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Brown cysts and white females of *Heterodera sacchari* from a rice field

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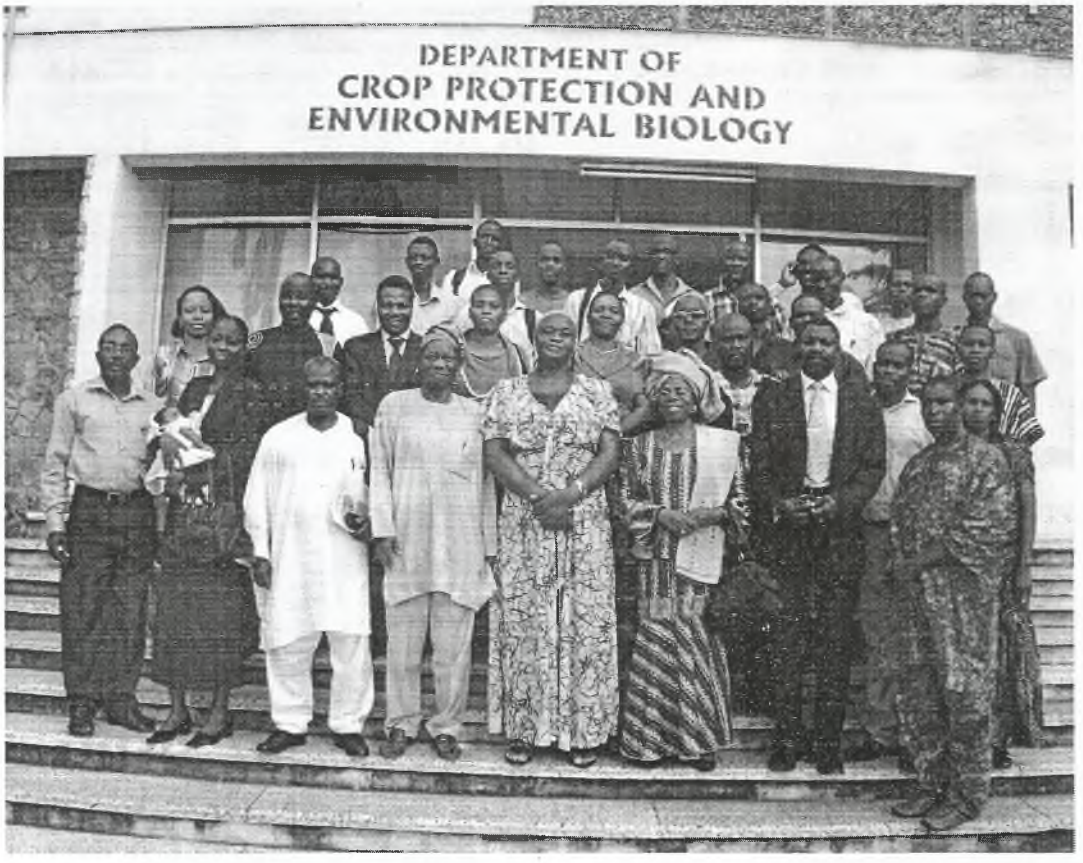
FOREWORD

Almost three years ago, specifically on September 15, 2011, practising Nematologists from all over Nigeria got together at the Department of Crop Protection and Environmental Biology, University of Ibadan (UI), Ibadan. They agreed, amongst others, to found a Nigerian Society of Nematologists (NISON). About a year after this, NISON was formally inaugurated at a ceremony held at the Conference Centre, UI, on October 16, 2012. The inaugural conference thereafter followed and the articles in this maiden issue of the *Nigerian Journal of Nematology* (NJON) are some of the papers presented at that inaugural conference. It is my hope that NISON will grow into a vibrant association and that the NJON will evolve into a qualitative outlet for the findings of the research of Nematologists, not only in Nigeria but in the West African sub-region and, indeed, sub-Saharan Africa.



Nigerian Nematologists that met on September 15, 2011

Bamidele Fawole, PhD.
Professor of Nematology
President, NISON

FROM THE EDITOR-IN-CHIEF

The Nigerian Journal of Nematology (NJON) is a learned peer reviewed publication of the Nigerian Society of Nematologists (NISON). It is positioned to strengthen nematology activities, showcase Nematology as a science, and encourage its growth and development in Nigeria and sub-Saharan Africa.

The Nigerian Journal of Nematology (NJON) will cover all aspects of the study of parasitic and non-parasitic (free-living) nematodes. It is our hope that it would be a repository that would provide needed information for development of national and international relevance. In NJON, manuscripts shall be categorised under six broad sections, namely: Taxonomy and Morphometry, Survey; Biotechnology and Molecular Biology; Host-Parasite Interaction; Nematode Management (including Bio-control, chemical control, use of biocides, cultural control, integrated pest management, etc); Environmental Nematology [Application of Free-Living nematodes in environmental management such as pollution monitoring and environmental clean-up, which, sometimes, may overlap into use as entomopathogens (biocontrol)] and; Animal-Parasitic Nematodes (host-parasite interactions, control, etc). This Maiden Issue features in three sections, some of the papers presented at the Inaugural Conference of the Nigerian Society of Nematologists, which held at the Conference Centre of the University of Ibadan between 16th and 17th October, 2012. Abstracts of other papers presented at the conference are presented immediately after the full-length papers.

Monioluwa O. Olaniyi, PhD
Associate Professor of Nematology
Editor-In-Chief, NIJON

TABLE OF CONTENTS

SELECTED FULL LENGTH ARTICLES OF PAPERS

PRESENTED AT THE INAUGURAL CONFERENCE OF

THE NIGERIAN SOCIETY OF NEMATOLOGISTS

HELD FROM 16TH TO 17TH OCTOBER, 2012

AT THE CONFERENCE CENTRE OF THE UNIVERSITY OF IBADAN, IBADAN. NIGERIA

Survey

Plant-Parasitic Nematodes Associated With Pepper (*Capsicum* spp) in Benue State

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ABSTRACT. This study was carried out to provide some information on the occurrence, diversity and abundance of plant-parasitic nematodes on pepper in Benue State. A survey of pepper-producing farms was carried out between July and September, 2009. Eleven Local Government Areas (LGA) were surveyed, two villages per Local Government Area and two farms per village that produced large quantities of pepper. Five soils samples were taken with hand trowel per farm, at 10-15cm from the base of the plants and at depth of 15-20cm. Pepper plants were uprooted with a hand trowel, five root samples per each farm were taken. A total of 220 samples were collected, bagged and labelled. The nematodes were extracted from the soil samples using the Pie-pan method, and maceration and pie-pan methods were used for the roots. The extracted nematodes were fixed, quantified and identified to genera at the Nematology Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan. Nematode population density and percentage nematode populations were estimated. The identified nematode genera were *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, *Tylenchus*, *Paratylenchus*, *Aphelenchus*, *Aphelenchoides*, *Scutellonema*, *Hoplolaimus*, Criconemoides, *Rotylenchulus*, *Tylenchulus* and *Hirschmanniella*. The most prominent genera in the soils and root samples were *Pratylenchus, Meloidogyne, Helicotylenchus.*

Keywords: *Helicotylenchus*, *Meloidogyne,* pepper, plant-parasitic nematodes, *Pratylenchus*

INTRODUCTION

Pepper, *(Capsicum spp.*), is one of the fruit vegetables grown in Benue and other States of Nigeria, for human consumption, supply of raw materials and commercial activities (Fayemi, 1999; FAOStat. 2009). Pepper is used also for medicinal, storage and pest control purposes (Fayemi, 1999; Nworgu, 2006; Odugbemi and Akinsulire, 2006). Pepper production is being threatened by biotic and abiotic factors such as insects, fungi, bacteria, weeds, viruses, nematodes, soil type, soil fertility and soil moisture (Terry-Kelly and Boyhan, 2009).

Plant-parasitic nematodes are an extremely important factor in vegetable production worldwide and in many areas a major factor requiring the use of pesticides (Sikora and Femandez, 2005; Baimey *et al.* 2009). Yield losses of 74-78% and 100% in pepper production due to root-knot nematodes have been reported (Sogut and Elekcioglu, 2007; Olabiyi and Oyedunmade, 2008).

There is the lack of information on the occurrence, diversity and distribution of plant-parasitic nematodes on pepper in Benue State and Nigeria; thus the need for this study.

MATERIALS AND METHODS

Sampling sites and procedure

The survey was carried out in Benue between July and September 2009. Eleven Local Government Areas namely; Apa, Agatu, Buruku, Gboko, Guma, Konshisha, Makurdi, Ohimini, Otukpo, Tarka and Ushongo which were selected on the basis of production and the number of cultivars grown (Baimey *et al.*, 2009). Two villages per Local Government Area and two farms per village were visited. Five soil samples, 200 ml soil each taken in zig-zag nature from the pepper plots with the aid of hand trowel 10-15cm from the base of the plants, at the depth of 15-20cml Ali and Sharma, 2003). The pepper plant were uprooted with the aid of hand trowel (Coyne *et al.*, 2007), and five root samples per farm were taken. The soil and root samples were bagged and labelled, and a total of two hundred and twenty-two samples were collected.

Nematode extraction and procedure

Nematodes were extracted from soil and root samples for 24- 48 hrs. Each soil sample, 200 $cm^3$was thoroughly mixed and the Pie-pan method (Whitehead and Hemming, 1965) was used, and the root samples washed, cut into small pieces and maceration method (Coyne *et al.*, 2007) and Pie-pan method (Whitehead and Hemming, 1965) were used. The extracted nematodes were killed and fixed by adding equal amounts of boiling water to the nematode suspension with four percent formaldehyde (Fourie *et al.*, 2001; Baimey *et al.*, 2009).

Nematode Identiïication

The extracted nematodes were identified to the genera level at the Nematology Laboratory of the International Institute for Tropical Agriculture (IITA) Ibadan, using the schemes of Mai and Lyon (1975), Mai and Mullin (1996), Stirling *et al.* (2002) and Hunt *et al.* (2005) with the aid of Olympus compound microscope. The nematode suspensions were mixed using a magnetic stirrer and one 2 ml aliquot was drawn from each suspension into a counting slide for the identification and quantification of the extracted nematodes.

Percentage frequency and percentage nematode population were calculated as follows:

Percentage frequency = n/NX100%

Where n= number of times an individual nematode occurred in all samples

N= sample size

Percentage nematode population =ln/TNx100%

Where in= Individual nematode population of all the same (Ononuju and Fawole, 1999; Adegbite *et al.*, 2006).

RESULTS

The frequency of occurrence of the plant-parasitic nematode genera associated with pepper in Benue State was found to vary (Table 1). In the soils of Apa LGA *Meloidogyne* (31.4%) and *Pratylenchus* (20.7%) were the most frequently encountered and *Mesocriconema* (1.4%) was the least frequently encountered.

Table 1. Mean percentage frequency rating of identified plant-parasitic nematodes associated with pepper in 11 LGAS of Benue State (200ml soil and 10g roots)

	Plant-parasitic nematodes genera																							
LGA	Meloid		Praty		Helico		Tylen		Aphel		Aphelen		Hoplo		Paraty		Scute		Hirsch		Tylench		Rotyl		Cricon	
	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root
Apa	31.4	38.8	20.7	26.3	12.6	10.6	10.6	12.6	8.5	6.5	3.4	-	3.6	9.2	2.6	-	6.5	-	-	-	-	-	-	-	1.4	-
Agatu	35.2	34.4	21.8	22.5	12.5	12.6	10.5	4.6	6.0	10.0	3.4	-	3.4	8.4	2.6	4.6	4.6	2.1	-	-	-	-	-	-	-	-
Otukpo	30.3	30.3	24.4	35.5	14.4	12.4	10.6	8.3	6.4	6.8	3.6	-	3.6	5.1	2.6	1.6	3.6	-	-	-	-	-	-	-	-	-
Ohimini	34.6	35.4	24.2	26.6	16.4	14.8	10.5	11.7	5.4	4.4	2.0	-	2.0	7.4	1.0	3.0	4.2	-	-	-	-	-	-	-	-	-
Gboko	30.6	38.4	22.6	26.4	12.4	11.4	8.4	6.5	6.4	5.1	2.2	-	5.2	8.4	3.2	-	6.4	4.0	-	-	2.5	-	-	-	-	-
Tarka	23.1	38.6	31.4	24.5	14.6	16.4	10.4	10.4	4.8	6.0	4.2	-	3.2	5.1	2.1	-	5.2	-	-	-	1.0	-	-	-	-	-
Guma	32.9	35.6	29.4	26.4	15.4	11.4	-	9.8	6.5	5.4	2.7	-	3.6	3.8	4.0	3.0	4.2	4.2	1.3	-	-	-	-	-	-	-
Buruku	30.5	36.6	24.6	30.4	10.4	16.8	15.1	10.5	8.2	2.5	3.2	-	2.5	3.2	2.4	-	4.6	-	2.0	-	-	-	-	-	-	-
Ushongo	31.6	30.6	26.4	28.8	13.0	12.6	8.6	10.2	7.2	6.4	2.0	-	4.4	-	2.0	2.8	4.8	6.8	-	-	-	-	-	1.2	-	-
Makurdi	29.6	33.5	30.2	30.2	12.8	11.5	10.2	8.4	5.8	4.8	2.0	-	3.8	5.2	2.1	2.5	4.7	2.4	-	-	-	-	-	-	-	-
Konshisha	33.6	35.2	27.0	26.4	14.3	13.4	8.4	10.8	4.8	9.8	1.4	-	3.6	3.2	2.0	2.0	4.0	-	-	-	-	-	-	-	-	-
Total -	343.4	387.3	282.7	303.8	148.8	143.9	103.3	103.8	70	67.7	30.1	-	40.1	59	26.6	195	52.8	19.5	3.3	-	3.5	-	-	1.2	1.4	-
X -	31.2	35.2	25.7	27.6	13.5	13.0	9.3	9.4	6.3	6.1	2.7	-	9.0	5.3	2.4	1.7	4.8	1.7	0.3	-	0.3	-	-	0.1	0.1	-
SD -	11.2	9.5	11.0	24.0	5.2	6.5	11.	47.2	5.1	6.9	2.7	-	2.8	8.8	2.3	5.0	5.4	7.4	2.1	-	2.4	-	-	1.1	1.3	-
SE -	3.3	2.9	3.3	7.2	1.2	1.9	3.42	2.2	1.5	2.1	0.8	-	0.8	2.6	0.7	0.5	1.6	0.2	0.1	-	0.1	-	-	0.0	0.0	-

Key: Meloid=*Medoidogyne*, Praty=*Pratylenchus*, Helico=*Helicotylendus*, Tylen= *Tylenchus*, Aphel=*Aphelenclus*, Aphelen=*Aphelenchoides*, Hoplo=*Hopolaimus*, Paraty=*Paratylenchus*, Scute=*Scutellonema*, Hirsch=*Hirschmmaniella*, Tylench=*Tylenchulus*, Rotyl=*Rotylenchulus*, Crico=*Criconemoides*

From the roots *Meloidogyne* (38.8%) and *Pratylenchus* (26.3%) were the most frequently encountered and *Aphelenchus* (6.5%) was the least frequently encountered (Table 1). The most frequently encountered from the soils of Agatu LGA were *Meloidogyne* (35.2%) and *Pratylenchus* (21.8%) and *Paratylenchus* (2.6%) was the least frequently encountered while, from the pepper roots the most frequently encountered were *Meloidogyne* (34.4%) and *Pratylenchus* (22.5%) and *Scutellonema* (2.1%) was the least frequently encountered. In the soils of Otukpo LGA the most frequently encountered were *Meloidogyne* (30.3%) and *Pratylenchus* (24.4%) and *Paratylenchus* (2.6%) was the least frequently encountered, while from the roots *Meloidogyne* (30.3%) and *Pratylenchus* (35.5%) were the most frequently encountered and *Paratylenchus* (1.6%) was the least frequently encountered.

The most frequently encountered from the soils of Ohimini LGA were *Meloidogyne* (34.6%) and *Pratylenchus* (24.2%) and *Paratylenchus* (1.0%) was the least frequently encountered, while *Meloidogyne* (35.4%) and *Pratylenchus* (26.6%) were the most frequently encountered and *Paratylenchus* (3.0%) was the least frequently encountered from pepper roots. The most frequently encountered from soils of Gboko LGA were *Meloidogyne* (30.6%) and *Pratylenchus* (22.6%), and *Aphelenchoides* (2.2%) was the least frequently encountered, while *Meloidogyne* (38.8%) and *Pratylenchus* (26.4%) were the most frequently encountered and *Scutellonema* (4.0%) was the least frequently encountered from the pepper roots. The most frequently encountered from soils of Tarka LGA were *Pratylenchus* (31.4%) and *Meloidogyne* (23.1%) and *Tylenchulus* (1.0%) was the least encountered, while the most frequently encountered from the pepper roots were *Meloidogyne* (38.6%) and *Pratylenchus* (24.5%) and *Hoplolaimus* (5.1%) was the least frequently encountered.

