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EMERGENCE OF *GRACILACUS* SPECIES AS ONE OF THE KEY PLANT-PARASITIC NEMATODES ASSOCIATED WITH CASSAVA IN EAST SENATORIAL DISTRICT, RIVERS STATE, NIGERIA

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ABSTRACT

The type, distribution and population of plant-parasitic nematodes on crops change over time and space. It is pertinent to know their current status to ensure effective management. A survey was carried out to determine the occurrence and population of plant-parasitic nematodes associated with cassava in the East Senatorial District of Rivers State, Nigeria. Multi-stage random sampling method was used to collect soil and root samples from 56 farms growing cassava in monoculture across four Local Government Areas. Plant-parasitic nematodes in samples were extracted using the modified Baermann's method, then later identified and counted. Data obtained were processed using descriptive statistic. Five genera of plant-parasitic nematodes were found to be associated with cassava namely: *Helicotylenchus*, *Pratylenchus*, *Tylenchus*, *Gracilacus* and *Meloidogyne*. *Gracilacus* with a frequency of occurrence (FOC) 39.3% and *Meloidogyne* (FOC 44.5%) were the predominant plant-parasitic nematodes in soil and roots of cassava, respectively. *Meloidogyne* species (FOC 32.7%) and *Gracilacus* species (FOC 48.5%) were the most abundant in soil and roots of cassava, respectively. The findings of this study gave credence to the emergence of *Gracilacus* species as one of the key plant-parasitic nematodes associated with cassava in Rivers State.

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INTRODUCTION

Cassava, *Manihot esculenta* Crantz is the second principal food crop in the Sub Saharan Africa for more than 200 million Africans (IITA, 1990; Tsegia and Kormawa, 2002). It is a source of income for millions of Africans, Asians and Latin Americans (Guillaume-Gentil, 2015). The uses of cassava vary as it is being consumed by man, animals, and serves as a source of raw materials for many industries (Plucknett *et al*., 1998; Guillaume-Gentil, 2015). Nigeria is the largest producer of cassava in the world with a production statistic of 53 million metric tonnes (MT) in 2013 (FAOSTAT, 2015). The other top four producers of cassava in the world are Thailand (30.2 million MT), Indonesia (23.9 million MT), Brazil (21.5 million MT) and Democratic Republic of Congo (16.5 million MT) (Faostat, 2015). However, Nigeria is not among the five top countries delivering highest yields. The five countries are India, Suriname, Malaysia, Cook Islands, and Lao's People Democratic Republic (Faostat, 2015). The low yield both in quality and quantity of cassava in Nigeria could be attributed to many factors including pests and pathogens amongst others (Coyne, 1994; Banito *et al*., 2007). Notable among these pests and pathogens are insects (such as cassava mealy bug, cassava green spider mite, termites, variegated grasshopper), viruses (cassava mosaic virus), bacteria (cassava bacterial blight), fungi (Anthracnose, Cercospora leaf spot and Cassava root rot) and plant-parasitic nematodes (Coyne, 1994; Banito *et al*., 2007). Plant-parasitic nematodes are responsible for between 87-98% yield losses in cassava (Caveness, 1982; Coyne *et al.*, 2012) Many plant-parasitic nematodes have been associated with cassava, but few are reported to have caused economical damage (Coyne, 1994; Nicol, 2002). Plant-parasitic nematodes often associated with cassava include *Meloidogyne incognita, Meloidogyne javanica, Pratylenchus brachyurus, Rotylenchulus reniformis, Helicotylenchus erythrinae and*

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Helicotylenchus dihystera (McSorley *et al*., 1983). Many workers have reported the root-knot nematodes, *Meloidogyne* species, as the major genus of plant-parasitic nematodes associated and affecting cassava production in most growing areas (McScorley *et al*., 1983). In order to curtail the menace of plant-parasitic nematodes on cassava, there is need to properly identify them. It is after proper identification that the contribution of each nematode singly or in relationship with others as a pest will be rightly determined and appropriate management strategies be developed. Also, nematode types, population and distribution varies across space and time due to many factors such as change in cropping patterns, landuse, climate change amongst others (Nicol, 2002). Nematodes that were viewed as non-damaging on crops are now assuming destructive roles on many crops due to climate change causing factors (Nicol, 2002). It is important that nematodes associated with crops be known across time and space to ensure their management. Some workers have given reports on nematode pests of cassava in Nigeria, but not in the recent and some areas were left out (Coyne, 1994).

