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Biodiversity of plant-parasitic nematodes of sugarcane in Bacita, Nigeria

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Plant-parasitic nematodes of sugarcane have been variously reported to be associated with decline in sugarcane production. A field survey of the sugarcane plantation at Bacita, Nigeria was conducted to identify the diverse nematode species associated with the soils and roots of sugarcane and to determine their population densities on the field. Twelve species of plant-parasitic nematodes were found in association with the roots and rhizosphere of the sugarcane plants. They include: *Pratylenchus zeae, Helicotylenchus dihystera, Rotylenchulus reniformis*, *Meloidogyne* sp., *Heterodera sacharri, Criconemoides limitaneum, Tylenchus* sp., *Tylenchorynchus annulatus, Hemicyclophora* sp., *Paratrichodorous* sp., *Trichodorous* sp. and *Longidorus* sp. *Pratylenchus zeae* was the most ubiquitous, occurring at a 100% frequency rating in all the samples. *Longidorous* sp. was not identified from the root samples. The investigation revealed a large diversity of important parasitic nematodes on the sugarcane plantation, thus there is a need for nematode control for improved yield.

Key words: Sugarcane, nematode diversity, population density, frequency of occurrence, Bacita, Nigeria.

INTRODUCTION

Sugarcane (*Sacharrum officinarum*) is an important food crop of the tropics and subtropics (Sivanesan and Waller, 1986). It is an essential food commodity with great potentials for foreign exchange earnings. The main product which is sugar is used universally as sweeteners, blender and as preservatives. It has also become an essential part in many diets, and almost indispensable in the food manufacturing and pharmaceutical industries (NSDC, 2003; Girei and Giroh, 2012).

In Nigeria, sugarcane is the major raw-material for sugar production as it is in other tropical countries. Although there are vast potentials for the commercial production of this crop, its processing industry did not come into existence in Nigeria until the early 1960's (Abdullahi, 2000) with the establishment of two major integrated sugar plants, Nigerian Sugar Company (NISUCO) at Bacita, Kwara state and Savannah Sugar Company (SSCL) at Numan, Adamawa state, in 1961 and 1977, respectively. The two plants had a combined installed capacity of 105,000 tonnes/annum or which is

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10% of the country annual requirement. However, production oscillated around 50,000 tonnes (NSDC 2003). This low sugar production by the sugar companies could only satisfy about 5% of the nation's requirement, thus creating a wide gap which is continually filled with massive importation of sugar. Presently, production seems to have stagnated with the two companies undergoing transformation from public to private ownership. However, the involvement of some private investors in the industry has improved sugar production in recent times. According to FAOSTAT (2011), Nigeria's accumulated import of sugar and sugar products in the last 50 years is valued at $8.18B (N1267.6B) showing that much of the sugar needs have been met through importation and with the ever growing population, the demand for sugar and sugar products is expected to rise.

Girei and Giroh (2012) described a number of factors that could be responsible for the low production of sugarcane from the sugar industries. One of the outstanding problems of sugarcane cultivation caused by nematodes has been the rapid yield decline of successive ratoon crops (Bock et al., 1968). Research elsewhere indicates that many years of monocropping with no fallow period can lead to build up of soil borne pests and diseases, including nematode pests to levels that may be economically important. This may result in a gradual decrease in yield commonly referred to as "yield decline", which is defined as "the loss of productive capacity of sugarcane growing soils under long term monoculture" (Garside et al., 1997).

Nematode diversity on sugarcane is greater than most other cultivated crops, with more than 310 species and 48 genera of endo-and ectoparasitic nematodes reported from the root and rhizosphere of the plant (Spaull and Cadet, 1991). *Meloidogyne* and *Pratylenchus* are the two species of plant-parasitic nematodes most frequently reported as highly pathogenic to sugarcane world-wide (Michel et al., 2005). Castille (1973) reported *Meloidogyne incognita, Heterodera sacharri* and *Pratylenchus* spp. as nematodes associated with yield reduction of sugarcane at fields in Bacita, Nigeria. He noted that damage due to the feeding activities of these nematodes on sugarcane resulted in, stunted plants with fewer mature tillers which appear to be wilting and plants with very patchy appearance. The sugarcane roots also became stunted, pitted and clubbed, thus, leading to a great reduction in the quality and quantity of the harvested sugarcane. Odihinrin (1977) however reported total crop failure on the sugarcane fields due to damage by plant-parasitic nematodes.

