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NEMATODE PARASITES OF WATERLEAF (*Talinum triangulare)* IN OBIO-AKPOR AND IKWERRE LOCAL GOVERNMENT AREAS OF RIVERS STATE, NIGERIA

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ABSTRACT

Waterleaf, *Talinum triangulare* is cultivated in home gardens and fields to improve nutritional quality for the family and a source of livelihood for farmers. However, the role of *T. triangulare* is often counteracted by plant-parasitic nematodes (PPNs). Effective management of PPNs will depend on the identification and determination of their population and distribution. A survey was conducted in two Local Government Areas (LGAs), Ikwerre and Obio-Akpor of Rivers State to identify plant-parasitic nematodes associated with waterleaf. Multistage random sampling technique was employed in the collection of 60 bulked soil and root samples across six waterleaf monoculture farming communities. The nematodes were extracted, identified and their population determined using standard procedures. Occurrences, relative importance values (RIV) and diversity indices were determined. Other data were analysed using descriptive statistics and analysis of variance. Six plant-parasitic nematodes genera were encountered in this study, *Helicotylenchus, Pratylenchus, Paratylenchus, Meloidogyne, Scutellonema* and *Tylenchulus*. *Pratylenchus* and *Helicotylenchus* were the most frequently occurring nematode pest genera with equal relative frequency of occurrence of 38.89%. *Pratylenchus* species with RIV of 61.34% was the most important nematode pest associated with waterleaf across the six farm communities. The dominance index of 0.71 for the nematode genera showed that some nematode species dominated others. Alakahia farms had the highest population of plant-parasitic nematodes (2,780) across the six farm communities, whereas Omagwa farms had the lowest (250). The study showed that nematode pests are associated with waterleaf and could be contributing to yield losses in this crop.

Keywords: Diversity indices, Nematode pests, Occurrence, Waterleaf, Survey.

INTRODUCTION

Waterleaf, *Talinum triangulare* has been reported to be of both South America or Africa origin and common throughout the humid tropics with abundance reported in several countries in West and Central Africa (Grubben, 2004; Edet *et al*., 2007). *Talinum triangulare* is considered a volunteer crop found growing immediately after the first rains of the year (Ekpe and Obiefuna, 1977). Waterleaf cultivation is one of the occupations of the Efik and Ibibio in Nigeria where it is in high production (Van Epenhuijesen, 1974). Several *Talinum* species including the closely related *Talinum portulacifolium* and *Talinum fruticosum* abound in West and Central Africa (Grubben, 2004). Waterleaf is eaten as a vegetable in many countries in West and Central Africa and of wide cultivation in Nigeria and Cameroon (Ekpo *et al*., 2013). The vegetable is best recognized by its triangular peduncle and it is collected from the wild during the dry season when other more popular vegetables are scarce and expensive (Schippers, 2000).

Talinum triangulare is grown because of the variety of uses of its young leaves and tender shoots. In Nigeria, the crop is important for its palatability and nutrients as it is often used in combination with other vegetables for local vegetable soup (Akoroda, 1990). It is a source of income for subsistent farmers (Van Epenhuijsen, 1974). In Cameroon, waterleaf is used as a treatment for measles, whereas in India, it is used to treat diabetes. Waterleaf performed well as a fodder for raising giant snails (Grubben, 2004). As a result of these, waterleaf is becoming increasingly important in ensuring food and nutrition security as the production serves as a balancing source of revenue to subsistent farmers. Since the increased popularity of Afang (*Gnetum africana*) in Cameroon, Southern and Eastern part of Nigeria, from around 1990 onwards, the demand for waterleaf has steadily risen and now a cherished vegetable in local market in Nigeria (Akoroda, 1990).

Direct or indirect losses in yield of *Talinum triangulare* due to pests is a major threat to income and food security of thousands of rural families (Savary and Willocquet, 2014). Although the agronomic practices that favour waterleaf production are less demanding and the yield is between 10-60 t/ha (Grubben, 2004), farmers still experience low yield of waterleaf attributable to pests and parasites (Savary and Willocquet, 2014). Plant-parasitic nematodes are major pathogens of fruits, vegetables and food crops in different

parts of the world (Osei *et al*., 2011). *Talinum triangulare* is highly susceptible to attack by nematode pests, especially in the tropics (Grubben, 2004; Sikora and Fernandez, 2005). This underscores the need to understand the implication of plant-parasitic nematodes on waterleaf cultivation (Savary and Willocquet, 2014).