Asimiea *et al*. (2015) carried out a preliminary investigation on nematode pests of cassava in three Local Government Areas in Rivers State. They reported the presence of *Gracilacus* species which has not been reported on cassava before in Nigeria. Also, Tanimola *et al.* (2016) reported *Gracilacus* species as one of the major nematodes associated with cassava in the West Senatorial District of Rivers State. This study was carried out to cover more cassava growing areas in Rivers State with a view to determining recent status of plant-parasitic nematodes in terms of occurrence and population on cassava cassava in East Senatorial District, Rivers State, Nigeria.

MATERIALS AND METHODS

Research sites

The survey was conducted between January and February 2015 in Rivers East Senatorial District (RESD), Rivers State, Nigeria. The RESD comprises eight Local Government Areas (LGAs); Port Harcourt City, Obio/Akpor, Ogu/Obolo, Ikwerre, Etche, Omuma and Emohua LGAs. However, four LGAs in the District were randomly selected for collection of samples. The four LGAs selected were; Obio/Akpor, Ikwerre, Etche, and Emohua (Fig. 1). The farming communities where samples were collected from were presented in Table 1.

Collection of soil and root samples

The sampling method used was Multi Stage Random Sampling Method (Adesoye, 2011). Four LGAs were randomly selected and three farming communities per LGA were also selected. Lastly, five farms growing cassava in monoculture were also selected per farming community. The four Local Government Areas with their respective sampled farming communities are presented in Figure 2. In each cassava farm, 15 cassava stands were randomly selected for collection of soil samples. The soil samples were collected within the rhizosphere of each cassava stand to a depth of 15 cm using a hand trowel. Soil collected from the rhizosphere of the 15 cassava stands in a farm were bulked together in a polythene bag and labeled appropriately. The bulked soil samples in insulated box were later moved swiftly to the laboratory for extraction. Fifteen cassava stands within a farm were randomly selected for collection of root samples in similar manner to collection of soil samples. The tender roots of cassava were collected to a depth of 15 cm per cassava stand using hand trowel. The collected root samples from the 15 cassava stands in a farm were bulked together with soil earlier collected in a polythene bag. In the same manner, root samples were collected from other farms in the District. The bulked roots with soil samples helped to prevent the roots from drying before further processing in the laboratory.

Extraction of plant-parasitic nematodes from soil and root samples

The extraction of plant-parasitic nematodes from cassava soil and root samples was carried out at the Department of Crop and Soil Science Laboratory, Faculty of Agriculture, University of Port Harcourt. The modified Baermann's method (Whitehead and Hemming, 1965; Coyne *et al*., 2007) was used in the extraction of plant parasitic nematodes from soil samples. Soil in each plastic bag were placed in a different labeled dish and sieved to remove stones and dirt and then mixed thoroughly. The procedure include placing a facial tissue in a plastic sieve with an extraction tray under it, 200 ml soil sample was poured on the facial tissue, then water was added to the extraction plate. The extraction set up was allowed to stay for 48 hours after which the sieve was removed and the nematode suspension was poured into a labeled beaker. The suspension was allowed to settle for six hours after which it was decanted and the suspension containing nematodes were poured into 10 ml vials and preserved in the refrigerator at 4^0C prior to identification and counting. The vials containing nematode suspension were later sent to Nematology Research Laboratory, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria in heat-insulated boxes for identification and counting.

Extraction of plant-parasitic nematodes was done using the same method as for soil samples. Cassava roots collected from each farm were rinsed gently under tap to get rid of dirt and soil particles. The roots were chopped into small parts of 1-2 cm and samples from each sampling unit mixed together. 10 g of the chopped root samples was weighed out using an electronic balance and blended with an electric blender with water above the blades of blender. The root mixture obtained after blending was poured on the facial tissue in the sieve. The extraction set up was allowed to stay for 48 hours after which the sieve was removed and the nematode suspension was poured into a beaker. The suspension was allowed to settle for six hours after which it was decanted and the suspension containing nematodes was poured into 10 ml vials and preserved in the refrigerator at 4^0C prior to identification and counting. The vials containing nematode suspension were later sent to IITA Nematology Research Laboratory in heat-insulated boxes for identification and counting.