The initial report of nematodes associated with sugarcane in Bacita (Castille, 1973) indicated *M. incognita, H. sacharri,* and *Pratylenchus* spp. as important nematode pests of sugarcane causing significant damage on the field. Reports from Hawaii sugarcane fields also indicated *M. incognita* as being frequently encountered and the genus most likely to cause economic damage (Schenck and Holtzmann, 1990). However, numerous nematode genera have been shown to be pathogenic on sugarcane, with *Meloidogyne* and *Pratylenchus* being the most important worldwide (Spaull and Cadet, 1991). Crow (2004) also described *Belonolaimus longicaudatus, Trichodorus* spp., *Paratrichodorus* spp., *Criconemoides* spp., *Tylenchorhynchus* spp., *Pratylenchus zeae, Hoplolaimus* spp., and *Meloidogyne* spp. as important pests of sugarcane fields in Florida

Currently, there is little information on the diverse species of plant-parasitic nematodes associated with sugarcane plantation at Bacita, Nigeria and their population density on the crop. The aim of this study therefore, was to provide an update on the diversity and population distribution of plant-parasitic nematodes associated with sugarcane in Bacita, Kwara State of Nigeria.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the sugarcane plantation of Nigerian Sugar Company in Bacita, (Longitude 4.97° 21'E, Latitude 9.067°N) between January and April, 2013. Bacita is located in the Edu Local Government Area of Kwara State, Nigeria (Figure 1). This ecological zone is within the Sudan savanna, with an annual rainfall of about 800-1000 mm. The sugarcane plantation in Bacita is characterized by cool, wet sandy loam soil which is often irrigated.

Soil sampling

Sugarcane plants were randomly selected for sampling. 30 soil samples were collected from the rhizosphere of each plant using a soil auger of diameter 1.9 cm. Eight core soil samples were taken per plant to a depth of 15-30 cm. A spade was used to dig up plant roots (Coyne et al., 2007). The soil samples were sealed in polythene bags and kept away from sun. The samples were properly labeled and taken to Nematology Research Laboratory, Nigeria Agricultural Quarantine Service, Ibadan for analysis and identification of plant-parasitic nematodes.

Nematode extraction from soil

Composite soil sample per sugarcane plant was gently mixed by hand and two hundred grams (200 g) sub-soil was taken for nematode assay using the Whitehead and Hemming (1965) tray modification of Baermann technique. Two hundred grams soil was put into a set up that has two plastic sieves with extractor tissue sandwiched in between.

The plastic sieves with the soil were thereafter placed in a plastic bowl and water was added to the extraction bowl just enough to wet the soil. The set-up was left undisturbed for 24 h. Thereafter, the plastic sieve containing the soil was removed briskly, and the nematode suspension in the bowl was poured into a Nalgene wash bottle and allowed to settle (Caveness, 1975). The supernatant was siphoned out, and the suspension containing nematodes

