AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Studies on the Distribution of Plant-parasitic Nematodes Associated with Pineapple in Delta, Imo and Cross River states of Nigeria

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ARTICLE INFO

Article history:
Received 2 February 2014
Received in revised form
8 April 2014
Accepted 28 April 2014
Available online 25 May 2014

Keywords:
Pineapple, Survey, Population distribution, Plant-Parasitic Nematodes, Nigeria

ABSTRACT

Background: Plant-parasitic nematodes are important pests of horticultural crops causing damage and significant reduction in the crop yield of pineapple**.** A survey was conducted to determine the types, frequency and population distribution of plant-parasitic nematodes associated with pineapple in some pineapple-producing states in Nigeria. A total of 30 farms were sampled from Delta, Imo, and Cross-River States using the Agricultural Development Project in each state as a pilot to locate representative farmlands in the States. Soil samples for the survey with were collected from the rhizosphere of the pineapple plants with the aid of a soil auger to a depth of about 15 cm and within a 25 cm radius from the base of the pineapple plants. Vermiform nematodes were extracted from 200g each of the composite samples using a modified Baermann extraction tray set-up. **Objective:** To provide an update on the diversity and population distribution of plant-parasitic nematodes associated with pineapple in Delta, Cross River and Imo states of Nigeria. **Result:** Fourteen species of Plant-Parasitic Nematodes (PPN) were found associated with pineapple from the fields surveyed as follow: *Pratylenchus brachyurus, Meloidogyne incognita, Rotylenchulus reniformis, Helicotylenchus dihystera, Scutellonema brachyurum, Tylenchus* sp., *Hoplolaimus pararobustus, Criconemoides limitaneum, Paratylenchus minutus, Gracilacus* sp., *Hemicycliophora* sp., *Aphelenchus* sp., *Aphelenchoides* sp. and *Xiphinema nigeriense*. The most prominent phytophagus nematode found in association with pineapple on all the fields was P. *brachyurus* with frequency ratings of 100%, 92% and 87% from Delta, Imo and Cross-river states respectively. *Meloidogyne* sp., *Pratylenchus reniformis* and *Rotylenchulus reniformis* were also frequently encountered in the three states. *Grasilacus* sp. was recorded on pineapple for the first time in Nigeria. **Conclusion:** The study indicated a widespread distribution of PPN on pineapple in south-south and south-east Nigeria which could be a factor responsible for low yields recorded in some pineapple fields. Therefore, there is an obvious need for pineapple farmers to control plant-parasitic nematodes for improved crop yield.

To Cite This Article: Fisayo Daramola and Steven Afolami, Studies on the Distribution of Plant-parasitic Nematodes Associated with Pineapple in Delta, Imo and Cross River states of Nigeria. ***Aust. J. Basic & Appl. Sci.,*** *8(7): 248-256, 2014*

INTRODUCTION

Pineapple (*Ananas comosus*. Meer) is an important delectable tropical fruit. It is the only source of bromilain (Bartholomew, *et al*., 2003), a protolytic enzyme that offers immense health benefits due to its natural anti-inflammatory properties, ability to inhibit the growth of malignant cells and also serve as antithrombotic, and fibrinolytic agents (Bhattacharyya, 2008; Joy, 2010). Pineapple is a rich source of vitamin C with considerable calcium, potassium and fiber and it has a variety of uses in diets as desserts, concentrates, marmalades, salads, fresh juice and canned drinks (Purseglove, 1972; Joy, 2010). Production and trade in fresh fruits and pineapple juice from manufacturing companies has great potential to advance foreign exchange earnings, apart from providing employment opportunities for the ever teeming population in developing countries.

A great limitation to world-wide production of pineapple is damage caused by plant-parasitic nematodes (Rohrbach and Apt, 1986). They have been described as important pests of horticultural crops (Stirling and Pattison 2008) causing significant reduction in pineapple yield (Gianessi, *et al*., 2002; Sipes , *et al*., 2005). Among the most important plant-parasitic nematodes of pineapple that has been reported world-wide are *Meloidogyne* sp., *Rotylenchulus reniformis* and *Pratylenchulus brachyurus* (Rohrbach and Apt, 1986).