The varying uses of the leaves of *T. triangulare* underscore the need for proper identification, speciation and occurrence of nematode pest taxa associated with it. It is vital to evaluate the effect of these nematode parasites on yield of waterleaf since they have been reported as major pathogens of vegetables in different parts of the world and quite destructive on vegetables (Khan and Khan, 1994). Information on their association with waterleaf in the Niger Delta, specifically in the Rivers State of Nigeria is scarce. This study was carried out to provide information on the occurrence, speciation and dominant nematode pests associated with waterleaf (*T. triangulare*) in two Local Government Areas of Rivers State, Nigeria.

MATERIALS AND METHODS

Description of the Study Area

This study was carried out in two Local Government Areas (LGAs), Obio Apkor and Ikwerre in Rivers State, Nigeria (Figure 1). Rivers State lies on coordinates 4º 44' 59.06" N, 6º 49' 39.58" E with a total land area of 11,077 km^2 and with a mean annual rainfall which ranges from 4,700 mm on the coast to about 1,700 mm (Sobrasuaipiri, 2016). The State occupies lowland area of Niger Delta with dense and thick tropical rainforest vegetation. It is characterized by high atmospheric (ambient) temperature that ranges between 25 ºC to 38 ºC.

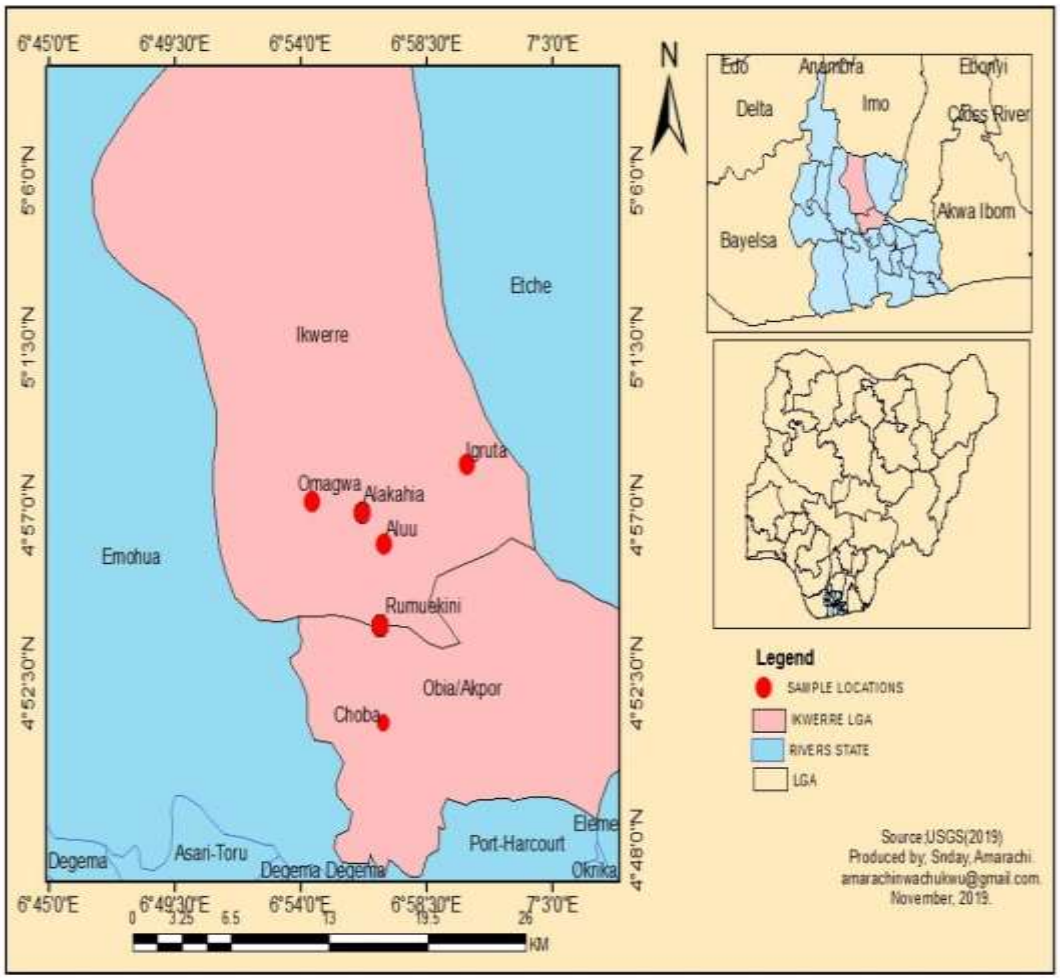

Figure 1: Map showing study locations with their co-ordinates and Local Government Areas