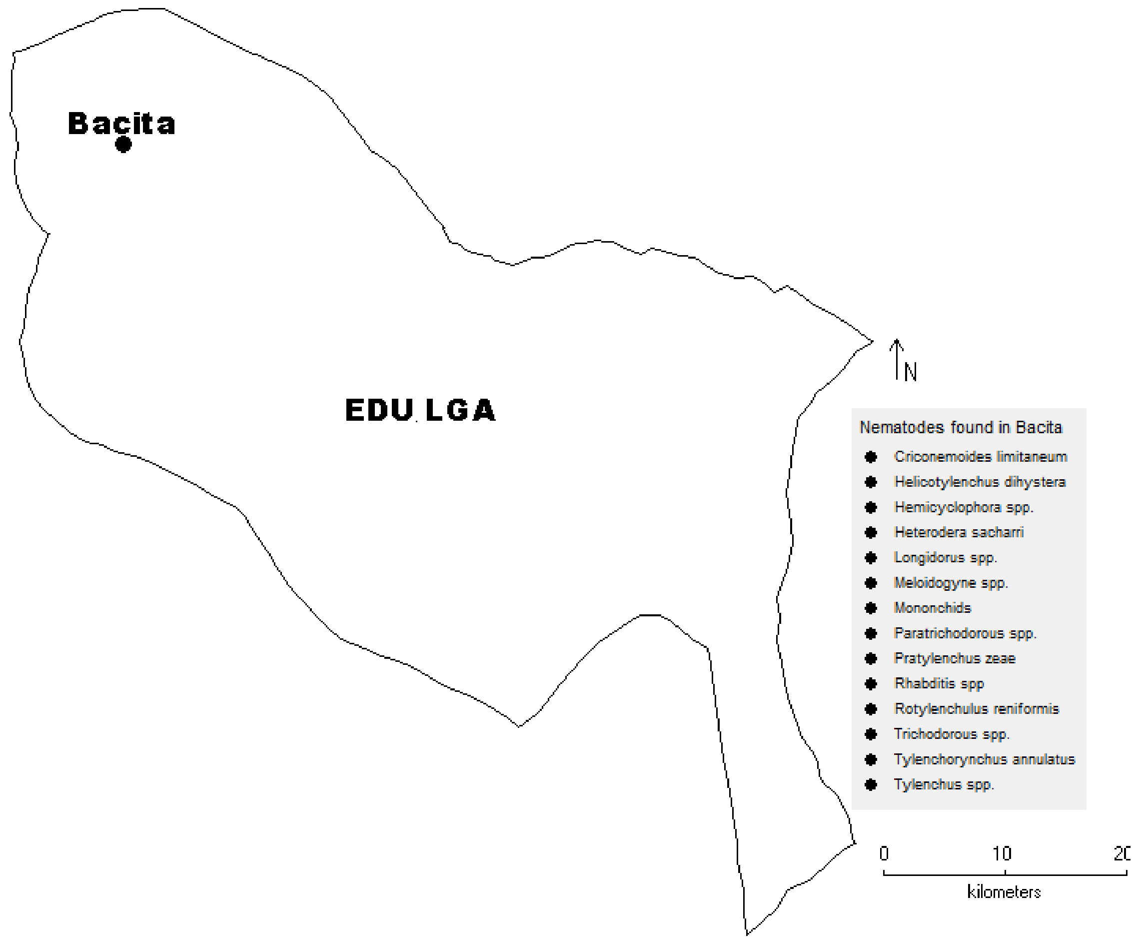


Figure 1. Map of Edu LGA of Kwara State indicating the location of Bacita sugarcane plantation.

was then poured into the Doncaster (1962) nematode counting dish and examined under stereo and compound microscopes.

Nematode extraction from plant roots

Root samples were taken by lifting a whole plant from the soil using a spade (Coyne et al., 2007) so that the galls, cysts and root lesions could be observed from the roots. Roots were collected at the same location as for soil, and were combined in the same sample bag, so that the soil prevented degeneration of the roots before taking them to laboratory.

Total root samples collected per plant were free of soil, washed under a gentle stream of cool tap water and weighed in the laboratory. The roots were separated into live (functional) and dead (non-functional) roots.

Thereafter live roots were cut transversely with scissors into about 1-2 cm length, mixed carefully and 10 g sub-sample was assayed for nematode using the Whitehead and Hemming (1965) tray modification of Baermann technique. A 10 g sub-sample root was put in a blender and macerated for 30 s. The macerated suspensions was then poured into a set up that had two plastic sieves with extractor tissue sandwiched in between them as previously described.

Nematodes identification

Identification of plant-parasitic nematodes was done with the aid of a compound microscope using the simplified pictorial nematode key of Mai and Lyon (1975) and the Crop Protection Compendium Nematode key of CABI (2003).

Data analysis

Percentage frequency of occurrence was determined using the formula.

$n/N \times 100$

n = number of times an individual nematode occurred in all the samples; N = sample size (30).
Also, the percentage nematode population was calculated using the formula.

$In/TN \times 100$

In = individual nematode population in all the samples, while TN = the total population of all the nematodes extracted in all the

Table 1. Population density of nematode species from the rhizosphere of sugarcane at Bacita Sugarcane Plantation, Nigeria.

Nematode species	Nematode population/200 g soil	Nematode population (%)
Mononchids	4,360	49.33
Pratylenchus zeae	910	10.3
Longidorous spp.	862	9.75
Helicotylenchus dihystera	604	6.83
Tylenchus spp.	592	6.7
Criconemoides limitaneum	256	2.9
Hemicyclophora spp.	241	2.73
Paratrichodorous spp	238	2.69
Rhabditis spp	189	2.14
Trichodorous spp.	146	1.65
Rotylenchulus reniformis	132	1.49
Meloidogyne spp.	122	1.38
Tylenchorynchus annulatus	101	1.14
Heterodera sacharri.	84	0.95
Total	8,837	

Sample size (N) = 30.

