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Plant Parasitic Nematodes Associated with Three African Indigenous Vegetables in Southwest Nigeria

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ABSTRACT

Background and Objective: African indigenous vegetables are rich in nutrients and medicinal properties that are important for health and vitality, but their availability is on the decline partly due to attacks by plant parasitic nematodes, hence field surveys were conducted in 2016 and 2017 to investigate the distribution of plant parasitic nematodes and their interactions with free-living nematodes in fields planted to three African indigenous vegetables in Southwest Nigeria. **Materials and Methods:** A total of 180 soil samples were taken from 180 farms in all Local Government Areas (LGAs) visited in the four States in Southwest Nigeria. Samples were taken from three vegetable fields in all the LGAs visited. Nematodes were extracted from 200 mL sub-samples; the nematodes were counted and identified under a compound microscope using a pictorial guide. **Results:** The results showed that 16 genera of plant parasitic nematodes were found associated with *Amaranthus cruentus, Solanum macrocarpon and Telfairia occidentalis*. *Meloidogyne*, *Helicotylenchus*, *Rotylenchulus*, *Xiphinema*, *Pratylenchus* and *Hoplolaimus* were the most abundant nematode species encountered in vegetable fields in the study areas. The correlation analysis shows an antagonistic relationship between free-living nematodes and plant parasitic nematodes. **Conclusion:** There is a need to cultivate vegetable crops to suppress the populations of plant parasitic nematodes in Southwest Nigeria.

KEYWORDS

Free living nematodes, plant parasitic nematode, *Telfairia occidentalis*, *Amaranthus cruentus*, *Solanum macrocarpon*

INTRODUCTION

Telfairia occidentalis, *Solanum macrocarpon* and *Amaranthus cruentus* are among the premium African indigenous vegetables grown in Southwest Nigeria. Their presence in nearly every market in this region describes how useful and important they are in the diet of the people. They are known to contain important minerals, vitamins, proteins and medicinal constituents that are relevant to human growth

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and well-being[1,2]. *Amaranthus cruentus* is rich in vitamin A and important for vision, particularly in children[1]. *Solanum macrocarpon* is known to be bitter and used as worm expellant for the treatment of respiratory and cardiovascular diseases[3,4].

Telfairia occidentalis is rich in iron, hence it is in high demand by lactating mothers and anemic patients[5]. Generally, these vegetables are leafy vegetables and as such are used in making soups and stews that can be eaten with various staples, thereby, providing essential balance diets to many Sub-Saharan Africans, who cannot afford meat, milk, egg and other proteins of animal origin [6,7]. It is because of the nutritional and medical importance of these vegetables that World Health Organization recommends that their consumption rate should be about 400 g per person per[8]. They are also important source of income, particularly to rural poor farmers[9,10].

However, their production is limited by both marginal soils and diseases. A number of nematodes have been reported on many vegetables[11-15]. Adekunle *et al.*[14] reported a significant reduction in biomass yields of *T. occidentalis* following attack by *Meloidogyne incognita*, *Tylenchus* spp., *Helicotylenchus* spp., *Hirschmaniella* spp. and *Longidorus* spp., while Ogundele *et al.*[15] reported the reduction of leaf yields of *A. cruentus* and *T. occidentalis* by *Meloidogyne incognita*, *Helicotylenchus* spp. and *Dolichodorus* spp. Many nematodes are soil-borne pathogens, they infect plants from the roots and through their feeding habits, they deform the roots of plants, hence interfering with the normal functions of the root system. They can also form disease complexes with other pathogens thereby increasing the severity of disease and making control difficult [16,17].

The populations of plant parasitic nematodes can be affected by the availability of free-living soil microbes including free-living nematodes and opportunist microorganisms[18,19]. Khan and Kim[18] reported a decrease in the populations of *M. incognita* following an increase in the populations of *Mononchoides striatus,* however; this relationship depends on soil conditions. More so, Amulu e*t al.*[19] reported that they were high populations of free-living nematodes in comparison with the populations of plant parasitic nematodes in fadama fields rich in organic matter. They proposed that the activities of the free-living nematodes might have contributed to the low populations of plant parasitic nematodes. This study investigated the distributions of plant parasitic nematodes and their relationship with free living nematodes associated with three African indigenous vegetables grown in organic soils in Southwest Nigeria.

MATERIALS AND METHODS

Field surveys were conducted in four states in Southwest Nigeria. Soil samples were taken from 180 farms planted to *A. cruentus*, *S. macrocarpon* and *T. occidentalis* in four States of Southwest Nigeria namely, Oyo, Osun, Ekiti and Ondo from April, 2016 to October, 2016. They comprised seven Local Government Areas (LGAs) in Osun (Odo-Otin, Ife-East, Ilesa-East, Ilesa-West, Ife-Central, Ife South and Oriade LGAs) 3 in Ekiti (Ikole, Ilejemeje and Ido-Osin LGAs), three in Oyo (Surulere, Ogbomosho South and Ibadan Northwest LGAs) and two in Ondo (Akoko Northeast and Akoko Southwest LGAs). A total of fifteen LGAs were visited for soil sampling. In each LGA, samples were taken from four farms. Sixty soil samples were taken in fields sown to *A. cruentus*, *S. macrocarpon* and *T. occidentalis* giving a total of 180 samples in all the States visited.

The number of plots in a farm ranged from two to six plots of 17×11 m each per farmer. The vegetables were cultivated on 2×3 m beds. The farmers were provided with pelletized organic fertilizer (sunshine, manufactured by Ondo State Government). The organic fertilizer was applied to all plots in each farm at the same rate (10 ton/ha) by all the farmers in each LGA. Soil samples were taken six weeks after planting.