Sampling Procedures for the Collection of Root and Soil Samples of Waterleaf

Multistage random sampling method was employed in the collection of root and soil samples from monoculture farms of waterleaf in which three agrarian communities were selected per LGA. In Obio-Akpor LGA, Choba, Alakahia and Rumuekini communities were selected, while Aluu, Igwuruta and Omagwa communities were chosen in

Ikwerre LGA. Five farms cultivating waterleaf in monoculture were chosen per community. Ten plantain stands were randomly selected per farm and soil and root samples were collected from them using hand trowel, then bulked. Five bulked samples of soil and roots were collected from the five farms in each community. A total of 15 bulked soil and root samples were therefore collected from each LGA, making a total of 30 soil samples and 30 root samples from waterleaf in the two LGAs for this study.

Extraction and Isolation Method for Plant-parasitic Nematodes

The nematodes were extracted using pie-pan method (Whitehead and Hemming, 1965). Soil samples collected were thoroughly mixed; stones and other debris were removed through sieving. 200 cm^3 of sieved soil sample were poured on a facial tissue in a plastic sieve and water carefully added to the extraction plates by the side. Also, the roots of waterleaf per bulked sample were chopped with knife to 1-2 cm length and thoroughly mixed. Ten grams of the chopped roots were later poured into the facial tissue placed in a plastic sieve and water also added by the side. The set-ups were left for 48 hours and the sieves were later removed. The nematode-water suspension in the extraction plates was then transferred into a beaker and allowed to settle down and after which the supernatant water was carefully decanted and the concentrated sample poured into sample bottle for analysis.

Identification of Plant-Parasitic Nematodes

Plant-parasitic nematodes were identified as described by Dropkin *et al*. (1960). The nematode samples were preserved by 4% of formaldehyde pending identification and counting. Aliquot of 2 ml suspension was transferred by pipette into Doncaster counting dish (Doncaster, 1962) and the counting dish was placed under a dissecting microscope and compound microscope synchronously for identification and counting using Bell's key (Bell, 2004). Multiple tally counters were used in the counting of the nematodes.

Data Processing and Analyses

Frequency of occurrence of nematodes pests associated with waterleaf was calculated with;

$$AFO = \frac{Number\ of\ samples\ containing\ species}{Number\ of\ samples\ collected}\ x\ \frac{100}{1}$$

$$RFO = \frac{Frequency\ of\ a\ species}{Frequecy\ of\ all\ species}\ x\ \frac{100}{1}$$

Where AFO = absolute frequency of occurrence; RFO = relative frequency of occurrence

Determination of relative importance values of nematodes associated with waterleaf

The method of Kent and Coker (1996) was used to determine relative importance values of plant-parasitic nematodes associated with waterleaf in Obio Akpor and Ikwerre LGAs

$$RD = \frac{Density\ of\ individual\ species}{Total\ density\ of\ all\ species}\ x\frac{100}{1}$$

$$RIV\ (\%)\ = \frac{RD + RF}{2}$$

Where RD = relative density, RF = relative frequency, RIV = relative importance value

Determination of Diversity of Nematodes

Paleontological Statistical Tool (PAST) was used to determine the diversity of nematode communities (Hammer *et al*., 2001). Some indices calculated were:

Genera or Species Richness (S)

Shannon-Wiener index (H')= -$\sum$ (pi)(Inpi); Where pi = the proportion of individuals or the abundance of the i^{th} species expressed as a proportion of total abundance of all species [pi= n/N]; n_i = number of individuals in the i^{th} species; N= total number of individuals in the sample; ln= 2.303 $\log_{10}$

Evenness index (J): = H'/InS; Where, S = Number of species enumerated in the community.

Dominance index= $\sum (n_i/n)^2$

Nematode counts were prior transformed on $\log_{10}$ (x+1), then analyzed with analysis of variance (ANOVA) and means were separated using Fisher's Least Significant Difference at probability of 5% with Statistical Analysis System (SAS, 2007) package. The back-transformed means were presented in the results.