Identification of plant-parasitic nematodes

The identification of plant-parasitic nematodes was carried out as described by Dropkin and Smith (1980). Nematodes in the suspension from the soil and roots of cassava were killed with heat. They were later preserved using 4% of formaldehyde pending identification and counting. Aliqout of 2 ml nematode suspension was collected using a pipette into a Doncaster counting dish (Doncaster, 1962). The Doncaster counting dish was placed under a dissecting microscope and compound microscope alternately for identification and counting of plant-parasitic nematodes using Bell's key (Bell, 2004).

Identification was done up to genus level and multiple tally counter was used in the counting of nematodes. Nematodes mean number counted from each aliquot of nematode suspension was multiplied by the total suspension volume to obtain the total number in the nematode suspension.

Data analysis

Descriptive statistics (% frequency and mean) were used to present information on occurrence of plant-parasitic nematodes and their abundance. Frequencies of occurrence of plant-parasitic nematodes in the samples collected were also determined using the formula stated by Norton (1978):

$$\text{Absolute Frequency} = \frac{\text{No. of samples containing a species}}{\text{No. of samples collected}} x \frac{100}{1}$$

$$\text{Relative Frequency} = \frac{\text{Frequency of occurence of a species}}{\text{sum of frequency of all species}} x \frac{100}{1}$$

RESULTS

Plant-parasitic nematodes in soil and roots of cassava in Emohua Local Government Area (LGA), Rivers State

The most frequently occurring plant-parasitic nematode in soil at Emohua Local Government Area (LGA) was *Meloidogyne* species with relative frequency of occurrence (RFOC) of 40.7%, followed by *Gracilacus* species (RFOC 18.5%), whereas *Pratylenchus* species and *Helicotylenchus* species recorded the same RFOC of 14.8% (Figure 3).

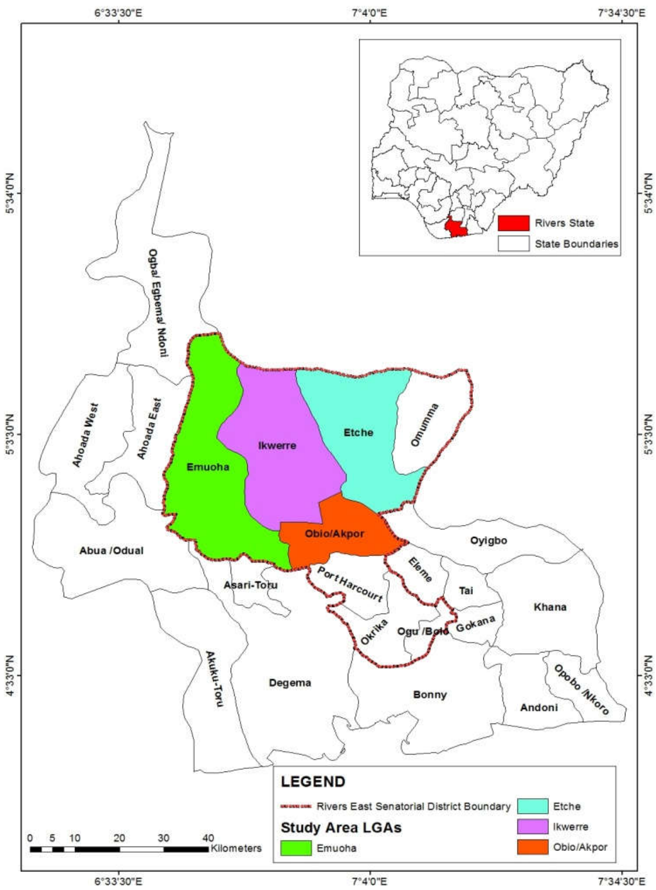

Figure 1. Map showing surveyed Local Government Areas of East Senatorial District, Rivers State

Tylenchus species recorded the least relative frequency of occurrence of 11.1%. In cassava roots, *Meloidogyne* species had the highest relative frequency of occurrence of 100%, but *Pratylenchus* species, *Helicotylenchus* species, *Tylenchus* species and *Gracilacus* species were not found (Figure 3).

Plant-parasitic nematodes in soil and roots of cassava in Obio/Akpor Local Government Area, Rivers State

In soil samples collected in cassava farms in Obio/Akpor LGA, *Meloidogyne* species (RFOC 34.62%) was the most occurring plant-parasitic nematodes, followed by *Helicotylenchus* species (RFOC 19.23%) and *Gracilacus* species (RFOC 19.22%), whereas *Pratylenchus* species and *Tylenchus* species recorded RFOC of 15.39% and 11.54%, respectively (Figure 4). In cassava roots collected from Obio/Akpor LGA, *Meloidogyne* species, *Helicotylenchus* species and *Tylenchus* species had the same relative frequency of occurrence of 33.33%, whereas, *Pratylenchus* species and *Gracilacus* species were not found (Figure 4).