Soil samples were collected from the root zones of 20 vegetable crops using zigzag sampling method in all the farms hat were visited in the study area. This was done using a soil auger that has a depth and diameter of 15 and 1.9 cm respectively. Soil samples collected in each farm were added together to form a composite sample and 200 mL sub-sample was taken from the composite sample for nematode analysis. Nematodes were extracted from the 200 mL sub-sample using the modified Baermann tray method[20]. Recovered nematodes from the soil samples in water suspension were killed by adding equal quantity of boiling water into the nematode suspension and fixed in 4% (w/v) formaldehyde[21]. Nematodes were placed in a counting dish and counted under a stereomicroscope (100×) (Motic SMZ-160M Motic-Group, China). After these nematodes were identified individually from each sample under a compound microscope (100×) (Motic microscopes; model: 61400131, Motic-Group, China) to genus level, with the aid of identification manual to genera of nematodes[20,22,23]. The sampling was repeated in 2017, during the same months and at the same farms, as the first year sampling was carried out. Percentage frequency of nematode distribution and percentage nematode population density were calculated using the following formula:

$$\text{Frequency of occurrencce (\%)} = \frac{n}{N} \times 100$$

Where, n is the number of times an individual nematode occurred in all the samples and N is the sample size:

$$\text{Nematode population} = \frac{In}{TN} \times 100$$

Where, In is the individual nematode population in all the samples and TN is the total population of all the nematodes extracted from all the samples. Nematode population counts were log-transformed [Log10 (x+1)] and subjected to Pearson's correlation analysis to establish relationships among nematode species using the Statistical Analysis System (SAS) package[24]. Nematode genera were classified as outlined by Hunt *et al.*[25].

RESULTS

Distribution of plant parasitic nematodes: Plant parasitic nematodes belonging to three orders Rhabditida, Dorylaimida and Triplonchida, 3 sub-orders Tylenchina, Dorylaimina and Diphtherophorina, four sub-families Aphelenchoiddoidea, Tylenchoidea, Dorylaimoidea and Diphtherophoroidea and ten families Aphelenchidae, Tylenchidae, Dolichodoridae, Hoplolaimidae, Meloidogynidae, Pratylenchidae, Criconematidae, Hemicycliophoridae, Longidoridae and Trichodoridae were encountered and identified in Southwest Nigeria. Sixteen genera of plant parasitic nematodes were encountered in soil samples taken from around the root zones of the three African indigenous vegetables in Southwest Nigeria in 2016.

Meloidogyne spp., *Helicotylenchus* spp. and *Rotylenchulus* spp. were the most abundant and most frequently occurring nematode species on *A. cruentus* in Ekiti and Ondo State in 2016 and 2017 (Table 1 and 2). The nematode population and occurrence in *S. macrocarpon* is similar to *A. cruentus*. In *T. occidentalis* fields, *Helicotylenchus* spp. and *Pratylenchus* spp. were the most abundant nematode species in soil samples taken from around the root zones of the vegetable with 1574 and 1421/200 mL soil respectively in Ekiti State. *Meloidogyne* spp. had high frequency rating of 83% but low population density of 313/200 mL soil. The populations of other nematode species including *Hoplolaimus* spp., *Aphelenchus* spp., *Longidorus* spp., *Hemicycliophora* spp., *Criconemoides* spp. and *Hemicriconemoides* encountered in this State (Ekiti) were low ranging from 11-141/200 mL soil. The populations of *Hoplolaimus* spp. were however, high in *S. macrocarpon* fields in 2016 and 2017.

Table 1: Plant parasitic nematode genera associated with three indigenous vegetables in Ekiti State