Nigeria on the global scene always had the largest land area (about 17% of global land area harvested under pineapple) for pineapple production (Asopa, 2003). However, outputs from this vast land, reflected low

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yields when compared with production from other countries. Nigeria has the largest land area for pineapple production yet ranked 7th largest producer with no record of exportation (FAOSTAT 2012). In recent times pineapple cultivation in Nigeria has increased substantially with active participation from the private sector thereby resulting in large acreages of land being planted to pineapple in order to meet the increasing demands of the local manufacturing industries (BGNL 2004).

In Hawaii, a significant reduction of about 60% - 74% in the crop yield of pineapple was reported (Sipes 2000) as a result of nematode infection. The most prevalent species of nematodes on pineapple were *Rotylenchulus reniformis, Helicotylenchus dihystera, Paratylenchus minutus, Pratylenchus brachyurus* and *Meloidogyne javanica. R. reniformis,* was the most significant of them all (Schenck and Holtzmann, 1990; Caswell , *et al*,. 1990). In Australia, *Meloidogyne javanica* is reported to be the most damaging of all nematodes in Queensland pineapple fields (Stirling, 1993). *Pratylenchus brachyurus and Rotylenchulus reniformis* have also been reported to cause major losses while *Helicotylenchus dihystera* and the ring nematode, *Criconemella ornata*, are also common on pineapple fields, although without evidence of economic damage (Raski and Krusberg, 1984; Stirling, 1993). Caveness (1965) gave a cursory report on plant-parasitic nematodes associated with pineapple in Nigeria. Daramola, *et al.* (2013) also reported a wide spread distribution of parasitic nematodes of pineapple in some southern states of Nigeria. This study therefore aims at providing an update on the diversity and population distribution of plant-parasitic nematodes associated with pineapple in Delta, Cross River and Imo states of Nigeria.

MATERIALS AND METHODS

Survey of Plant-Parasitic Nematodes Associated with Pineapple in Some Pineapple-Producing States in South-eastern Nigeria

Collection Site:

A survey of the plant-parasitic nematodes associated with pineapple was conducted in Delta, Cross River and Imo states, three major pineapple-producing states in Nigeria. Pineapple farms were purposively selected from each state using the Agricultural Development Project (ADP) structure as a guide to locate representative pineapple fields in each state. The criteria for selection of the farms to sample were based on accessibility, availability of farmer or escort, and the willingness of the farmers to allow sample collection from their farms. A total of 30 farms were sampled from the three states.

Sample Collection:

Soil samples for the survey were collected from the rhizosphere of the pineapple plants with the aid of a soil auger to a depth of about 15 cm and within a 25 cm radius from the base of the pineapple plants. Thirty soil core samples per hectare were randomly taken from each of the pineapple fields and bulked together to form a composite sample. Each composite sample was thoroughly mixed and passed over a 10mm diameter mesh sieve to remove all stones and debris that might be present, and were taken to the laboratory for nematode extraction. Soil samples were collected in sealed bags and properly labeled with attention given to the date of collection, time, location and field from which the samples were collected.

Extraction of Nematodes from Soil:

The Extraction Tray method of Whitehead and Hemming (1965) was used for the extraction of vermiform nematodes from the soil samples. Two hundred grams (200g) of each of the composite samples were placed in the upper sieve of a modified Baermann tray set-up which was made up of a double-ply facial tissue, sandwiched between a pair of plastic sieves and placed in a bowl of water with about 500 ml of water in it. The set-up was allowed to remain undisturbed for 24 hours, after which the sieves were gently lifted off. The resulting nematode suspension in the bowl were poured into a 500 ml Nalgene wash bottle and left undisturbed for 5 hours. The suspension was concentrated to 25 ml by siphoning excess water (supernatant) using the settling-siphon method (Caveness, 1975) and the remaining nematode suspension were poured into a Doncaster (1962) counting dish for identification and counting of the different nematode genera and species.