Table 2. Population density of nematode species from roots of sugarcane at Bacita Sugarcane Plantation, Nigeria.

Nematode species	Nematode population/10 g root	Nematode population (%)
Tylenchus spp.	56	24.14
Pratylenchus zeae	51	21.98
Meloidogyne spp.	32	13.79
Rotylenchulus reniformis	24	10.34
Helicotylenchus dihystera	20	8.62
Trichodorous spp	13	5.6
Heterodera sacharri	11	4.74
Hemicyclophora spp.	10	4.31
Paratrichodorous spp	7	3.02
Criconemoides limitaneum	5	2.16
Tylenchorynchus annulatus	3	1.29
Total	232	

Sample size (N) = 30.

samples.

RESULTS

Nematode species isolated from soil and roots of sugarcane

Twelve species of parasitic nematodes were found associated with the soil and roots of sugarcane in Bacita, Nigeria. They include: *Pratylenchus zeae, Helicotylenchus dihystera, Rotylenchulus reniformis, Meloidogyne* sp., *Heterodera sacharri, Criconemoides limitaneum, Tylenchus* sp., *Tylenchorynchus annulatus, Hemicyclophora* sp., *Paratrichodorous* sp., *Trichodorous* sp. and *Longidorus* sp. (Figure 4).

Population densities of nematode species associated with sugarcane

The population densities of the nematode species recovered from the soil and roots sugarcane plants is described in Tables 1 and 2. High populations of *P. zea*

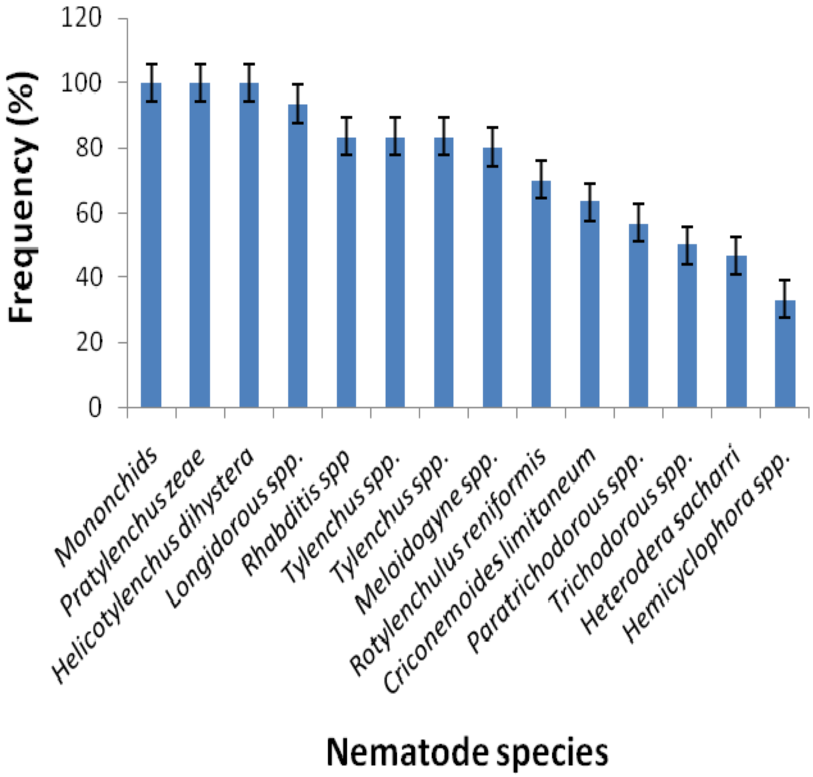


Figure 2. Frequency rating of nematode species associated with rhizosphere of sugarcane in Bacita, Nigeria during a field survey in 2013.

were observed from the soil and root samples, constituting about 22% of the total nematode species associated with the root samples. Also found in high populations were root-knot nematodes (*Meloidogyne* species) from the root samples. *Logidorous* spp., *Helicotylenchus dihystera* and *Criconemoides limitaneum* were found in high populations in the soil however, *Longidorus* spp. was not recorded in the root samples. *Tylenchorynchus annulatus* was also recorded in low populations from the soil and root samples.

