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OCCURRENCE AND POPULATION DISTRIBUTION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH ROSELLE (*Hibiscus sabdariffa* L.) IN NORTHERN NIGERIA

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ABSTRACT

Pests including nematodes have been reported as a limiting factor to the production of roselle worldwide.A survey of rosellefarmsinsix Northern Sates of Nigeria was conductedin 2013 to identify the diverse nematode species associated with the soils and roots of the plant and determine their population densities on farmers' fields.Eightsoil and ten plant samples were randomly collected from each of twenty farms from which nematodes were extracted and identified. Four genera of plant-parasitic nematodes (Meloidogyne spp.,Helicotylenchus spp., Rotylenchulus spp.and Tylenchus spp.) were found in association with roots and rhizosphere of roselle plants. Meloidogyne were most populous (62.29%) on the roots while Tylenchuswas least (1.68%). Helicotylenchus spp appeared in highest density (50.05%) in plant rhizosphere, but not identified from the roots. The highest percentage of nematode population (22.7 %) wasfrom Katsina, followed by Sokoto (21.6%), Jigawa (17.4%), Zamfara (16.5%), Kano (15.1%) and Kaduna states (6.7%).The generally low nematode density observed might be due to low precipitation and high soil temperature of the six states.Occurrence of varied species of nematodes demands effective control measures for improved roselle productivity. Kano, Zamfara and Jigawawith low nematode population densities can be considered for further research towards the establishment of pest-free areas or area of low pest prevalence for roselle nematodes in Nigeria, useful in the agricultural export trade. Further studies are required on the determination of density economic threshold and the definitive roles of nematodes in causing low productivity of roselle.

Keywords: Export value, *Hibiscus sabdariffa,* nematodes, population densities, pest-free area

INTRODUCTION

Roselle (*Hibiscus sabdariffa*L.) also called rosella, sorrel or java jute is a fibre crop of the family *Malvaceae*. It is probably native to West Africa although known in the West Indies early in the 16th century and was growing in Asia by the 17th century (EBI, 2017). Its extensive cultivation in Indonesia began in the 1920s under a government-subsidized program established to obtain fibre for sugar-sack manufacture. It is commercially propagated in different parts of the world including USA, United Kingdom and India while Benin, Sudan, Cote D'Ivoire, Ghana, Niger, Burkina Faso and Nigeria were reported as major areas of roselle cultivation in Africa (Oyewole and Mera, 2010; Babatunde and Mofoke, 2006)India, Java, and the Philippines are the world major producers (EBI, 2017; Orwa *et al.*, 2009).

The plantis an erect, bushy herbaceous sub-shrub propagated from seed, widely grown in the tropics and growing to 3 m height (Fern, 2012). Roselle is usually grown as an annual plant. It is referred to as "zobo" in western Nigeria (the Yorubas call the white variety "Isapa") and "Zoborodo" in Northern Nigeria. The two main varieties of s*Hibiscus sabdariffa* are *H. sabdariffavar. altissima* and *H. sabdariffa* var. *sabdariffa* race *ruber.* The variety *H. sabdariffa* has red or pale yellow inflated edible calyces but a poor quality fibre