Data Analysis:

Percentage frequency of occurrence (frequency rating) was determined using the formula.

n/N x 100/1

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

Also, the percentage nematode population was calculated using the formula.

In/TN X 100/1

Where In = Individual nematode population, Tn = Total number of nematodes extracted in the samples.

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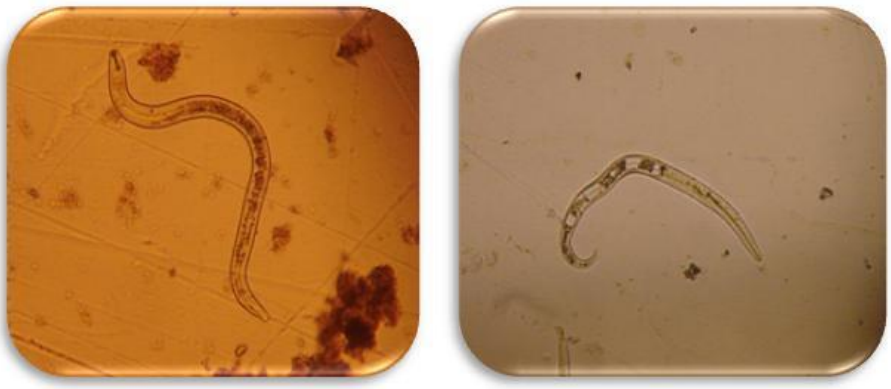
Result:

Plant-parasitic nematodes Associated with pineapple:

Fourteen species of plant-parasitic nematodes were identified and recorded in association with pineapple from Delta, Cross River and Imo States of Nigeria. The plant-parasitic nematodes recovered from soil in the pineapple fields include; *Pratylenchus brachyurus, Tylenchus* sp., *Helicotylenchus dihystera, Scutellonema brachyurum, Rotylenchulus reniformis, Meloidogyne incognita, Hoplolaimus pararobustus, Criconemoides limitaneum, Paratylenchus minutus, Grasilacus* sp., *Hemicycliophora* sp., *Aphelenchus* sp., *Aphelenchoides* sp. and *Xiphinema nigeriense* (Plate 1). The occurrence and widespread distribution these nematode species on pineapple fields in the three states surveyed is as described in table I.

a. Male *Rotylenchulus reniformis* b. Young female *Rotylenchulus reniformi* (Infective stage)

c. *Scutellonema brachyurum* d. *Helicotylenchus dihystera*

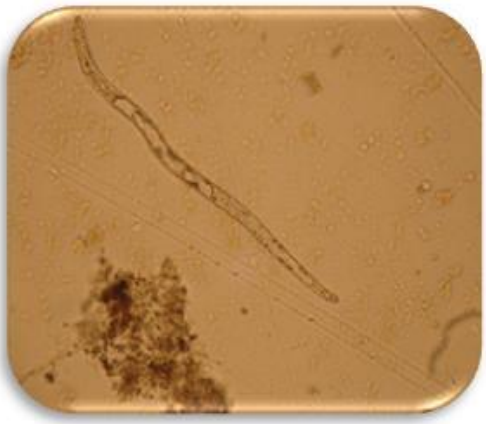


e. *Pratylenchus brachyurus* f. *Gracilacus* sp.

Plate 1: Micrographs of plant-parasitic nematodes found on pineapple in Delta, Cross River and Imo states during a field survey in 2009 (x 100).

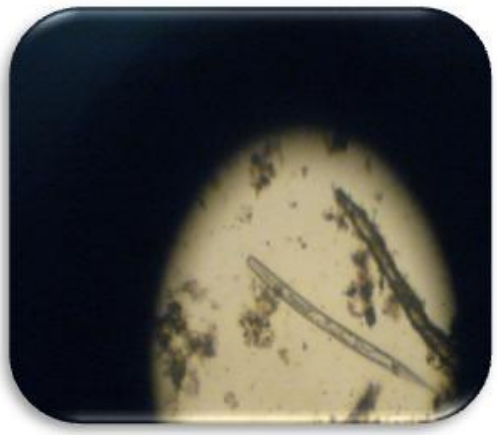
g. *Paratylenchus minutus*

h. *Criconemoides limitaneum*



i. *Aphelenchus* sp.

j. *Xiphinema nigeriens*



k. *Hemicycliophora* sp.

l. *Meloidogyne* sp. (infective stage)

Plate 1: continued: Micrographs of plant-parasitic nematodes found on pineapple in Delta, Cross River and Imo states during a field survey in 2009 (x 100)

Table I: Occurrence and geographical distribution of plant-parasitic nematodes of pineapple from Delta, Cross River and Imo states of Nigeria.