Nematode genera/ Vegetables	2016 *FO (%)	2016 Total nematode populations/ 200 mL soil	2017 *FO (%)	2017 Total nematode populations/ 200 mL soil
Amaranthus cruentus				
Meloidogyne	92.00	1582.0 (26.5)	92.00	1782.0 (24.8)
Helicotylenchus	92.00	1573.0 (26.4)	92.00	1730.0 (24.0)
Hoplolaimus	83.00	562.00 (9.4)	83.00	573.00 (8.0)
Xiphinema	58.00	576.00 (9.7)	58.00	575.00 (8.0)
Pratylenchus	58.00	558.00 (9.4)	58.00	558.00 (7.8)
Rotylenchulus	50.00	1454.0 (12.6)	50.00	1552.00 (21.6)
Tylenchus	50.00	141.00 (2.4)	50.00	168.00 (2.3)
Aphelenchus	50.00	22.00 (0.4)	50.00	42.00 (0.6)
Tylenchorhynchus	50.00	19.00 (0.3)	42.00	29.00 (0.4)
Paratrichodorus	33.00	143.00 (2.4)	33.00	150.00 (2.1)
Longidorus	17.00	14.000 (0.2)	8.000	14.000 (0.2)
Hemicycliophora	17.00	12.000 (0.2)	17.000	13.000 (0.2)
Criconemoides	8.000	11.000 (0.2)	8.000	12.000 (0.2)
Solanum macrocarpon				
Meloidogyne	92.00	1995.0 (21.7)	92.00	1886.0 (23.1)
Helicotylenchus	92.00	1982.0 (21.5)	92.00	1875.0 (22.9)
Hoplolaimus	67.00	1261.0 (13.7)	67.00	1197.0 (14.6)
Xiphinema	75.00	1129.0 (12.3)	75.00	132.0 (1.6)
Pratylenchus	83.00	1148.0 (12.5)	83.00	1152.0 (14.1)
Rotylenchulus	83.00	1469.0 (16.0)	83.00	1509.0 (18.4)
Tylenchus	58.00	137.00 (1.5)	58.00	151.00 (1.8)
Aphelenchus	42.00	14.00 (0.5)	42.00	115.00 (1.4)
Tylenchorhynchus	25.00	19.000 (0.2)	25.00	12.00 (0.1)
Paratrichodorus	25.00	18.000 (0.2)	25.00	118.000 (1.4)
Longidorus	-	-	-	-
Hemicycliophora	8.000	12.000 (0.1)	8.000	12.000 (0.1)
Criconemoides	17.00	12.000 (0.1)	8.000	12.000 (0.1)
Hemicriconemoides	8.000	12.000 (0.1)	8.000	11.000 (0.1)
Telfairia occidentalis				
Meloidogyne	83.00	313.000 (6.0)	83.00	277.0 (5.7)
Helicotylenchus	90.00	1574.0 (30.2)	92.00	1579.0 (32.7)
Hoplolaimus	75.00	593.00 (11.4)	75.00	499.00 (10.3)
Xiphinema	0.00	500.000 (9.6)	0.00	430.0 (8.9)
Pratylenchus	83.00	1421.0 (27.3)	83.00	1296.0 (26.8)
Rotylenchulus	75.00	272.00 (5.2)	75.00	288.0 (6.0)
Tylenchus	58.00	91.000 (1.7)	58.00	93.00 (1.9)
Aphelenchus	67.00	65.000 (1.2)	67.00	104.0 (2.2)
Tylenchorhynchus	25.00	11.000 (0.2)	25.00	13.00 (0.3)
Paratrichodorus	25.00	328.00 (6.3)	25.00	226.00 (4.7)
Longidorus	17.00	13.000 (0.2)	25.00	3.000 (0.1)
Hemicycliophora	8.000	11.000 (0.2)	8.000	12.000 (0.2)
Criconemoides	8.000	13.000 (0.2)	8.000	14.000 (0.3)

*FO: Frequency of occurrence, Figures in parenthesis are means of percentage nematode populations and sample size[12]

In 2016 and 2017, *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp., *Hoplolaimus* spp., *Xiphinema* spp. and *Pratylenchus* spp. were the most abundant and most frequently encountered nematode species in soil samples taken around the roots of *A. cruentus* and *S. macrocarpon* in Osun State (Table 3). *Helicotylenchus* spp., *Xiphinema* spp. and *Pratylenchus* spp. were the most abundant and frequently occurring nematode species in soil samples taken from the base of *T. occidentalis* in 2016 and 2017. The populations of *Aphelenchus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Paratrichodorus* spp., *Longidorus* spp., *Criconemoides* spp., *Scutelonema* spp. and *Hemicycliophora* spp. were low, ranging from 2-478/200 mL soil for the three vegetables in the same region.

Table 2: Plant parasitic nematode genera associated with three indigenous vegetables in Ondo State

Nematode genera/ Vegetable	2016 *FO (%)	2016 Total nematode populations/ 200 mL soil	2017 *FO (%)	2017 Total nematode populations/ 200 mL soil
Amaranthus cruentus				
Meloidogyne	63.00	1320.0 (24.5)	63.00	1284.0 (25.5)
Helicotylenchus	63.00	1142.0 (21.2)	63.00	1223.0 (24.3)
Rotylenchulus	63.00	1104.0 (20.5)	63.00	997.00 (19.8)
Aphelenchus	63.00	31.00 (0.6)	50.00	55.00 (1.1)
Tylenchorhynchus	50.00	18.00 (0.3)	38.00	16.000 (0.3)
Pratylenchus	38.00	524.00 (9.7)	38.00	523.00 (10.4)
Xiphinema	38.00	516.00 (9.6)	38.00	520.00 (10.3)
Hoplolaimus	25.00	500.0 (9.3)	25.00	205.000 (4.1)
Paratrichodorus	25.00	200.0 (3.7)	13.00	204.000 (4.1)
Dolichodorus	13.00	4.000 (0.1)	13.00	6.000 (0.1)
Tylenchus	13.00	2.000 (0.0)	13.00	1.000 (0.0)
Solanum macrocarpon				
Meloidogyne	88.00	1363.0 (24.4)	88.00	1347.0(22.6)
Helicotylenchus	88.00	1132.0 (20.3)	88.00	1160.0 (19.4)
Tylenchus	75.00	191.00 (3.4)	75.00	90.00 (1.5)
Aphelenchus	75.00	174.00 (3.1)	75.00	75.00 (1.3)
Rotylenchulus	50.00	1125.0 (20.2)	50.00	1133.0 (19.0)
Pratylenchus	50.00	530.00 (9.5)	50.00	635.00 (10.6)
Tylenchorhynchus	50.00	22.00 (0.4)	50.00	44.00 (0.7)
Hoplolaimus	25.00	314.00 (5.6)	25.00	554.00 (9.3)
Xiphinema	13.00	509.000 (9.1)	13.00	610.00 (10.2)
Paratrichodorus	13.00	215.000 (0.6)	13.00	318.000 (5.3)
Telfairia occidentalis				
Helicotylenchus	88.00	229.0 (7.5)	88.00	284.0 (9.0)
Rotylenchulus	88.00	215.0 (7.1)	88.00	289.0 (9.1)
Pratylenchus	75.00	1188.0 (39.1)	75.00	1217.0 (38.5)
Hoplolaimus	75.00	395.0 (13.0)	75.00	212.0 (6.7)
Meloidogyne	63.00	161.0 (5.3)	75.00	72.00 (2.3)
Aphelenchus	63.00	35. (1.2)	63.00	61.00 (1.9)
Tylenchus	50.00	82.0 (2.7)	50.00	83.00 (2.6)
Xiphinema	38.00	519.0 (17.1)	38.00	725.00 (22.9)
Tylenchorhynchus	38.00	4.0 (0.1)	13.00	5.000 (0.2)
Paratrichodorus	13.00	213.0 (7.0)	25.00	216.000 (6.8)