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while variety *altissima* has red or green, spiny calyces which are inedible and grown for its jute-like fibre (Fern, 2012).At the base of each flower is a fleshy calyx (sepal of the flowers) which is the part that is harvested and used (Harrinson, 2010). In many tropical areas, the red, somewhat acid calyxes of the variety *altissima* are used locally for beverages, sauces, jellies, and preserves while the leaves and stalks are consumed as salads or cooked vegetables and used to season curries (EBI, 2017). In Nigeria, roselle cultivation has gained wide acceptability among farmers due to its medicinal (Olaniran *et al.*, 2013) and industrial importance (Aoshima *et al.*, 2007). It is used as a digestive agent, purgative and adiuretic (Osuntogun and Aboaba, 2004) and as afolk medicine for cancer, obesity, diabetes and hypertension (Tabuti *et al.*, 2003). It is also used in food production such as local non-alcoholic beverages, industrial wine, jam, marmalade and tea (Aoshima *et al.*, 2007). Production of non-alcoholic beverage (Zoborodo) from dried red roselle calyces is very popular in Nigeria. A strong fibre obtained from the stem is used for various household purposes including making sackcloth, twine and cord (Bolade *et al.*, 2009). Roselle is cultivated in various agro-ecological zones of Nigeria but highly concentrated in the North Eastern, North Western and Middle belt regions (Oboh and Elusiyan, 2004). However, despite the high economic importance of roselle, especially its potential as acrop with high export value, little attention has been paid to the crop in the areas of important pests and diseases as well as research for improvement. Diseases have been reported as a limiting factor to the production of roselle worldwide (Ooi and Saleh 1999). Many fungal and few bacterial diseases of roselle have been reported from various parts of the world including Nigeria and these include damping-off, vascular wilt, leaf spot, stem and foliar blight, leaf, stem, fruit and root rot (Amusa *et al.*, 2005,& Nwaukwu andAtaga, 2013). Ogunsola *et al.* (2016) also reported anincidence of leaf blight, leaf spot, stem wilt, flower decay and leaf discolouration in roselle plants cultivated in northern Nigeria. Apart from fungi, apathogenic bacterium, *Bacillus solanacearum*, has been isolated from roselle (Orwa *et al.*, 2009).

Nematode infestation has also caused economic yield loss in major crops (Orwa *et al.*, 2009). The root-knot nematode (RKN), *Meloidogyne* spp. has been reported as one of the most damaging agricultural pests attacking a wide range of crops (Wesemael *et al.*, 2011) and can cause dramatic yield losses, mainly in tropical and sub-tropical agriculture (Moens *et al.*, 2009). The cumulative effect of the feeding damage caused by nematodes could have a negative impact on the plant vigour, thereby constituting serious impediments to the growth and yield of the roselle which is the ultimate to farmers (Afolami, 2000). Due to the insidious nature of damage caused by plant-parasitic nematodes (PPN), farmers are in most cases not aware of them and often times do not control nematodes on the field, making yield decline unavoidable. Despite the reported occurrence of some RKN such as *Meloidogyne arenaria, M. incognita and M. javanica* (Orwa *et al.*, 2009; Adegbite *et al.*, 2014) and *Heterodera rudicicola* (McClintock and Tahir, 2004), there is limited knowledge of parasitic nematode diseases of roselle, especially from the commercial roselle producing parts of Nigeria. Such information is vital for the establishment of pest free areas (PFA) or area of low pest prevalence (ALPP) in Nigeria, which is used in agricultural export trade provided such area is under phytosanitary measures (FAO, 1995; ISPM, 1999). Thus, due to the high commercial and export values of roselle with inadequate documented information on the status of the quarantine nematode pests in Nigeria, this study was conducted to investigate the occurrence and distribution of PPN of roselle in six Northern states of Nigeria.

MATERIALS AND METHODS

Survey of nematodes associated with roselle

Survey of PPN associated with roselle was carried out in six major roselle producing States in Nigeria (Kano, Kaduna, Zamfara, Sokoto, Katsina and Jigawa) between April and May, 2013. Twenty – two (22) roselle farms were arbitrarily selected in thirteen Local Government Areas (LGAs) of the six states (Figure 1). Within each roselle field, ten (10) roselle plants were randomly selected along a 'W' shaped path irrespective of the size of the farm, making a total number of two hundred and twenty (220) roselle plants sampled for nematode extraction. Extension agents from Agricultural Development Projects (ADPs) of each of the States were employed to overcome language barrier, enhance locating farms, and access to the roselle farmers in the states. Theinter-personal interview was also carried out between scientists/extension officers and the farmers to obtain their demographic data and cropping history, including information on nematode infestation and control measures. Geo-references of each point surveyed (location /farm name and farm size) were determined using global positioning system equipment (eTrex, Garmin, 12 channels GPS Corporation, Taiwan).