The most frequently encountered from the soils of Guma were *Meloidogyne* (32.9%) and *Pratylenchus* (29.4%) and *Hirschmmaniella* (1.3%) was the least frequently encountered, while from the pepper roots *Meloidogyne* (35.6%) and *Pratylenchus* (26.4%) were the most frequently encountered and *Paratylenchus* (3.0%) was the least frequently encountered. The most frequently encountered from the soils of Buruku LGA were *Meloidogyne* (30.5%) and *Pratylenchus* (24.6%) and *Hirschmmaniella* (2.0%) was the least frequently encountered, while from the pepper roots the most frequently encountered were *Meloidogyne* (36.6%) and *Pratylenchus* (30.4%) and *Aphelenchus* (2.5%) was the least frequently encountered. The most frequently encountered from the soils of Ushongo LGA were *Meloidogyne* (31.6%) and *Pratylenchus* (26.4%) and the least frequently encountered were *Aphelenchoides* and *Paratylenchus* (2.0%) each, while from the pepper roots the most frequently encountered were *Meloidogyne* (30.6%) and *Pratylenchus* (28.8%) and *Rotylenchulus* (1.2%) was the least frequently encountered.

The most frequently encountered from the soils of Makurdi LGA were *Pratylenchus* (30.2%) and *Meloidogyne* (29.6%) and *Aphelenchoides* (2.0%) was the least frequently encountered, while *Meloidogyne* (33.5%) and *Pratylenchus* (30.2%) were the most frequently encountered and *Scutellonema* (2.4%) was the least frequently encountered from pepper roots. The most frequently encountered from the soils of Konshisha LGA were *Meloidogyne* (33.6%) and *Pratylenchus* (27.0%) and *Aphelenchoides* (1.4%) was the least encountered, while *Meloidogyne* (35.2%) and *Pratylenchus* (26.4%) were the most frequently encountered and *Paratylenchus* (2.0%) was the least frequently encountered among from the pepper roots.

The nematode population of the plant-parasitic nematode genera associated with pepper from 200 ml soils and 10g pepper roots were not even (Table 2). In the soils from Apa LGA the highest in population were *Meloidogyne* (2,200 J_2) and *Pratylenchus* (2,000 J_2) and the least was *Criconemoides* (200 J2), while *Meloidogyne* (160 J_2) and *Pratylenchus* (100 J_2) were highest and *Helicotylenchus* and *Hoplolaimus* (40 J_2) the least respectively from pepper roots. The nematode population from the soils of Agatu LGA were *Meloidogyne* (2,400 J_2) and *Helicotylenchus* (1,800 J_2) were the highest, and *Paratylenchus* (200 J_2) the least, while from the pepper roots were *Meloidogyne* (170 J_2) and *Pratylenchus* (90 J_2) were highest and *Hoplolaimus* and *Scutellonema* (20 J_2) respectively were the lowest. The nematode population of the plant-parasitic nematodes from the soils of Otukpo LGA were *Meloidogyne* and *Helicotylenchus* (1,600 J_2) respectively were the highest, and *Paratylenchus* (400 J_2) was the least, while *Meloidogyne* (110 J_2) and *Pratylenchus* (100 J_2) were the highest, and *Hoplolaimus* (30 J_2) was the least from pepper roots. The nematode population from the soils of Ohimini LGA the highest were *Pratylenchus* (2,800 J_2) and *Meloidogyne* (1,800 J_2) and the least was *Paratylenchus* (400 J_2), while from the roots the highest were *Meloidogyne* (90 J_2) and *Pratylenchus* (70 J_2) and *Aphelenchus* and *Hoplolaimus* (10 J_2) respectively were the least. The highest mean population from the soils of Gboko LGA were *Meloidogyne* and *Pratylenchus* (1,600 J_2) respectively, and *Tylenchulus* (200 J_2) was the least, while from the roots the highest were *Meloidogyne* (110 J_2) and *Pratylenchus* (100 J_2) and *Scutellonema* (10 J_2) was the least. The highest nematode population from the soils of Tarka LGA were *Pratylenchus* (2,400 J_2) and *Meloidogyne* (2,200 J_2), and *Tylenchulus* (200 J_2) was the least, while *Meloidogyne* (80 J_2) and *Pratylenchus* (70 J_2) were the highest and *Hoplolaimus, Tylenchus* and *Aphelenchus* (20 J_2) were the least respectively, from pepper roots. In the soils of Guma LGA, the highest nematode population were *Pratylenchus* (1,600 J_2) and *Meloidogyne* and *Helicotylenchus* (1,200 J_2) respectively.

Table 2. Mean population density of identified plant-parasitic nematodes association with pepper in 11 LGAS of Benue State (200ml soils and 10g roots)

	Plant-parasitic nematodes genera																							
LGA	Meloid		Praty		Helico		Tylen		Aphel		Aphelen		Hoplo		Paraty		Scute		Hirsch		Tylench		Rotyl		Cricon	
	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root
Apa	2200	160	2000	100	1400	40	2000	70	1000	50	1000	-	600	40	400	-	800	-	-	-	-	-	-	-	200	-
Agatu	2400	170	1200	90	1800	40	2200	30	1600	50	400	-	600	20	200	30	600	20	-	-	-	-	-	-	-	-
Otukpo	1600	110	1400	100	1600	40	1400	40	1400	40	1400	-	1000	30	400	40	800	-	-	-	-	-	-	-	-	-
Ohimini	1800	90	2800	70	1600	30	2400	30	1600	10	1000	-	800	10	400	40	1200	-	-	-	-	-	-	-	-	-
Gboko	1600	110	1600	100	1400	20	2000	40	1000	20	800	-	800	30	600	-	800	10	-	-	200	-	-	-	-	-
Tarka	2200	80	2400	70	800	40	1800	20	1600	20	400	-	1200	20	1000	-	800	-	-	-	200	-	-	-	-	-
Guma	1200	80	1600	80	1200	30	-	20	1200	20	200	-	800	40	200	10	800	20	200	-	-	-	-	-	-	-
Buruku	1600	90	1400	100	1200	10	2400	40	1200	30	600	-	600	10	600	-	600	-	200	-	-	-	-	-		-
Ushongo	2200	110	1400	70	1200	20	2400	20	1000	30	400	-	800	-	400	30	600	20	-	-	-	-	-	10	-	-
Makurdi	1400	110	1600	90	1600	20	1600	40	1000	20	400	-	1000	30	600	20	600	10	-	-	-	-	-	-	-	-
Konshisha	1600	100	1400	80	1400	30	1400	20	800	30	200	-	1000	10	400	20	600	-	-	-	-	-	-	-	-	-
Total -	19800.0	1200.0	18800.0	950.0	15200.0	320.0	19600.0	370.0	13400.0	320.0	6800.0	-	9200.0	240.(	5200.0	190.0	8200.0	80.0	400	-	400	-	-	10.0	200.0	-
X -	1800.0	109.0	1709.0	86.3	1381.8	29.0	1781.8	33.6	1218.1	29.0	618.1	-	836.3	21.8	472.7	17.2	745.4	7.2	36.3	-	36.3	-	-	0.9	18.1	-
SD -	1232.8	94.2	2326.1	29.2	27 50.2	33.0	2217.2	47.4	914.5	33.0	1215.0	-	620.8	41.9	708.3	51.1	572.0	28.6	255.8	-	25.5	-	-	9.5	190.6	-
SE -	373.6	28.5	704.8	8.8	833.3	10.0	671.9	14.3	277.1	10.0	368.1	-	188.1	12.7	214.6	15.5	173.3	3.6	7.5	-	7.5	-	-	0.1	7.7	-

Key: Meloid= *Meloidogyne*, Praty= *Pratylenchus*, Helico= *Helicotylenchus*, Tylen= *Tylenchus*, Aphel= *Aphelenchus*, Aphelen= *Aphelenchoides*, Hoplo= *Hoplolaimus*, Paraty= *Paratylenchus*, Scute= *Scutellonema*, Hirsch= *Hirschmmaniella*, Tylench= *Tylenchulus*, Rotyl= *Rotylenchulus*, Cricon= *Criconemoides*.

The least were *Aphelenchoides, Paratylenchus* and *Hirschmmaniella* (200 J_2) respectively, while the highest were *Meloidogyne* (80 J_2) and *Pratylenchus* (70 J_2) and the least were *Hoplolaimus, Tylenchus* and *Aphelenchus* (20 J_2) respectively were from pepper roots. The highest nematode population from the soils of Buruku LGA were *Meloidogyne* (1,600 J_2) and *Pratylenchus* (1,400 J_2), and *Hirschmmaniella* (200 J_2) was the least, while *Pratylenchus* (100 J2) and *Meloidogyne* (90 J2) were the highest and *Helicotylenchus* and *Hoplolaimus* (10 J_2), respectively were the least from pepper roots.

The highest nematode population from the soils of Ushongo LGA were *Meloidogyne* (2,200 J_2) and *Pratylenchus* (1,400 J_2) and *Aphelenchoides* and *Paratylenchus* (400 J_2) respectively were the least, while *Meloidogyne* (100 J_2) and *Pratylenchus* (70 J_2), and *Rotylenchulus* (10 J_2) was the least from pepper roots. The highest nematode population from the soils of Makurdi LGA were *Pratylenchus* and *Helicotylenchus* (1,600 J_2) respectively and *Meloidogyne* (1,400 J_2) and *Aphelenchoides* (400 J_2) was the least, while *Meloidogyne* (110 J_2) and *Pratylenchus* (90 J_2) were highest and *Helicotylenchus* and *Scutellonema* (20 J_2) respectively were the least from pepper roots. The highest nematode population from the soils of Konshisha LGA were *Meloidogyne* (1,600 J_2), *Pratylenchus* and *Helicotylenchus* (1,400 J_2) respectively and *Aphelenchoides* (200 J_2) was the least, while *Meloidogyne* (110 J_2) and *Pratylenchus* (90 J_2) were the highest, and the least was *Scutellonema* (10 J_2) from pepper roots. The highest nematode population from 200 ml soils and 10g roots are shown in Table 2.

DISCUSSION

The plant-parasitic nematodes identified to be associated with pepper in Benue State where not evenly distributed and of varying population densities (Olabiyi *et al.*, 2009), and it is generally assumed that the presence of the host plant is the main determinant of the population density of plant-parasitic nematodes, including soil type and cropping history (Baimey *et al.*, 2009). The majority of farmers cropping pepper in the State have small farm sizes, with continuous cropping of pepper either as sole cropping or mixed cropping with many years of farming. Nematodes are abundant in the soils of Nigeria, many which are parasites of plants including food crops and causing losses in both quantity and quality (Olabiyi *et al.*, 2009).

Pepper is a good host for *Meloidogyne* and *Pratylenchus* (Adesiyan *et al.*, 1990; Sikora and Fernandez, 2005; Fawole, 2009) and recorded as important nematode pests on this crop (Sikora and Fernandez, 2005). *Meloidogyne* occurred in all the soil and root samples which conforms to the report that the root-knot nematodes occur whenever and wherever susceptible crops are grown, thus with a wide host range (Castagnose-Sereno, 2002; Barker, 2003). The dominance of *Meloidogyne*

over other plant-parasitic on vegetable nematodes have been reported (Machado and Inomoto, 2001; Sikora and Fernandez, 2005). *Meloidogyne* have been identified to be associated with other vegetables and crops (Waliullah, 1992; Fourie *et al.*, 2001; Baimey *et al.*, 2009; Bhan *et al.*, 2010; Bao and Neher, 2011).

Pratylenchus has been recorded to be associated with vegetables and identified as a nematodes pest of pepper (Adesiyan *et al.*, 1990; Waliullah, 1992; Baimey *et al.*, 2009). *Pratylenchus* was identified from all the soil and root samples in this study. All species of *Pratylenchus* should be considered of potential importance when encountered within the roots (Sikora and Fernandez, 2005), and the greatest damage on crops occurs when the lesion nematode interacts with the wilt-causing fungus, *Verticillium* Spp (Bao and Neher, 2011).

The reniform nematode *(Rotylenchulus)* though record lowest in the nematode population density among the identified plant parasitic nematodes associated with pepper in Benue state is the most important nematode affecting pepper after *Meloidogyne* but it is often neglected or overlooked where it occurs concomitantly with *Meloidogyne* (Sikora and Fernandez, 2005).

Helicotylenchus, Scutellonema, Hoplolaimus and Aphelenchoides have been reported to be associated with vegetable crops (Waliullah, 1992; Sikora and Fernandez, 2005; Baimey *et al.*, 2009) but their damage to vegetable production have not been determined (Sikora and Fernandez, 2005). *Hoplolaimus* is an important and widely distributed ectoparasite causing root damage and reduction of crop yield (Khan *et al.*, 2008).

In a survey conducted for a two year period on pepper crop, the resident plant-parasitic nematodes in order of occurrence and population were *Meloidogyne incognita, Pratylenchus brachyurus* and *Hoplolaimus* spp (Nwanguma *et al.*, 2011).

In conclusion, the plant-parasitic nematodes associated with pepper in Benue State were *Pratylenchus, Meloidogyne, Helicotylenchus, Tylenchus, Paratylenchus, Aphelenchus, Aphelenchoides, Scutellonema, Hoplolaimus, Criconemoides, Rotylenchulus, Tylenchulus* and *Hirschmanniella* with varying population densities and population.

Implication of the study is that the cultivation of crops in Benue state stands a serious risk of decline in production due to attack and infection by the plant-parasitic nematodes which are abundant in the soils. There is the need for nematologists to be involved in the planning, management and production of crops in the State and Nigeria. Also, enlightenment of the farmers about plant-parasitic nematodes, on their effects and

management should be encouraged in Benue State and Nigeria in general.

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Impact of Organic Amendments on *Meloidogyne* spp. and Yield Improvement of Soybean

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Abstract. Field experiments to investigate the impact of organic amendments on *Meloidogyne* spp. and yield improvement of soybean was carried out in 2009 and repeated in 2010 to confirm observed data. Composts prepared from fresh leaves of *Chromolaena odorata, Tithonia diversifolia* and *Carica papaya* stacked with manure from either poultry or cattle sources were applied at the rate of 6 tha^{-1}. Each time, the experiment was laid out in a Randomized Complete Block Design with six replications. Treatments included six composts while untreated plots served as the control. A root-knot nematode-susceptible soybean variety, TGx 1019-2EN was used for the trial. One-week-old soybean seedlings were thinned to one plant per stand and inoculated with 5,000 eggs of *Meloidogyne* spp. seven days after emergence to augment the population in the soil. Data on growth parameters were collected bi-weekly while those on population of *Meloidogyne* spp. were recorded 60 days after inoculation. Matured pods were harvested, threshed, winnowed and weighed. Data were analyzed for variance and means separated. Results showed that composted manure of both poultry and cattle origins significantly (P = 0.05) reduced the root-knot nematode populations by 94.9%. This nematode control strategy resulted in attendant soybean yield increase by up to 60%. Increased biological activities led to increase in grain yield across all treated plots compared with the control. This result provides an impetus for development of environmental-friendly measures for the management of root-knot nematode problem mitigating large scale soybean production towards ensuring sustainable improvements in grain yield production.

Keywords: Compost, grain yield, nematode management, organic manure, root-knot nematodes

INTRODUCTION

Tropical soils comprise of several micro and macro-organisms such as bacteria, fungi, nematodes, mites, earthworms, millipedes and centipedes living and interacting among themselves and with their environment (ecosystems). While some are beneficial to crop and crop ecology, the pathogenic ones constitute nuisance to plant growth and development either through direct injuries or direct infection of host plants. Plant-parasitic nematodes are economically-important agricultural pests owing to the damage and damage

potentials they pose on major susceptible crops wherever such crops are cultivated. They are enormous and capable of causing losses of up to 100 % in the yield of crops (Oyediran, 1992); a loss level which no country in the world can afford.

Soybean, (*Glycine max.* (L.) Merril) is the most important grain legume in terms of total production, consumption, and international trade. It is an oilseed, which provides a cheap, and balanced diet (Ogundipe *et al.*, 1989) with proximate analysis of 40 % protein, 32 % carbohydrate, 20 % edible oil, 5 % minerals and 3 % fibre (Singh, 1987). United States of America (USA) is the worlds leading producer of soybean accounting for 49 % of the world's output of 34 %; Asia produced 14 % while Africa produced less than one percent of the world's output. The world average yield in 2000 was 2,210 Kg per hectare, ranging from about 3,520 Kg per hectare in Western Europe to 2,650 Kg per hectare in the USA and 990 kg per hectare in Africa (FAO, 2009).

Meloidogyne incognita (root-knot nematode) was documented as a major constraint to large scale soybean production. (Atungwu *et al.*, 2012). Their infestation often results into economic and socio-economic problems arising from low protein intake, insufficiency in food supply, increase in cost of living as well as cost of production. Nematodes cause direct impairment of physiological functions making nematode-infected plants show distinctive symptoms, such as retarded growth and poor inter-node development due to poor nutrient uptake, wilting as a result of poor water uptake, hyperplasia and hypertrophy of cells leading to galling, poor yield and consequent crop loss (Atungwu *et al.*, 2012)

The search for suitable and sustainable control method for organisms like plant-parasitic nematodes led to discovery of nematicides. Though, significant increases in crop yield have been recorded with the use of these nematicides, the withdrawal of many nematicides from the market due to environmental concerns and constraints of use such as the high cost (about US $ 500 per hectare per year) have drawn attention to the development of alternative methods, which besides being environmentally-friendly would also be within the purchase capacity of resource-poor farmers worldwide (Gowen, 2000). For instance, traditional nematicides such as fumigant 1, 3-dichloropropene, the carbamates, aldicarb and oxamyl, and the organophosphate (fenamiphos) when applied correctly, will increase crop yield if initial nematode population densities exceeds damage thresholds (Whitehead, 1998). However, there is no long-term suppression of nematode population densities with their use (Starr, 2001).