RESULTS

The occurrence and relative importance values of plant-parasitic nematodes associated with waterleaf in Obio-Akpor and Ikwerre Local Government Areas are shown in Figure 2. *Helicotylenchus* and *Pratylenchus* with the same relative frequency of occurrence (RFOC) are the most frequently occurring nematodes in Obio-Akpor Local Government Area (LGA) having the highest RFOC of 39.29% among the six nematode genera found in both soil and roots of waterleaf in (Figure 2). However, *Pratylenchus* species with relative importance value (RIV) of 61.81% is the most important nematode pest associated with waterleaf in Obio Akpor LGA. The least frequently occurring nematode pest genera were *Scutellonema* and *Paratylenchus* with the same RFOC of 3.57%. *Scutellonema* had the least RIV of 1.91% among the six nematode genera on waterleaf in Obio Akpor LGA (Figure 2).

In Ikwerre Local Government Area, *Pratylenchus* which was the most frequently occurring nematode pest had

RFOC of 40.00%, whereas *Scutellonema* had least frequency of occurrence of 26.67%. *Pratylenchus* with RIV of 63.64% was the most important nematode pest of waterleaf in Ikwerre LGA, followed by *Helicotylenchus* (RIV 20.76%). The least important nematode genus was *Scutellonema* (RIV 15.61%).

Figure 3 shows the diversity indices of plant-parasitic nematodes of waterleaf in Obio-Akpor and Ikwerre LGAs. In Obio-Akpor, 1383.33 nematode pest individuals were encountered which were higher than 366.67 nematode individuals encountered in Ikwerre LGA. Plant-parasitic nematodes associated with waterleaf in Obio-Akpor LGA had Dominance value of 0.72 compared with 0.77 obtained in Ikwerre LGA. In terms of Simpson values, nematode pest genera in Obio-Akpor LGA have a value for 0.28, but a lower value of 0.23 in Ikwerre LGA. The Shannon Wiener index for nematode pests of waterleaf in Obio-Akpor was 0.61 which was higher than a value of 0.53 in Ikwerre LGA. The evenness index for plant-parasitic nematodes in Obio-Akpor was 0.31 while that of Ikwerre LGA was 0.53 (Figure 3).

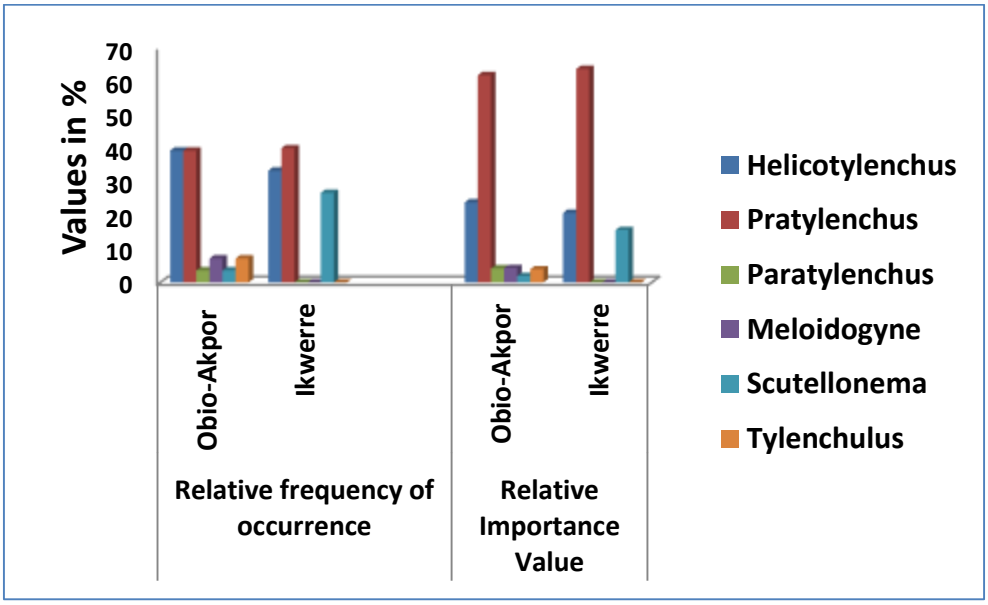

Figure 2: Occurrence and importance values of plant-parasitic nematodes associated with waterleaf in Ikwerre and Obio-Akpor Local Government Areas, Rivers State, Nigeria