Sampling of soil around roselle plant

Eight core soil samples were taken per farm to a depth of 15-30 cm around the plant root using soil auger of diameter 1.9 cm. The soil samples were sealed in polythene bags and kept away from sun. Samples were properly labelled and taken to Nematology Research Laboratory, Nigeria Agricultural Quarantine Service, Moor Plantation Ibadan for analysis and identification of PPN.

Extraction and identification of nematode from soil

Bulked sample per roselle plant was gently mixed by hand and two hundred grams (200 g) of sub-soil was taken for nematode assayusing the Whitehead and Hemming (1965) tray modification of Baermann technique described as follows: Two hundred grams (200 g) of soil was put into a set up that has two plastic sieves with double-ply tissue sandwiched in between them. The plastic sieves with the soil were thereafter placed in a plastic bowl and water was added to the extraction bowl just enough to wet the soil. The set-up was left undisturbed for 48 hours in the laboratory. Thereafter, the plastic sieve (of mesh size 45 µm)containing the soil was removed briskly, and the nematode suspension in the bowl was poured into a 500 ml Nalgene wash bottle and allowed to settle (Caveness, 1975). The supernatant was siphoned out with a rubber tube, and the suspension containing nematodes was then poured into the Doncaster (1962) nematode counting dish and examined under a stereomicroscope. Nematodes were transferred with a sharpened broomstick to a slide containing a drop of water, covered with a coverslip and examined under a compound microscope (Navite Olympus, China) with X40 and X 100 magnifications for identification.

Sampling of roselle roots for nematode

Root samples were taken by lifting whole roselle plant from the soil using a spade (Coyne *et al.,*2007) so that the galls and root lesions could be observed from the roots in situ. Roots were collected at the same location as for soil and were combined in the same sample bag, such that the soil prevented degeneration of the roots before laboratory processing. Both roots and soil samples were placed in ice-box. The samples were then transported to the laboratory and stored at 10^{o} C for thorough symptomatology and nematode laboratory assay.

Extraction and identification of nematode from plant roots

Whole root system collected per plant were freed of soil, washed under a gentle stream of cool tap water, mopped dry and weighed in the laboratory. The roots were separated into live (functional) and dead (non-functional) roots. Thereafter, live roots were cut transversely with scissors into about 1-2 cm pieces, mixed carefully and 10 g sub-sample was assayed for nematode using the Whitehead and Hemming (1965) tray modification of Baermann technique. A 10 g sub-sample root was put in a blender with some quantity of water and macerated for 30 seconds. The macerated suspension was then poured into a Whitehead and Hemming (1965) set up as previously described. Thereafter, the plastic sieve containing the macerated roots was removed briskly, and the nematode suspension in the bowl was poured into a 500 ml nalgene wash bottle and allowed to settle (Caveness, 1975). The supernatant was siphoned out with arubber tube, and the suspension containing nematodes was then poured into the Doncaster (1962) nematode counting dish and examined under a stereo and compound microscope as previously described. Identification of PPN was done with the aid of a compound microscope using the simplified pictorial nematode key of Mai and Lyon (1975). Nematode population was determined by counting and population data were expressed in percentage.

RESULTS

Roselle farmers in the surveyed area were between 20 and 68 years old with 10 to 54 years of farming experience. Their farm sizes ranged from 0.04 hectare in Daura, Katsina state to 3.45 hectares in Tsuburowa, Jigawa state (Table 1). The seed for planting were sourced from open markets and fellow farmers, with few from ADPs offices. Roselle plants on surveyed farms were 2 to 3 months old and both mixed cropping (with either sorghum, maize or cowpea) and sole cropping were practised by the farmers. Many farmers in the study areas shifted from cultivating roselle to other crops due to poor marketing and while only one roselle farm per LGAs was found in some locations, two or three farms were observed in others. Foliar symptoms of insect pest infestation, disease infection and root galls were observed in some of the plants on the fields while other farms looked apparently healthy.