Nematode species	States		
	DELTA	IMO	CROSS RIVER
Meloidogyne incognita	-	x	x
Tylenchus sp.	x	x	x
Pratylenchus brachyurus	x	x	x
Paratylenchus minutus	-	x	x
Helicotylenchus dihystera	x	x	x
Scutellonema brachyurum	x	x	x
Hoplolaimus pararobustus	x	x	x
Rotylenchulus reniformis	-	-	x
Gracilacus sp.	x	x	x
Criconemoides limitaneum	-	x	x
Hemicycliophora sp.	-	x	x
Aphelenchus sp.	x	x	x
Aphelenchoides sp.	x	-	-
Xiphinema nigeriense	x	-	-

X=Present;
- = Absent

Population Densities of Nematode species associated with Pineapple:

The population densities of the individual nematode species associated with pineapple in the three states surveyed are illustrated in TABLES II-IV. In Delta state, high populations of *Tylenchus* sp., *P. brachyurus* and *Grasilacus* sp. were recorded while *Hemicyclophora* sp. occurred in very low population (TABLE II). A large diversity of plant-parasitic nematodes was recorded from Cross River state with high populations of *Tylenchus* sp., *H. dihystera*, *P. brachyurus*, *Paratylenchus minutus* and *Grasilacus* sp. Also recorded in low populations were *S. brachyurum* and *Criconemoides* species (TABLE III) *Melodogyne* and *Rotylenchulus* species were also recorded in soil samples from the state. In Imo state however, high populations of *Meloidogyne incognita*, *P. brachyurus* and *Grasilacus* sp. were recorded. Low populations of *Hoplolaimus pararobustus* was also recorded from the state.

Table II: Population density of nematode species associated with pineapple in Delta State, Nigeria.

Nematode species	Population/200ml soil	% population
Mononchids	3022	62.6
Tylenchus sp.	756	15.7
Pratylenchus brachyurus	239	5.0
Helicotylenchus dihystera	108	2.2
Scutellonema brachyurum	46	1.0
Gracilacus sp.	233	4.9
Hemicycliophora sp.	2	0.04
Aphelenchus sp.	34	0.7
Eutylenchus sp.	12	0.3
Dorylaimids	373	7.7
Total	4825	

Sample size (N) = 9

Table III: Population densitties of nematode species associated with pineapple in Cross River State, Nigeria.

Nematode species	Population/ 200ml soil	%population
Mononchids	3874	43.8
Meloidogyne incognita	124	1.4
Tylenchus sp.	1442	16.3
Pratylenchus brachyurus	463	5.2
Paratylenchus minutus	442	5.0
Helicotylenchus dihystera	1164	13.5
Scutellonema brachyurum	42	0.5
Hoplolaimus pararobustus	206	2.3
Rotylenchulus reniformis	120	1.4
Gracilacus sp.	396	4.5
Criconemoides limitaneum	36	0.4
Hemicycliophora sp.	80	0.9
Aphelenchus sp.	110	1.2
Dorylaimids	353	4.0
Total	8852	

Sample size (N) = 8

Table IV: Population density of nematode species associated with pineapple in Imo State, Nigeria.