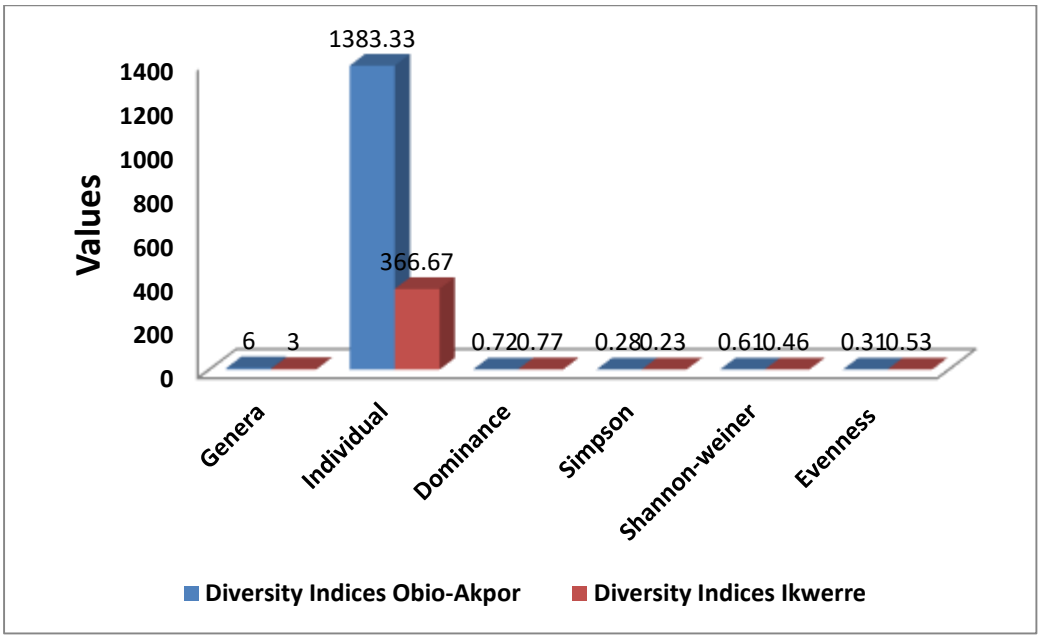

Figure 3: Diversity indices of plant-parasitic nematodes associated with waterleaf in Ikwerre and Obio-Akpor Local

Government Areas, Rivers State, Nigeria.

The population of plant-parasitic nematodes associated with waterleaf in Ikwerre and Obio-Akpor is presented in Table 1. In Ikwerre Local Government Area, *Pratylenchus* species with mean population of 50 obtained in Aluu waterleaf farms were the highest, but not significantly higher than the nematode pest population in Omagwa (20) and Igwuruta (20). Similar trend was observed for *Pratylenchus* (Table 1). There was no significant difference in the mean population of *Meloidogyne* obtained in Omagwa waterleaf farms, Aluu and Igwuruta. Aluu waterleaf farms (550) had the highest mean population of nematode pests associated with waterleaf among the three farm communities and this was not significantly higher than those of Omagwa (250) and Igwuruta (300) (Table 1).

Furthermore, Obio-Akpor LGA nematode data showed that *Helicotylenchus* species had significantly the highest population in Alakahia (250) compared with Choba (60) and Rumuekini (40). *Pratylenchus* population in Alakahia (2500) ranks highest and was significantly higher than those of Choba (600) and Rumuekini (400) among *Pratylenchus* on waterleaf farms in Obio-Akpor LGA. (Table 1). *Paratylenchus* species (200) were only found in Rumuekini waterleaf farm community, whereas *Meloidogyne* was the only nematode pest genus found in Choba waterleaf farms. *Tylenchulus* species with a mean population of 30 in Alakahia was not significantly higher than those at Choba and Rumuekini. Alakahia waterleaf farms recorded the highest mean plant-parasitic nematodes of 2780 which was significantly higher than total plant-parasitic nematode in Choba (720) and Rumuekini (650) (Table 1).

Figure 4 reveals the occurrence and importance values of plant-parasitic nematodes associated with waterleaf across the six waterleaf farm communities in Obio-Akpor and Ikwerre LGAs. *Pratylenchus* was the most frequently occurring nematode pest genera (RFOC 38.89%), then *Helicotylenchus* (RFOC 38.89%) across the six farm communities. In this table, *Pratylenchus* has a relative importance value of 61.34% and this shows that *Pratylenchus* is the most important nematode pest associated with waterleaf across the six farm communities. The least frequently occurring nematode genera were *Paratylenchus* and *Meloidogyne* with the same RFOC of 5.56%. However, *Tylenchulus* had the least RIV of 3.15% among the nematode genera (Figure 4).

Figure 5 shows the diversity of plant-parasitic nematode pests associated with waterleaf across the six farm locations. A total of 100 individual nematodes were encountered across the six waterleaf farm communities. The dominance index of nematode genera across the six farm communities was 0.71, Simpson index value of 0.29 was obtained, 0.62 for Shannon Weiner index and Evenness index of 0.37.