*FO: Frequency of occurrence, Figures in parenthesis are means of percentage nematode populations and sample size[8]

In Oyo State, *Meloidogyne* spp. (1122/200 mL soil), *Helicotylenchus* spp. (1107/200 mL soil), *Xiphinema* spp. (1104/200 mL soil), *Rotylenchulus* spp. (1180/200 mL soil), *Paratrichodorus* spp. (1141/200 mL soil) and *Pratylenchus* spp. (739/200 mL soil) were the most abundant and most frequently occurring nematode species on the three vegetables in Oyo State in 2016 and 2017 (Table 4). However, *Meloidogyne* spp. had low-frequency ratings (25%) and low populations (164/200 mL soil) on *T. occidentalis* in both years in Oyo State.

Correlation analysis between populations of plant parasitic and free-living nematodes

In *Amaranthus cruentus* field: In 2016 and 2017, *Hoplolaimus* correlated positively with *Meloidogyne*, *Helicotylenchus* and *Rotylenchulus* (Tables 5 and 6). *Rhabditis* correlated negatively with *Meloidogyne* and *Hoplolaimus* positively with *Mononchus* and *Dorylaimus*. *Dorylaimus* also correlated negatively with *Meloidogyne*, *Hoplolaimus* and *Rotylenchulus* and positively with *Mononchus* in 2017.

In *Solanum macrocarpon* field: In 2016, *Helicotylenchus* correlated negatively with *Meloidogyne*, *Hoplolaimus* and *Rotylenchulus* and positively with *Mononchus* (Table 5). *Hoplolaimus* correlated positively with *Meloidogyne* and negatively with *Mononchus*. *Rotylenchulus* correlated positively with *Meloidogyne*

Table 3: Plant parasitic nematode genera associated with three indigenous vegetables in Osun State

Nematode genera	2016 *FO (%)	2016 Total nematode populations/ 200 mL soil	2017 *FO (%)	2017 Total nematode populations/ 200 mL soil
Amaranthus cruentus				
Meloidogyne	82.10	2018 (29.7)	82.00	2037 (31.0)
Helicotylenchus	82.10	1987.0 (28.8)	82.00	1948.0 (29.0)
Rotylenchulus	78.60	1507.0 (14.8)	79.00	1516.0 (16.0)
Xiphinema	75.00	1335.0 (9.8)	75.00	1336.0 (10.0)
Hoplolaimus	71.40	1161.0 (4.7)	68.00	1156.0 (4.7)
Pratylenchus	53.60	1223.0 (6.5)	54.00	1159.0 (4.8)
Aphelenchus	50.00	172.00 (2.1)	39.00	161.00 (1.8)
Tylenchorhynchus	46.40	146.00 (1.4)	43.00	49.00 (1.5)
Tylenchus	35.70	149.00 (1.4)	39.00	35.00 (1.1)
Paratrichodorus	17.90	119.000 (0.3)	11.00	226.000 (0.2)
Longidorus	17.90	18.000 (0.2)	18.00	14.000 (0.4)
Criconemoides	14.30	18.000 (0.2)	14.00	7.000 (0.2)
Scutellonema	7.100	13.000 (0.1)	7.000	4.000 (0.1)
Solanum macrocarpon				
Meloidogyne	85.70	2724 (34.8)	86.00	2932 (36.6)
Helicotylenchus	82.10	2238 (23.6)	82.00	2238 (22.7)
Rotylenchulus	78.60	2029 (18.8)	79.00	2062.0 (19.1)
Xiphinema	71.40	1532 (7.4)	71.00	1416.0 (6.2)
Tylenchus	64.30	373.00 (3.7)	57.00	346.0 (2.7)
Hoplolaimus	57.10	1366 (3.6)	57.00	1300.0 (3.8)
Pratylenchus	42.90	1331.0 (2.8)	43.00	1239.0 (2.6)
Tylenchorhynchus	39.30	251.00 (0.9)	36.00	345.00 (1.3)
Paratrichodorus	21.40	349.00 (0.9)	21.00	340.00 (0.7)
Aphelenchus	17.90	214.00 (0.1)	71.00	308.0 (4.0)
Longidorus	3.600	211.000 (0.0)	4.000	114.000 (0.0)
Telfairia occidentalis				
Helicotylenchus	95.80	1935.0 (32.1)	95.80	2266 (38.3)
Xiphinema	91.70	1623.0 (26.9)	83.30	1639.0 (19.3)
Meloidogyne	87.50	394.000 (6.5)	79.20	381.0 (11.5)
Rotylenchulus	75.00	375.000 (6.2)	75.00	263.0 (8.0)
Pratylenchus	75.00	1259.0 (20.9)	70.80	1244.0 (7.4)
Aphelenchus	58.30	122.000 (0.2)	58.30	94.00 (2.8)
Hoplolaimus	54.20	175.00 (34.8)	45.80	478.0 (8.4)
Tylenchus	45.80	88.000 (1.5)	45.80	94.00 (2.8)
Tylenchorhynchus	45.80	31.000 (0.5)	45.80	14.00 (0.4)
Hemicycliophora	20.80	5.0000 (0.1)	20.80	6.000 (0.2)
Paratrichodorus	16.70	8.0000 (0.1)	16.70	311.00 (0.3)
Longidorus	12.50	5.0000 (0.1)	12.50	12.00 (0.4)
Hemicriconemoides	8.300	4.000 (0.1)	4.200	1.000 (0.0)
Criconemoides	8.3.00	2.000 (0.0)	8.300	2.000 (0.1)