Nematode species	population /200ml soil	% population
Mononchids	8,570	65.7
Meloidogyne incognita	703	5.4
Tylenchus sp.	1287	9.9
Pratylenchus brachyurus	684	5.2
Paratylenchus minutus	156	1.2
Helicotylenchus dihystera	108	0.8
Scutellonema brachyurum	106	0.8
Hoplolaimus pararobustus	36	0.3
Rotylenchulus reniformis	170	1.3
Gracilacus sp.	387	3.0
Criconemoides limitaneum	126	1.0
Hemicycliophora sp.	86	0.7
Aphelenchus sp.	126	1.0
Dorylaimids	506	3.9
Total	13051	

Sample size (N) = 13

Frequency ratings of Nematode species Associated with Pineapple:

Pratylenchus brachyurus was the most ubiquitous phytophagous nematode having been found in large populations and occurring in all the farms at frequency ratings of 100%, 92% and 87% in Delta, Imo and Cross River states respectively (TABLE II; Fig. 1a-c). *Tylenchus* sp., *Aphelenchus* sp., *Helicotylenchus dihystera* and *Scutellonema brachyurum* were also numerous and frequently encountered in the three states surveyed (Fig.1a-c).

In south-south Nigeria, the prominent plant-parasitic nematodes found in association with pineapple in Delta State included *Tylenchus* sp., *P. brachyurus, H. dihystera, Aphelenchus* sp. and *S. brachyurum. P. brachyurus* was the most prevalent phytophagous nematode species occurring at 100% frequency rating in all the soil samples from the state. *Scutellonema* sp. *and H. dihystera* were also common and frequently encountered in the soil at frequency ratings of 78% and 67% respectively. *Meloidogyne incognita* and *R. reniformis* were not recorded from the pineapple fields, however low populations of *Gracilacus* sp. was recorded at a frequency rating of 33% (Fig. 1a).

In Cross River State, the most frequently encountered nematode species found in association with pineapple were *P.brachyurus* and *H. dihystera* at frequency ratings of 87% and 75%. *M. incognita* and *R. reniformis* occurred in low populations and frequency ratings of 12.5% and 5% respectively. *Gracilacus* sp., *C. limitaneum* and *Hemicycliophora* sp. also occurred at frequency ratings of 35% and 25% respectively.

In the south-east Imo state, higher populations of *Gracilacus* sp., *C. limitaneum* and *Hemicycliophora* sp. occurred at frequency ratings of 46%, 39% and 39% respectively (Fig. 4c). Low frequency ratings were also observed for *M. incognita* and *R. reniformis* at 39% and 31% of occurrence while the prevalent phytophagous nematodes *(P. brachyurus, S. brachyurum* and *H. dihystera)* occurred at frequency ratings of 92%, 79% and 54% respectively.

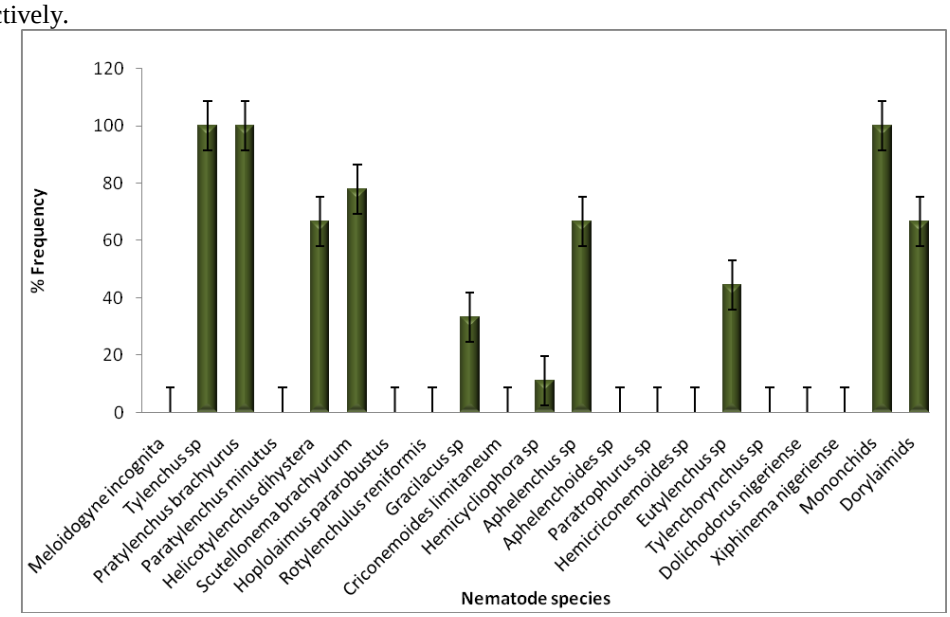


Fig. 1(a): Frequency rating of nematodes found in association with pineapple in Delta state of Nigeria during a field survey in 2009.