The recent gradual shift from "conventional" to "organic" agriculture and quests for ecologically-friendly procedures for managing problems of nematode pests mitigating against production of soybean quantitatively and qualitatively, that would also be within the reach of resource-poor farmers led to the discovery of potencies of some organic materials administered as amendments in recent times. Organic materials such as composted manure of animal and green manure of plant origin have been used since the advent of agriculture to improve soil fertility, soil biological and physical properties, and recycle nutrients for expected increase in

crop yield (Rodriguez-Kabana *et al.*, 1987).

Though sometimes filthy, bulky and slow in the rate and time of nutrient release, constituents of organic materials according to Riegel and Noe, (2000) have been found to enhance microbial activities through the development of nematode antagonists (Riegel *et al.* 1996; Atungwu *et al.*, 2012) and the production of nematicidal substances thus leading to a significant increase in crop yield.

Use of organic materials as amendment offers solution to the environmental problems associated with the use of toxic chemicals including nematicides, and there are many organic materials which have been found to compare favourably with synthetic nematicides in terms of crop yield, environmental friendliness, compatibility with other cultural practices and their nematicidal properties.

More research grounds need to be covered in the area of combining these cheap and readily-available materials into a single form called "composts". The need to further prepare and test the efficacies of different sources of composts on nematode reproduction and survival as well as their impacts on grain yield in a nematode-infested soil informed this study.

MATERIALS AND METHOD

Locations of the experiment

The experiment comprised two field trials, the first was located at the experimental plots of the Organic Agriculture Project in Tertiary Institutions in Nigeria (OAPTIN) at the Federal University of Agriculture, Abeokuta, Ogun State and the second at the Olusegun Obasanjo Centre for Organic Agricultural Research and Development (OOCORD), Ibadan. The study spanned 4th June to December 2009 and was repeated in 2010.

Soil sampling

Soil sampling was carried out with a soil auger at 15cm depth. Twelve core samples were taken per plot, bulked and made to form six composite samples as representative samples. The samples sealed and labelled in polythene bags were transported to FUNAAB's Central Laboratory for routine and chemical analysis while initial population of root-knot nematodes was analyzed at Crop Protection laboratory in the same University.

Compost preparation

Six composts of different origin were prepared using fresh leaves of *Chromolaena odorata, Tithonia diversifolia* and *Carica papaya* stacked with poultry and cattle manure in ratio 3:1 for a period of six months. The various composts were: *C. odorata* + poultry manure (A1), *T. diversifolia* + poultry manure (B1), C. *papaya* + poultry manure (C1), *C. odorata* + cattle dung (A2), *T. diversifolia* + cattle dung (B2), and *C. papaya* + cattle dung (C2). The composts were turned regularly within the holding units with a garden fork in order to ensure even distribution of substrates and heat generated during decomposition. This was followed by curing by air-drying for seventy-two (72) hours.

Compost application, layout of experiment and inoculation of seedling

Each compost was applied at the rate of 6 t/ha. The experiment was laid out in a Randomized Complete Block Design with six replicates. The treatment

included the six composts while no compost added. Soybean variety, TGx 1019-2EN, susceptible to *Meloidogyne incognita* was planted and thinned to one plant per stand. Seven days after emergence, stands were inoculated with 5,000 eggs of *Meloidogyne incognita* earlier maintained on a pure culture of *Celosia argentea* and extracted through Hussey and Baker (1975) sodium hypochlorite method. This was considered necessary as a way of augmenting the inherent soil populations to 5000±5 eggs per plant.

Estimation of Nematode population

Final population of *Meloidogyne incognita* was estimated from sample of 250 g soil, which was extracted through the Whitehead and Hemmings (1965) tray method 60 days after inoculation.

Data collection

Data were collected on stem girth, number of leaves and branches bi-weekly while pod numbers, seed weight per hectare, root parameters as well as the final population of root-knot nematodes were taken at harvest. All statistical analyses were carried out using the Statistical System Analysis (SAS) guide (SAS, 2000).

RESULTS

Combined analysis of location effect showed that there were no significant differences in leaf number of plant, stem girth (cm) and branch number in the two locations. However, assessment of the results by location revealed that in Ibadan, all the amended plots showed a significantly ($P<0.05$) higher leaf number than the control while the treatment Cattle manure + *Tithonia diversifolia* gave a significantly higher number of leaves than all other untreated plots served as the control with treatments including the control (Table 1).

Fresh root weight (g) and root length (cm) varied significantly in Ibadan. There was a significant difference ($p<0.05$) in fresh root weights (g) of crops in Abeokuta and Ibadan (Table 2). All the amendments stimulated higher root growth when compared with the control. Root-shoot ratio at the two locations was higher across all the amendment treatments than the unamended control. (Table 2).

In table 3, effect of treatment was significant $P<0.05$ on root-knot nematodes population density. This also corresponded with the higher root-knot nematode population obtained from the control plots than the amended plots.

Percentage increase in pods on amended plots obtained from the two locations compared with the unamended control plots showed that poultry manure + *Carica papaya,* Cattle manure + *Thitonia diversifolia* and cattle manure + *Carica papaya* gave pods that were more than 50% higher than what was obtained from unamended control plots in Ibadan (Table 4).

Increase in percentage pod number of amended plots over the control also varied in all treated plots in Abeokuta but, Poultry manure + *C. odorata*, Poultry manure + *Carica papaya* and cattle manure + *C. papaya* gave a higher percentage pod number than other amended plots. Grain yields were also higher on all the amended plots in Ibadan than the control but not in the first experiment in 2009.

Table 1. Mean leaf number, stem girth and branch number per plant of soybean inoculated with *Meloidogyne incognita* in compost-amended plots weeks after inoculation (WAI).

COMPOSTS	ABEOKUTA			IBADAN		
	No. of Leaves	Stem girth (cm)	No of Branches	No. of Leaves	Stem girth (cm)	No. of Branches
Poultry manure + *Chromolaena odorata*	23b	2.93a	6a	48a	2.80a	9a
Poultry manure + *Tithonia diversifolia*	30ab	2.13c	7a	37a	2.13abc	7ab
Poultry manure + *Carica papaya*	37ab	2.83ab	6a	55a	2.23abc	7ab
Cattle manure + *Chromlaena odorata*	37ab	2.63abc	9a	39a	1.80bc	10a
Cattle manure+ *Thitonia diversifolia*	44a	2.48abc	9a	45a	1.80bc	9a
Cattle manure+ *Carica papaya*	32ab	2.40abc	8a	35a	1.67c	8ab
No Compost (Control)	36ab	2.83ab	7a	28a	1.93ab	8ab

Means with same letters within column are not significantly different from one another at 5% probability

Table 2. Mean fresh root weight (gm), shoot weight (gm) and root length (cm) of inoculated soybean plants amended with different compost

TREATMENT	ABEOKUTA				IBADAN			
	Fresh Root (R)	**Fresh Shoot (S)**	**Ratio (R: S)**	**Root length** (cm)	**Fresh Root (R)**	**Fresh Shoot (S)**	**Ratio (R: S)**	**Root length** (cm)
Poultry manure + *Chromolaena odorata*	9.15a	88.37a	1:9	29.83ab	10.30a	129.25a	1:11	36.00ab
Poultry manure + *Tithonia diversifolia*	7.73a	77.92a	1:10	35.50a	7.73a	86.77a	1:11	27.00c
Poultry manure + *Carica papaya*	10.68a	93.23a	1:9	35.17a	8.73a	117.17a	1:13	38.00a
Cattle manure + *Chromolaena odorata*	11.70a	111.97a	1:10	33.17ab	7.97a	111.47ab	1:13	33.00ab
Cattle manure+ *Tithonia diversifolia*	9.63a	96.32a	1:10	28.50ab	10.50a	115.07a	1:11	38.67a
Cattle manure+ *Carica papaya*	8.73a	70.03a	1:8	32.67ab	11.43a	123.00a	1:11	31.67ab
No Compost (Control)	10.83a	89.87a	1:7	29.00 ab	10.10a	54.43b	1:5	34.67ab

Means with same letters within column are not significantly different from one another at 5% probability.

Table 3. Mean *Meloidogyne incognita* contained in 250g soil of inoculated soybean fields amended with different compost types 60 days after inoculation and its reproduction factor.

	Meloidogyne incognita population	
TREATMENT	ABEOKUTA	IBADAN
Poultry manure + ***Chromolaena odorata***	2c	7abc
Poultry manure + ***Tithonia diversifolia***	5b	9abc
Poultry manure + ***Carica papaya***	8ab	11ab
Cattle manure + ***Chromolaena odorata***	7abc	11ab
Cattle manure + ***Tithonia diversifolia***	7abc	11ab
Cattle manure + ***Carica papaya***	4b	11ab
No Compost (Control)	12a	27a

Table 4. Percentage (%) increase in pod number and grain yield (g) of inoculated soybean plants amended with different compost

TREATMENT	ABEOKUTA		IBADAN	
	Pod Number/ ha	**Grain Weight** tons / ha	**Pod Number/** ha	**Grain Weight** tons / ha
Poultry manure + *Chromolaena odorata*	37	11	10	24
Poultry manure + *Tithonia diversifolia*	7	3	19	26
Poultry manure + *Carica papaya*	24	3	51	17
Cattle manure + *Chromolaena odorata*	4	3	43	15
Cattle manure+ *Tithonia diversifolia*	5	3	63	9
Cattle manure+ *Carica papaya*	31	13	54	19
Control	0	0	0	0

DISCUSSION

Soybean growth response observed in this study was in line with the report of Atungwu and Kehinde (2008) where organic-based fertilizer reduced nematode populations and gave a significant increase, which was comparable to that of Furadan, a synthetic nematicide, in growth and yield of soybean. Higher grain yields obtained on all amended plots in Ibadan compared with the control in 2010 is suspected to be as a result of increased vegetative growth in plants at the expense of pod formation in Abekuta compared with plants in Ibadan.

Increase in the yield of soybean grown on organic-amended soils have been attributed to the roles of organic substances as: plant-parasitic nematode suppressant (Akhtar and Mahmood 1996), provider of suitable environment for crop root growth, a good source of nematostatic and nematotoxic substances like ammonia (Rodriguez-Kabana *et al.*, 1996) and booster of nematophagus organisms such as some fungi species (e.g Arbuscular micorrhizal fungi).

Results obtained from this present work showed that carefully-composted manures applied on nematode-infested soybean fields reduced the population of root-knot nematodes by 94.9%, affirming the report by Atungwu and Lawal (2008) while testing the efficacy of organic-based fertilizer in the management of *M. incognita* in soybean. In addition to enhancement of microbial population and interaction, previous experiments have proven that organic amendments are substances whose regulated and timely application can reduce population densities of plant-parasitic nematodes in infested crop fields and also improve crop yields (Atungwu and Lawal, 2009; Orisajo *et al.*, 2006).

Efficient recycling and amendment of agricultural soils with organic substances that could otherwise be regarded as "wastes" through proper composting as demonstrated by this study has proven to be promising and vital to improving the antagonistic status of soils to the root-knot nematode in South-Western Nigeria. Therefore, collection of organic materials with proven nematostatic or nematicidal properties and the combination of these substances in composted form would not only improve soybean growth (Atungwu *et al.*, 2009), but would also lead to significant increase in yield of the crop.

This study has demonstrated that amending soils with compost would likely provide fertile and healthy soils which can suitably ensure better crop yield, wholesome enough to meet the nutritional and dietary needs of the ever-increasing human and livestock populations quantitatively and qualitatively.

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Comparative effects of *Acalypha wilkesiana* leaf extract, hot and boiling water on plantain growth response and nematode damage

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Abstract. This research was carried out to investigate the comparative effect of pre-plant treatment of plantain suckers in red *Acalypha* leaf extract, hot water and boiling water on plant emergence and nematode damage. The treatments were paring and dipping in crude water extract of red Acalypha leaf for 5, 10, 15 and 20 min duration, hot water treatment at 52±2°C, boiling water treatment at 100°C and non-pared control. Cumulative plant emergence count was taken every 14 days till 56 days after planting. Destructive sampling was carried out 55, 84 and 111 days after planting to assess root and rhizome damage, identify and count nematode species densities. Pre-plant dip in extract for 20 min was phytotoxic relative to plant emergence. Highest percentage of plants that emerged was in the hot water treatment while the extract treated materials recorded the least percentage emergence with the 20 min dip being the poorest. There was inconsistency in the root damage results but with an indication that 15 min dip in crude water *Acalypha* leaf extract could reduce root damage and also nematode densities.

Keywords: Boiling water, control, *Helicotylenchus multicinctus*, hot water, leaf extract plant parasitic nematode, plantain, *Pratylenchus coffeae*

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INTRODUCTION

Plantains are useful components in mixed farming systems providing continuity of food, income and employment throughout the year. Plantains may be grown as permanent crop or on a system of replanting every 3-8 years or longer (Stover and Simmonds, 1987). Plantain productivity is however often impaired for reasons related to soil structure, soil fertility, drainage, and severity of diseases and pest (Stover and Simmonds, 1987), so frequent that replanting is necessary. Plant parasitic nematodes are important pathogens and the major constraint to plantain production in Nigeria (Speijer *et al.*, 2001) estimated to cause on average, 50% yield loss in the southeastern part of the country (Olaniyi, 2011). The plant parasitic nematodes species most frequently associated with plantain in Nigeria are *Helicotylenchus multicinctus*, *Hoplolaimus pararobustus*, *Meloidogyne* spp., *Pratylenchus* spp. and *Radopholus similis* (Rotimi *et al.*, 1999; Speijer *et al.*, 2001).

Planting of infested materials is one of the major ways in which nematode species of economic significance like *Radopholus similis* (Cobb), *Helicotylenchus multicintus* (Cobb) and *Pratylenchus goodeyi*, the banana weevil (*Cosmopolite sordidus*) and panama disease *Fusarium oxysporum* are spread. A number of options exist to improve the quality of planting materials. Suckers selected for transplanting can be pared by removing roots and affected rhizome tissues. By detaching the roots, the nematode inocula present in the roots is removed and to an extent the inocula in the rhizome (Gowen and Queneherve, 1990) and the adhering soil (Olaniyi, 2006). Pared suckers may further be immersed in hot water held at a constant temperature of 55°C for a period of 15 – 25 minutes (Bridge, 1975; Stover, 1972). Boiling water treatment of pared planting materials for a very brief period of 30 sec has been shown to also be effective in cleaning planting materials in an experiment conducted at Ogotun, Ekiti state of Nigeria. (Coyne *et al.*, 2010).

Among several control measures available in nematode management, the use of synthetic nematicides has been the most immediate practical solution to nematode problem worldwide. With the subsistent farmer however, the use of synthetic nematicides is plagued with several limitations. Among these are high cost and lack of technical expertise in their application. Less specialized production serving the local markets may not justify the high cost of chemical treatment. There are other limitations in chemical treatment method: their use has negative impact on the environment and general public health. As a result, there is growing interest in methods of nematode management that are economically viable and environmentally friendly.

The use of botanicals in the control of plant parasitic nematodes has received global attention. When used as extracts, some botanicals have proved to be comparable with synthetic nematicides. Some of the botanicals may be applied as root dips, soil amendment, root exudates, aqueous extracts, chemical extracts, seed powders and cakes among others (Olaniyi and Moens, 2007). One of such plants is *Acalypha wilkesiana* (red *Acalypha*), a common hedge plant in Nigeria. It is a perennial ornamental herbaceous plant and in some other tropical environments where it is present, it is commonly referred to as

copperleaf and is a feature of parks and gardens.

Water extract of the red or brown varieties of *A. wilkesiana* has been used for decades by the local inhabitants for paediatric treatment of skin diseases. Mothers pluck the leaves and boil in hot water, the decoction is then used to bath the affected area on their baby's skin. The baby is also given some quantity to drink. To date, no side effect of its use has been documented.

Used as soil amendment, red *acalypha* was effective in the control of *Meloidogyne incognita,* significantly resulting in more males than female nematodes and exhibiting better efficacy than leaves of Neem, *Azadirachta indica* (Olaniyi *et al.*, 2005). This implied that it reduced the damage potential of the nematode. Rotimi and Moens (2005) documented the efficacy of crude water extract of the plant leaves in the control of *Meloidogyne incognita* in the screen house. However, the authors claimed that 20 min dip of roots of tomato seedlings in the extract prior to transplanting was phytotoxic on the test plant.

This study was, therefore, initiated to establish the duration of exposure of plantain suckers to red *Acalypha* leaf extract that would not be phytotoxic, and compare the effects of red *Acalypha* leaf extract, hot water and boiling water treatments of plantain suckers on the field establishment and protection against nematode infection.