Table 2 shows the poulation of plant-parasitic nematodes associated with waterleaf across the six locations of Obio-Akpor and Ikwerre Local Government Area, Rivers State. Alakahia waterleaf farm community had the highest population of *Helicotylenchus* species (250) which was not significantly higher than the population obtained at Choba (60) (Table 2). The highest population of *Pratylenchus* species (2500) was obtained at Alakahia waterleaf farm community, this was not significantly higher than populations at Rumuekini (400) and Choba (600). Alakahia had the highest population of *Paratylenchus* species among the farm locations, but no significant difference exists in their populations.

Table 1: Population of plant-parasitic nematodes associated with waterleaf in Ikwerre and Obio-Akpor Local Government Areas, Rivers State

Local Government Area/Farm Community	Plant-parasitic nematode genera						Total plant-parasitic nematodes
	Helicotylenchus	*Pratylenchus*	*Paratylenchus*	*Meloidogyne*	*Scutellonema*	*Tylenchulus*	
Ikwerre							
Aluu	50.00	500.00	0.00	0.00	0.00	0.00	550.00
Omagwa	20.00	200.00	0.00	0.00	30.00	0.00	250.00
Igwuruta	20.0	260.00	0.00	0.00	20.00	0.00	300.00
LSD (≤0.05)	80.55	800.74	0.00	0.00	41.72	0.00	880.56
Obi-Akpor							
Alakahia	250.00	2500.00	0.00	0.00	0.00	30.00	2780.00
Choba	60.00	600.00	0.00	60.00	0.00	0.00	720.00
Rumuekini	40.00	400.00	200.00	0.00	10.00	0.00	650.00
LSD (≤0.05)	145.62	1456.21	355.8	65.37	17.79	35.58	1639

Rumuekini recorded the population of 200 for *Meloidogyne* which was significantly different from Alakahia, Omagwa and Choba. Omagwa had a population of 30 for *Scutellonema* and this was not significantly higher than populations at Igwuruta (20) and Rumuekini (10). Only Alakahia had 30 nematode pest individuals of *Tylenchulus* (Table 2).