Frequency of occurrence of plant-parasitic nematodes from soil samples

The prominent plant-parasitic nematodes found on the rhizosphere of the sugarcane plants were *P. zeae* and *Helicotylenchus dihystera* which were most ubiquitous with 100% frequency ratings in all soil samples. *Meloidogyne* sp. and *Rotylenchulus reniformis* were also frequently encountered at frequency ratings of 80 and 70%, respectively (Figure 2). Low population of *T. annulatus* and *Hemicyclophora* sp. (Table 1) was recorded at frequency ratings of 40 and 33.33%, respectively.

Frequency of occurrence of plant-parasitic nematodes from the roots of sugarcane

The most prevalent plant parasitic nematodes extracted from the root samples were *P. zeae, Meloidogyne* sp., *R. reniformis* and *H. dihystera*. They occurred at frequency ratings of 76.67, 73.33, 60 and 53.33%, respectively. *Heterodera sacharri* and *Trichodorous* sp. occurred at 33.33% while *Tylenchorynchus annulatus*, *Hemicyclophora* sp*., Paratrichodorous* sp. *Criconemoides* sp. occurred in low frequency ratings (Figure 3). *Longidorus* sp. was not found in association with the root samples.

DISCUSSION

The result of this study revealed a large diversity of ecto- and endo-parasitic nematodes in association with sugarcane in Bacita sugarcane plantation. They include important nematode species such as *P. zeae*, *Meloidogyne* sp., *R. reniformis*, *H. saccharri, H. dihystera, T. annulatus, Criconemoides, P. longidorous* and *Trichodorus* species which have been frequently associated with yield reduction in sugarcane production

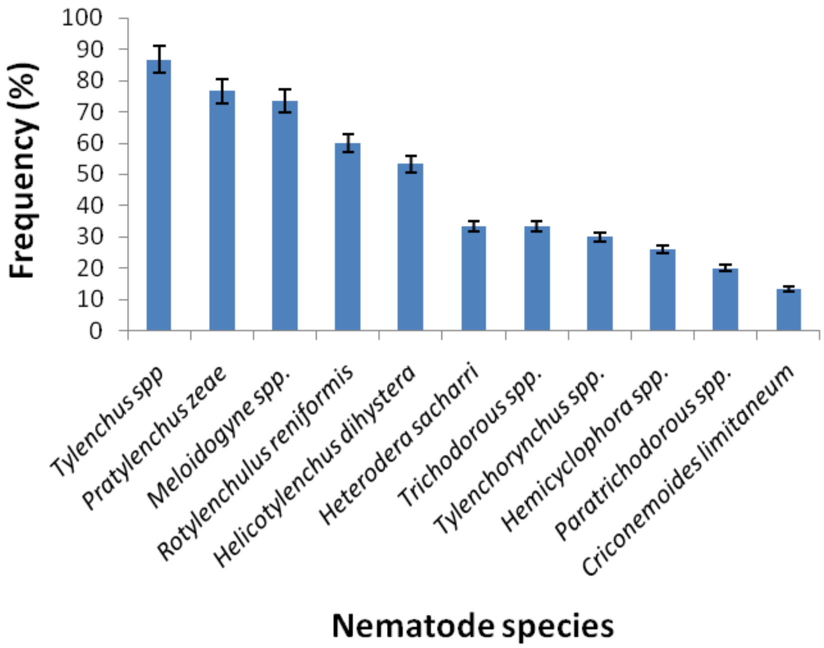


Figure 3. Frequency rating of nematode species associated with roots of sugarcane in Bacita, Nigeria during a field survey in 2013.

world-wide (Schenck and Holtzman, 1990; Crow, 2004). These nematode pests were also encountered in populations that could be detrimental to plant health and good yield which according to Afolami (2000) is the ultimate goal of farmers. Reports from other parts of the world (Lamberti et al., 1987; Spaull and Cadet, 1991; Blair et al., 1999; Bond et al., 2000) have indicated these nematode species as important pests of sugarcane causing plant debilitation and poor growth.