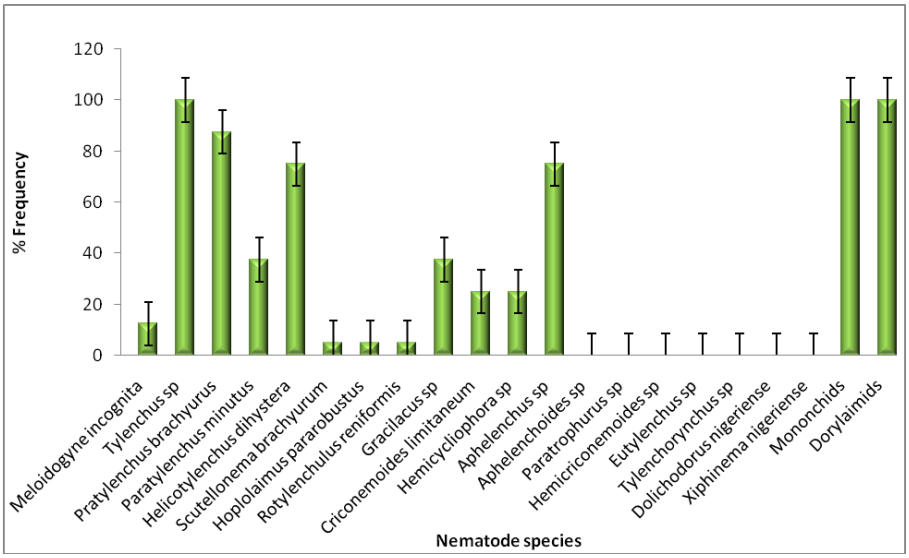


Fig. 1(b): Frequency rating of nematodes found in association with pineapple in Cross River state of Nigeria during a field survey in 2009**.**

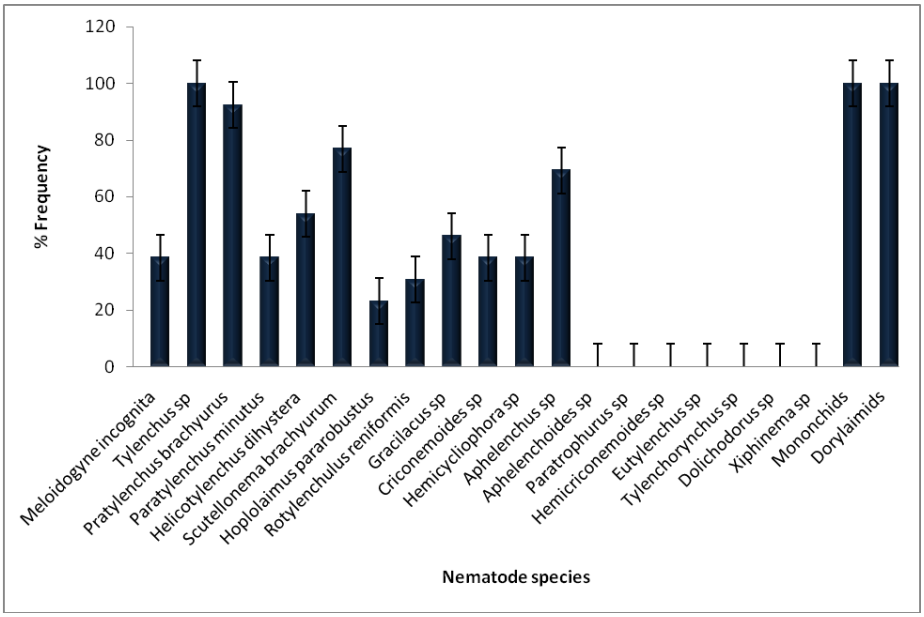


Fig. 1(c): Frequency rating of nematodes found in association with pineapple in Imo state of Nigeria during a field survey in 2009.