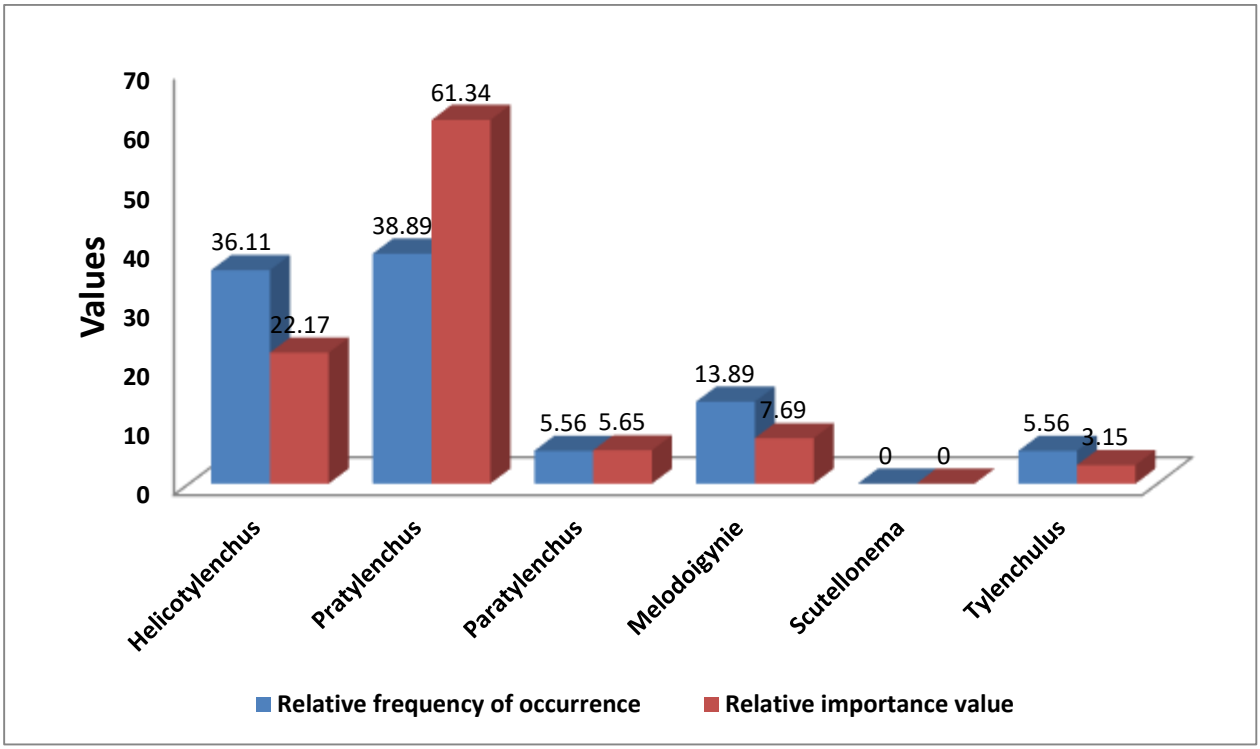

Figure 4: Occurrence and importance values of plant-parasitic nematodes associated with waterleaf in six farm communities of Obio-Akpor and Ikwerre Local Government Areas

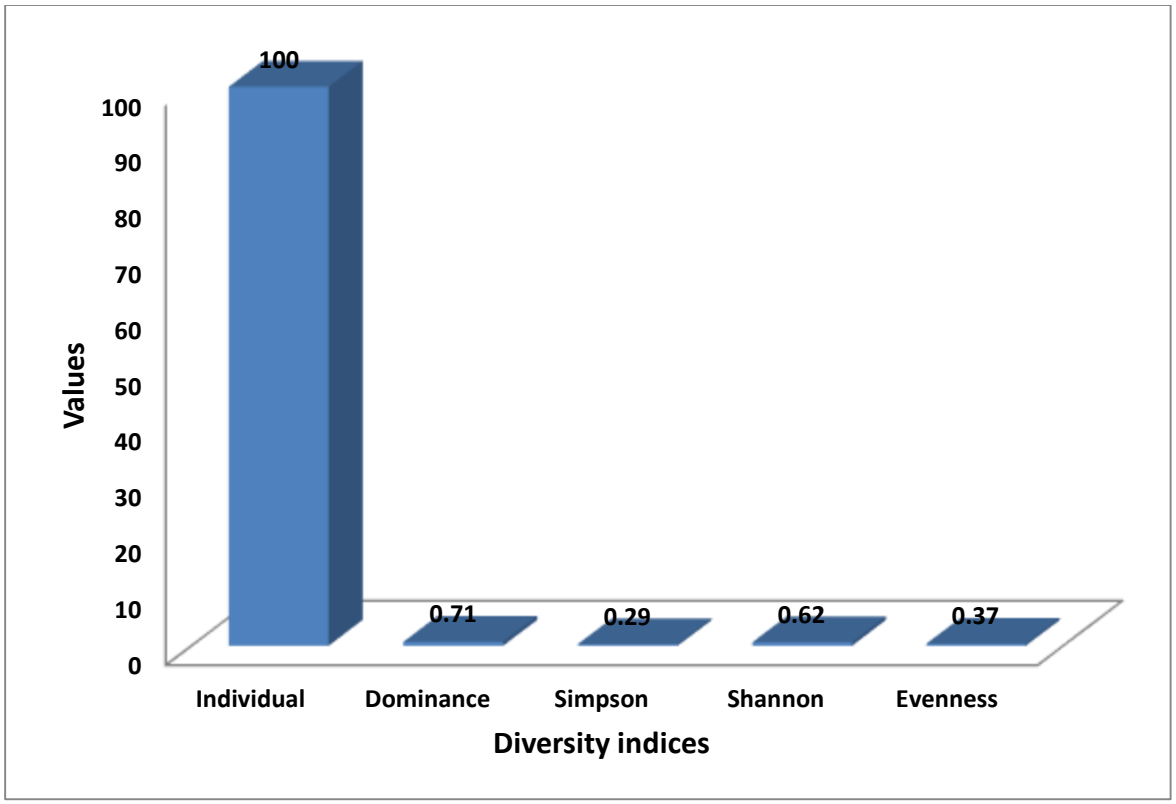

Figure 5: Diversity indices of plant –parasitic nematodes associated with waterleaf across six farm communities in both Obio-Akpor and Ikwerre Local Government Areas

Table 2: Populations of plant-parasitic nematodes of waterleaf across the six communities of Obio-Akpor and Ikwerre Local Government Areas, Rivers State

