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Prevalence of Root-knot Nematode (*Meloidogyne* Species) on Waterleaf (*Talinum triangulare*) in Three Locations in University of Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DOE and ICB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EIE and BRA managed the analyses of the study. Author DOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A comprehensive survey of root-knot nematodes was conducted in three locations (Unical farm, Biological Science and Botanical Garden) between June, 2018 to August, 2018 to determine the intensity of *Meloidogyne incognita* and *Meloidogyne hapla* on the three locations of University of Calabar, Nigeria.
Methodology: A randomized sampling pattern was used during sampling, were a total of 60 samples (30 infected roots and 30 infected soil) samples were collected from the three major locations. In each location, 10 sampling points were selected randomly. Data were collected on Number of galls per root system, Gall index (G.I), Nematode density in soil and Number of nematode per plant. Data was analyzed using descriptive statistics of chi-square.

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Results: The prevalence of Root-knot nematodes was recorded with varying degree in all locations. The highest prevalence of Root-knot nematodes (*M. incognita*) 62.25% with (G.I = 4.00) was recorded from Botanical garden followed by 39.12% with (G.I = 4.00) in Biological Science block compared to Unical farm which recorded minimum prevalence of 15.41% with (G.I = 3.00). The result of the survey obtained showed that (*M. incognita* and *M. hapla* are prevalence in all the three locations. However, Unical farm had the least Root-knot infection of (*M. hapla*) compare to *(M. incognita*).

Conclusion: The results of this study showed that the composition of nematode communities (plant-parasitic and free-living) may be used as bio indicators of soil health or condition. This further suggests that magnitude of nematode problem needs serious consideration to tackle by the use of useful nematode management strategies.

Keywords: Survey; Meloidogyne species; waterleaf; soil health; University of Calabar.

1. INTRODUCTION

Root-knot nematodes (*Meloidogyne* species) are parasitic nematodes which are found in the soil and roots of infected plants. The genus *Meloidogyne* have about 98 species and the common spices encountered by farmers are *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria* [1]. They can exist either in hot climates or short winters around the world. In a report by Gill and Mcsorley [2], root-knot nematode is one of the most destructive groups of plant-parasitic nematodes and is pests of almost all major crops. Karajeh et al. [3], stated that about 5% of the world crop production is destroyed by *Meloidogyne* species every year. According to Sasser [4], more than 2,000 plants species have been designated as hosts to root-knot nematodes, and most cultivated crops are attacked by at least one root-knot nematode species. In 2003, the host range already encompasses more than 3000 plants species.

Abad et al., [5] which shows an increasing number of hosts infected with root-knot nematode. The host range is extensive that it is difficult to find common crops that are not hosts [6]. The hosts include trees, bedding plants, grasses, shrubs, numerous weed and vegetables especially waterleaf (*Talinum triangulare*).

Waterleaf (*Talinum triangulare*) is a vegetable crop of the family Portulacea and is consumed in parts of West and Central Africa, including Nigeria and Cameroon [7]. Waterleaf grows well in humid conditions at temperature of 30^{o}c, but faster during the wet season, and slows down during the dry season [8]. As a short duration vegetable, waterleaf has some natural characteristic which makes it attractive to farmers and consumers. Waterleaf has high quality of crude fiber (11.12%), crude ash (33.98%) and protein (22.1%) [9]. As a result of these, waterleaf is becoming increasingly important in ensuring food and nutrition security as the production serves as a balancing source of revenue of farmers [7]. However, waterleaf is highly susceptible to nematode attacks, especially in the tropics [10].

The presence of these nematode populations puts Agricultural production in Africa at a significant risk, given the fact that most farmers do not know the actual Nematode species present in their farms. Root-knot nematode is a major pathogen of fruits, vegetables and other food crops in different parts of the world [11]. The short life cycle of 6 to 8 weeks enables root-knot nematode population to survive well in the presence of a suitable host and their population builds up to maximum usually as crops reach maturity [12]. Cavenass [13], noted that about 75% of Agricultural soil in Nigeria are dominated by different species of *Meloidogyne;* and they remain the most destructive group of nematode [14]. The most visible symptom is the appearance of swellings or Giants galls on the roots of affected plants [15,16] causing the deformation of the root system which affects nutrient and water uptake by plants roots.