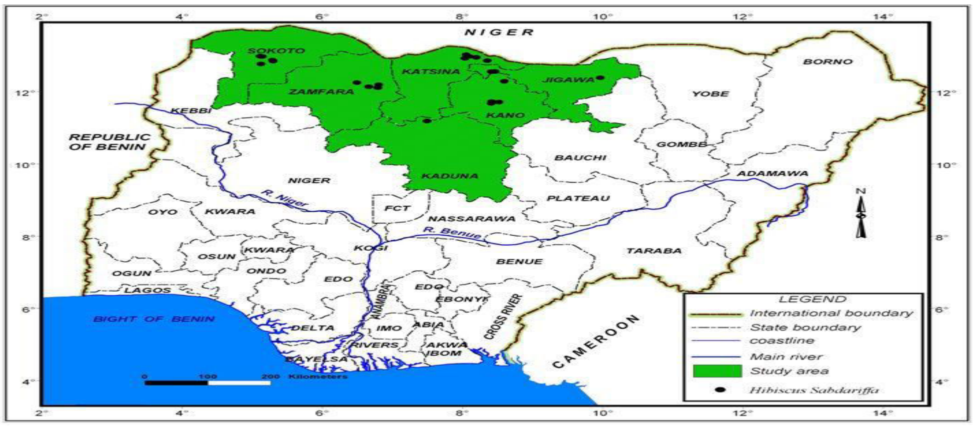

Figure 1: Map of Nigeria showing the six states surveyed for plant parasitic nematodes

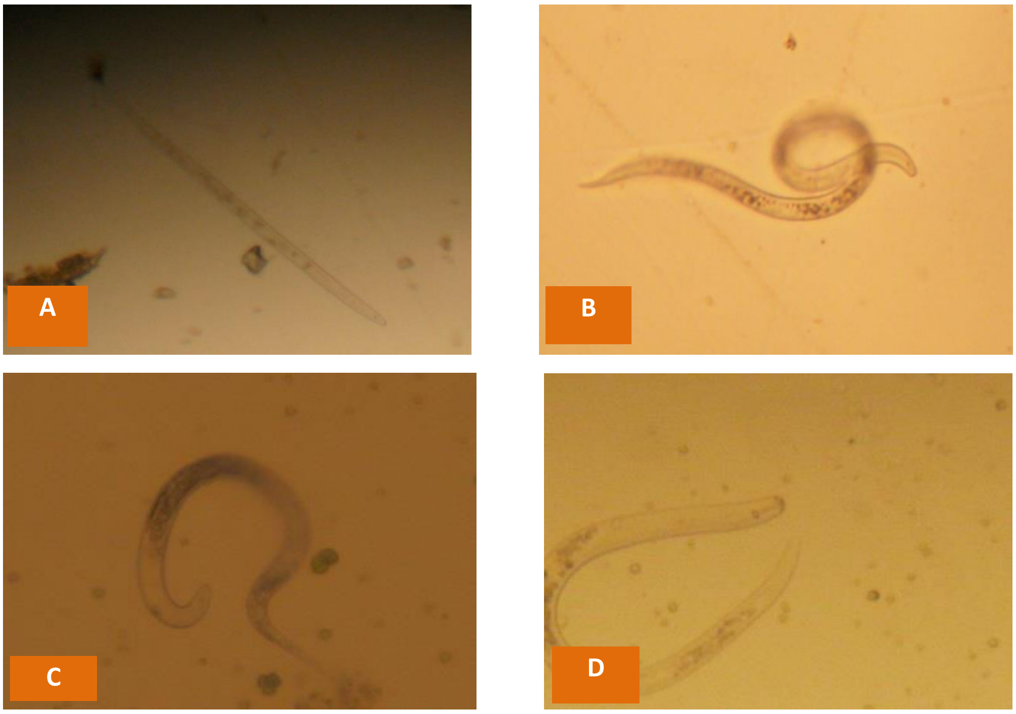

Plate 1 (A - D): Micrographs of plant-parasitic nematodes extracted from roselle plants.(A) *Meloidogyne* spp(male), (B)*Rotylenchulus* spp, (C) *Tylenchus* spp and (D)*Helicotylenchus* spp (X 60)

