

SURVEY AND DISTRIBUTION OF PLANT PARASITIC NEMATODES INHABITING FARMLANDS AROUND KWARE LAKE

ABSTRACT

A survey was conducted on nematodes associated with soils of farmlands around Kware Lake, Kware local government area of Sokoto State. The aim of this research is to determine the species composition and distribution of nematodes in farmlands around Kware Lake. Sampling was carried out from July to September, 2016. Soil samples were collected to a depth of 0-15m. Centrifugation technique was used for the extraction of nematodes and viewed under the microscope. Results indicate that seven species were detected whose richness varied with respect to sites. The nematodes detected were *Xiphinema spp.*, *Heterodera spp.*, *Trichodorus spp.*, *Meloidogyne spp.*, *Rotylenchus spp.*, *Longidorus spp.*, and *Pratylenchus spp.* The most abundant nematodes isolated were *Meloidogyne spp.* (29.63%) while *Pratylenchus spp.* were found to be least abundant (2.22%). This is because *Meloidogyne* was very widely distributed and affect a wide variety of crops while *Pratylenchus* was a migratory endoparasite and can be ascertained by its high population in the root. There was no significant difference on nematode species occurrence between the three sampling sites at $p < 0.05$. The result showed there was wide diversity of nematodes inhabiting the study area with diversity index of 1.77. The presence of plant parasitic nematodes in the soils of farmlands around Kware Lake highlights need for prevention and control of nematode species, so as to reduce the risk of losses to agricultural yield.

Keywords; Nematode, Kwarelake, Parasitic, Plants, Soil.

INTRODUCTION

Nematodes constitute one of the most numerically important components of the soil fauna[1] and thus significantly impact nutrient cycling and primary productivity in diverse ecosystem. Nematodes are microscopic worm like organisms that make up the phylum Nematoda and are among one of the most abundant multicellular animals on the earth after arthropods[2] they live in water films and water filled pore spaces in the soil. Members of the phylum Nematoda (roundworms) have been in existence for an estimated one billion years, making them one of the most ancient and diverse types of animals on earth [3]. They are thought to have evolved from simple animals, some 400 million years before the “Cambrian explosion” of invertebrates able to be fossilized [4].

Typically, they are found abundantly in the upper soil layers where organic matter, plant roots and other resources [5]. The soil is a particularly rich habitat for nematodes with about 26 percent described genera inhabiting soil as bacterivores, fungivores, omnivores, predators or plant parasites [6].

Most species of nematodes are free living and sustain themselves by consuming bacteria, fungi or other microscopic organisms; others are parasites of plants or animals. Free living nematodes are abundant multi-cellular animals that participate in fundamental ecological processes in the soil such as decomposition and nutrient cycling [7]. Free living soil nematodes are sensitive to ecosystem disturbances [8]. They also possess several attributes that make them excellent indicators for evaluating soil conditions [9].

A great limitation to worldwide production of food crops is damage caused by plant parasitic nematodes [10]. Plant parasitic nematodes are recognized as one of the greatest threats to crops throughout the world [11]and can devastate a wide range of crops plants, causing billions of dollars of agricultural losses each year [12]. All plant parasitic nematodes are obligate parasites, feeding exclusively on cytoplasm of living plant cells [13].

Although nematodes are generally regarded as silent enemies, losses up to 80 percent have been associated with them in vegetable fields that are heavily infested [14]. They have been described as important pests of horticultural crops [15] causing significant reduction in yield.

Comment [F1]:

what is the background to doing research at FARMLANDS AROUND KWARE LAKE?

Has there been an outbreak of nematode disease?

What is the impact caused by nematode parasites?