Plants parasitic nematodes affect plants growth and yield [16]. Root-knot nematode affect both the standard and measurable quality of marketable value of most vegetables in Nigeria [17]. The most visible symptom is the appearance of swellings or giant galls on the roots of affected plants [15,16], causing the deformation of the root system which affects nutrient and water uptake by plant roots [18]. Infection caused by *Meloidogyne* species can lead to reduction in the formation of nodules by

nitrogen fixing bacteria [19]. They also interact with other plant pathogenic organisms, resulting to increased damage due to the opportunistic diseases, low yield and poor market value in affected crops [20].

The control of root-knot nematode has been a major problem because they are ubiquitous, with a very wide range [21]. The use of synthetic pesticides is considered as the most effective practical means of controlling plant-parasitic nematodes [22]. However, pesticide application as a means of pest control has become less attractive in recent times, due to high toxicity and persistence of the nematicides and environmental pollution [23].

Root knot nematodes are important pests of many cultivated plants and known to cause yield losses of over 5 – 43% globally and 25 – 50% for small holder farmers in developing countries [24]. Caveness [13], also noted that about 75% of Agricultural soils in Nigeria are dominated by different species of *Meloidogyne;* and they remain the most destructive group of nematode [14]. The study was carried out to check the occurrence and the widespread of *Meloidogyne* species on vegetables, especially waterleaf to identify the particular species that reduces the yield of waterleaf. The result from this study will serve as a baseline for effective control.

2. MATERIALS AND METHODS

2.1 Experimental Site

This research was carried out in the Department of Plant and Ecological Studies Research Laboratory, Faculty of Biological Science, University of Calabar. Other works was carried out in the Department of Zoology and Environmental Biology University of Calabar, Nigeria. The Survey was carried out to checkmate the prevalence of *Meloidogyne* species in three different locations from June to August 2018 at University of Calabar. The three locations are as follows Unical Farm, Botanical Garden and Biological Science Farm of the University of Calabar, Nigeria.

Calabar lies in the tropical high rainforest agro ecology of the equatorial climatic belt of Nigeria (Latitude 5°00' and 5°40'N, Longitude 8°04' and 8°62'E) and about 70m above the sea level [25]. It has a bimodal rainfall distribution that ranges from 2500 – 3500mm with a mean annual temperature range of 22.2°C to 38.2°C and a relative humility that ranges from 75 – 90%.

2.2 Sample Collection

The soil and root samples were collected using Randomized sampling pattern. The soil samples were collected at the depth of 0 – 15 cm below using a soil auger, placed in nylon bags and labelled carefully. Sample of fleshy roots of water leaf were also collected from the survey fields and was taken to Post Graduate Research Laboratory of Plant and Ecological Studies for nematodes extraction and identification.

2.3 Nematode Count in the Soil

Nematode count in the soil was done according to Coyne et al*.,* [26]. Ten (10) plastic sieves and plastic plates, tissue paper, masking tape, water, measuring cylinder, petri dishes, 1000ml beakers, Binocular microscope, automatic pipette, single tally counter and a sensitive weighing balance was used for the trail. Sieve were line up with tissue paper placed on plastic on plastic plates. 200 grams of soil was weighed using sensitive weighing balance. The weighed soil was placed on the tissue paper and it was ensured that the soil remains on the tissue and does not spill over the edges. Labelling was done according to the different sample locations by using masking tape. Water was carefully poured gently into the plates and it was ensured that the water poured, went down the gap between the plastic plate and the sieve.