Four genera of plant-parasitic nematodes were observed to be associated with roselle plants in Kano, Kaduna, Zamfara, Sokoto, Katsina and Jigawa states of Nigeria. The nematodes, extracted from the soil and roselle roots in twenty-two roselle farms were *Meloidogyne* spp, *Helicotylenchus* spp, *Rotylenchulus* spp and *Tylenchus* spp (Plate 1). These nematodes were identified from the soil or roselle root samples in all the six states but with avaried population. The population densities of all the four nematode genera (Table 1) showed highest percentage nematode population (22.7 %) from Katsina state, followed by Sokoto (21.6 %), Jigawa (17.4 %), Zamfara (16.5 %), Kano (15.1 %) and Kaduna state (6.7 %). Nematodes were observed in both soil and root in most of the farms studied but few farms showed anabsence of nematode in either soil or root while a farm (Shargale) in Katsina state had no *Rotylenchulus* spp. in both soil and roots. Some farms in Sokoto (Maganawy I), Zamfara (Kotor Koshi) and Kano states (Danbatta) showed low nematode densities whereas others within the same state or LGAs [(e.g. Sokoto (Tsebe/Lugan1) and Zamfara states (Bungudu)] or in other states [e.g. Jigawa (Tsuburowa)] produced high nematode population. Higher nematode population was observed in the soil than plant roots. *Helicotylenchus* spp., *Tylenchus* spp.,*Meloidogyne* spp. and *Rotylenchulus* spp.

were extracted from the soil at population densities of 50.05 %, 19.56 % 16.67 %, and 13.70 % respectively (Table 2). However, *Meloidogyne* spp., *Rotylenchulus* spp. and *Tylenchus* spp. were abundant in roots of roselle plants at 62.29 %, 36.03% and 1.68% population respectively (Table 3). The genus *Helicotylenchus* spp., though most prominent in the soils of the farms across the six states, was not observed in roselle roots in all the farms (Tables 1 and 3). Meanwhile, *Meloidogyne* spp. population was highest from the roselle roots. Out of the total nematode population, 92.86 % were extracted from the soil while only 7.14 % nematode population were from plant roots (Tables 2 and 3). Higher soil (97.05 %) to root (2.95 %) population ratio of the four nematode genera was observed in Kaduna state while the lowest ratio (87.33 to 12.67 %) was found in Zamfara state.

DISCUSSION

A detection survey of roselle farms in six northern states of Nigeria revealed some diversity of types and population levels of PPN in soils and roots of roselle plants. The roselle farmers have long (10 to 54) years of farming experience which might enhance commercial roselle production for local and international markets. Four nematode genera: *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp.and *Tylenchus* spp. were detected from the twenty-two farms investigated. The results are similar to earlier reports (Michel *et al.*, 2005) that roselle is frequently grown in environments that are conducive to root knot and reinform nematodes. This study showed that while the highest population of *Helicotylenchus* spp. was observed in the soils across the six states, *Meloidogyne* spp were most abundant in the roots of roselle plants. Asimilar result was reported on the two nematode genera in northern Nigeria (Agbenin and Ogunlana, 2006) which also agrees with the findings of Orwa*et al.* (2009), and Adegbite *et al.* (2014) that *Meloidogyne arenaria, M. incognita and M. javanica* were the main parasitic nematodes of roselle. Nematode population was higher in the soils of roselle farms than in the roots. This has been earlier reported in northern Nigeria (Agben in and Ogunlana, 2006). Surprisingly, *Helicotylenchus* spp. that was most prominent in the soils of the farms across the six states, was not found in roselle roots from any of the farms. This rare occurrence might be due to the genus *Helicotylenchus* being usually ectoparasites (van der Putten *et al.*, 2005) though some are semi-end parasites (Yeates *et al.*, 1993) or to some level of resistance to nematodes which has been reported in roselle (Heffes *et al.*, 1991).