Discussion:

The study on the occurrence and distribution of the plant-parasitic nematodes associated with pineapple from the Delta, Cross River and Imo states provides an update on the findings of Caveness (1965). The results of this study showed that *Meloidogyne incognita*, *Pratylenchus brachyurus* and *Rotylenchulus reniformis* which have been implicated world-wide as important nematode pests of pineapple are common and abundant in pineapple fields in Nigeria. The spiral nematode *(Helicotylenchus dihystera)* which was found in large numbers in the pineapple soils, as indicated by the results of this investigation, has also been reported to be important in pineapples (Babatola, 1985; Rohrbach and Apt, 1986; Ko and Schmitt, 1993).

Tylenchus and *Aphelenchus* species were found occurring in large numbers and at high frequency ratings in the soil samples examined from the pineapple fields. The abundance of these nematode species in soil has been variously reported but their importance in agricultural fields is yet to be established (Bafokuzara, 1996; Sharma and Amabile, 1999). Adding to the parasitic load in the soil are *Scutellonema brachyurum*, *Hoplolaimus pararobustus*, *Criconemoides limitaneum*, *Hemicycliophora* sp., *Paratrophurus* sp., *Hemicriconemoides* sp. and *Tylenchorynchus* sp. Most of these nematodes are of limited or unknown pathogenicity on pineapple (Sipes , *et*

al., 2005). However, the presence of these numerous nematode species in pineapple could form an additional pressure of parasitism and their occurrence in large populations pose a threat to pineapple production. Plant-parasitic nematodes have been identified as one of the limiting factors responsible for the decline suffered in the pineapple industry in Hawaii, a leading pineapple-producing country, (Sipes and Schmitt, 2000).

Report on the association of *Paratylenchus*, *Paratrophurus*, and *Gracilacus* species with pineapple and their damage on the plant has not previously been reported from Nigeria fields (Daramola , *et al*., 2013). *Paratylenchus* sp. had however been reported as a major nematode pest of economic importance of pineapple in Malaysia and has been recorded in exceedingly high numbers in pineapple fields in Hawaii (Hassan , *et al*., 2006). The results of this study revealed the occurrence of *Paratylenchus minutus* at low frequency ratings in the pineapple-producing states surveyed with the exemption of Delta state. The survey also revealed the presence of high population of *Gracilacus* sp. in association with pineapple for the first time in Nigerian fields.

The widespread distribution of these plant-parasitic nematodes that have been known to cause plant debility and poor yields in Nigeria is a matter of concern and could be a factor in the low yield of pineapple recorded in the country (Asopa, 2003). Factors responsible for the temporal and spatial distribution of plant-parasitic nematodes in agricultural soils have been well-documented (Sipes , *et al*., 2005). Some of these factors could be linked directly to the 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. All these have contributed significantly to the distribution of plant-parasitic nematodes in pineapple fields surveyed. Of great concern also, is the fact soil factors that promote good growth of pineapple seem to also favor rapid multiplication of nematodes.

The insidious nature of damage caused by these nematodes make their damaging potential to be underestimated and often mistaken for those caused by other plant pathogens. In most cases farmers are not aware or adequately informed and equipped to combat this menace to crops.

Conclusion:

This study shows that there is a widespread distribution of important plant-parasitic nematodes on the pineapple fields surveyed from the three states in Nigeria. The presence of these parasitic nematodes even at low populations in the soil is significant, as population build-up could eventually result in great reduction of crop yield. It is therefore imperative that farmers be provided with nematode-free suckers and planting materials. Also, awareness programs should be created for the farmers on the damaging potential of plant-parasitic nematodes on agricultural crops.

ACKNOWLEDGEMENT

The authors are grateful to the Management of National Horticulture Research Institute, Idi-isin Ibadan, Nigeria, for the financial support and assistance with logistics in the course of the field trip.

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