The stored extract from sample plates were kept for 2 days, closely monitored to ensure that the samples remain wet and not dry out due to evaporation. After two days, the sieves were lifted up for proper draining of water and the release of some settled nematodes in the soil.

The drained water collected from different sample locations sieve plates were poured into 1000 ml measuring cylinder to determine the volume of water. Readings of each volume of water from each sample sieves were recorded, while the soil and tissue papers of each fixed nematode sieve stands were discarded properly. Thorough rinsing of the plates into the measuring cylinder was done. The samples were left to settle for one hour. The suspension in the measuring cylinder was reduced by decanting, and 1000 ml was used to collect the suspension for assessing of nematodes. Automatic pipette was used to draw 2 ml of the suspension into the

petri dish for the count on Binocular microscope. A simple tally counter was used to count the nematodes. This procedure was repeated for all the fixed nematode sieve stand samples.

2.4 Nematode Extraction in the Root

Nematode count in the root was done according to Byrd et al., [27] staining technique. Fresh roots of water leaf were collected washed with tap water in the Laboratory. The roots were weighed using electronic weighing balance. One gram of the total weight from each plant was cut into pieces and used for the nematode count. The cut root was poured into 250 ml of conical flask with 70 ml of 1.5% sodium hypochlorite (half strength house hold bleach). This was bleached for five minutes with occasional stirring. Root was rinsed with water and soaked in 1% acetic acid for 15 minutes. Acid solution was drained off from the root and the root was placed in 30mg of distilled water. 1 mg of acid fuschin was added to the solution and heated over a low flame until it boils. Boiling was done gently for 30 seconds and then allowed to cool for 30 minutes at room temperature. Excess staining was removed by rinsing with water. The root was placed in 200mg acidified glycerol and heat to boiling temperature.

Acidified glycerol was obtained by dissolving 2 mg of hydrochloric acid in 300 mg of water and 700 mg of glycerol added to the solution. The root was removed from heat immediately boiling commences. This was allowed to cool quickly by standing the 250 ml conical flask containing the root in shallow water. The root was poured into petri dish containing glycerol and gently teased apart. This was viewed under Binocular microscope for nematode count. This procedure was repeated for all the entire ten survey locations.

2.5 Perennial Pattern of Root-knot Nematode Species

Mature females of root-knot nematodes were dissected out from the infected roots and were identified on the basis of female perennial patterns as described by Tayor and Netscher [28]. *Meloidogyne incognita* and *Meloidogyne hapla* was identified from the study area.

2.6 Number of Galls per Root System

The total number of galls per root system of each plants collected was counted and expressed as average per root.

2.7 Number of Nematodes per Plant

The total number of nematodes per plant was counted on Binocular microscope and expressed as average per plant.

2.8 Gall Index (G I)

The gall index (G I) was measured according to Tayor and Sasser [24], following the standard scale of 0-5. The scale is explained as follows where;

Zero Galls - - - 0	= Immune
1 or 2 Galls - - - 1	= highly resistant
3 – 10 Galls - - - 2	= highly resistant
11 – 30 Galls - - - 3	=moderate galling (moderately susceptible)
31 – 100 Galls- - - 4	= heavy galling (susceptible)
>100 Galls - - - 5	= very heavy galling (highly susceptible)

2.9 Statistical Analysis

The collected data was analyzed according to the prevalence of nematode using descriptive statistics of Chi-square.

3. RESULTS

A total of 60 samples (30 roots and 30 soil samples) were collected from 3 major locations in University of Calabar to determine the prevalence of root-knot nematodes on waterleaf. In each location, 10 sampling points were selected randomly. 30 samples of soil and roots were collected as shown in Table 1.

Result on the prevalence of root-knot nematodes from Unical Farm were recorded with varying degree. The prevalence according to the sampling points showed that plot A1 has 12.86%, plotA2 (8.75%), plotA3 (30.0%), plotA4 (32.69%), plotA5 (15.63%), plotA6 (11.27%), plotA7 (6.02%), plotA8 (5.0%), plotA9 (6.66%) and plotA10 (38.10%). The results are as shown in Fig. 1.