*FO: Frequency of occurrence, Figures in parenthesis are means of percentage nematode populations and sample size[28]

and *Hoplolaimus* and negatively with *Mononchus*. *Mononchus* correlated negatively with *Meloidogyne*. The results of the 2017 followed similar trend with those of 2016, except that *Pratylenchus* correlated positively with *Rhabditis*, *Dorylaimus* and *Mononchus* and negatively with *Hoplolaimus* (Table 6). *Rhabditis* correlated positively with *Mononchus*, *Dorylaimus* and negatively with *Hoplolaimus*. *Dorylaimus* correlated positively with *Helicotylenchus* and *Mononchus*.

In *Telfairia occidentalis* field: In 2016, *Helicotylenchus* correlated negatively with *Meloidogyne* and positively with *Rhabditis*, *Mononchus and Dorylaimus* (Table 5). *Rhabditis* correlated negatively with *eloidogyne* and positively with *Mononchus* and *Dorylaimus*. *Mononchus* correlated negatively with

Table 4: Plant parasitic nematode genera associated with three indigenous vegetables in Oyo State

Nematode genera/ Vegetable	2016 *FO (%)	2016 Total nematode populations/ 200 mL soil	2017 *FO (%)	2017 Total nematode populations/ 200 mL soil
Amaranthus cruentus				
Meloidogyne	42.00	1122.0 (15.7)	42.00	1558.0 (20.6)
Helicotylenchus	42.00	1107.0 (15.5)	42.00	1195.0 (15.8)
Xiphinema	42.00	1104.0 (15.4)	42.00	1102.0 (14.6)
Rotylenchulus	42.00	1180.0 (16.5)	42.00	1238.0 (16.4)
Paratrichodorus	42.00	1141.00 (16.0)	33.00	1137.0 (15.1)
Pratylenchus	33.00	793.00 (1.1)	33.00	694.0 (0.9)
Tylenchorhynchus	33.00	11.00 (1.8)	33.00	23.00 (3.7)
Hoplolaimus	25.00	685.00 (9.6)	25.00	589.00 (7.8)
Criconemoides	17.00	3.000 (0.0)	8.00	1.00 (0.0)
Hemicycliophora	8.00	1 .000(0.0)	17.00	3.00 (0.0)
Longidorus	8.00	1.000 (0.0)	25.00	9.00 (0.1)
Solanum macrocarpon				
Meloidogyne	42.00	1516.0 (25.0)	42.00	2435.0 (25.5)
Helicotylenchus	33.00	1262.0 (20.8)	33.00	1424.0 (14.9)
Rotylenchulus	33.00	1218.0 (20.1)	33.00	1223.0 (12.8)
Xiphinema	33.00	961.00 (15.8)	33.00	1123.0 (11.8)
Tylenchus	33.00	39.00 (0.6)	33.00	55.00 (0.6)
Aphelenchus	25.00	82.00 (0.1)	25.00	35.00 (0.0)
Pratylenchus	25.00	957.00 (15.8)	25.00	765.00 (8.0)
Tylenchorhynchus	25.00	25.00 (0.4)	25.00	27.00 (0.3)
Paratrichodorus	17.00	13.00 (0.0)	17.00	1216.00 (12.8)
Criconemoides	8.000	1.00 (0.0)	8.000	21.000 (0.2)
Hoplolaimus	-	-	25.00	1212.0 (12.7)
Telfairia occidentalis				
Helicotylenchus	33.00	1132.0 (20.0)	33.00	1130.0 (20.5)
Xiphinema	33.00	1137.0 (20.1)	33.00	1132.0 (20.6)
Hoplolaimus	67.00	1103.0 (19.5)	25.00	985.00 (17.9)
Rotylenchulus	33.00	196.00 (3.5)	33.00	192.00 (3.5)
Meloidogyne	25.00	164.00 (2.9)	25.00	153.00 (2.8)
Tylenchus	25.00	27.00 (0.5)	25.00	46.00 (0.8)
Pratylenchus	17.00	747.0 (13.2)	17.00	1141.0 (20.7)
Tylenchorhynchus	17.00	11.00 (0.2)	17.00	6.000 (0.1)
Paratrichodorus	17.00	1115 (19.7)	17.00	616.00 (11.2)
Longidorus	17.00	6.00 (0.1)	17.00	12.00 (0.2)
Hemicycliophora	17.00	4.000 (0.1)	17.00	4.000 (0.1)
Aphelenchus	8.000	23.00 (0.4)	8.000	87.00 (1.6)
Hemicrinemoides	8.000	2.000 (0.0)	8.000	2.000 (0.0)

*FO: Frequency of occurrence, Figures in parenthesis are means of percentage nematode populations and sample size[28]

Table 5: Correlation analysis between plant parasitic and free living nematodes in fields grown to 3 indigenous vegetables in Southwest Nigeria in 2016