Plant parasitic nematodes in soil and roots of cassava in Ikwerre Local Government Area, Rivers State

In soil collected from cassava farms in Ikwerre LGA, *Meloidogyne* species was the predominant PPN encountered (RFOC 40.74%), followed by *Gracilacus* species (RFOC 29.63%). *Pratylenchus* and *Helicotylenchus* had the same RFOC of 11.11%. *Tylenchus* species recorded the least RFOC of 7.41% (Figure 5). However, in cassava roots, *Meloidogyne* and *Pratylenchus* species were the predominant plant-parasitic nematodes with RFOC of 39.99%, followed by *Gracilacus* (RFOC 20.1%) while, *Helicotylenchus* and *Tylenchus* were not encountered in soils of cassava in Ikwerre LGA (Figure 5).

Plant parasitic nematodes in soil and roots of cassava in Etche Local Government Area, Rivers State

In Etche LGA, soil collected from cassava farms showed that *Gracilacus* species (RFOC 69.23%) were the predominant plant-parasitic nematodes encountered, followed by *Pratylenchus* species (RFOC 15.38%) (Figure 6). *Helicotylenchus* species and *Tylenchus* species had the same RFOC of 7.69%. *Meloidogyne* species were not encountered in soil collected from the cassava farms in Etche LGA. Also, all plant-parasitic nematodes found in the soil grown with cassava in Etche LGA were not found in the cassava roots.

Plant-parasitic nematodes associated with cassava across four Local Government Areas in East Senatorial District of Rivers State

Gracilacus species were the most frequently occurring plant-parasitic nematodes, followed by *Meloidogyne*, whereas *Pratylenchus*, *Helicotylenchus* and *Tylenchus* had frequencies of occurrence of 15.47%, 16.67% and 10.71%, respectively (Figure 7). However, the highest occurring plant-parasitic nematodes in roots were *Meloidogyne* species, followed by *Pratylenchus*. *Helicotylenchus* species, *Tylenchus* species and *Gracilacus* species showed the same occurrence (Figure 7).

Population of plant-parasitic nematodes in soil and roots of cassava across the four Local Government Areas in the East Senatorial District, Rivers State

Meloidogyne species had the highest population of plant-parasitic nematodes associated with cassava in soil that translated to 32.7% of the total population of plant-parasitic nematodes associated with cassava in East Senatorial District, Rivers State (Table 1).