Based on the results collected from Biological Science, the prevalence of root-knot nematode from the sampling points were as follows Plot B1 showed a prevalence of 46.66%, plotB2 (45.0%), plotB3 (25.0%), plotB4 (50.0%), plotB5 (42.86%), plotB6 (54.55%), plotB7 (44.19%), plotB8 (24.62%), plotB9 (44.44%) and plot10 (26.09%) as shown in Fig. 2.

The prevalence of root-knot nematode from the different sampling points of Botanical Garden showed that plot C1 had 50.00% prevalence, plotC2 (73.85%), plotC3 (66.67%), plotC4 (75.0%), plotC5 (47.06%), plotC6 (77.08%), plotC7 (65%), plotC8 (78.57%), plotC9 (56.34%) and plotC10 (70.73%). The results are as shown in Fig. 3.

4. DISCUSSION

The present study clearly indicates the prevalence and occurrence of root-knot nematodes in the three study locations of university of Calabar. The study revealed *Meloidogyne incognita* and *Meloidogyne hapla* in water leaf was prevalence in all locations. However, *M. Incognita* was more abundant in Botanical garden and Biological science compared to Unical farm. *M. hapla* was minimal in Unical farm and this could be due to the nature of the soil or poultry manure in the study area which might not be more suitable for *M. hapla.*

The results of this survey showed variations in the prevalence and severity of root-knot nematode in different study locations (Unical farm, Biological Science and Botanical Garden) in University of Calabar. Similar results were reported by Khan et al*.,* [29] and Shahid et al*.,* [30]. They confirmed the present findings regarding the prevalence of plant parasitic nematodes and occurrence of

Table 1. Nematode count, number of galls and galling index of root-knot nematode in University of Calabar

Locations	Sampling point	Nematode count in the root (1 g)	Nematode count in the soil (2 ml)	No. of galls	Gall index
Unical Farm	UF Plot A1	18	12	10	2
	UF Plot A2	20	21	5	2
	UF Plot A3	16	21	2	1
	UF Plot A4	10	22	4	2
	UF Plot A5	8	18	6	2
	UF Plot A6	7	13	7	2
	UF Plot A7	3	14	2	1
	UF Plot A8	5	16	2	1
	UF Plot A9	2	19	3	2
	UF Plot A10	9	11	4	2
Biological Science	BS Plot B1	18	30	22	3
	BS Plot B2	42	43	16	3
	BS Plot B3	32	27	24	3
	BS Plot B4	18	49	13	3
	BS Plot B5	24	42	41	4
	BS Plot B6	31	43	38	4
	BS Plot B7	28	48	21	3
	BS Plot B8	14	43	12	3
	BS Plot B9	19	47	14	3
	BS Plot B10	16	23	16	3
Botanical Garden	BS Plot C1	20	38	24	3
	BS Plot C2	48	50	42	4
	BS Plot C3	81	43	51	4
	BS Plot C4	75	74	81	4
	BS Plot C5	86	86	62	4
	BS Plot C6	38	48	75	4
	BS Plot C7	36	49	36	4
	BS Plot C8	69	42	68	4
	BS Plot C9	40	32	46	4
	BS Plot C10	31	63	69	4

Meloidogyne species on vegetables. FAO, [31] also reported that estimated overall annual yield loss of the world's major crops is due to damages caused by plants parasitic nematodes. There are reports which confirmed that prevalence and severity of root-knot nematodes are affected by soil type, soil pH and application of manure [32,33].

In terms of abundance, this study identified two species of Root-knot nematodes from the three study area, *M. Incognita* and *M. hapla. M. incognita* appeared to be more abundant than *M. hapla* and this could probably be as a result of the nature of the soil in the study area, which might be more suitable for the survival of *M. incognita* than *M. hapla*. It could also be that *M. incognita* is more resistant to stress and other environmental challenges than *M. halpa* which could generally give *M. Incognita* reproductive advantage over *M. hapla*. This study is in conformity with the works of Haroon and Zylstru [34].