Local Government Area/Farm Locations	**Plant-parasitic nematode genera**						**Total plant-parasitic nematodes**
	Helicotylenchus	***Pratylenchus***	***Paratylenchus***	***Meloidogyne***	***Scutellonema***	***Tylenchulus***	
Aluu	50.00	500.00	0.00	0.00	0.00	0.00	550.00
Omagwa	20.00	200.00	0.00	0.00	30.00	0.00	250.00
Igwuruta	20.00	260.00	0.00	0.00	20.00	0.00	300.00
Alakahia	250.00	2500.00	0.00	0.00	0.00	30.00	2780.00
Choba	60.00	600.00	0.00	60.00	0.00	0.00	720.00
Rumuekini	40.00	400.00	200.00	0.00	10.00	0.00	650.00
LSD(P≤0.05)	111.46	1113.1	238.32	43.78	30.38	23.83	1246.20

DISCUSSION

The result of this study shows that six major plant-parasitic nematode genera in order of importance: *Pratylenchus>Helicotylenchus> Paratylenchus> Melodoigynie >Scutellonema> Tylenchulus* were associated with waterleaf in the two study areas (Ikwerre and Obio Akpor Local Government Areas). The presence of these diverse nematode genera from the soil and root samples in all the farms visited shows that waterleaf is a likely susceptible host to plant-parasitic nematodes (PPNs). The outcome of this study is in conformity with the report of other workers who reported that plant-parasitic nematodes such as *Meloidogyne* species are one of the major pathogens associated with waterleaf (Sikora and Fernandez, 2005; Etim *et al*., 2020).

The outcome of this study revealed *Pratylenchus* species are associated with waterleaf in Obio-Akpor and Ikwerre Local Government Areas, this finding corroborated the research outcome by Sikora and Fernandez (2005) who reported *Pratylenchus* spp. to be one of the most economically important phytonematodes occurring in waterleaf with a wide host range of more than 350 hosts. Actual assessment of loss due to *Pratylenchus* species has not been made possible under vegetable field condition due to presence of other populations of nematode pests in the waterleaf farm (Eyo *et al*., 2000). *Pratylenchus* spp. being the most important nematode pest with the highest relative importance value (RIV) in this study has been reported as a major nematode pest of economic value and endoparasites that cause severe losses to waterleaf (Osei *et al*., 2011). They penetrate the roots of waterleaf making their way through the tissues and breaking down the cells as they feed. Lesions form in the roots as fungi and bacteria enter damaged tissues, and root rot often occurs (Osei *et al*., 2011).

Several factors such as biology and life cycle of the nematode, plant-host response, the environmental influences, farm history as well as the cultural practices embarked upon by farmers contribute to the extent of damage being observed on any vegetable and their distribution in time and space. Of great concern also, is the fact that soil factors that promote good growth of waterleaf seem to also favour rapid multiplication of plant-parasitic nematodes (Thompson *et al.,* 1975). The nature and symptoms of damage caused by these nematodes make their damaging potentials to be underestimated and often mistaken for those caused by other plant pathogens such as fungi and bacteria. In most cases, farmers are not aware or adequately informed and equipped to combat this menace to crops.

The higher population of plant-parasitic nematodes in waterleaf farms around urban areas than agrarian communities might be due to introduction of plant-parasitic nematodes associated with increase in human activities such as trading of farm produce.

Besides, this study has identified key nematodes that are of economic importance in waterleaf such as the root-knot nematodes, *Meloidogyne* species. Waterleaf has been reported as a susceptible host to root-knot nematodes (Grubben, 2004). However, the presence of *Meloidogyne* spp. in low population density in waterleaf farms in this study might be an indication of preference of other nematode pests for waterleaf ahead of *Meloidogyne* species. Also, climate change in the recent causes occasional pests to assume the status of key pests of most crops (Nicol, 2002). Generally, the low population of nematode pests of waterleaf across the farmlands visited could be due to cultural practices such as manuring and bush burning adopted by farmers which are unfavorable to these nematodes (Osei *et al*., 2011). The farming practices in these studied LGAs might have significantly influenced the occurrence, distribution and population of nematode pests (Nicol, 2002; Osei *et al*., 2011).

CONCLUSION

This study showed that waterleaf is a host to economically important plant-parasitic nematodes such as *Pratylenchus, Helicotylenchus*, *Meloidogyne* spp., *Scutellonema* spp., *Tylenchulus* spp. and *Paratylenchus* in Obio-Akpor and Ikwerre Local Government Areas. However, *Helicotylenchus* spp., *Pratylenchus* spp., *Paratylenchus* spp. are the most important nematode pests associated with waterleaf and could be a major limiting factor in waterleaf production. Further research is necessary to establish the pathogenicity of the reported nematode pests in order to quantify damages caused by individual nematode genera associated with waterleaf with an aim of designing a proper nematode management strategy.

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