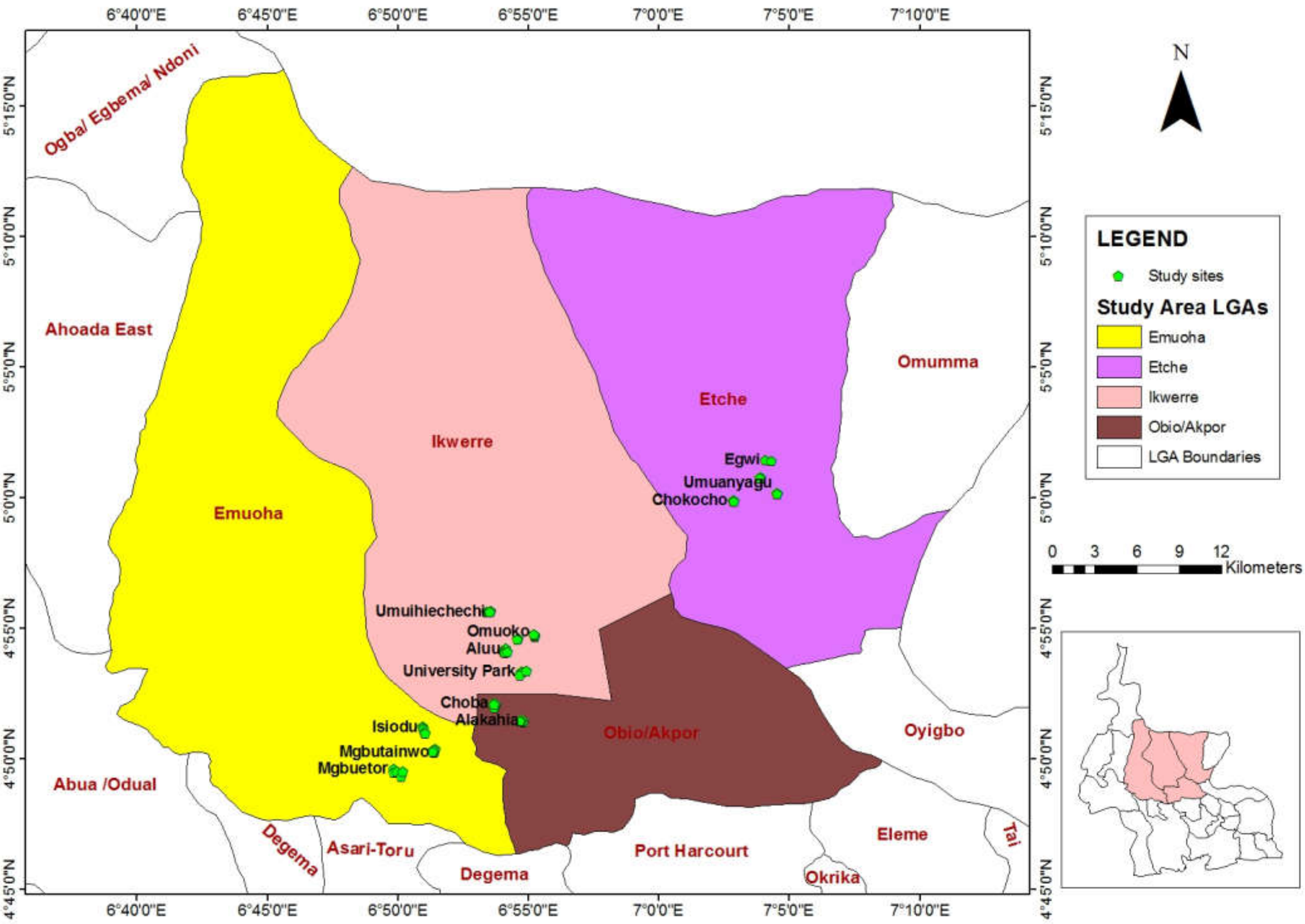

Figure 2. Cassava farming communities sampled in East Senatorial District, Rivers State, Nigeria

Table 6. Population of plant-parasitic nematodes in soil and roots of cassava in East Senatorial District of Rivers State, Nigeria

	Soil		Roots	
Nematode genera	Nematode population*	% Nematode population**	Nematode population/10g	% Nematode population**
Meloidogyne spp.	37.6	32.7	12.5	15.2
Gracillacus spp.	30	26.1	40	48.5
Scutellonema spp.	10	8.6	10	12.1
Pratylenchus spp.	9.62	8.4	10	12.1
Helicotylenchus spp.	27.9	24.2	10	12.1
Total	115.2	100	82.5	100
Sample size=56				

N = Sample size (56)
* Nematode population per 200 ml of soil
**In/TN x100/1 (In = individual nematode in all the samples and TN =Total Population of all nematodes extracted in all the samples)

Gracilacus species and *Helicotylenchus* species had plant-parasitic nematodes population of 30 and 27.9 per 200 ml soil, respectively. Their population densities translated to 26.1% and 24.2%, respectively. *Pratylenchus* species had the lowest population in soil with percentage population of 8.4% among the plant-parasitic nematodes associated with cassava in soil.

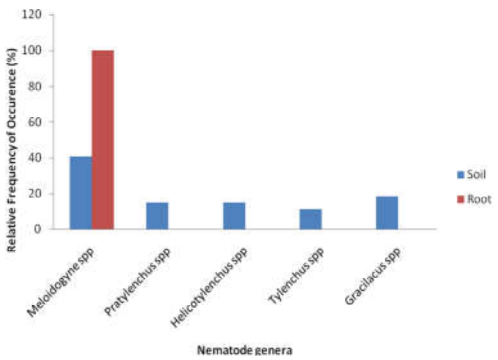

Figure 3. Occurrence of plant-parasitic nematodes in soil and roots of cassava in Emohua Local Government Area, Rivers State