The prevalence of root-knot nematodes in Unical Farm (15.41%) was quiet low. This might be because of the application of Poultry manure in Unical farm by farmers. This fact is supported by the findings of other researchers who found that the presence of organic matter content of the soil reduces the number of nematode [35,36]. Chindo and Khan [37] also reported that poultry manure application drastically reduced *Meloidogyne incognita* population and severity of root galling with resultant increase in yield of vegetables. The influence of poultry manure on the populations of the nematodes is due to increased hydrogen ion concentration (pH) and induced hypersonic solution released in the process of decomposition. This activity may have probably restricted nematode survival as earlier reported by Aslam et al. [38] in *Brassica oleracea*.

Biological Science had high root-knot nematode prevalence rate of 39.12% which might be because of the absence of nitrogenous compounds and the application of sawdust in the soil. This fact was confirmed by the findings of Nwanguma and Awoderu [39] which reported that sawdust amended soil significantly harbored the highest number of soil and root nematode populations while the lowest number of nematode occurred in poultry manure amended soil. The effect of sawdust on nematode population was low apparently due to low nitrogenous compounds in saw dust [33].

According to the result recorded Botanical Garden has the highest prevalence of 62.25% due some deficiencies in the soil. There were little or no application of poultry manure, sawdust and lack of nitrogenous compounds and organic matter in the soil. This fact is supported by the findings of other researchers who found presence of poultry manure organic matter content of the soil reduces the number of nematodes [35,36,40]. The results of this study indicate that root-knot nematodes had high prevalence on the three locations in University of Calabar but occurred in different degree.

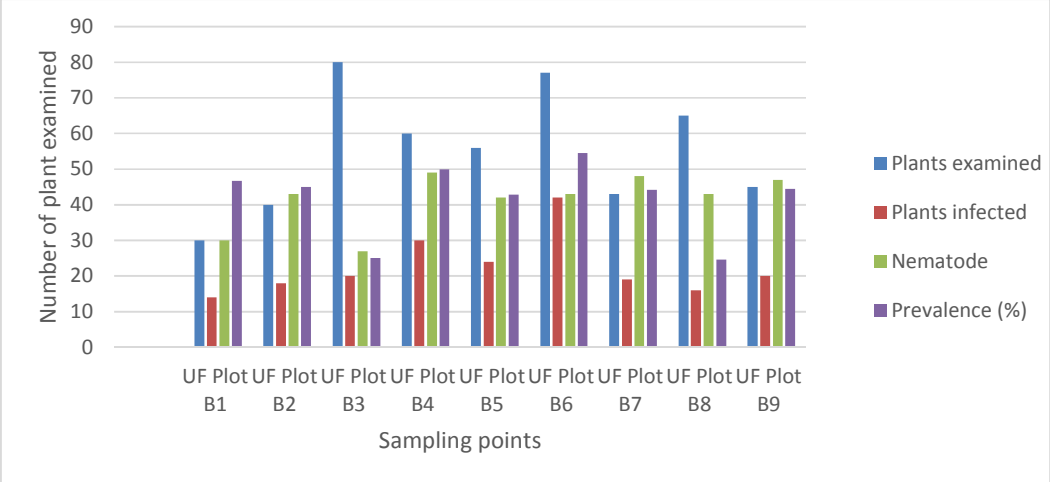

Fig. 1. Prevalence of root-knot nematode (*Meloidogyne species*) on water leaf in Unical Farm (UF)