Add related data

Among the most important genera of plant parasitic nematodes that have been reported worldwide are: *Meloidogyne* (root knot nematode) and *Heterodera* (cyst nematode). Both the cyst and root knot nematodes have complex interactions with their host, but there are characteristic differences in their parasitic cycles. They are adapted to parasitize on large number of plants [16] and are distributed worldwide over a wide range of geographical conditions of tropical, subtropical, and temperate regions of the world [17]. Others include: *Pratylenchus* spp. (lesion nematodes), *Ditylenchus* spp. (stem and bulb nematodes) and *Rotylenchus* spp. (reniform nematodes) [18].

Materials and Methods

Study Area

Soils of farmlands around KwareLake, Kware Local Government Area of Sokoto state were used for the study. Kware is located 20km northeast of Sokoto metropolis in Nigeria. It is located within longitude 5°16'N and latitude 13°15'E. Kware is flanked by Sahel savanna to the northeast and by eastern part of river Rima. The vegetation of the area is mostly dominated by grasses and tress (Sudan savannah). It is characterized by two distinct seasons, the short raining season which runs from May to September or October depending on the rainfall pattern of the year, the long dry season start from October to April. The humidity is less than 20% and temperature range from 20°C to 42°C.

Sample collection

Soil samples were collected from three difference farmlands in KwareLake. The sample was collected at the vicinity of the plant by digging to about 10cm to 15cm deep. The soil sample collected were placed in polythene bags separately and taking to laboratory for analysis. in addition, part of the root was also collected.

Isolation of parasites

The isolation of the plant parasitic was done in the laboratory using Cobb's decantation technique.

Comment [F2]: add location map

Comment [F3]: in abstract : Sampling was carried out from July to September, 2016.

Comment [F4]: state the name of the location and show it on the map

Comment [F5]: how many excavations were carried out

Comment [F6]: how many ?

Comment [F7]: name of laboratory

Cobb's decantation technique

This technique is used for the extraction of motile nematodes from soil. It was introduced by [19] and is mainly used for difference species of nematodes. In principle, soil will be washed in water and allow it to settle some minutes and latter decanted and nematodes will be collected on sieves of different aperture.

In this method, the soil samples brought to the laboratory were transferred into a container and half fill with water. The solution was mixed thoroughly and in the process any lumps were broken. The mixture stirred was then sieve by pouring it through sieves of varying mesh sizes into basin then the sieve was rinsed and the residue may contain very large nematodes.

In order to collect the residue, the sieve was turn upside down on the edge of the petridish, so that the residue was washed down using a gentle stream of water in to a petridish. This was then observed under dissecting microscope from here the nematodes that were found was put under in preserving bottle using a handling needle.

Isolation from root involves macerating the plant root which was transferred into a container with water. The solution was missed thoroughly and sieve as above. The residue was observed under microscope ($\times 10$).

Comment [F8]: microscope type?

Slides preparation

A drop of water was put on glass slide then, the forceps was used to pick the nematode from the petridish into the drop of water on the glass slide and a cover slip was used to cover it. Then a drop of iodine was used in staining the sample.

Nematode identification

The slides prepared were viewed using compound microscope and their pictures were taken using phone camera which later were compared using different picture of different genera of plant parasitic nematodes [20].

Comment [F9]: Microscope type?

Statistical Analysis

Analysis of variance (ANOVA) was used in analyzing the data obtained from three sampling site (A, B, and C) to ascertain the distribution of difference plant parasitic nematodes.

Comment [F10]: the name of the location?

Results

The results below show the distribution of nematodes in soils of farmlands around Kwarelake. A total number of 135 species of plant parasitic nematodes were examined in both sampling sites A, B and C. Whereas sampling site "A" has a total number of 40 different species of plant parasitic nematodes. *Meloidogynes* species has the highest percentage (17.5%) and the least frequently examined species were *Pratylenchus* species with a total percentage of (5.0%), it appears to be less because they are migratory endoparasites that feed and reproduce in the root and move around. Unlike the cyst and root knot nematodes, which stay in one place.

Comment [F11]: Species or individu?

Comment [F12]: Species or individu?