Nematode genera	*Meloidogyne*/ 200 mL soil	*Helicotylenchus*	*Hoplolaimus*	*Pratylenchus*	*Rotylenchulus*	*Rhabditis*	*Mononchus*	*Dorylaimus*
Amaranthus cruentus								
Meloidogyne	1							
Helicotylenchus	0.474	1						
Hoplolaimus	0.793*	0.708*	1					
Pratylenchus	0.129	0.700	0.357	1				
Rotylenchulus	0.617	0.325	0.708*	-0.248	1			
Rhabditis	-0.777*	-0.189	-0.538	-0.060	-0.252	1		
Mononchus	-0.535	0.190	-0.173	0.166	-0.032	0.756*	1	
Dorylaimus	-0.479	-0.068	-0.107	0.129	0.105	0.777*	0.625	1
Solanum macrocarpon								
Meloidogyne	1							
Helicotylenchus	-0.929**	1						
Hoplolaimus	0.939**	-0.939**	1					

Table 5: Continued

Nematode genera	*Meloidogyne*/ 200 mL soil	*Helicotylenchus*	*Hoplolaimus*	*Pratylenchus*	*Rotylenchulus*	*Rhabditis*	*Mononchus*	*Dorylaimus*
Pratylenchus	0.178	0.101	-0.068	1				
Rotylenchulus	0.833*	-0.833*	0.939**	-0.165	1			
Rhabditis	-0.262	0.214	-0.431	0.140	-0.429	1		
Mononchus	-0.874**	0.850**	-0.906**	0.038	-0.946**	0.228	1	
Dorylaimus	-0.452	0.595	-0.431	0.342	-0.429	-0.524	0.515	1
Telfairia occidentalis								
Meloidogyne	1							
Helicotylenchus	-0.769**	1						
Hoplolaimus	0.210	0.053	1					
Pratylenchus	-0.371	0.455	-0.263	1				
Rotylenchulus	0.392	-0.322	0.007	0.469	1			
Rhabditis	-0.867**	0.755**	0.004	0.503	-0.270	1		
Mononchus	-0.832**	0.881**	-0.119	0.378	-0.497	0.720**	1	
Dorylaimus	-0.853**	0.692*	-0.284	0.594*	-0.098	0.846**	0.741**	1

*Significant at 5% level of probability,**Significant at 1% level of probability, -: Negative correlation and +: Positive correlation

Table 6: Correlation analysis between plant parasitic and free-living nematodes in fields grown to three indigenous vegetables in Southwest Nigeria in 2017

Nematode genera	*Meloidogyne*	*Helicotylenchus*	*Hoplolaimus*	*Pratylenchus*	*Rotylenchulus*	*Rhabditis*	*Mononchus*	*Dorylaimus*
Amaranthus cruentus								
Meloidogyne	1							
Helicotylenchus	0.025	1						
Hoplolaimus	1.523**	-0.466	1					
Pratylenchus	-0.522	-0.340	-0.213	1				
Rotylenchulus	1.523**	-0.428	1.611**	-0.092	1			
Rhabditis	-0.882	0.617	-1.233*	0.301	-1.031	1		
Mononchus	-0.905	0.819	-1.258*	0.169	-0.943	1.498**	1	
Dorylaimus	-1.350**	0.335	-1.570**	0.725	-1.426**	1.300**	1.292**	1
Solanum macrocarpon								
Meloidogyne	1							
Helicotylenchus	0.139	1						
Hoplolaimus	1.690**	0.108	1					
Pratylenchus	-1.006	0.265	-1.121*	1				
Rotylenchulus	1.204*	0.461	1.381**	-0.920	1			
Rhabditis	-1.157*	0.365	-1.172*	1.220*	-1.003	1		
Mononchus	-1.145*	0.819	-1.211*	1.309**	-0.889	1.246*	1	
Dorylaimus	-0.781	1.246*	-0.839	1.296**	-0.423	1.069*	1.498**	1
Telfairia occidentalis								
Meloidogyne	1							
Helicotylenchus	-1.384**	1						
Hoplolaimus	0.378	0.095	1					
Pratylenchus	-0.668	0.819	-0.473	1				
Rotylenchulus	0.706	-0.580	0.013	0.844	1			
Rhabditis	-1.561**	1.359**	0.007	0.905	-0.486	1		
Mononchus	-1.498**	1.586**	-0.214	0.680	-0.895	1.296**	1	
Dorylaimus	-1.535**	1.246*	-0.511	1.069*	-0.176	1.523**	1.334**	1

*Significant at 5% level of probability,**Significant at 1% level of probability, -: Negative correlation and +: Positive correlation

Meloidogyne and positively with *Dorylaimus*. *Dorylaimus* correlated positively with *Pratylenchus* and negatively with *Meloidogyne*. The result of the 2017 followed a similar trend (Table 6).