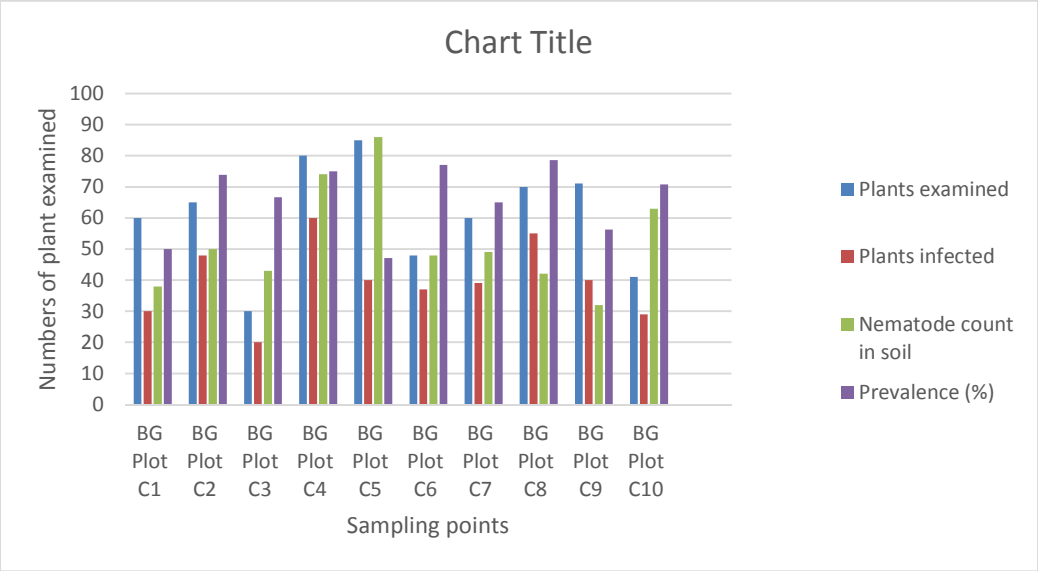

Fig. 2. Prevalence of root-knot nematode (*Meloidogyne species*) on water leaf in Biological Science (BS)

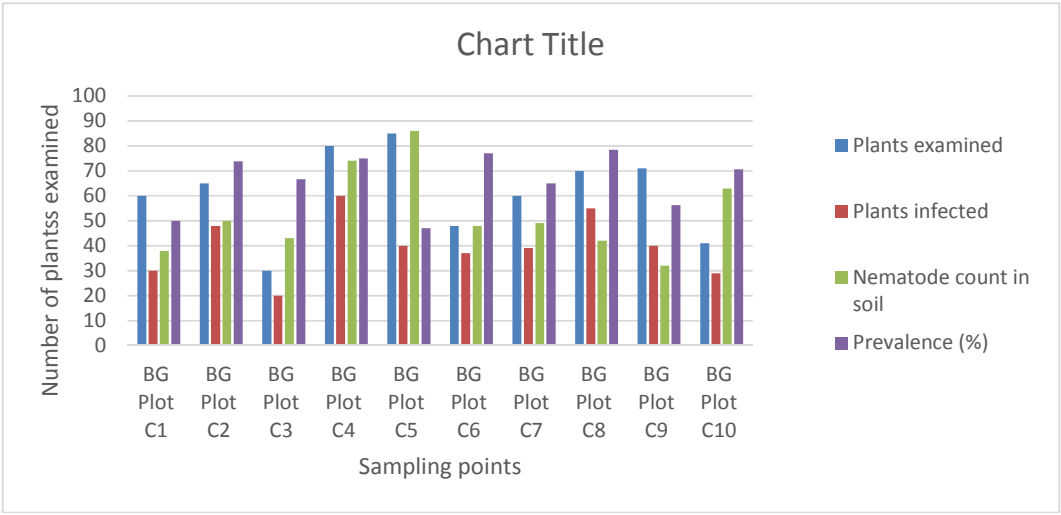

Fig. 3. Prevalence of root-knot nematode (*Meloidogyne species*) on water leaf in Botanical Garden (BG)

5. CONCLUSION

Findings also showed that the composition of nematode communities (plant-parasitic and free-living) may be used as bio indicators of soil health or condition because composition correlates well with nitrogen cycling and decomposition, two critical ecological processes in soil. This study further suggests that magnitude of nematode problem needs serious consideration to tackle by the use of useful nematode management strategies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jones JT, Haegemen A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Palomares-Rius JE, Wesemael WML, Perry RN. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 2013;14:946-961.