While the sampling site "B" also has a total of 36, the most abundant plant parasitic nematodes observed were *Xiphinema* species with total percentage of (27.78%), whereas the least species were *Rotylenchus* species and *Trichodorus* species with a total percentage of (13.89%). The genus *Rotylenchus* prefers sandy soil to survive in fallow soil for up to 6 months with mortality usually not exceeding 50%, it causes damage which affect size and shape of corns. Whereas the sampling site "C" has the highest number with a total of 56 different genus of nematodes, and also *Meloidogyne* species were observed to be the most dominant species with a total percentage of (30.56%). The root knot nematodes feed and mature inside the root of plants. Their feeding induces abnormal enlargement of the root, called galls. And they are also the most abundant plant parasitic nematodes found in worldwide and where as the least species were *Pratylenchus* species with a total percentage of (1.79%).

Comment [F13]: ??

Comment [F14]: ???

Table 1: Species of nematodes isolated from soil samples collected from Site A of KwareLake, Sokoto State.

| Species | Number isolated from each soil sample | | | | | Total | Percentage |
|--------------------------|---------------------------------------|----------|----------|----------|----------|-----------|------------|
| | I | II | III | IV | V | | |
| <i>Xiphinema spp.</i> | 2 | 0 | 3 | 0 | 2 | 7 | 17.5 |
| <i>Meloidogyne spp.</i> | 3 | 4 | 1 | 2 | 1 | 11 | 27.5 |
| <i>Heterodera spp.</i> | 1 | 1 | 2 | 0 | 1 | 5 | 12.5 |
| <i>Longidorus spp.</i> | 2 | 0 | 2 | 3 | 1 | 8 | 20.0 |
| <i>Trichodorus spp.</i> | 1 | 3 | 1 | 2 | 0 | 7 | 17.5 |
| <i>Pratylenchus spp.</i> | 1 | 1 | 0 | 0 | 0 | 2 | 5.0 |
| Total | 10 | 9 | 9 | 7 | 5 | 40 | 100 |
| Species richness | 6 | 4 | 5 | 3 | 4 | 6 | |

Comment [F15]: ???

Table 2: Species of nematodes isolated from soil samples collected from site B of KwareLake, Sokoto State.

| Species | Number isolated from each soil sample | | | | | Total | Percentage |
|-------------------------|---------------------------------------|-----------|----------|----------|----------|-----------|------------|
| | I | II | III | IV | V | | |
| <i>Xiphinema spp.</i> | 0 | 2 | 2 | 5 | 1 | 10 | 27.78 |
| <i>Meloidogyne spp.</i> | 3 | 2 | 2 | 0 | 2 | 9 | 25.00 |
| <i>Longidorus spp.</i> | 2 | 4 | 1 | 0 | 0 | 7 | 19.44 |
| <i>Trichodorus spp.</i> | 1 | 0 | 0 | 2 | 2 | 5 | 13.89 |
| <i>Rotylenchus spp.</i> | 1 | 2 | 0 | 2 | 0 | 5 | 13.89 |
| Total | 7 | 10 | 5 | 9 | 5 | 36 | 100 |
| Species richness | 4 | 4 | 3 | 3 | 3 | 5 | |

Comment [F16]: ???

Table 3: Species of nematodes isolated from soil samples collected from Site C of KwareLake.

| Species | Number isolated from each soil sample | | | | | Total | Percentage (%) |
|--------------------------|---------------------------------------|----|-----|----|----|-------|----------------|
| | I | II | III | IV | V | | |
| <i>Xiphinema spp.</i> | 3 | 0 | 3 | 1 | 2 | 9 | 16.07 |
| <i>Meloidogyne spp.</i> | 5 | 4 | 2 | 5 | 1 | 17 | 30.56 |
| <i>Heterodera spp.</i> | 0 | 1 | 0 | 2 | 0 | 3 | 5.36 |
| <i>Longidorus spp.</i> | 2 | 1 | 0 | 1 | 3 | 7 | 12.5 |
| <i>Trichodorus spp.</i> | 1 | 2 | 1 | 2 | 4 | 10 | 17.85 |
| <i>Rotylenchus spp.</i> | 2 | 3 | 4 | 0 | 0 | 9 | 16.07 |
| <i>Pratylenchus spp.</i> | 1 | 0 | 0 | 0 | 0 | 1 | 1.79 |
| Total | 14 | 11 | 10 | 11 | 10 | 56 | 100 |
| Species richness | 6 | 5 | 4 | 5 | 4 | 7 | |

Comment [F17]: ????