DISCUSSION

The results of the current study show that sixteen genera of plant parasitic nematodes were isolated and identified in fields grown to *A. cruentus*, *S. macrocarpon* and *T. occidentalis* in Southwest Nigeria. *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp., *Xiphinema* spp., *Hoplolaimus* spp. and *Pratylenchus* spp. were the most prevalent nematode species encountered in the study area. The results of these findings agreed with the findings of Atungwu *et al.*[13], who revealed the presence of five nematode genera in fields usually planted to three leafy indigenous vegetables in Ogun State Nigeria. The identified nematodes were; *Tylenchus*, *Pratylenchus*, *Helicotylenchus*, *Meloidogyne* and *Rotylenchulus* spp. on *Celosia*, *Amaranthus* and *Corchorus*. Similarly, Adekunle *et al.*[14] and Ogundele *et al.*[15] identified the presence of 3 genera of plant parasitic nematodes including *M. incognita*, *Dolichodorus, Longidorus*, *Helicotylenchus* and *Xiphinema* species each affecting *T. occidentalis* and *A. cruentus* in vegetable fields in Osun State Nigeria. The former reported significant reduction in biomass of *T. occidentalis* planted in fields infested with *M. incognita*, *Longidorus* and *Xiphinema* species while the latter reported significant reduction in leaf yields of *A. cruentus* and *T. occidentalis* grown in fields infested with *M. incognita*, *Helicotylenchus* and *Dolichodorus* spp. In Ebonyi State, Southeast Nigeria, Ngele and Kalu [26] revealed the presence of seven genera of nematodes including *M. incognita* (35.5%), *Pratylenchus* spp. (20.83%), *Heterodera* spp. (15%), *Xiphinema* spp. (41.62%), *Dolichodorus* spp. (33.3%) and *Trichodorus* spp. (25%) in fields planted to a number of vegetables.

Large numbers of these plant parasitic nematodes were extracted from soil samples taken around the roots of the vegetables across the four States. These nematodes have consistently been encountered in a number of vegetable fields[27-29]. The root-knot nematode, *Meloidogyne* spp. had been reported to parasitize and cause significant yield losses on a number of vegetables globally[30,31]. *Meloidogyne incognita* is the most common root-knot nematode species in Nigeria and the most damaging on a worldwide basis[32].

About 80% yield losses due to this nematode on vegetables had been reported in heavily infested soils[33]. Severe damage to vegetables by the root knot nematode, *M. incognita* has been reported in many African countries[34]. Significant yield reductions of okra by the nematode have been reported in Nigeria[35].

A recent study in Southwest, Nigeria showed that the interactions between *Helicotylenchus* and other plant parasitic nematodes resulted in `significant yield losses in some vegetable fields[14,15]. The nematode is known to prefer soils rich in organic matter[36]. This is probably because their proliferation and reproduction are enhanced by nitrogen[37]. This might have contributed to the high populations recorded in various communities, where samples were taken as organic fertilizer (sunshine) rich in nitrogen was used by the farmers.

Rotylenchulus spp. was encountered in large numbers in many of the communities where these vegetables were grown. It is known that after *Meloidogyne* spp., *R. reniformis* is the most economically important nematode affecting vegetable production in the tropics[38]. This may be attributable to the fact that the nematode also induces gall-like swellings known as syncytia on root systems like *Meloidogyne* spp.; hence many workers and farmers might have misdiagnosed the infections caused by *Meloidogyne* spp. In the present study, the nematode was among the most widely occurring and abundant nematode species encountered in the study areas. In Nigeria, the nematode is not recognised as important pest of vegetables[38]. The nematode has been found parasitizing *Solanum* spp., *Amaranthus* spp. amongst other indigenous vegetables in Nigeria and elsewhere[30-32].

Pratylenchus spp. was also found across the states in *A. cruentus, S. macrocarpon* and *T. occidentalis* fields. The most reported *Pratylenchus* spp. in Nigeria is *P. brachyurus* and it has been found in large numbers in vegetable fields[38]. Damage caused by *Pratylenchus* spp. has been documented in a number of vegetable crops including tomato, eggplant and cucumber in the temperate regions[23]. However, the nematode is not recognised as an important pest of vegetables in Nigeria due to the overriding importance of root-knot nematode in vegetable fields[38]. In several studies conducted in many African countries, *Pratylenchus* spp. were found to be associated with a number of vegetables and in some cases it was recorded as a major pest in the study areas[13-27].

Xiphinema, *Longidorus* and *Paratrichodorus* sp. were the most important ectoparasitic nematodes reported to be associated with the three vegetables in some of the States sampled. *Xiphinema* spp. were the most abundant and the most widely distributed in the study areas when compared with *Paratrichodorus* spp. Soil texture is one of the main edaphic factors affecting the distribution of these nematodes. They are known to be more active and reproduce effectively in lighter soils[39]. The highest population densities of these pests were recorded in derived savannah areas of the region. This area is characterised by light soil and fairly low rainfall and this might have favoured their activities and reproduction. Apart from their direct effects on crop plants, they can also transmit viruses through their feeding habits thereby, exacerbating disease infections[28,40]. They are however, not recognised as important pests of agricultural crops in Nigeria[38]. These nematodes have been reported associated with a number of vegetable crops including tomato, cucumber, aubergine, *S. melongena* and sweet pepper, green beans and squash[41].

Dolichodorus spp. was encountered in communities in Ondo State. The nematodes are not common in vegetable fields in Nigeria and as such little or no information is known with reference to their effects on vegetables in Nigeria. However, the nematode can cause damage as severe as those caused by the sting nematode *Belonolaimus longicaudatus*, however, their activities are limited to moist habitats, hence yield losses caused by the nematode is not widely spread[42]. In this study the nematodes were encountered in hydromorphic fields. The nematode has been shown to reduce the yields of *T. occidentalis* and *A. cruentus* in Nigeria[15].

Scutellonema species were encountered in low populations and were among the least distributed and least abundant. The nematodes were however, encountered only in *A. cruentus* fields. This pest is not known to cause damage in vegetable fields in the tropics[43-44] but a number of authors have reported the devastating effects of *Scutellonema Brady* in tuber crops particularly yam tubers[43,44]. The presence of *Scutellonema* spp. in vegetable fields could be due to farming practices and cropping history as some of the farmers practice mixed cropping, where they combined these vegetables with other crops. However, *Scutellonema clathricaudatum* was reported as third most prevalent nematode affecting vegetable crops in Benin[27]. The authors reported that the nematode infected 53.9% of the crops sampled and was observed on vegetable crops in 44.4% of surveyed communities.