MATERIALS AND METHODS

Site Description and Trial Establishment

The trial was conducted at the Teaching and Research Farm (Crop section) of the Federal University of Technology, Akure in Nigeria. Akure lies within the Tropical rainforest belt between latitude 5°N and longitude 15°E of the equator, with an annual mean temperature of about 27°C. The dry season is usually witnessed in Akure between November and March, while the rainy season ranged from March/April to October/November.

The experimental site covered a total area of 960m^2. Previously, the site was used for a mulch trial to study the vegetative response of plantain to two organic mulch types. The experiment had been terminated five months earlier and left to re-vegetate naturally before it was opened for this present study. Pre-plant soil nematode densities were assessed. Pre-plant sucker parameters were also measured. The site was slashed and burned before marking out and establishing the field. The experiment was laid out in a completely randomized design (CRD) of eight treatments and ten replicates (plants) per treatment. The spacing used was 3 metres between the rows and 2 metres within the rows, there were 10 suckers per row and eighty suckers in all for the experiment.

Preparation of Red *Acalypha* Leaf Extracts and Sucker Treatment

Plantain suckers were purchased from farmers in a village that adjourns the University campus while the red *Acalypha* was sourced from Owena, a suburb of Akure, Ondo State, Nigeria and the pseudostem length were reduced to about 30cm in cases where they were longer than that. Air-dried leaves of red *Acalypha* plant were pulverized, 100g of the powder was homogenized in 9 litres of cold water and left to stand for 30 minutes. Thereafter, 10 pared suckers were separately dipped into four suspensions and left to stand for 5, 10, 15 and 20 minutes. For the hot water treatment, 10 pared suckers were dipped in water at 52°C ±2°C for 20 minutes and thereafter, left to cool, while for the boiling water treatment, 10 suckers

were dipped in water at 100°C for 30 seconds. Ten suckers, which were only pared without any further treatment served as the pared control, while 10 others were neither pared nor further treated and served as the non-pared control. The treatments were then denoted as: T1: pared control, T2: pared suckers with 5 min dip in *Acalypha* extract, T3: pared suckers with 10 min dip in *Acalypha* extract, T4: pared suckers with 15 min dip in *Acalypha* extract, T5: pared suckers with 20mins dip in *Acalypha* extract, T6: Hot water treatment at 52°C for 20 min, T7: Boiling water treatment at 100°C for 30 sec and T8: non-pared control. Suckers were planted in a 30 x 30 x 30 cm planting holes on 21 December 2006, 24 hrs after treatments have been assigned. Due to cessation of rainfall during this period of the year, manual irrigation of the plant once in two days was adopted until the resumption of rainfall in late March, 2007.

Data Collection

Plant establishment and growth parameters: Cumulative plantain sucker establishment count was taken at 14 days interval up till 56 days after planting (DAP). Newly emerged pseudostem with a primordial leaf was counted as newly established sucker.

Root Damage Assessment: Root necrosis was assessed as percentage of lesion on the roots according to Speijer and De Waele (1997). Three plants per plot making a total of nine plants per treatment were uprooted on 55, 84 and 111 days after planting to assess root damage and other parameters.

Nematode Extraction and Identification: Nematodes were extracted from five grams sub-samples of the roots assessed for necrosis, with a modified Bearmann tray technique (Gowen and Queneherve, 1990). Soil samples were taken from each plant hole at the time of uprooting and nematodes extracted from each sample using the modified Baermann tray technique. Plant parasitic nematodes were identified to species level with the light microscope and all developmental stages of the nematodes species were counted, except for the root knot nematode, which was identified only to genus level (since only vermiform juveniles and males could be extracted with the extraction technique). Root densities were presented as numbers per 100g root fresh weight while the soil densities per one litre soil.

RESULTS

Pre-Plant Sucker Parameters

Sucker fresh weight ranged from 150g-2.5kg with a mean value of 474.31g before paring. After paring, the value of the upper range reduced to 1.15kg with an average of 335.92g (Table 1).

Table 1. Summary of pre-plant parameters of plantain (cvr Agbagba) suckers.

Parameters	Min.	Max.
Fresh weight (g) B/P	150	2500
Fresh weight (g) A/P	120	1150
Sucker length (cm)	7	47
Inner Rhizome length (cm)	0	16
Outer rhizome length (cm)	4	19
Girth (cm)	8	48
Total root (number)	0	28
Rhizome circumference (cm) B/P	21	49
Rhizome circumference (cm) A/P	17	45
Root bases (number)	4	60
Small lesions (number)	0	5
Large Lesions (number)	0	2
Eyes/bud on rhizome (number)	0	5

B/P: before paring; A/P: After paring

The pre-plant nematode density in the soil was low with an average of one *Pratylenchus coffeae* per litre soil and an average of two *Helicotylenchus multicintus* per litre soil.

Plant Emergence

Hot water treatment (T6) had the highest percentage emergence 56 days after planting (Figure 1) with a value of 90% while pared control (T1) and boiling water treatment (T7) had 80% emergence each at 56 days after planting (DAP). The 20 minutes dip in *Acalypha* extract treatment (T5) having only 60% emergence.

Effects of Treatments on Root Damage

At 84 DAP, the highest percentage root necrosis was recorded on the pared control (T1) and it was only different statistically from the 10 minutes (T3) and 20 minutes (T5) dip in extract treatment (Figure 2b), while at 55DAPand 111DAP, there were no differences across the treatments (Figure 2a and 2c).

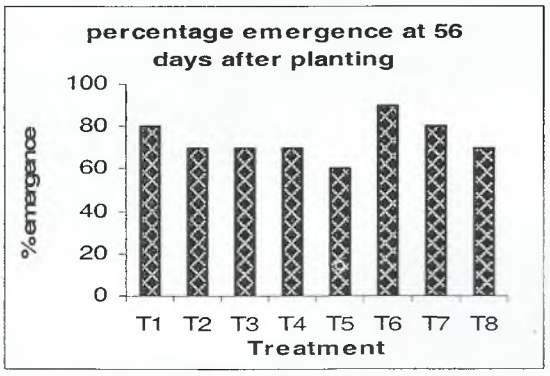

Figure 1. Effect of the duration of pre-plant exposure to *Acalypha wilkesiana* leaf extract, hot and boiling water on percentage emergence of plantain suckers
T1: Pared only (control); T2: 5 mins dip in *Acalypha* extract; T3: 10 mins dip in *Acalypha* extract; T4: 15 mins dip in *Acalypha* extract; T5: 20 mins dip in *Acalypha* extract; T6: Hot-water treatment for 20 mins; T7: Boiling-water treatment for 30 sec; T8: Non-pared control

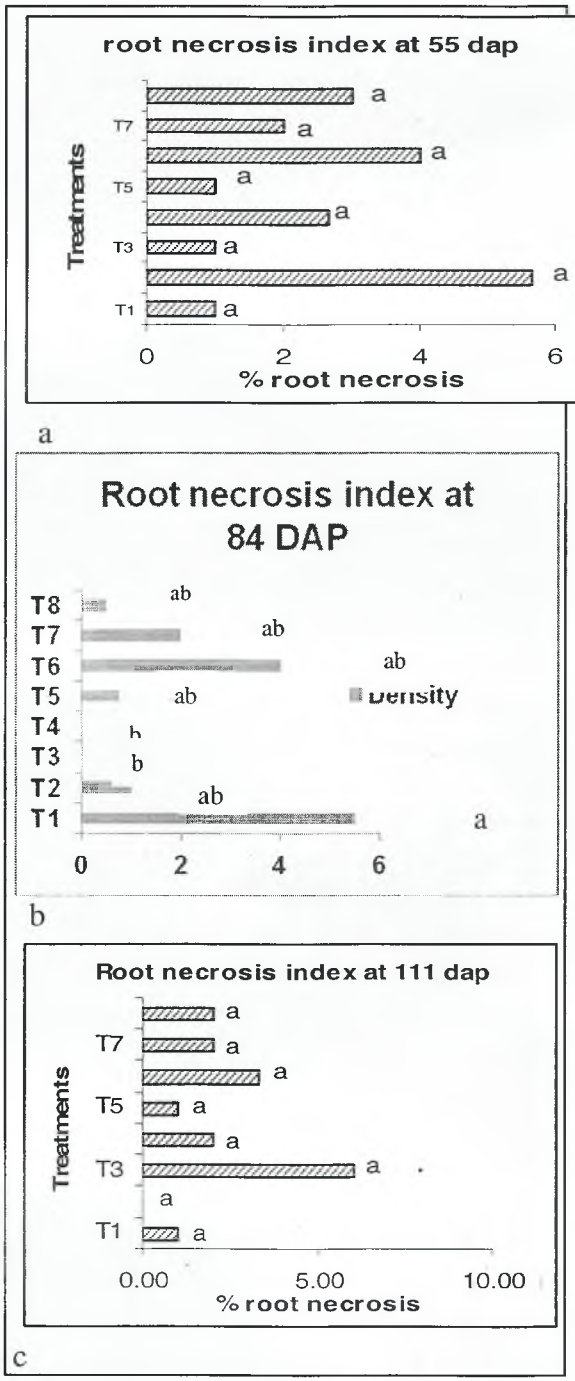

Figure 2. Effects of treatments on root necrosis index at 55, 84 and 111 (a, b and c respectively) days after planting (DAP).
T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.

Effects of Treatments on Plant Nematode Species and Densities Recovered

At 55DAP (Figure 3a), there was combination of *Meloidogyne* spp and *Radopholus similis* recovered from the root in the 15 minutes dip in *Acalypha* extract (T4), at 84 DAP, nematodes were recovered only from the 20 minutes dip in extract (T5) and the non-pared control (Figure 3b), while at 111DAP, the nematode population recovered from the roots had reduced relatively to the earlier population densities observed in the preceding samplings (Figure 3a, b and c).

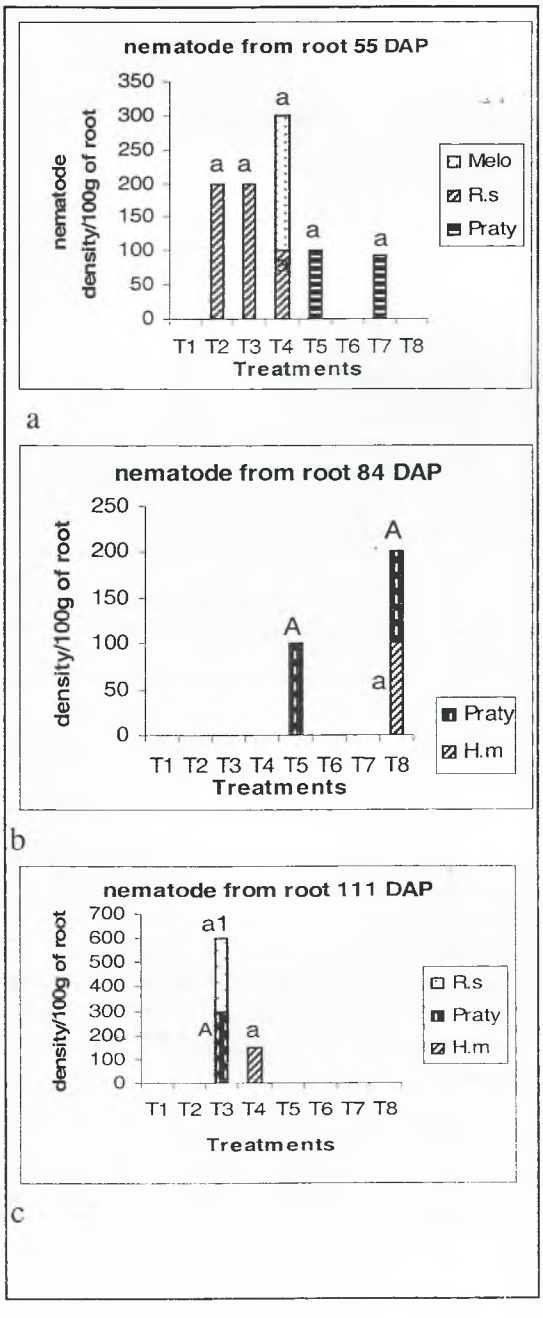

Figure 3a-c. Effects of treatments on root nematode densities at 55, 84 and 111 days after planting (DAP).

T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.

Hm:*Helicotylenchus multicinctus*, Praty: *Pratylenchus coffeae*, R.s: *Radopholus similis*

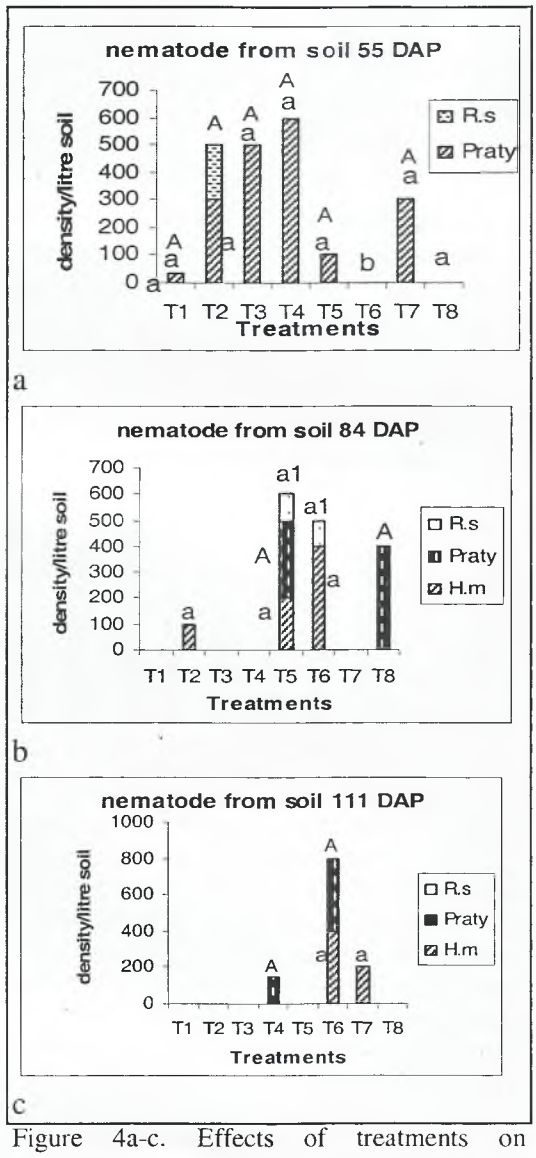

Figure 4a-c. Effects of treatments on rhizosphere nematode densities at 55, 84 and 111 days after planting (DAP).

T1: Pared control (no dip treatment), **T2**: 5 minutes dip in *Acalypha* extract, **T3**: 10 minutes dip in *Acalypha* extract, **T4**: 15 minutes dip in *Acalypha* extract, **T5**: 20 minutes dip in *Acalypha* extract, **T6**: Hot-water

treatment for 20 minutes, **T7**: Boiling-water treatment for 30 seconds, **T8**: Non-pared control.
Hm:*Helicotylenchus multicinctus*, Praty: *Pratylenchus coffeae*, Radopholus *similis*

At 55DAP, only the 5 minutes dip in extract (T2) had a combination of two species of nematodes namely *Radopholus similis and Pratylenchus coffeae* (Figure 4a), at 84DAP, the highest population density and species combination was observed in the 20 minutes dip in extract (T5) (Figure 4b) while at 111DAP, the nematode population recovered from the roots had reduced relatively to the earlier population densities observed in the preceding samplings (Figure 4c).

DISCUSSION

The low population density of nematodes in the pre-plant sampling might be due to the dryness of the soil because moisture is an important factor in nematode population dynamics. It may also be related to the slow development and build up of plant parasitic nematodes over time (Olaniyi *et al.*, 2005). The low percentage plant emergence recorded in the 20 minutes dip in *Acalypha* extract (T5) might imply the phytotoxicity of the treatment. Rotimi and Moens, (2005) also observed phytotoxicity of 30 minutes root dip of 4 weeks old tomato seedlings in standard concentration of red *acalypha* leaf extract. It was generally observed that plants treated with *Acalypha* extract took a longer time to emerge compared with the controls and the hot water treatments.

The variations obtained per treatment in the nematode densities across the three sampling dates established the rationale and the importance of sampling several times so that correct assertions can finally be made. Also, sampling only the root may not give a correct diagnosis, soil sampling should be included in accord with Rotimi *et al.* (2005). Generally, sampling several times across seasons as suggested by Rotimi *et al.* (2004) would limit the level of error in the conclusion about nematode incidence in any study and better guide nematode management decisions.

The short duration of the study might be responsible for failure to obtain clear statistical differences in the treatments, especially the 15 minutes dip in Acalypha extract and the hot water treatments, which appeared to favour plant emergence. Only the 20 minutes dip in extract showed a trace of phytotoxicity in the percentage plant emergence. Effect of red *Acalypha* extract, hot and boiling water dips on root rhizome damage was not consistent over time but the effects of these treatments implied that 15 minutes dip of planting materials in red acalypha extract showed promise in reducing nematode damage and densities on plantain. The inconsistency recorded in this study may be due to the wide variation in the size of planting materials coupled with the short duration of the study. There is however the need to monitor plant response till yield sage.