2. Gill HK, Mcsorley R. Cover crops for managing root-knot nematodes. university of florida, IFAS Extension, ENY. 2011; 3:1–6.
3. Karajeh M. Interaction of root-knot nematode (*Meloidogyne javanica*) and tomato as affected by hydrogen peroxide. J Plant Prot Res. 2008;48(2):2.
4. Sasser JN. Root-knot nematodes: A global menaced to crop production. Plant Disease. 1980;64(1):36-41.
5. Abad P, Favery B, Rosso MN, Castagnone-Sereno P. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Mol Plant Pathology. 2003; 4:217–224.
6. Olsen MW. Root-knot Nematode. University of Arizona, Arizona Cooperative Extension, AZ. 2000;1187:1–3.
7. Udoh EJ, Etim NA. Measurement of farm level efficiency of waterleaf (*Talinum triangulare*) production among city farmers in Akwa Ibom State, Nigeria. Journal of Sustainable Development in Agriculture and Environment. 2008;3(2):47-54.
8. Nyananyo BL, Olowokudego JD. Taxonomic studies in the genus *Talinum* in Nigeria. Wildnowia. 1986;15:455-463.
9. Akachuku CO, Fawusi MAO. Growth characteristics, yield and nutritional value of waterleaf, *Talinum triangulare* (Jacq) wild in a semi-wild environment. Discovery and Innovation. 1995;7:163-172.
10. Sikora RA, Fernandez E. Nematode parasites of vegetables. Pp 319-392. In Luc M, Sikora RA, Bridge J, (eds). Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd Edition. CAB International; 2005.
11. Khan MT, Khan MW. Effect of ammonia and root-knot nematode on tomato. Agriculture Ecosystem and Environment. 1994;53:71-81.
12. Shurleff MC, Averre, CW. Diagnosing plant disease caused by plant parasitic nematodes. The American Phytopathol Society. 2000:187.
13. Caveness FE. End of tour progress report on Nematology. Ministry of Agriculture and Natural Resources; 1967.
14. Fawole B, Egunjobi OA, Adesiyan SO, Babatola OA, Idowu AA. The biology and control of nematode pests of food crops in Africa. Heineman Educational Books (Nig) ltd. 1992:67-98.
15. Osei K, Addico R, Nafeo A, Edu-Kwarteng A, Agyemang A, Danso Y, Sackey-asante j. effect of some organic waste extracts on hatching of meloidogyne incognita eggs. Afr. Jour. of Agric. Res. 2011;6(10):2255-2259.
16. Stonton J. Tomato root knot nematodes: Biology and control. Department of Primary Industries and Fisheries. G. Stirling (edn.), Biological Crop Protection; 2001.
17. Widmer TL, Ludwig JW, Abawi GS. The northern root-knot nematodes on carrot, lettuce and onions in New York. New York Food and Science Bulletin; 2005.
18. Sikora RA, Greco N. Nematode parasites of food legumes In: Plant parasitic nematodes in sub-tropical agriculture. CAB International Institute of Parasitology Walling Food, United Kingdo. 1990; 8(4):523-629.
19. Mussarrat J, Haseeb A. Agrichemicals as antagonist of lectin-mediated rhizobium-legume symbiosis: Paradigms and Prospects. Curr. Sci. 2000;78:793-797.
20. Adesiyan SO. Nematode pests of tropical crops. Ibadan, Oyo State: Heinemann Educational Books, Nigeria Limited; 1990.
21. Akpheokhai IL, Cladius-Cole AO, Fawole B. Evaluation of some plant extracts for the management of meloidogyne incognita on Soybean (Glycin max). World J. Agric. Sci. 2012;8(4):429-435.
22. Adesiyan SO. The efficacy of carbofuran (furadan) in controlling *Meloidogyne incognita* in water leaf (*Talinum triangulare*). Nigerian Journal of Agricultural Sciences. 1992;1(1),22-27.
23. Alalade OA, Matanmi BM, Olaoye IJ, Adegoke BJ, Olaitan TR. Assessment of pests control methods and its perceived effect on agricultural production among farmers in Kwara State, Nigeria. Journal of Tropical Agriculture, Food, Environment and Extension. 2017;16(1):42-47.
24. Taylor AL, Sasser JN. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University, USAID, NC Graphics, Raleigh; 1978.
25. Iwena OA. Essential geography, Ibadan, Oyo, Tonal Publisher Limited; 2008.
26. Coyne DL, Nicol JM, Claudius-Cole B. Practical plant nematology: A field and laboratory guide. Benin, Cotonou, International Institute of Tropical Agriculture. (IITA) Printing Press; 2014.