It was also observed that none of the farms in the study areas was free of nematodes and the population densities of the four extracted nematode genera varied with location and type. Factors contributing to such widespread distribution of the nematodes might include over cultivation of a single common cultivar and other environmental factors such as soil type as well as the presence of a suitable alternate host (Bafokuzara, 1996). Also, farm practices such as continuous cropping and monoculture with a single cultivar, which is prevalent with Nigerian farmers, might result to build up of nematode pests to levels that may be economically important. It has been reported that species of *Meloidogyne* are found more frequently in sandy soils than in finer texture soil (Spaull and Cadet, 1991). The slight increase in nematode populations in the four states in the Sudan savanna area than Kaduna and Kano statesmay probably be due to ease of movement of the nematodes through the large soil pore diameter and soil particle size (Idowu, 1981), which are typical properties of a generally sandy soil. This could also account for different population densities of the PPN found in soil and roots of roselle in different locations surveyed. The generally low nematode density observed in the six states might be due to low precipitation and high soil temperature of the survey areas. Beneficial or negative effects of climate on nematode population have been reported earlier (Colagiero and Ciancio (2011).Increase nematode population due to moisture was previously reported (Jordan *et al.*, 1989). Similarly, Gbadeges in *et al.*, (1993) observed a depressed nematode population in dry season in the savanna areas of Nigeria which later increased in the rainy season and got to the peak between August and October. Negative correlation between temperature and nematode abundance has also been reported (Kandel *et al.*, 2013).These climatic and edaphic factors play important roles in determining the abundance and distribution of nematode species. For instance, several *Meloidogyne* species are known to attack different crops and tend to favour light soil and warm temperatures (Micheal *et al.*, 2005).Thus, the varied population densities among the six northern states might be due to the differences in rainfall distribution and the characteristic soil types of the states surveyed which are located within the Sudan and Guinea savanna zones of Nigeria.

Table 1: Population density of plant parasitic nematodes extracted from 200 g soil and 10 g root samples on roselle farms from six states in the northern Nigeria

State	LGA	farm	Farm	Meloid		Helico		Rotyle		Tylenc		Total		% Po
		location	size (ha)	soil	root	soil	root	soil	root	Soil	root	farm	state	
Sokoto	Wamako	Maganawy I	0.36	33	4	45	0	4	17	10	0	113	879	21.6
		Maganawy II	1.10	61	3	95	0	13	5	22	2	201		
	Bodinga	Dogon karfe	1.04	84	17	16	0	42	0	20	0	179		
	Denge Shuni	Tsehe/Lugan 1	0.42	52	22	96	0	24	5	30	0	229		
		Tsehe/Lugan 2	0.32	46	16	71	0	17	2	5	0	157		
Zamfara	Bungudu	Kotor Koshi	0.31	19	6	46	0	15	2	32	0	120	671	16.5
		Gada	0.50	22	14	93	0	11	13	41	1	195		
		Bungudu	0.29	8	43	104	0	21	0	20	0	196		
	Gusau	Gebawa	0.36	27	6	65	0	42	0	20	0	160		
Kano	Gwulum	Kura	0.09	14	2	72	0	18	3	81	0	190	614	15.1
	Danbata	Danbatta	0.14	1	7	63	0	3	9	40	0	123		
		Ajumawa	0.62	5	6	93	0	5	11	12	0	132		
		Barebari	0.66	3	4	124	0	5	2	31	0	169		
Katsina	Sandamu	Sabongari	0.14	62	1	53	0	21	5	36	0	178	920	22.7
	Daura	Daura	0.04	2	1	142	0	11	3	5	0	164		
		Shargale	0.25	4	3	92	0	0	0	43	0	142		
	Mashi	Doka I	0.34	4	5	130	0	84	4	70	0	297		
		Doka II	0.06	2	1	84	0	13	7	32	0	139		
Jigawa	Hadejia	Tsuburowa	3.45	94	4	152	0	40	8	63	1	362	707	17.4
	Kazaure	Firji	0.20	0	2	74	0	95	3	12	1	187		
		Kurare	0.07	3	5	52	0	31	6	61	0	158		
Kaduna	Markarfi	Markarfi	0.74	83	6	126	0	2	2	52	0		271	6.7
Total				629	178	1888	0	517	107	738	5		4062	