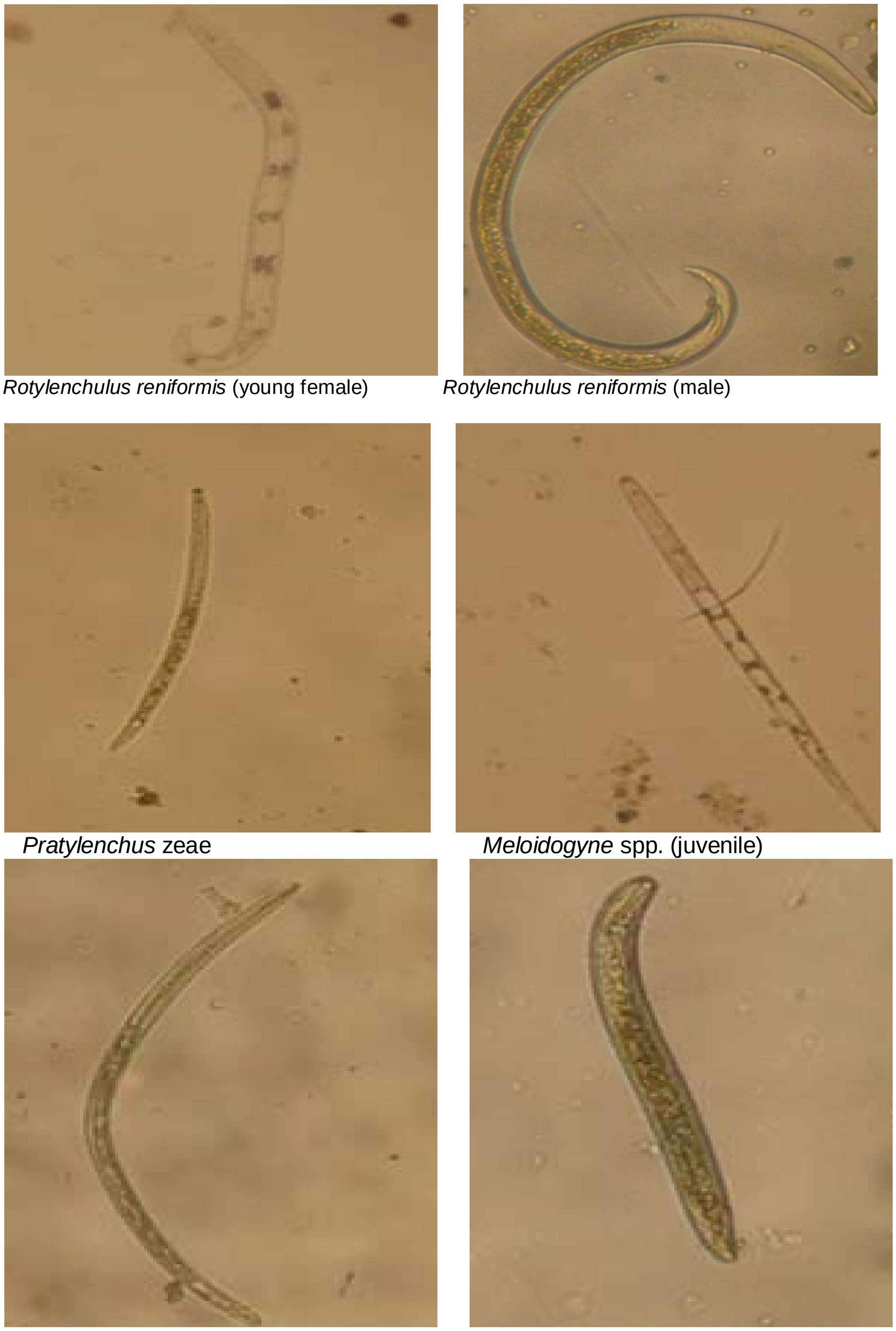
The present study also confirms *Meloidogyne* species, *P. zeae and H. sacharri* as frequently encountered and abundant in the irrigated fields of Bacita sugarcane plantation as indicated by Castille (1973). The study also shows that *R. reniformis* and *H. dihystera* are abundant and frequently encountered in the plantation. The high population of these nematode species on the crop is an indication that the sugarcane is a suitable host which is grown in an environment that favors and supports the development of the nematode pests on the crop.

Factors that could have contributed to the distribution of these nematode pests in the sugarcane plantation at Bacita include cropping system, type of soil, plant age and cultural practices among other environmental influences. According to Taylor (1971), soil type plays an important role in nematode distribution. Plant-parasitic nematodes have been reported to be more abundant and cause greater damage in sandy soils than in the heavier soils (Schenck and Holtzmann, 1990). Bond et al. (2000) also recorded greater occurrence of *Meloidogyne* spp. and *Paratrichodorus* spp. in soils with a very low clay content, majority of which had high sand content. The sugarcane plantation in Bacita is characterized by cool, wet sandy irrigated soil thus providing a suitable and enabling environment for the development of these nematode pests.