The wide variation in the size of the planting materials used is a major limitation of plantain study, as Olaniyi (2011) also noted this limitation. There is need for a technique to produce more uniform planting materials for research purposes. Tissue culture has provided solution to this problem but tissue cultured materials are not within the reach of the peasants who cultivate the crop. A commercial tissue culture laboratory is also yet to be established in Nigeria. Macro propagation is an alternative, which could be explored (Baiyeri, 2005; Baiyeri & Aba, 2004). It is necessary to encourage the development of a rapid multiplication

technique for producing large quantities of plantain planting materials that would yield a more uniform set of planting materials for pathogenicity studies on the field.

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Morphometric Characterization of Nematophagous Fungi Isolated From Some Orchards around Zaria, Nigeria

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Abstract. Nematophagous fungi are natural enemies of nematodes. They are currently being used for biological control of plant-parasitic nematodes with resounding success. In view of their potential as biocontrol agents against plant-parasitic nematodes, a survey was carried out in some orchard plants around Zaria with the view to identifying nematophagous fungi that can be deployed for bio-control of plant parasitic nematodes in Nigeria. Samples of decayed debris were collected from underneath mango, cashew and orange trees. Five samples collected from different locations under each tree were thoroughly mixed together and packaged into labelled polyethylene bags. Debris from each sample was plated on water agar supplemented with goat excreta. Each sample was replicated four times. Nematophagous fungi grew from the debris in culture between 10-14 days. The isolated fungi were sub-cultured in corn meal agar medium enriched with goat excrete and about 100 juveniles of *Meloidogyne incognita* were pipetted unto the medium. After 7 days, all the petri dishes were examined under the stereoscopic microscope for morphological identification of the fungi from the debris collected from Botanical Garden, ABU, Samaru, Wusasa area, Jos road and Kano road around Zaria metropolis respectively. The fungi identification was done on the basis of their morphological structure and trapping devices (three-dimensional adhesive nets and constricting rings), non-stalk constricting ring and adhesive sticky knob. Fungi identified from the cultures were *Arthrobotrys*, *Dactylella*, *Nematoctonus* and *Monacrosporium* species.

Keywords: *Arthrobotrys* spp., *Dactylella* spp., *Nematoctonus* spp., Nematophagous fungi, *Meloidogyne* spp., *Monacrosporium* spp., isolation

INTRODUCTION:

Nematodes are obligate plant parasites, most of them are pathogens that destroy the plant on which they feed. The most important plant parasitic nematodes include root-knot nematodes (*Meloidogyne spp.*,), cyst nematodes (*Heterodera spp.*, and *Globodera spp.*,), root lesion nematodes (*Pratylenchus spp.*,) stem nematodes (*Ditylenchus dipsaci*), burrowing nematodes (*Radopholus similis*) etc. Nematode population densities can be controlled by several measures. First and foremost is the use of synthetic nematicides. A short coming in their use is that nematicides are toxic to human beings, cause environmental hazards, too costly for

use especially by our local farmers and the developing world.

Biological control which is an alternative to nematicides has been gaining ground and is becoming important in recent years, most especially the use of natural enemies within the same environment to control plant parasitic nematodes and such natural enemy of nematodes that their population is abundant in all types of soils is the predacious fungi. These fungi have a significant contact with nematodes in their vicinity and thus, can constantly destroy nematodes in nearly all soils at different geographical areas (Siddiqui and Mahmood, 1996). The primary function of these fungi appears to be that of plant decay to obtain carbon and hence they are cellulolytic or lingo-cellulolytic fungi (Barron, 2003). In such environment where plant debris is in abundance with high carbon, nematodes might serve as an important source of nitrogen during growth on carbohydrate containing substrates. These predacious fungi are commonly found in natural soils, agricultural soils and all kinds of decaying manures.

Nematophagous fungi have been the subject of research over several decades in fundamental studies of their ecology, distribution and systematic, and as potential biological control agents of nematode pathogens of plants and animals (Li *et al*., 2000; Liu and Zhang, 2003; Dong *et al*., 2004; Li *et al*., 2005). Many species in the genera *Pleurotus* and *Hohenbuehelia* are nematophagous. This is made possible by hyphae that may have adhesive knobs that attach to passing nematodes and secret nematoxic compounds (Thorn *et al*., 2000 and Koziak *et al*., 2007). The predacious hyphomycetes in *Arthrobotrys*, *Corda* and related genera, some with teleomorphs in *Orbilia* Fr. (*Ascomycota*, *Orbiliaceae*), destroy nematodes using several kinds of trapping devices: stalker and sessile adhesive knobs, two- or three-dimensional adhesive nets, and constricting and non-constricting hyphal rings (Scholler *et al*., 1999).

Since the discovery of predacious activity in *Arthrobotrys oligosporal* (Zopf, 1888), the nematode-trapping fungi have attracted much interest amongst mycologists probably due to the spectacular trapping method, in the physiology involved with it and, last but not the least, in their potential economic importance as biocontrol agents. *Arthrobotrys* species are known to produce a range of nematicidal compounds, including Linoleic acid (Anke *et al*., 1995) and Oligosporons (4', 5'-dihydrooligosporon, hydroxyoligosporon, and 10', 11', -epoxyoligosporon) (Anderson *et al*., 1995).

Although, many of the trap-forming and egg-parasitic fungi can survive in soil saprophytically, the endoparasites are mostly more dependent on nematodes for nutrients source. Aiming to improve on the biocontrol process and to have alternative to chemical nematicides that are toxic to human beings and cause environmental hazards, there is need to control these plant parasitic nematodes using some eco-friendly techniques and biocontrol agents such as predaceous and endoparasitic fungi that are safe, sustainable and have no deleterious effect on the environment as well as the plant (Goswami and Uma, 1995).

MATERIALS AND METHODS

Collection of Samples and Isolation of Nematophagous Fungi

Isolation of different isolates of the fungi was carried out using the

method of Bandyopadhyay and Singh (2000). From different locations 250gm of decayed leaf debris were collected in separate labelled polyethylene bags from different locations within Zaria town, which is about 80 km north of Kaduna. Zaria is located between longitude 7° 44' East, and latitude 11° 6' North of the equator (Duze and Ojo, 1990).

The locations were Samaru, Wusasa, Sabon-Gari and Basawa. In each location, three economic orchard trees were selected for sampling, *Mangifera indica* (Mango), *Anacardium occidentale* (Cashew) and *Citrus* species (Orange). Underneath each tree, samples of decayed leaf debris were randomly collected at five different points and mixed together in a labelled polyethylene bags to represent a sample from each tree. All the samples collected were transported to Department of Crop Protection, nematology laboratory, ABU, Zaria and stored in the cool room for future use.

Preparation of Agar Medium and Inoculation with decayed Leaf Samples

Twenty grammmes of agar-agar was dissolved in a conical flask in 1,000 ml tap water on a hot plate for 15 to 20 minutes. The mixture was continuously stirred with a clean glass rod to dissolve the agar-agar. Few drops of streptomycin was added the resulting solution to prevent bacteria growth before autoclaving at 15psi pressure and 121°C for 15 minutes. Afterwards it was allowed to cool for a few minutes and dispensed gently into 9 cm Petri-dishes to cover about 2/3rd area of each plate. All the Petri-dishes were labelled to correspond with each tree sample. From each sample, 2 g of decayed leaf debris were carefully sprinkled onto the solidified medium and 3 g of ground goat excrete pellets were added. Each selected sample had four replicates inoculated for the sporulation of nematophagous fungi. The inoculated plates were arranged on laboratory bench to incubate and routinely observe for two weeks for the presence of nematophagous fungi under stereoscopic binocular microscope.

Isolation of Nematophagous fungi and nematodes from Decayed Leaf Debris of Mango, Cashew and Orange

Corn meal agar was prepared by adding 20 g each of maize powder and agar-agar in 1,000 ml distilled water, streptomycin was added to prevent bacteria growth. The sterilized CMA was poured into several Petri-dishes and allowed to solidify. Similarly, goat excrete agar was prepared and thin layer poured over the CMA to boost the nutrient level of the medium. Subcultures were made from the conidial heads of individual sporulated fungi into each Petri-dish of CMA. Nematodes observed to be trapped by fungi were picked with the help of a sterilized needle into Petri-dishes containing corn meal agar medium (CMA).All the Petri-dishes were incubated on the laboratory bench in the clean room, for 10 days.

Identification of the fungi

For identification of different isolates of the nematophagous fungi, conidia, conidiophores, hyphae, trapping devices formed on the growing mycelium and directly formed on the spores as well as nematodes captured were recorded and compared with the original description given by Drechsler (1937) and Cook and Godfrey (1963). Isolates from each location were tested for their ability to capture nematodes by introducing *Meloidogyne* spp. juveniles into separate CMA plates.

Inoculation of *Meloidogyne incognita* and observation of fungal trapping devices

Freshly hatched second stage juveniles of *M. incognita* were collected in large numbers from egg masses of root galls of *Solanum lycopersicum*. The juveniles were washed five times with sterilized water. A 10 µl drop containing 100 nematodes was transferred into each CMA plate containing nematophagous fungi. All the Petri-dishes were incubated for 24 hours for trapping or capturing and killing of nematodes. After 24 hours, the Petri-dishes were observed under the stereoscopic microscope for nematodes captured, predaceous fungi, capturing organs, fungi mycelia, conidia and spores. The observation was routinely carried out daily for 8 days. All the morphological futures that can help in the identification of the fungi were recorded.

RESULTS

After 14 days of inoculation, it was observed that the sprinkled decayed leaf debris from different locations within Zaria inoculated on water agar medium harboured nematophagous fungi. On the second day of inoculation, the Petri-dishes were observed under the microscope. Nematodes were in abundance in the samples collected. The presence of nematodes might have stimulated the induction of capturing devices of the nematophagous fungi.

The isolates from all the locations had more of *Arthrobotrys* species than *Dactylella* spp., *Nematoctonus* spp., and *Monacrosporium* spp.

At the species level the type of trapping organ is considered to have high taxonomic significance. As a rule, a species can only form one type of trapping organ. Three modifications of this rule were noted in this study:

a) Non-constricting rings may be accompanied by stalked knobs.
b) Within the knob-forming fungi a variation in the stalk can be observed. The proliferation of knobs, as seen in *M. parvicolle*, sometimes resembles the hyphal branches of *M. gephyropagum*.
c) The simple hyphal branches (*M. cionopagum*) tend to fuse, forming a two-dimensional network (*M. gephyropagum*).

Below are some pictures depicting the morphological structure and capturing devices of nematophagous fungi isolated from these plant debris (Plates 1 – 9).

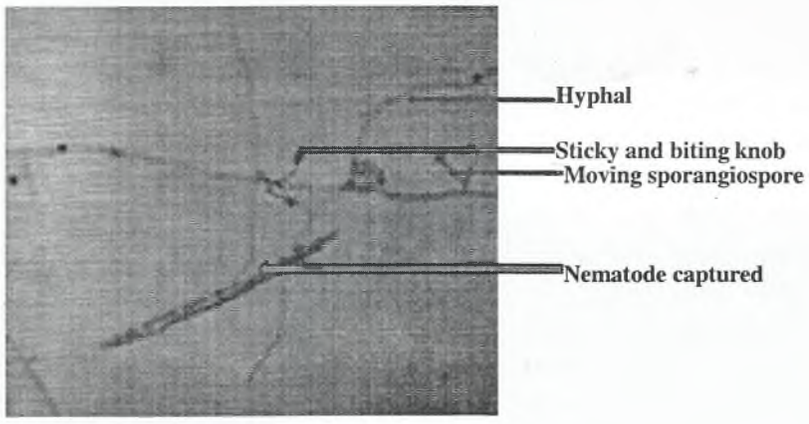


Plate 1. ***Monacrosporium*** **species that captured nematode**

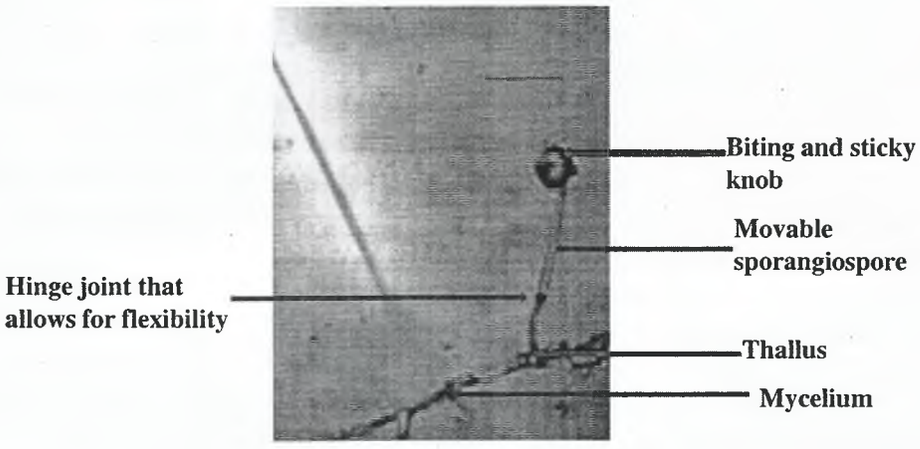


Plate 2. Matured predaceous fungi: ***Monacrosporium*** **spp**

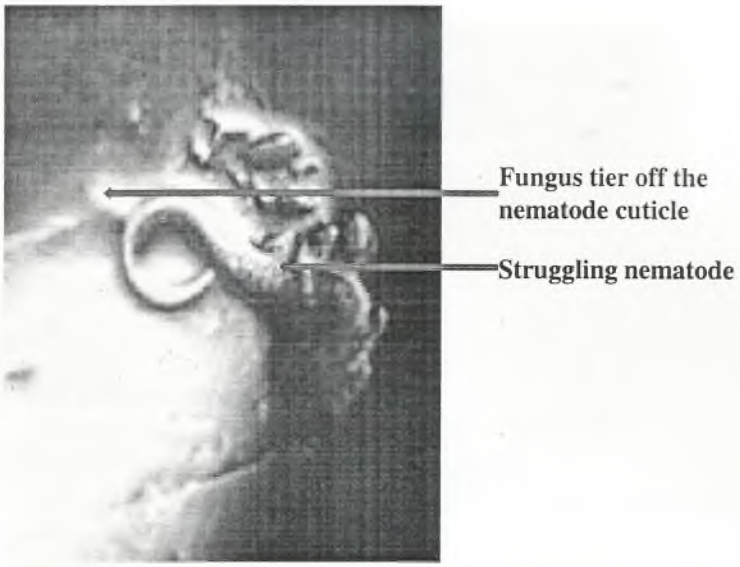
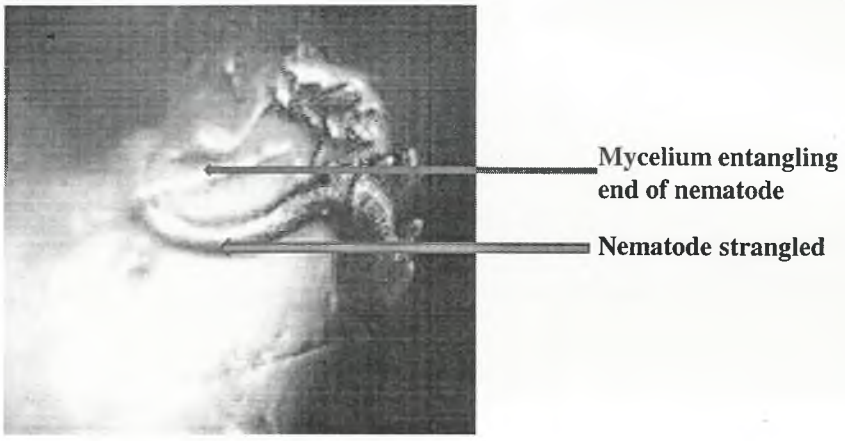


Plate 3. ***Monacrosporium*** **spp. destroyed the nematode cuticle**



Plare 4. ***Monacrosporium*** **spp. entangling the nematode after tearing off the cuticle**

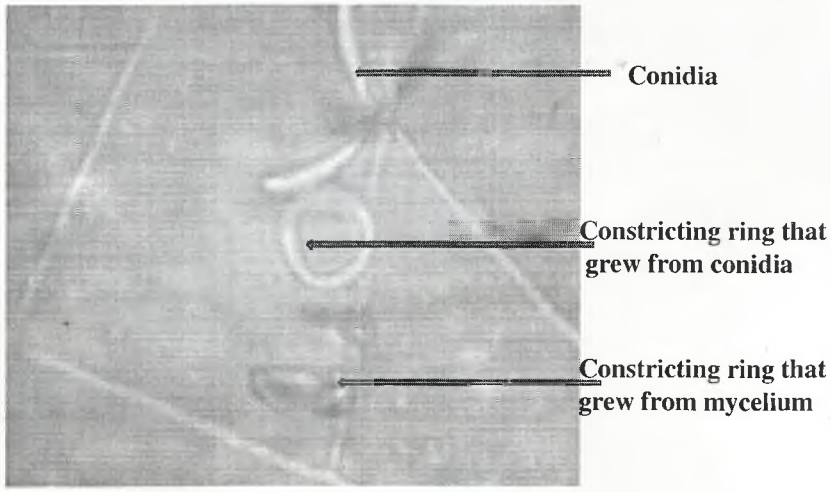


Plate 5. ***Dactylella*** **spp.**

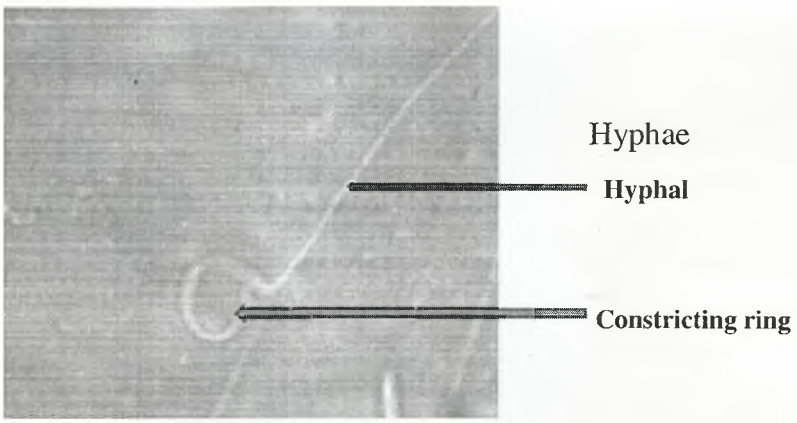


Plate 6. Constricting ring of ***Dactylella*** **species**

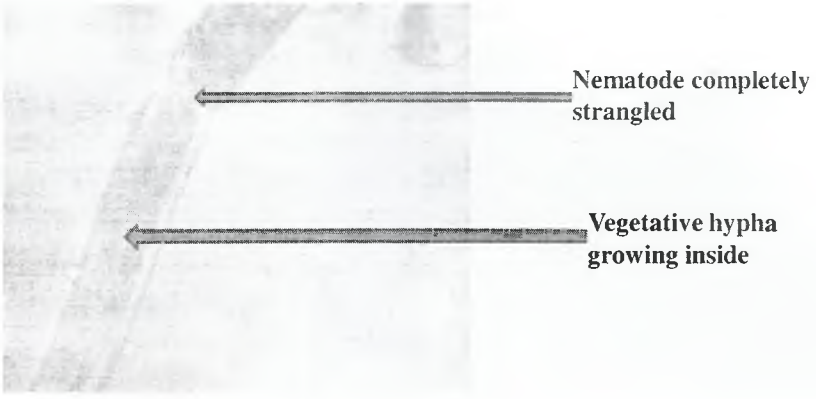


Plate 7. Nematode strangled with vegetative hypha *Dactylella* spp. growing inside the nematode

Are we to assume the nemae of the fungi???