Other nematodes that were encountered in the study areas were *Hoplolaimus*, *Criconemoides*, *Hemicriconemoides*, *Tylenchus*, *Tylenchorhynchus*, *Aphelenchus* and *Hemicycliophora* species. Though they are not considered as important nematode pests affecting vegetables in Nigeria, but have been reported on a number of vegetables in both the tropical and subtropical regions of the world including Nigeria[27,41,45].

The frequency of occurrence of these nematodes on crops including vegetables differs. This may be attributable to a number of factors including root exudates produced by some of these vegetables and soil conditions. Take for instance *Xiphinema* was not reported in soil samples taken from around the roots of *T. occidentalis* but were reported in those taken from around the roots of *A. cruentus* and *S. macrocarpon* in Ekiti State. It is possible that the nematode does not prefer feeding on this vegetable due to its antagonistic nature. Authors have demonstrated that some plant roots produce substances that antagonistic to the nematode.

The numbers of nematodes in this current study are generally low; this may be due to the fact that soil sampling periods were during the growth period of the investigated crops and some of the nematode species are endoparasitic in their feeding habit hence method of extraction may not display the whole plant parasitic community.

The correlation analysis results in the present study further confirm the antagonistic relationship between free living and plant parasitic nematodes. Fields sown to the three vegetables in the three States revealed that *Rhabditis, Mononchus* and *Dorylaimus* spp. population densities correlated negatively with those of some genera of plant-parasitic nematodes including *Meloidogyne*, *Hoplolaimus* and *Rotylenchulus* spp. The applications of the organic fertilizers by the farmers might have influenced this interaction. This finding agrees with those of Moosavi and Zare[46], who reported significant increase in the populations of bacteria feeding nematodes, fungi feeding nematodes, omnivorous feeders and predatory nematodes with corresponding decrease in the populations of plant parasitic nematodes following the application of sun hemp as green manure in plots sown to cucumber. Our earlier study also showed that the incorporations of sun hemp and Mexican sunflower seedlings at 10 or 20 seedlings/plot each significant increase the population of *Rhabditis*, *Mononchus* and *Dorylaimus* spp. in comparison with fallow control plots sown to *S. macrocarpon, A. cruentus* and *T. occidentalis* in soils amended with sun hemp and Mexican sunflower[47]. The populations of the free living nematodes might have been stimulated by the organic fertilizers used by the farmers. Authors have demonstrated that the applications of organic materials in soils can enhance the build-up of free living nematodes; hence, it is possible that the organic fertilizer enhanced the populations and activities of the free living nematodes in this study, which in turn affected the populations of the plant parasitic nematodes[48].

Mononchus spp., may affect the nematode population directly by feeding on the plant-parasitic nematodes, but *Rhabditis* (bacteria feeders) and *Dorylaimus* (omnivorous feeders) spp. may not directly affect the populations of plant parasitic nematodes but their feeding activities may result in the release of natural chemical substances that may be lethal to plant parasitic nematodes. *Dorylaimus* is known to play important role in the degradation of organic matters and during this process a number of toxic metabolites such as ammonia, flavonoids, nimbin, azadirachtin and phenols are released into the soil and these in turn create unfavorable conditions for plant-parasitic nematodes[48]. The predatory nematode, *Mononchus* spp. can affect nematode populations directly; it is known to possess massive teeth-like structure known as onchia which they use in killing plant parasitic nematodes.

In the correlation analysis, there are some positive correlations between plant parasitic nematodes such as *Hoplolaimus* and *Meloidogyne* (0.939), *Rotylenchulus* and *Hoplolaimus* (0.939) this is an indication that the activities of one plant-parasitic nematode may also increase the populations of another plant-parasitic nematode.

Continuous cultivation of vegetables in a particular field could trigger and facilitate the buildup of plant parasitic nematodes and this might consequently affect crop yields. The cultivation of vegetables could be beneficial in organic soils, fadama fields or soils amended with organic materials. The reason being that, these soils favor the multiplication of free living nematodes and other microorganisms that are antagonistic to plant parasitic nematodes and this will in turn keep their population below economic thresholds.

CONCLUSION

Among the sixteen genera of plant parasitic nematodes found associated with the vegetables, *Meloidogyne*, *Helicotylenchus* and *Rotylenchulus* spp. *Pratylenchus* spp. and *Xiphinema* spp. were the most prominent nematode pests in the region. Free-living nematodes significantly correlated negatively with plant parasitic nematodes. Farmers are therefore advised to cultivate their vegetables in ways and manner that will not support the build-up of nematode pests beyond economic thresholds. One way of achieving this is through the use of organic amendments in growing their crops as this can act as both fertilizers and natural nematicides and can also facilitate the build-up of free-living nematodes. Further study should be conducted to measure the damage and yield losses these nematode pests can cause on the three African indigenous vegetables.

SIGNIFICANCE STATEMENT

This study investigated the distributions of plant parasitic nematodes and their relationship with free living nematodes associated with three African indigenous vegetables grown in organic soils in Southwest Nigeria. The result revealed that 16 genera of plant parasitic nematodes were found associated with three African indigenous vegetables in Southwest Nigeria. The correlation analysis shows an antagonistic relationship between free living nematodes and plant parasitic nematodes.

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