Irrigation which is a regular practice on the sugarcane plantation could have also contributed to the widespread distribution of *H. sacharri*. Odihinrin (1977) reported those heterodera cysts are easily carried in the irrigation canals by flood waters as a means of dissemination.

In Bacita, Nigeria, sugarcane is usually grown as a continuous monoculture with very few months of break between removing the old ratoon crop and replanting the field. These conditions also tend to favour the development and accumulation of relatively large populations of nematode communities in the sugarcane field. Studies that characterized nematode populations in sugarcane soil and have demonstrated that monoculturing of sugarcane can foster the accumulation of diverse nematode communities (Spaull and Cadet, 1991; Hall and Irey, 1990).

Factors such as plant age and cropping system also play important roles in determining the abundance and distribution of nematode species. The Bacita sugarcane plantation has been cultivated for about fifty years with very few years of fallow. According to Bond et al. (2000), nematode population are found to be greater in sugarcane plants that were planted shortly after the



Rotylenchulus reniformis (young female) *Rotylenchulus reniformis* (male)

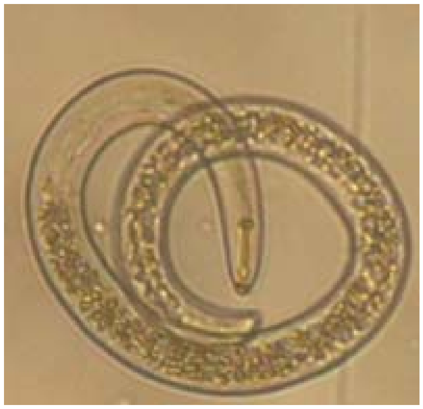
Pratylenchus zeae *Meloidogyne* spp. (juvenile)

Tylenchorynchus annulatus (female) *Criconemoides limitaneum*

Figure 4. Micrographs of plant-parasitic nematodes found on sugarcane in southwestern states and Bacita, Kwara State of Nigeria during a field survey in 2013 (60x).

harvest of final ratoon crops than those following a fallow period, also higher community densities of nematodes are often recorded in the ratoon crops than in the plant cane. This suggests that the sugarcane plantation at Bacita is a likely reservoir for diverse nematode species.

The study also reveals the presence of important soil nematodes such as *Trichodorus*, *Paratrichodorus*, *Criconemoides* and *Longidorous* species which were not



Helicotylenchus dihystera (Adult female)

Trichodorous spp.

Heterodera sacharri (immature female)

Paratrichodorous spp.

Hemicyclophora spp.

Longidorus spp.

Figure 4. Contd.

indicated in the initial report (Castille, 1973) from the sugarcane plantation. This could be attributed to the increased plant productivity and climatic changes experienced on the plantation over a period of about 50 years. According to Van der Wal et al. (2009), diversity of soil nematodes increased with increasing plant productivity after nitrogen addition. As one of the most important soil biota, soil nematodes are widespread and highly diverse, occupying multiple trophic positions in the soil food web and are increasingly used as bio-indicator of soil environmental change. Their responses also play a key role in the feedbacks of terrestrial ecosystems to climate change (Neher, 2001; Wei et al., 2012). Climatic changes and global warming could have contributed significantly to the incidence and increase in the biodiversity of plant-parasitic nematodes on the Bacita plantation.

Nematodes have been reported to constitute serious impediments to intensified production of sugarcane in various part of the world (Michel et al., 2005). Estimates for *M. incognita* or *Pratylelenchus* spp. in Australia indicate that where the number of individuals in soil exceed 100/200 g of soil before planting or 200/200 g of soil at mid-season, there may be a significant reduction in cane yield (Stirling and Blair, 2000).

Afolami (2000) stated that the ultimate goal of the farmer is the yield, which unfortunately could be negatively impacted by the cumulative effect of the feeding damage caused by these nematodes. Therefore managing parasitic nematodes in sugarcane fields such as Bacita sugarcane plantation is expedient for improved crop yield which could ultimately boost local sugar production in the country and reduce dependence on importation.

Conclusion

There is a need to educate local farmers on the large diversity of parasitic nematodes associated with sugarcane and their damage potentials by creating awareness programmes. Screening of seed plants and regular plant quarantine services should be employed prior to the introduction of new varieties to sugarcane farmers and also, effective nematode management strategies should be considered in order to improve yield in sugarcane plantations.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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