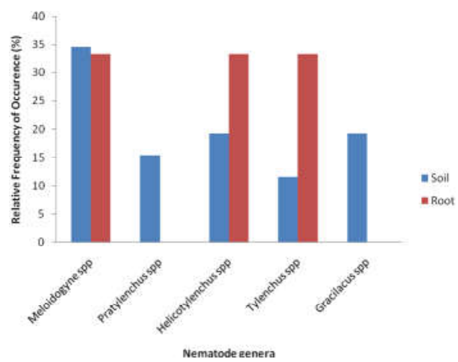

Figure 4. Occurrence of Plant parasitic nematodes in soil and roots of cassava in Obio/Akpor Local Government Area, Rivers State, Nigeria

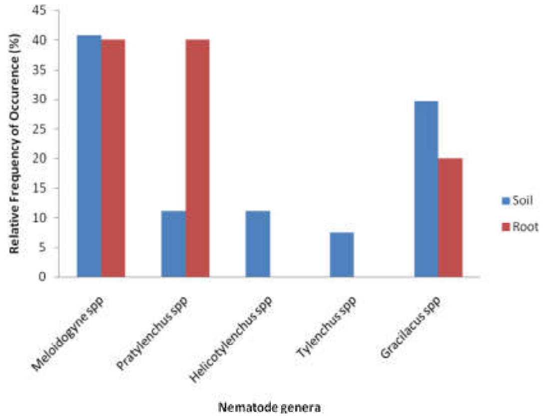

Figure 5. Occurence of plant parasitic nematodes in soil and roots of cassava in Ikwerre Local Government Area, Rivers State

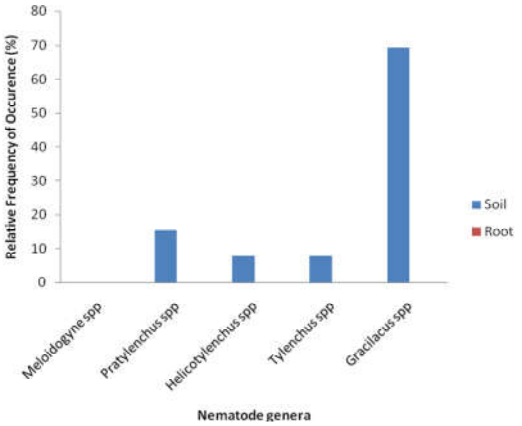

Figure 6. Occurrence of plant-parasitic nematodes in soil and roots of cassava in Etche Local Government Area, Rivers State

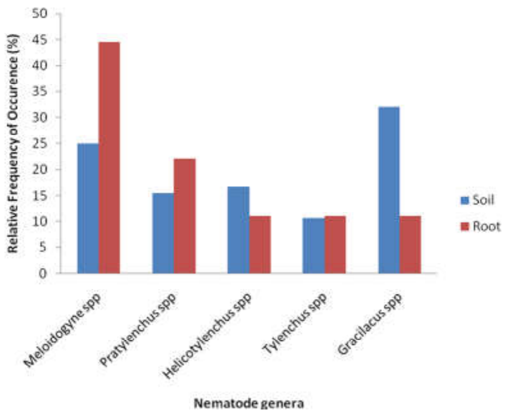

Figure 7. Occurrence of plant parasitic nematodes associated with cassava across four Local Government Areas in East Senatorial District of Rivers State, Nigeria

Plate 1. *Gracilacus* species magX40

This was followed by *Meloidogyne* spp. with a population of 12.5/10 g of cassava roots. However, *Helicotylenchus*, *Gracilacus* species had the highest plant-parasitic nematodes population of 40/10 g of cassava roots across the four Local Government Areas (Table 1) (Plate 1). *Pratylenchus* and *Tylenchus* had the same population of 10/10 g of cassava roots. The percentage population of these plant-parasitic nematodes on cassava roots in the RESD showed that *Gracilacus* species (48.5%) had the highest percentage population, then *Meloidogyne* species (15.2%), where as *Helicotylenchus* species, *Pratylenchus* species and *Tylenchus* species had the same percentage population of 12.1% (Table 1).

