In Nigeria, Duru *et al*. (2015) surveyed yam farms in Awka-North local government in Anambra state and reported that seven genera were isolated from only one treatment, yam farm. A similar finding was reported on nematodes associated with strawberries in Prana, Brazil (Krezanoski *et al*., 2020). *Meloidogyne* spp. (the root-knot nematodes) was the most widely distributed, most commonly encountered and most abundant in all the treatments evaluated. Several studies have reported similar findings on the wide geographical distribution of the genera (Khan *et al*., 2005; Onkendi *et al*., 2014; and Schwarz *et al*., 2020). This is due largely to the wide host range of species that make up the genera. In Africa, several staple crops have been reported to be host to one or more species of *Meloidogyne*. *Meloidogyne incognita, M. javanica, M. enterolobii* and *M. arenaria* have all been reported to be important pests of yam (Kolombia *et al*., 2017). The susceptibility of some maize cultivars to infestation by

M. incognita and *M. javanica* in South Africa was reported by Ngobeni *et al*. (2011). In Egypt, Banora and Almaghrabi (2019) evaluated selected tomato genotype for susceptibility to infestation by *M. javanica* and reported on the susceptibility of three of the genotypes. Aside from *Meloidogyne*, three other genera isolated in this study, *Heterodera, Pratylenchus,* and *Xiphinema* have been reported to be among the 10 top PPNs of scientific and economic importance globally (Jones *et al*., 2013). The fallow land had the highest overall nematode population and the highest population of *Meloidogyne*, *Heterodera* and *Pratylenchus* genera among all the treatments evaluated. This may be due to the high diversity of plants in this treatment. The relatively stable nature of the treatment may have aided the population growth of these nematode genera (Eche *et al*., 2013). The results from the fallow land indicate that weeds / uncultivated plants also susceptible to nematode infestation and can be very important alternate or collateral hosts of the parasites. This view is validated by reports from literatures (Kokalis-Burelle and Rosskopf, 2012; Giraldeli *et al*., 2017 and Rocha *et al*., 2021). It also points to the fact that land fallowing can bring about a build-up in the population of diverse plant-parasitic nematode genera. This may result in significant crop failure and yield loss if susceptible crops are planted after the fallow period. The rice field presented an interesting scenario. Contrary to the report of Namu *et al.* (2018) about the predominance of different nematode genera in rice fields, only two genera were isolated from the evaluated fields, namely; *Meloidogyne* and *Scutellonema*. Additionally, *Hirschmanniella and Aphelenchoides* which are well-known parasites of the crop (Coyne *et al*., 2000; Udo *et al*., 2011; and Thio *et al*., 2017) were absent. It is not clear what may be responsible for this, but an investigation revealed that one of the fields was put under rice cultivation for the first time, and the rice plants were only a few weeks old at the time of sample collection. The excess water may also have brought about a decline in nematode diversity. Finally, the diversity of weed population and the stable nature of sugarcane, cocoa and plantain fields, being perennial crops, may have accounted for the diversity of nematode genera isolated from the treatments.

5. Conclusion

Results from this study showed that each treatment is infested by at least two or more PPNs. Species belonging to the genera *Meloidogyne, Heterodera*, *Pratylenchus* and *Helicotylenchus* were the most abundant and widespread. Crop history, agronomic practices and the non-adoption of nematode management strategies may have contributed to the diversity and population structure. The presence of these parasitic nematodes, even at a low population, in the treatments evaluated is worrisome. This is because a large population build-up can occur within a short period if susceptible crops are planted. It can be recommended, therefore, that appropriate and adequate nematode management strategies be included and adopted by farmers, and concerned individuals, in their crop production plan within the study area of Akure. Other locations with similar agro-ecological conditions and crops may need to adopt these management strategies as well.

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