27. Byrd DW, Kirkpatrick T, Baker KR. An improved technique for clearing and staining plant tissue for detection of nematodes, Journal of Nematology. 1983; 15:142-143.
28. Taylor DP, Netscher C. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica. 1974; 20:268-269.
29. Khan HU, Mukhtar T, Ahmad R. Geographical distribution of root-knot nematodes (*Meloidogyne* spp.) in the Punjab Province of Pakistan. Pakistan Journal of Nematology. 2005;23(1):133-140.
30. Shahid M, Rehman AU. Khan AU, Mahmood A. Geographical distribution and infestation of plant parasitic nematodes on vegetables and fruits in the Punjab province of Pakistan. Pakistan Journal of Nematology. 2007;25(1):59-67.
31. FAO. Food and Agricultural Organisation of the United nation on-line and Multilingual Database; 2006. Available:http://faostat.fao.org/foastat Retrieved 25th August, 2017.
32. Sasser JN, Carter CC. The international *Meloidogyne* project- its goals and accomplishment. Annual Review of Phytopathology. 1985;21:271-288.
33. Singh RS, Sikora RA, Beniwal SP. Observation on the effect of sawdust on the incidence of root- knot nematode and yield of okra and tomatoes in nematode infested soil. Plant Disease Reporter. 1967;51:861-863.
34. Haroon TH, Zylstru SN. Rapid identification of genetic relationship of the *M. incognita* population by polymerase chain reaction RAP makers Egyptian. Journal of Agro-nematology. 2003;32(4):18-20.
35. Netsher C. Observation and preliminary studies on the occurrence of resistance breaking biotypes biotypes of *Meloidogyne* specie on tomato. Cah. ORSTOM. Serial Biology. 1985;11:173-178.
36. Aung T, Prot JC. Effects of crop rotations on *Praryletdiils zeae* and yield of rice cultivar UPL Ri-5. Revue de Nématologie. 1990;13:445-448.
37. Chindo PS, khan FA. Effects of soil organic amendment with poultry manure on the damage caused by the Root-knot nematode *Meloidogyne incognita* on tomato. International Network Newsletter, 1986;3(4):30-33
38. Aslam SN, Newman MA, Erbs G, Morrissey KL, Chinchilla D, Boller T, Jensen T, De Castro C, Ierano T, Molinaro A, Jackson RW, Knight MR, Cooper RM. Bacterial polysaccharides suppress induced innate immunity by calcium chelation. Curr. Biol. 2008;18:1078–1083.
39. Nwanguma EI, Awoderu JB. The relevance of poultry and pig droppings as nematode suppressants on okra and tomato in Ibadan, South Western Nigeria. Nigerian Journal of Horticultural Sciences. 2002;6: 67-69.
40. Floret C, Serpantié G. La jachère en Afrique de l'Ouest. ORSTOM, Colloques et Séminaires, Paris; 1993.

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