DISCUSSION

In order of significance, five genera of plant-parasitic nematodes were associated with cassava in the East Senatorial District of Rivers State; *Meloidogyne, Gracilacus, Pratylenchus, Helicotylenchus* and *Tylenchus.* Environmental constraints have been identified as one of the reasons for the presence of 3-6 species of plant-parasitic nematodes found in a field at a time (Norton *et al*., 1982; Atungwu *et al*., 2013). This might be the reason also for the presence of five genera of plant-parasitic nematodes identified in this study. The predominance of *Gracilacus* species in the roots of cassava in the study areas gave credence to the emerging importance of this nematode on cassava. Although previous report ranked *Meloidogyne* spp. (McSorley *et al*., 1983) as the most important plant-parasitic nematode associated with cassava in most cassava growing regions in the world. *Gracilacus* has not been associated with cassava before now in Nigeria and most cassava growing regions of the world until recently when Asimiea *et al*. (2015) reported the presence of *Gracilacus* species on cassava in Rivers State. The deviation in the types and population of plant-parasitic nematodes associated with cassava in the district might be due to changes in cropping systems such as the cultivation of resistant varieties that could have been a source of resistance to some of the earlier reported nematodes associated with cassava (Nicol, 2002). Other factors culpable for the changes observed in the study could also be climate change and land-use patterns. Differences in the types of soil and properties across the study locations might be another reason for variation observed in occurrence and abundance of nematodes associated with cassava in this study.

Nematode abundance in root and soil samples when compared shows that *Gracilacus* was the predominant in roots while *Meloidogyne* was the most predominant in soil cultivated with cassava. *Gracilacus* is reported to be a migratory ectoparasite (Berry and Coop, 2000) which justifies its presence in the soil also in this study. However, it is endoparasitic in its feeding habits entering the exit points of lateral roots, but do not create their own entrance point as with typical endoparasites and that might be the reason for being found more in the roots of cassava as well as soil (Rhoades and Linford, 1961; Berry and Coop, 2000). *Gracilacus* had also been associated with grape fruits in Germany and some few species had been described in Brazil (Raski, 1962). Some *Gracilacus* species feed primarily in cortical tissue (Inserra and Vovlas, 1977). Their long and robust stylets facilitate their penetration into many cells where they become permanently attached to the root surface without their bodies penetrating into the root tissues. However, detail identification to species level and pathogenic status of *Gracilacus* species on cassava in Nigeria and most cassava-growing regions is yet to be determined. The polyphagous and ubiquitous nature of *Meloidogyne* species explains its occurrence and abundance in roots and soils of cassava. They are sedentary endoparasitic in their feeding habits with very wide host range that ensures their survival on many crops including cassava (Goossens, 1995). They attack feeder roots of cassava causing small galls. The second-stage juveniles found their way into the soil more often and this explains why they were more abundant in the soil. *Helicotylenchus* species are among the most ubiquitous plant-parasitic nematodes worldwide with wide host range (William, 2013). This explains their presence in virtually all samples in roots and soil of cassava in this study. *Helicotylenchus* occurs in both cultivated and uncultivated soils. Their feeding habits classification as ectoparasites, semi-endoparasites and even migratory endoparasites (Decraemer and Hunt, 2006) supported their survival in either the soil or roots of cassava. Luc *et al*. (2005) reported this nematode to be associated with cassava. In this survey, *Helicotylenchus* species were more abundant in soil cultivated with cassava than in roots and thus tend to be ectoparasitic in feeding habit.

However, there is scarcity of reports on damage being inflicted on crops by *Helicotylenchus* species worldwide. *Tylenchus* species are ectoparasites commonly occurring in most soils and feed on algae, mosses, lichens and plant roots (Namaplex, 1999). Yeates *et al*. (1993) described *Tylenchus* spp. as "plant associated", indicating that they were found in the rhizosphere of plants. However, there is little information on the extent of damage of this nematode on cassava. *Pratylenchus* species rank third behind root-knot and cyst nematodes as the nematode of greatest economic impact on crops worldwide (Davis and MacGuidwin, 2000). This is not only due to their wide host range, but their distribution in almost every temperate and tropical environment (Davis and MacGuidwin, 2000). The ability of *Pratylenchus* species of having wide host range might have ensured their presence and survival on cassava in this study. This study shows that *Gracilacus* is emerging significantly as one of the major nematodes of cassava alongside *Meloidogyne* species in East Senatorial District, Rivers State. This outcome corroborated the report of Asimiea *et al*. (2015) and Tanimola *et al*. (2016) on the presence of *Gracilacus* species on cassava in three LGAs and West senatorial district, Rivers State, Nigeria. The species of *Gracilacus* isolated on cassava should be properly identified and their pathogenicity on cassava should be carried out because not all plant-parasitic nematodes associated with any crop are responsible for significant economic damage.

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