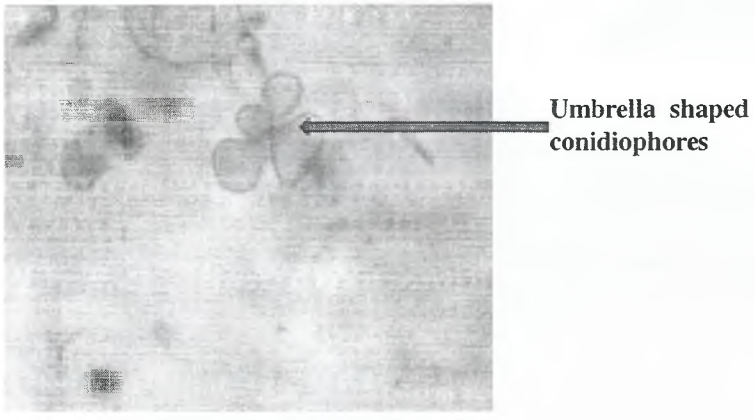


Plate 8. *Arthrobotrys oligosporal* isolated from decayed leaf of mango

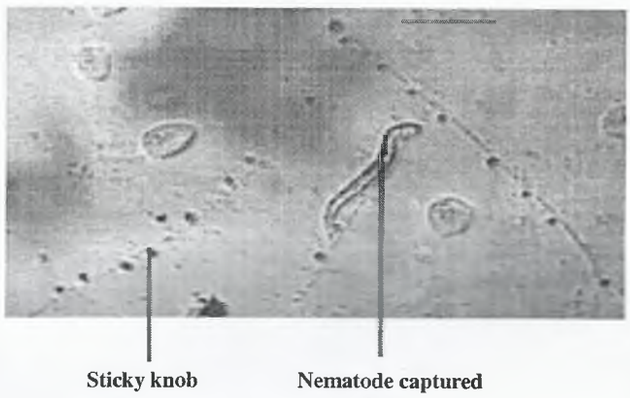


Plate 9. **Nematode captured by *Nematoctonus* spp.**

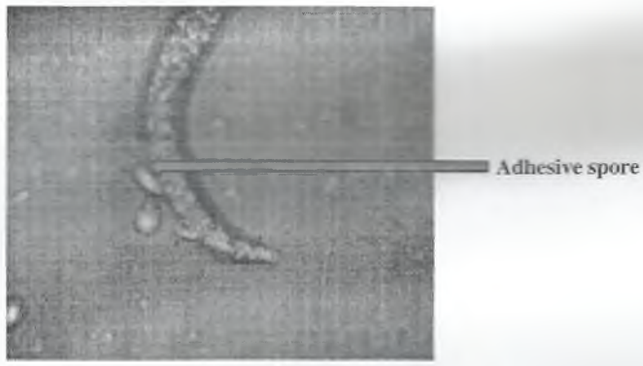


Plate10. **Spores of *Nematoctonus* species attached to nematode**

After 8 days of observation, it was noted that nematophagous fungi isolated from decayed mango leaf debris from Botanical garden, Samaru captured about 90 juveniles of the nematodes introduced and the least was 66 juveniles from the isolate of mango leaf litter from Wusasa in an average of 100 juveniles introduced as indicated in Table 2

Number of Nematodes Captured by Nematophagous Fungi in different locations in Zaria

High numbers of nematodes were recorded captured in mango, followed by cashew and orange tree respectively as shown in Table 1. There was a significant difference at ($p \leq 0.05$) between the leaf debris of the three orchard trees irrespective of their locations. Significant difference was observed between the debris collected from all the orchard trees after 8 days. The mango tree has the highest number of nematodes (77.75) captured, followed by cashew (74.56) and orange (71.31) tree respectively (Table 1). However, with respect to location or point of collection, it was observed that, there was a significant difference between all the locations after 4 days, Samaru (58.50) having the highest number of nematodes captured and

lowest in samples collected from Jos road (21.50). After 6 and 8 days of observations, there was a significant difference between Samaru and Kano road after 6 and 8 days. The nematodes captured were highest in Samaru location at both 6 and 8 days (82 and 92) as indicated in Table 2.

Table 1. Average Number of Nematode Captured by Nematophagous Fungi in different leaf debris in Zaria

	Sampling period (Days)			
Treatment(Tree)	2	4	6	8
Mango	31.00[a]	55.19[a]	76.06[a]	77.75[a]
Cashew	28.75[b]	48.82[b]	71.31[b]	74.56[b]
Orange	22.44[c]	31.88[c]	63.88[c]	71.31[c]
LSD(≤0.05)	1.34	1.74	1.34	1.57

Means in column followed by different letter are significantly different (P≤0.05) using (LSD) least significant difference.

Table 2. Average Number of Nematode Captured by Nematophagous Fungi in different locations in Zaria

	Sampling period(Days)			
Treatment(Location)	2	4	6	8
Samaru	31.92[a]	58.50[a]	81.75[a]	92.17[a]
Kano road	30.25[b]	53.00[b]	73.67[b]	75.08[b]
Wusasa	25.92[c]	37.08[c]	63.75[c]	65.50[c]
Jos road	21.50[d]	32.58[d]	62.50[c]	65.42[c]
LSD(P≤0.05)	1.54	2.00	1.58	1.80

Means in column followed by different letter are significantly different (P≤0.05) using (LSD) least significant difference.

In respect of locations and orchard tree types, at 4 days of observation, there were no significant differences between cashew and mango decayed leaf debris in Jos road location as well as Samaru location, but there was a significant difference between the two tree debris and orange debris in these two locations. However, there was no significant difference between the nematodes captured in orange debris in Jos road and Wusasa, as well as between Kano roads Samaru (Table 3).

Similarly, it was observed that nematodes captured in leaf debris of cashew, orange from Jos road and Wusasa location, there was no significant difference between the two locations. However, leaf debris collected from cashew and mango trees from Botanical garden Samaru captured more nematodes than the other locations (Table 3).

Table 3. Interactive effect of Nematode Captured by Nematophagous Fungi after 4 days in different locations and types of trees in Zaria

	Orchard Trees		
Treatment(Location)	Cashew	Mango	Orange
Jos road	33.75[e]	36.25[ea]	27.75[f]
Kano road	58.50[c]	63.75[b]	36.75[e]
Samaru	68.75[a]	70.75[a]	36.00[e]
Wusasa	34.25[e]	50.00[d]	27.00[f]

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Table 4: Interactive effect of Nematode Captured by Nematophagous Fungi after 8days in different locations and type of trees in Zaria

	Types of trees		
Treatment(Location)	Cashew	Mango	Orange
Jos road	65.25d	65.50d	65.75d
Kano road	74.00c	86.50b	64.75d
Samaru	94.50a	93.00a	89.00b
Wusasa	64.50d	66.66d	65.75d

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

After 8 days of incubation, more than 90 out of the 100 nematodes introduced into the CMA petri dishes were captured from the isolate of the leaf debris of cashew and mango collected from Samaru location, however there was a significant difference between these trees and orange debris. For all the orchard trees, nematophagous fungi from Samaru tends to be more virulent in capturing nematodes based on the number of nematodes cannibalised by these fungi. These predaceous fungi were mostly *Arthrobotrys species*. Table IV also indicated that, there was no significant difference between Jos road and Wusasa locations for all the three orchard trees.

DISCUSSION

The nematophagous fungi isolated from this leaf debris were found to develop sophisticated hyphal trapping structures such as hyphal nets, three-dimensional adhesive nets, constricting rings, and adhesive knobs to capture nematodes. However, there are more than 160 species of predacious fungi that are able to capture and kill nematodes in soil and plant debris (Dijksterhuis *et al.*, 1994; Siddiqui *et al.*, 1996; Thorn *et al.*, 2000). The high number of nematodes captured by the nematophagous fungi isolated in Samaru compare to other locations may be as result of high concentration of organic waste piled up over years or a different virulent of *Arthrobotrys* spp. Mankau (1968) reported that *A. oligosporal* and *A. dactyloides,* which produce constricting ring are common fungi that were frequently detected in soils amended with organic amended soils. Drechsler (1937) also reported that *Arthrobotrys oligosporal* is the

most widespread. This fungi has been isolated from plenty of different substrates, e.g. from compost, decomposing wood and animal excrements. The upright conidiophores is said to bear 20-30 groups of 5-20 two celled, 16-30µm long and 8-16 µm broad conidia clearly indented at the septa, whose distal cell is about twice as large as the proximal cell (Haard, 1968).

Similarly, the type of nematode-trapping structure formed depends on species or even strains of species as well as on environmental conditions, both biotic and abiotic factors. The most important biotic factor is living nematodes, which not only induce the formation of trapping structures when it touches fungal mycelium but also serve as food source for the fungi after they might have been invaded by the fungi (Nordbring-Hertz, 2004). Thus the nematodes induce the formation of the structures in which they are later consumed, serving as an additional food source. The nematophagous fungi isolated from these different locations were similar to some reports on the isolation and characterization of nematophagous fungi from various sources including soil, dung, compost and fresh faeces of some animal species at different geographical areas (Chandrawathani *et al.*, 2001; Sanyal, 2000). Nematophagous fungi are said to be carnivorous fungi that specialize in trapping and digesting nematodes, and there exist both species that live inside the nematodes from the beginning and others that catch them mostly with glue traps or in rings. Some species possess both types of traps. Another technique employed by the fungi is to stun the nematodes using toxins, which is a method usually employed by *Coprinus comatus* and the family *Pleurotaceae* (Thorn *et al.*, 2000).

In conclusion, use of chemical in the control of nematodes, which requires application of large amounts of chemicals and knowledge of special equipments to control root-knot nematodes on various crops should be discouraged. If successfully done, it would also check the effect on climate change. In view of this, there is need to replace highly toxic and potentially polluting chemicals used for the management of plant parasitic nematodes. Some of these nematophagous fungi that are non-phytotoxic and commonly found within the rhizosphere should be put into use. Farmers should be encouraged to use organic amendments in the production of their crops in order to facilitate the growth of these nematophagous fungi, hence control of plant-parasitic nematodes.

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Comparative Analysis of Mitochondrial COI and 18S rRNA Genes in the Identification of Marine Nematodes

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Abstract. Free-living nematodes are the most abundant metazoan organisms in marine sediments. They are important in many ecological processes and are used as bio-indicators. Yet, they remain the least described taxon, because morphological diagnostic features are difficult to observe due to their small body size. DNA barcoding may overcome the problems associated with morphology and may lead to a quicker identification of marine nematodes. In this study, the amplification and sequencing success of both gene fragments were compared in a wide range of marine nematodes. 73 species belonging to 56 genera were sampled from Paulina Polder (the Netherlands) and Belgium North Sea. Our results demonstrate that 18S is easily amplified in marine nematodes compared to COI (57% vs 43% amplification success). Sequencing success was higher for 18S rRNA genes than for COI (61% vs 39%) genes. Neighbor joining analysis using the K2P-model showed that both genetic markers cluster into well-defined clades congruent with known taxonomic families and orders that have been delineated based on morphology. Pairwise genetic distance for the 18S sequences showed that ≈74% of intraspecific comparisons showed a genetic divergence ≤3% while about 77% of interspecific comparisons were above 3%. For COI sequences, 98% of intraspecific comparisons showed a genetic divergence of ≤8% and >8% was observed for about 94% of all interspecific comparisons. This study establishes the fact that although, the 18S rDNA may prove invaluable in the identification of marine nematodes, COI gene may be a better choice. However this gene is hampered by low amplification and sequencing rates.

Keywords: DNA Barcoding . COI . 18rDNA . Molecular Identification. Marine nematodes.

INTRODUCTION

Nematodes have been reported to be the most abundant metazoans on the surface of the earth with about 10^8 individuals distributed within a square meter (Decraemer & Hunt, 2006). In marine sediments alone, about 10^5 -10^7 free-living nematodes have been reported to exist in every square meter (Lambshead, 2004). Yet, the number of described nematode species is estimated at only 27,500 species (Hallan, 2007) out of the over one million species predicted by Lambshead (2004). It is a well-known fact that marine nematodes play an important role in many ecosystems. Their roles in bioturbation, nutrient decomposition and nutrient mineralization as well as in food web

interactions have been well reported (Moens and Vincx, 1997; Lawton *et al.*, 1998). Their sensitivity to changes in the environment caused by pollution has been exploited in their use as bio-indicators in the environment (Austen and McEvoy, 1997; Boyd *et al.*, 2000). Despite their important roles, discriminating species remains a difficult task that requires great taxonomic expertise, leaving a greater part of knowledge on nematode species diversity in a state of enigma. According to Hammond (1992), it has been estimated that since only few taxonomists can identify more than 0.01% of the estimated 10-15million nematode species, about 15,000 taxonomists will be required to identify life if our reliance on morphological diagnosis is to be sustained.

While identification based on morphological diagnosis is possible given the availability of an in-depth taxonomic skill and high throughput equipment, it is often saddled by certain limitations. First, there is a high chance of species misidentification due to both phenotypic plasticity as well as genetic variability (Hebert *et al.*,2003a). Second, certain life stages or sex may be needed before a complete identification is possible in some cases; hence in the absence of such a criterion, identification is either impossible or based on guess work (Knowlton, 1993; Jarman and Elliott, 2000). Third, the identification of cryptic species i.e. two or more distinct species that were classified as the same due to morphological similarity (Bickford *et al.*, 2007).

DNA Barcoding, has over the years since its discovery in 2003 continued to unfold the indecipherable taxonomic bottlenecks associated with identification of nematodes in marine sediments as well as in those associated with plants and other large metazoans (Floyd *et al.*, 2002; De Ley *et al.*, 2005; Bhadury *et al.*, 2006a, b;Holterman *et al.*, 2008; Derycke *et al.*, 2010a). Although various segments of the genome have been used as molecular identification markers in taxonomic and ecological studies, cytochrome *c* oxidase subunit I gene (*cox*-1 = COI) of the mitochondrial DNA (mtDNA) was proposed as the standard barcode for identifying many animals (Hebert *et al.*, 2003a). This is chiefly due to two reasons; the availability of robust universal primers that can amplify the COI gene in a wide range of animals (Folmer *et al.*, 1994; Zhang and Hewitt, 1997). It has been shown that COI can provide species identification with more than 90% resolution (Hajibabaei *et al.*, 2006a; Hajibabaei *et al.*, 2006b; Hajibabaei *et al.*, 2007; Meusnier *et al.*, 2008). Hence, effort towards expanding the standard sequence reference database of COI continues to increase (Marshall, 2005; Silva *et al.*, 2010). Barcoding studies in nematodes have traditionally used a fragment of the ribosomal 18S gene, however, its resolution is may not be sufficient for species-level identification especially when closely related species are involved (Derycke *et al.*, 2010b). It is against this background that this study was conducted to compare the reliability and performance of the two barcode genes – 18S and COI in species identification for marine nematodes through the use of sequence alignment, calculation of the number of differences between species and constructing phylogenetic trees.