% Po - Population percentage, Meloid - *Meloidogyne species*, Helico - *Helicotylenchus species*, *Rotyle* = *Rotylenchus species,* Tylenc - *Tylenchus species,* LGA - Local government area

Table 2: Population density of plant parasitic nematodes extracted from soil (200 g) samples on roselle farms from six northern Nigeria states

Nematode	Sokoto		Zamfara		Kano		Katsina		Jigawa		Kaduna		Total	
	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po
Meloidogyne sp	276	35.11	76	12.96	23	4.04	74	8.32	97	14.33	83	31.56	629	16.67
Helicotylenchus sp	323	41.09	308	52.56	352	61.75	501	56.29	278	41.06	126	47.91	1888	50.05
Rotylenchulus sp	100	12.72	89	15.19	31	5.44	129	14.49	166	24.52	2	0.76	517	13.7
Tylenchus sp	87	11.07	113	19.28	164	28.77	186	20.9	136	20.09	52	19.77	738	19.56
Total	786	89.42	586	87.33	570	92.83	890	96.74	677	95.76	263	97.05	3772	92.86

Po - nematode population, % Po - population percentage

Table 3: Population density of plant parasitic nematodes extracted from roselle root (10 g) samples from six northern Nigerian States

Nematode	Sokoto		Zamfara		Kano		Katsina		Jigawa		Kaduna		Total	
	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po	Po	% Po
Meloidogyne sp	62	66.67	69	81.18	19	43.18	11	6.18	11	36.67	6	75.00	178	61.37
Helicotylenchus sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotylenchulus sp	29	31.18	15	17.65	25	56.82	19	63.33	17	56.67	2	25.00	107	36.89
Tylenchus sp	2	2.15	1	1.18	0	0	0	0	2	6.67	0	0	5	1.72
Total	93	10.58	85	12.67	44	7.17	30	3.26	30	4.24	8	2.95	290	7.14

Po = nematode population, % Po = population percentage

Arcent survey on collection and evaluation of roselle germplasm in Nigeria has shown Kaduna and Jigawa as parts of the four states with the highest roselle accessions, suggesting areas of greatest diversity of genetic resources in Nigeria (Dauduet *al*., 2015). Such high crop intensity have been reported to result into increased nematode density (Kandel *et al*.,2013) which might be responsible for the higher nematode population observed in Jigawa than Kano state. Highest nematode population was observed in Katsina state with lowest from Kaduna. However, since only one farm was accessed in Kaduna state due to limited roselle farms, Kano, Zamfara and Jigawa states, with low nematode pests in many locations, can be considered for further research towards establishment of pest free areas (PFA) or area of low pest prevalence (ALPP). The establishment of such PFA is important in agricultural export trade (FAO, 1995; ISPM, 1999).

CONCLUSION

Findings from this study indicate the presence of four genera of plant-parasitic nematodes (*Helicotylenchus* spp, *Tylenchus* spp. *Meloidogyne* spp,and *Rotylenchulus* spp) associated with roselle in the northern Nigeria. This suggests the need for roselle farmers in Nigeria to source for planting materials (seeds) from ADPs or farmers shops and not from established farmlands in their locality or local markets which can expose their farmlands to the danger of infection by nematode pests resident in those fields. This could result in the further spread and distribution of the pathogen in the country and its consequence reduction in roselle's productivity. Kano, Zamfara and Jigawa states with low nematode densities are recommended for further research towards the establishment of pest-free areas or area of low pest prevalence for nematodes in Nigeria, which is useful in the agricultural export trade. Further studies are required on the determination of density economic threshold, the definitive roles of nematodes in low productivity of roselle and development of disease-resistant seed varieties.

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