MATERIALS AND METHODS

Taxon Sampling

Sediments were collected from six stations in Paulina Polder which is located in the polyhaline zone of The Westerschelde estuary (The Netherlands) during low tide in

September, 2011. The stations included: Canal Sediment (51° 20.913□ N, 3° 43.354□E), Before Experimental Plot Sediment (51° 21.0171□ N, 3° 43.496□E), After Experimental Plot Sediment (51° 20.984□ N, 3° 43.484□E), Silt Sediment (51° 21.149□N, 3° 43,911□E), Biofilm-based Sediment (51° 21.068□ N, 3° 43.854□E) and Sea Grass Plot Sediment (51° 20.998□ N, 3° 43.897□E). Three replicates of the top 5 cm were collected for all sampled stations. All samples were immediately fixed in a compound of 20% DMSO (Dimethyl sulfoxide), 0.25 M disodium EDTA (Ethylenediaminetetraacetic acid) and saturated with NaCl (Sodium chloride) known as DESS (Yoder *et al,*. 2006). Samples from the subtidal zone of the Belgium North Sea collected from a previous study, was further added to increase the chances of obtaining more species for this study.

Nematode Extraction and Sample Preservation

Each sediment sample was washed twice with tap water after removing DESS using a doubly-arranged set of sieves (1mm and 38µm respectively) until the water passing through the set of sieves became clear. The content of the 1mm sieve was properly rinsed until only fractions larger than 1mm in width were left. Ludox™ (specific gravity 1.18) was used to saturate the washed sample in the 38 µm sieve and left for 3 minutes. The Ludox-added sample was then distributed into tubes and centrifuged after been calibrated with a weight balance. Centrifugation was maintained at 3000 rpm for 12 minutes. Supernatants from all the tubes were poured into the 38µm sieve, then into a clean labeled empty sample pot. The residues were twice subjected to the same procedure each time pouring the supernatant into the sample pot but after the third centrifugation, all collected supernatants were poured into the 38µm sieve and rinsed with tap water to get rid of the ludox. The content in the 38µm sieve was then immersed in DESS and poured into the rinsed labelled sample pot.

Morphological Identification and Vouchering of Specimens

Adult nematodes, especially males were picked-out from each sample pot one after the other under a dissecting microscope, rinsed off with distilled water and each individual specimen was mounted on a temporary slide and identified under a LEICA DMR research microscope. Identification, were possible was made to species level based on morphological dissimilarities and morphometric measurements using Nematotheek (A Collection of Publications on Marine Nematodes Descriptions) and identification guides (Warwick & Platt: Part I, II and III). Diagnostic features of the specimen were digitally captured using LEICA Application Suit. The vouchered specimen was then transferred into an Eppendorf tube containing 20 µL worm lysis buffer (50 Mm KCl, 10 mTris Ph 8.3, 2.5 mM MgCl2, 0.45% NP40, 0.45% Tween 20) and stored at -20°C.

DNA Extraction and Amplification of the COI and 18S regions

To extract genomic DNA of each specimen, proteinase K (1µl of 10g/ml) was added into each Eppendorf tube containing a single nematode in worm lysis buffer. This was followed by incubation at 65°C for one hour and the proteinase K was denatured at 95°C for 10minutes. Finally, the extracted DNA was centrifuged for one minute at 20°C and stored at 4°C. PCR-Mix was prepared for each

primer set separately in total volumes of 25 µL containing 15.13 µL distilled water, 2.5 µL of 10x PCR buffer, 2.5 µL dye, 2 µL MgCl2, 0.5 µL dNTP, 0.25 µL of each primer (25 nM), 0.125µL TopTaq polymerase (Qiagen) and 2 µL DNA. Three test samples were randomly chosen each time to test the amplification success of the primers based on the eight different primer sets used and PCR cycling conditions optimization (Table 1). To amplify CO1, JB3-JB5 primer set was chosen based on its amplification success under pre-set optimized condition of the PCR cycler. The conditions were: initial denaturation of 5 minutes at 94°C, 35 cycles of (94°C for 30s; 50°C for 30s; 72°C for 30s) and a final extension of 10 minutes at 72°C. To amplify 18S, G18S4-4R primer set was used under optimized PCR cycler conditions: initial denaturation of 5 minutes at 94°C, 45 cycles of (94°C for 30s; 54°C for 30s; 72°C for 1 min) and a final extension of 10 minutes at 72°C. PCR products were loaded on 1% agarose gels containing 0.003% ethidium bromide and visualized using BioDoct-ItTM Imaging System (UVP). Each gel contained one lane with 4 µL of DNA Mass Ladder (Invitrogen), one lane which contained 4µL of positive control (PCR product containing 2µL DNA of *Litoditis marina*), one lane which contained 4 µL of negative control (PCR product without DNA) while all other lanes contained 4µL PCR products of the specimens under scrutiny. Amplifications were considered successful when a band of the expected size was observed on agarose gel and when aspecific bands were absent. Primers used for amplification and sequencing are denoted by double asterisks while those used for only for sequencing are denoted by a single asterisk. Those without asterisks were only used to test amplification success.

Sequencing

Five (5) µL of PCR products which gave reliable bands and void of aspecific bands were added into 96-well plates containing 5 µL of primers: G18S4 and 4R were used as forward and reverse primers respectively to sequence the 18S gene while JB3 and JB5 were used to sequence the CO1 gene (Table 1). Sequencing plates were then sent to Macrogen for sequencing. To investigate the biological origin of the sequences as being nematode sequences, all sequences were first subjected to a Basic Local Alignment Search Tool search using BLASTn for 18S sequences and BLASTx for the CO1 sequences. The reliability of sequences was then checked using BioEdit sequence alignment editor (Hall, 1999) and EditSeq v. 7.1. The obtained chromatogram for each sequence was used as a yardstick in the selection of usable and non-usable sequences. Chromatograms with clearly defined nucleotide peaks and high fluorescent signals were considered usable while those with multiple peaks and low signals were considered non-usable. Forward and reverse sequences of COI and 18S were edited, assembled and merged into consensus sequences using SeqMan ProTM software (Lasergene v 8.0.3, DNASTAR®). All COI sequences were translated using the invertebrate mtDNA genetic code in SeaView v. 4 (Gouy *et al.*, 2010). 18S sequences were not translated since the 18S region is non-coding. Prior to alignment, sequences were manually trimmed to remove all ambiguous characters such as gaps and unresolved base calling errors.

Table 1: List of Primer sequences used in this study

Primer	Sequence	Reference
JB3 (F)**	5□ TTT TTT GGG CCT GAG GTT TAT 3□	Bowles *et al.*, 1992
JB5 (R)**	5□ AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG 3□	Bowles *et al.*, 1992
JB2 (F)	5□ ATG TTT TGA TTT TAC CWG CWT TYG GTG T 3□	Derycke *et al.*, 2007
JB5GED (R)	5□ AGC ACC TAA ACT TAA AAC ATA RTG RAA RTG 3□	Derycke *et al.*, 2007
G18S4**	5□ GCT TGT CTC AAA GAT TAA GCC 3□	Blaxter *et al.* 1998
4R**	5□ GTA TCT GAT CGC CKT CGA WC 3□	Blaxter *et al.* 1998
F04 (F)	5□ AGAGGT GAAATTCTTGGATC 3□	Blaxter *et al.* 1998
22R (R)	5□ GCC TGC TGC CTT CCT TGG A 3□	Blaxter *et al.* 1998
9R*	5□ AGC TGG AAT TAC CGC CGC TG 3□	Blaxter *et al.* 1998
G10 (F)	5□TCW ACW AAT CAT AAA GAT ATT GG 3□	LCOmod, *S. Derycke*
G11 (R)	5□ ACT TCS GGR TGA CCA AAA ATCA 3□	HCOmod, *S.Derycke*
18P (R)	5□ TGA TCC WMC RGC AGG TTC AC 3□	Blaxter *et al.* 1998

Data Analysis

Amplification and Sequencing success

Amplification success of each primer-set was calculated by dividing the number of successfully generated amplicons (those which gave reliable bands) by the total number of specimens ran over agarose gel for the specific primer-set. Sequencing success for all primers used to sequence the genes under study were calculated by dividing the sum of successfully sequenced reactions of the forward and reverse primers by the total sum specimens sequenced. To obtain sequencing success for each fragment, both forward and reverse percentages for individual primer-set were added. For intra- and inter-species-level analysis, nucleotide-sequence divergences were computed using the Kimura□s 2 Parameter (K2P) substitution model in MEGA v.5.0. Intra- and interspecific genetic distances were calculated using all COI and 18S sequences.

Sequence Alignment and Phylogenetic analysis

The CO1 and 18S rRNA sequences were separately aligned using Muscle (Edgar, 2004) as implemented in SeaView v. 4. Subsequently, both ends in all sequences containing the forward and reverse primers were trimmed off.

While it is true that this process may lead to the loss of some phylogenetically informative segments of the sequences, it also eradicates the inclusion of questionable characters that may lead to wrong phylogenetic inference (Floyd *et al.*, 2002). The aligned sequences were subsequently used to calculate pairwise distances using the K2P-model in MEGA v. 5. (Tamura *et al.*, 2011). Finally, neighbor joining (NJ) trees for both genes under study were constructed in MEGA v. 5 using the K2P-model because it is the most widely used model for DNA barcoding and in the inference of barcoding gaps (Hebert *et* al., 2003a). Node support was evaluated with 1000 bootstrap pseudoreplicates.

RESULTS

Taxon Sampling

A total of 170 specimens were screened and vouchered yielding 73 species belonging to 56 genera of marine taxa. About 33 bidirectional sequences were obtained for COI analysis from this study. In addition 60 COI sequences from previous studies were added to broaden the scope of phylogenetic analysis. Fifty-seven (57) bidirectional sequences were obtained for the 18S rRNA gene.

Amplification and Sequencing success of 18S rRNA and COI

The G18S4-4R primer set outperformed JB3-JB5 primer set in terms of number of specimens 18S gene amplified. Among the 170 specimens tested, 57% of the specimens were successfully amplified by G18S4-4R, although aspecific bands were produced in most lanes in the agarose gels due to primer dimers. JB3-JB5 had 43% amplification of the COI region without the production of aspecific products. Aspecific bands were produced when G18S4-4R primer set was used to amplify the 18S region in *Paroxystomina micoletzkyi, Parodontophora cobbi, Viscosia abyssorum, Theristus acer, Eleutherolaimus, Ptycholaimellus pandispiculatus, Odontophora* sp3, *Chromadorina,* sp1 *Antomicron* sp1 and *Chromadorita* sp1. However, distinct bands were produced when JB3-JB5 primer pairs were used to amplify the COI region of the same species of nematode. Sequencing success was calculated for the four separate primers used in the study: JB3(F), JB5(R), G18S4(F) and 4R(R). JB5 outperformed JB3 (46.22% vs 42.62%) in terms of sequencing success of the COI-amplified specimens. JB3 also produced more ambiguous chromatograms than the reverse primer. On the contrary, for the sequencing of 18S, G18S4 outperformed 4R (76.70% vs 68.46%) of the amplified specimens. Ambiguous chromatograms were more in 4R sequences than in sequences obtain using G18S4. Sequencing success of the COI fragment was clearly higher than the 18S- amplified genes (61% vs 39%).

Assembled COI sequences were subsequently compared with sequences in GenBank database to check if they originated from the phylum nematoda. Most of the hits reported hereafter had coverage of 99 or 100% and maximum identity higher than 85%. About 65.59% of all COI sequences blasted in GenBank matched with the exact nematode species sequences in the database while 34.41% of the sequence did not show resemblance to the exact nematode sequences. However, some of the sequences that did not match still showed that they are of nematode origin (Table 2) as revealed by the Taxonomy Section of BLASTx. The sequences of *Ascolaimus* sp1, *Bolboliamus* sp1, *Microlaimus* sp1 *Sabatiera* sp1 and *Enoplolaimus*

attenautes showed similarity with organisms from four different phylums: *Chattonella marina* (100%): *Onchrophyta, Cryptosporidum parva* (99%): Apicomplexa, *Colwellia psychrerythraea* (100%): Proteobacteria-gamma, *Megischus bicolor* (100): Arthropoda and *Thelyphonidae* sp (88%): Arthropoda respectively.

Molecular Phylogenetic Analysis of COI sequences

Nucleotide insertions were observed in the COI sequence alignment of *Daptonema setosum*_JB5, *Praeacanthonchus* sp1, *P. punctulatus*, *Praeacanthonchus* sp_sofsam and *Sphaerolaimus penicillus* while some nucleotide single deletions were observed in the nineteenth position in *Microlaimus sp1, M. punctulatus, Microlaimus* sp1_P and *Bolbolaimus sp.* There appeared to be a pattern of occurrence among species of *Praeacanthonchus*: Insertions with about nine amino acid residues were observed. In the case Sphaerolaimus-derived sequences, only *Sphaerolaimus penicillus* showed a seven-amino acid long insertion on the 84th position, no insertion was observed in the others. Based on the COI sequences, eighty sequences used in constructing the NJ tree showed congruence with those of known morphologically-derived families and orders of marine nematodes. However there were discrepancies at the phylogenetic level for species positioned at the basal "pseudoclade" of the NJ tree (Figure 1). *Theristus acer*_HL, *Daptonema setosum*_JB5 and *Sphaerolaimus hirsutus*_NX1 formed the "pseudoclade" since there was no basis for the clustering of their sequence. Another group of species also showed incongruent pattern of phylogenetic positioning within the tree: *Microlaimus* sp1_P, *M. punctulatus*_KM, *Metachromadora remanei, Theristus* sp1_TZ, *Theristus* sp1, *Araeolaimus* sp1, *Enoplus* sp1, *Bathylaimus australis* and *Sphaerolaimus penicillus*. This incongruence likely confirms the BLASTx similarity search as shown in Table 2 since the sequences of all these nine species are of a different origin. Although discrepancies exist in the position of a few species within the NJ tree, most of the branches formed eight well supported clades with bootstrap supports ranging from 93% to 100%. Low bootstrap support was however observed in the clades of Enoploidea (40%), Chromadorina (56%) and Monhysteridae. Pairwise genetic distance using K2P substitution model for the COI sequences showed that about 98% of intraspecific comparisons showed a genetic divergence ≤8% while about 94% of interspecific comparisons were above 8%.

Molecular Phylogenetic Analysis of 18S sequences

The multiple alignment of all 18S sequences showed that nucleotide insertions were noticed in *Monoposthia mirabilis*_NQ, *Bathylaimus* australis, *Bathylaimus* denticaudatus and *Anoplostoma viviparum*. Deletions were observed in the 20th position in *Monoposthia mirabilis*_NQ, on the 179th, 82nd and 67th positions in *Bathylaimus* australis, *Bathylaimus denticaudatus* and *Anoplostoma viviparum* respectively. No deletion was observed in all sequences except in *Anoplostoma viviparum*. Based on the aligned 18S sequences, 57 sequences used in constructing the NJ tree showed congruence with those of known morphologically-derived families and orders of marine nematodes. However there were discrepancies at the phylogenetic level for species

positioned at the basal "pseudoclade" of the NJ tree (Figure 2). *Monoposthia mirabilis*_NQ, *Microlaimus honestus*_Y3 and *Hypodontolaimus inaequalis*_H3 formed the "pseudoclade" given the fact there was no bases for their clustering. Another group of species also showed incongruent pattern of phylogenetic positioning within the tree: *Sphaerolaimus hirstus*_LK, *Metadasynemoides* sp1, *Ethmolaimus pratensis, Anoplostoma viviparum, Stylotheristus* sp1 and *Tubolaimoides tenuicaudatus*. These incongruence likely confirm the BLASTn similarity search since the sequences of all these nine species are of a different origin (Table 4). Although discrepancies exist in the position of a few species within the NJ tree, most of the branches formed nine well supported clades with 100% bootstrap support. A bootstrap support was however observed in the Xyalidae clade (90%). Pairwise genetic distance using the K2P substitution model for the 18S sequences showed that ≈74% of intraspecific comparisons showed a genetic divergence ≤3% while about 77% of interspecific comparisons were above 3%.

DISCUSSION

DNA barcoding is increasingly becoming important in the identification of marine nematodes just like their plant-parasitic nematode counterparts. Its application in environmental metagenetics has been largely exploited (Porazinska *et al.*,2009a; Porazinska *et al.*,2009b; Creer *et al.*, 2010; Porazinska *et al.*,2010; Bik *et al.*, 2011). The nuclear ribosomal 18S rRNA and mitochondrial COI genes have been employed in the search for a universal barcode in marine nematodes (Bhadury *et al.*, 2006a; Martinez-Arce and Rochas-Olivares 2009; Derycke *et al.*, 2010a). Nonetheless, arguments exist on which of these genetic markers are wholly representative. This study was undertaken to compare the reliability and performance of both genes in the identification of marine nematode species.

Polymerase chain reaction products were recovered from most of the specimens whose 18S rRNA were amplified than in those specimens whose COI genes were amplified. However the difference in amplification was surprisingly almost similar. There were no complications with the molecular methods; hence the higher amplification percentage of 18S rRNA could not have happened by chance. In fact higher values than 57% were expected since rRNA genes have been reported to be easily amplified by universal primers due to their highly conserved flanking regions (Blok and Power, 2009; Meyer *et al.*, 2010). Furthermore, their repetitive arrangement within the genome provides excessive amounts of template DNA for polymerase chain reactions (Floyd *et al.*, 2002; Bhadury *et al.*, 2008). Surprisingly, only a little more than half of the specimens were amplified in this study. This may have resulted from various possibilities. One of such possibilities may be a likely inadequacy in the amplification of the 18S rDNA of the particular organism, in this case marine nematodes. Although other 18S-designed primers including 18P, G10-G11 and F04-22R (Blaxter, 1998) were tested at the preliminary amplification stage, they hardly gave any bands and this makes G18S4-4R a better choice. Sequencing similarity analysis using GenBank database for comparing 18S-derived sequences allowed identification of the individual nematode sequences as closely related to sequences derived from named taxa in the database. All 18S derived-sequences appeared to be

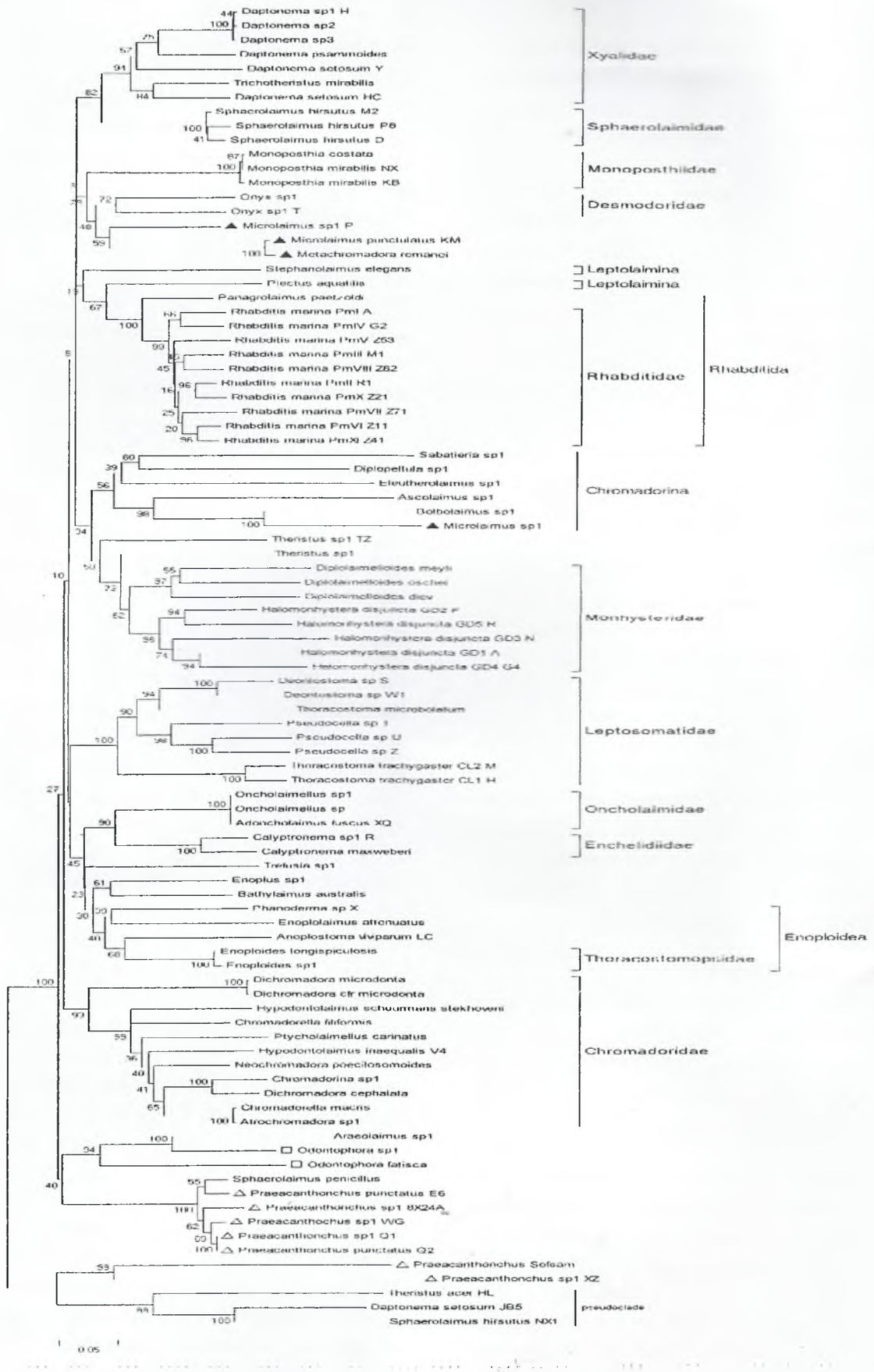

Figure 1: Phylogenetic resolution (with bootstrap values: 1000 replicates) of successfully sequenced specimens, based on COI sequences.

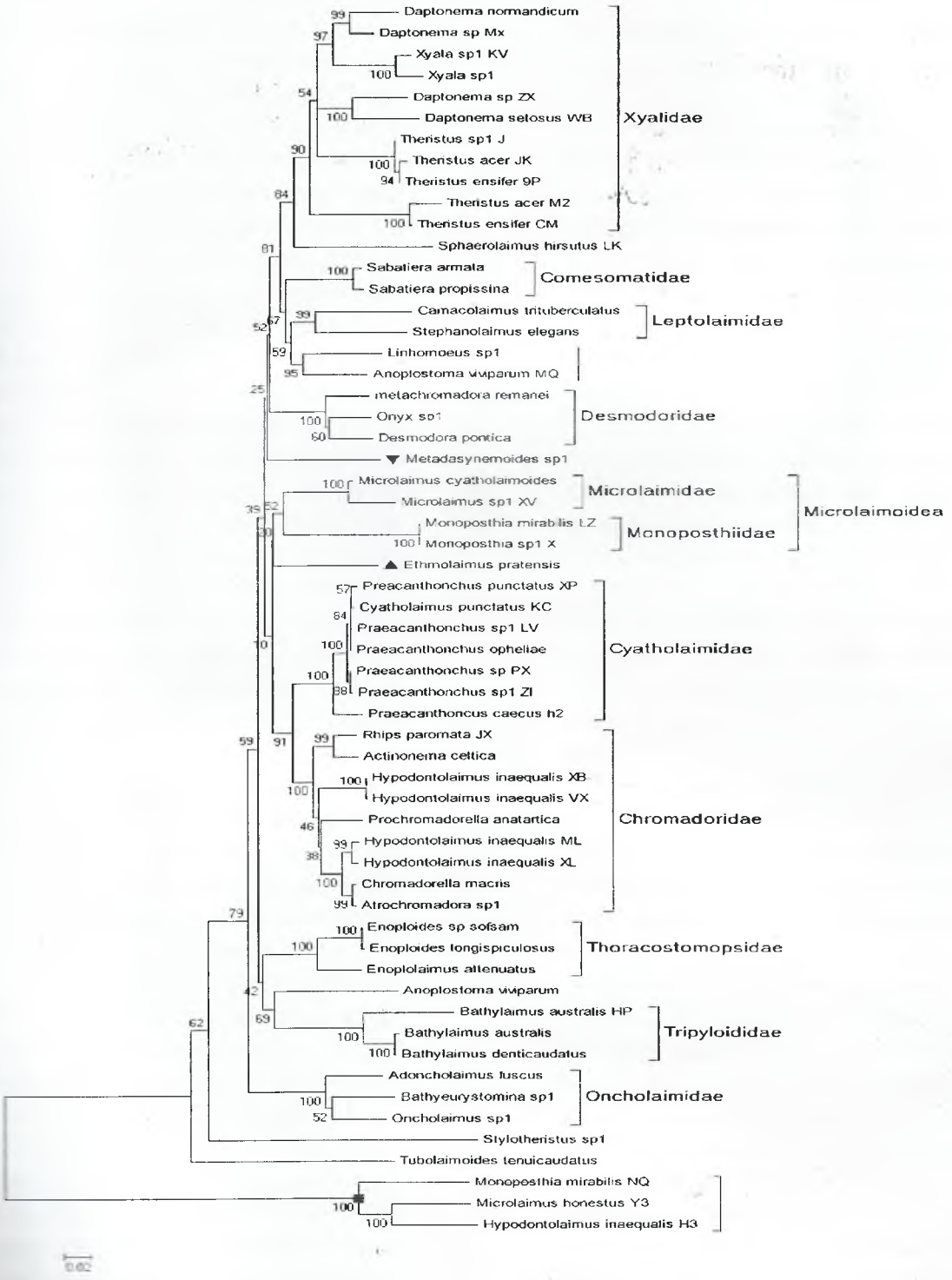

Figure 2: Phylogenetic resolution (with bootstrap values; 1000 replicates) of successfully sequenced specimens, based on nuclear ribosomal 18S sequences.

of a nematode origin hence gave some degree of reliability on the source of sequences used.

A phylogenetic analysis based on the 18S sequences discriminated groups into morphologically correct families and in some cases orders in this study. While popular opinion holds it that the discriminatory power of the 18S is

weak in the delimitation of nematode species (Fitch *et al.*, 1995; Silva *et al.*, 2010), the phylogenetic analyses in this study contradict such findings to a slight degree. Distinct monophelytic groups were recovered from the phylogenetic tree comprising species showing low divergence. Inner nodes in the tree were well supported with bootstrap values of either 99 or 100. Other works have shown that the semi-conserved or conserved areas in the 18S gene allow the unraveling of deep phylogenetic relationships within the phylum (Aguinaldo *et al.* 1997; Blaxter, 1998;
Bhadury *et al.*, 2006a and Meldal *et al.*, 2007). However, there were some exceptions (Figure 2). Three species: *Microlaimus honestus*_Y3, *Monosposthia* sp_NQ and *Hypodontolaimus inaequalis*_H3 were wrongly assigned and formed a "pseudo-clade" at the base of the NJ tree. Such a placement whether haphazardly assigned within or anywhere else on the tree may reduce its reliability of the tree topology (Bhadury *et al.*, 2007). This may perhaps be due to misidentifications caused as a result of distortion of morphological characters resulting from preservation in DESS or wrong judgments during morphological identification. After all, BLASTn search showed that *Microlaimus honestus*_Y3 and *Hypodontolaimus inaequalis*_H3 share a high resemblance to *Calomicrolaimus* sp and *Neochromadora.* Since the certainty in the morphological status is debatable, verifications may be required by using the initial vouchers/pictures taken of these specimens.

Bacterial contamination of genomic DNA could also possibly have been another reason for the wrong derivation. Therefore, amplification and sequencing of other genomic regions for these three specimens could provide vital information for subsequent assignment to correct genus or species as the case may be. Aside from the amplification and sequencing success, the 18S rDNA genetic distance matrix for the sequences tested, indicated that divergence values within species were ≤ 0.03. Although no standard threshold values exist for intra- and inter-specific variation among species of marine nematodes. A repetition of this study using a higher number of 18S rDNA sequences may confirm or in effect, disregard this value. In any case, the need to have a threshold in the delineation of species may be needful as this may ease species diagnosis (Hebert *et al.*, 2003b). Although the use of threshold value is highly debatable, it should be noted that these values may likely depend on the metrics used and number of congeneric taxa employed in a given study (Jansen and Vepsalainen, 2009).

Unlike the 18S rDNA, COI gene proved difficult to sequence (94.15% vs 55.20%). However some success of sequencing was achieved even though it was low. Low amplification and sequencing rates of COI fragments have been reported for marine nematodes (De Ley *et al.*, 2005; Bhadury *et al.*, 2006b; Derycke *et al.*, 2010b). The high number of variable regions and indels at the primer sites in COI-generated sequences may likely impair amplification (Creer *et al.*, 2010). Development of plausible primers that could

successfully amplify and sequence the COI fragment to high degree is therefore needful. However, the obtained COI-derived sequence was able to largely discriminate almost all morphological species. *Microlaimus* sp1, *Theristus* sp1 and *Theristus* sp1_TZ and *Sphaerolaimus penicillus* had random positions within the phylogenetic tree and at the base of the tree is a pseudoclade consisting *Sphaerolaimus hirsutus, Daptonema setosum*_JB5 and *Theristus acer*_HL. The non-clustering they show with their relative species or genera on the phylogenetic tree may be due to a contamination caused by bacteria such as *Wolbachia;* a bacteria which has been found to cause extreme COI divergence in infected organisms. Although this phenomenon of extreme divergence in the COI has been reported in insects (Gerth *et al.,* 2011; Smith *et al.,* 2012; Xiao *et al.,* 2012) and filariids in which they occur at an appreciable frequency (Bordenstein *et al.,* 2003). No report exists in marine nematodes yet. However, this has been reported in a large group of invertebrates and vertebrates (Avise *et al.*, 1994; Thomaz *et al.,* 1996; Walker *et al.,*1997; Waters and Burridge 1999; Lindell *et al.,* 2008). Since large-scale barcoding projects rely largely on mitochondrial COI sequences, a screening of marine nematofauna for possible occurrence of *Wolbachia*-COI interference may give more insights to the interpretations of phylogenetic inferences. Introspection of the use of DNA-Barcoding reveals some inherent shortcomings of this promising identification tool.

Another possibility for the non-clustering of the species may be due to the occurrence of nuclear mitochondrial DNA, abbreviated as NUMT (Song *et al.*, 2008). NUMTs are described as any transfer or transposition of cytoplasmic mitochondrial DNA sequence into the nuclear genome of a eukaryote organism (Lopez *et al.*, 1994). They are also referred to as pseudogenes. Although these NUMT„s remain transcriptionally inactive, reports have shown that they can be co-amplified with orthologous mitochondrial DNA, hence increase the likelihood of a misled amplification output based on the use of a universal or conserved primers (Bensasson *et al.*, 2001; Song *et al.*, 2008). NUMTs have been reported in a broad range of organisms (more than 82 eukaryotes according to Bensasson *et al.*, 2001) ranging from nematodes (Gibson *et al.*, 2007) to mouse and human beings (Richly & Leister 2004). The latter study showed that 99% of the mitochondrial sequences were transferred to the different parts of the nuclei in mouse and humans. Although there is no elaborate published description as well as characterization of NUMTs in marine nematodes yet. Regardless of the possible occurrence of NUMTs, a strict quality control of the COI sequences is needful. However, the COI genetic distance matrix for the sequences tested, indicated that divergence values within species were ≤0.08 K2P genetic distance. Although no standard threshold values in the COI exist for intra- and inter-specific variation among species of marine nematodes. Derycke *et al.* (2010a) suggested that an intraspecific divergence of 0.05

K2P distance will discriminate species within marine taxa. This discordance in values does not make any of the results incredible. In fact, it has been reported in a study by Jansen *et al.* (2009) that demarcating threshold for delimiting species may vary with metrics used and also the number of congeneric taxa. The success of the barcoding approach also depends on the distribution of genetic distances between conspecific individuals and heterospecific individuals given that failures in barcode clustering are proportional to the overlap between both distributions. It has been shown that lineages diversify more quickly within species than between species (Pons *et al.*, 2006). This is due to the fact that diversification within species is driven by mutation at a rate higher than speciation within lineages. Hence, the branch length between species tends to be much deeper than between conspecific individuals leading to a gap in the distribution of the pairwise distance between conspecific individuals and between species that has been referred to the barcoding gap (Meyer *et al.*, 2010).

In a nutshell, assessing the biodiversity of nematodes in marine sediments still poses a big challenge to many taxonomists due to the inherent limitations of the widely used morphology-based approach for identification. The implication of this deterrent is the under-estimation of species richness within marine environments. Although there is a trade-off between using 18S genes that are easy to amplify, but which by their nature are highly conserved and underestimate the true number of species, and using COI genes that give a better description of the number of species, but which are more difficult to amplify. The evaluation of the performances of these two genetic markers linked directly to their taxonomic placement unveils a novel approach for marine nematode identification instead of the use of operational taxonomic units (Porazinska *et al.*,2009a; Porazinska *et al.*,2009b).

This study has established the fact that DNA barcoding using 18S rDNA and COI can be used to identify marine nematode communities. The performance of 18S rDNA outperforms COI in terms of amplification and sequencing success. This has made the 18S rDNA a typical example of a gene employed in environmental metagenetics for biodiversity studies and analysis on eukaryotes, specifically nematodes (Porazinska *et al.*,2009a; Porazinska *et al.*,2009b; Creer *et al.*, 2010; Porazinska *et al.*,2010; Bik *et al.* 2011). However, COI showed higher resolution in the identification of species but the generation of high profile primers for the amplification and sequencing of its fragment may improve future results.

Therefore given the shortcomings of working with systems of identification separately, an integrative taxonomy in which a combination of molecular approach with vouchering of important structures, morphometric measurements as well as making judgments based on valid identification keys may solve the recurrent ambiguous identification challenges within some taxa in the marine nematode communities.

3. REFERENCES

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