Table 4: Soil nematode species identified from farmland around KwareLake, Kware Local Government Area, Sokoto State.

| Species | Number isolated from each sampling sites | | | Total | Percentage (%) |
|--------------------------|--|----|----|-------|----------------|
| | A | B | C | | |
| <i>Xiphinema spp.</i> | 7 | 10 | 9 | 26 | 19.25 |
| <i>Meloidogyne spp.</i> | 14 | 9 | 17 | 40 | 29.63 |
| <i>Heterodera spp.</i> | 5 | 0 | 3 | 8 | 5.93 |
| <i>Longidorus spp.</i> | 8 | 7 | 7 | 22 | 16.30 |
| <i>Trichodorus spp.</i> | 7 | 5 | 10 | 22 | 16.30 |
| <i>Rotylenchus spp.</i> | 0 | 5 | 9 | 14 | 10.37 |
| <i>Pratylenchus spp.</i> | 2 | 0 | 1 | 3 | 2.22 |
| Total | 43 | 36 | 56 | 135 | 100 |
| Species richness | 6 | 5 | 7 | 7 | |

Comment [F18]: my advice: use cluster analysis or correspondence analysis

F. Cal. = 0,714. df 2, 18 at P<0.05, F. Tab. = 3.56

Comment [F19]: ????

Table 5: Diversity index of nematodes isolated from farmland around Kwarelake.

| Species | Number isolated | P_i | | |
|--------------------------|-----------------|-------|--------|------|
| <i>Xiphinema spp.</i> | 26 | 0.193 | – 1.64 | 0.32 |
| <i>Meloidogyne spp.</i> | 40 | 0.296 | – 1.22 | 0.36 |
| <i>Heterodera spp.</i> | 8 | 0.059 | – 2.83 | 0.17 |
| <i>Longidorus spp.</i> | 22 | 0.163 | – 1.81 | 0.30 |
| <i>Trichodorus spp.</i> | 22 | 0.163 | – 1.81 | 0.30 |
| <i>Rotylenchus spp.</i> | 14 | 0.104 | – 2.26 | 0.24 |
| <i>Pratylenchus spp.</i> | 3 | 0.022 | – 3.82 | 0.08 |
| Total | 135 | | | 1.77 |

P_i = Number of individual species / Total number
Evenness = 0.9

Discussion

From the study, it can be observed that seven genera of nematodes were identified in total, with variations in species richness with respect to sampling sites. The highest density of nematodes occurred in site C (56 nematodes/400g of soil) with higher richness while site B was the least in abundance (36 nematodes/400g of soil). Some species detected in site C were absent in the other sampling sites. Detection of plant parasitic nematodes in soils could be due to function of soil as a medium by which the nematodes search for host plant [21]. This result agreed with the findings of [22] whereby nematodes were detected in the rhizosphere soils of farmlands in Nomansland and Salanta. The result also agreed with the findings of [23] in which some nematodes were found present in some sampling sites and absent in others.

The most occurring plant parasitic nematodes identified were *Meloidogyne spp.* (29.63%) and *Xiphinema spp.* (19.25%) while the least species isolated were *Pratylenchus spp.* (2.22%). *Meloidogyne spp.* was species with the highest abundance in site A and site C as the crops grown there are good hosts for the nematode [24]. High density of *Meloidogyne spp.* was in line with the fact that *Meloidogyne spp.* was the widely most abundant nematode present worldwide [17].

Comment [F20]: please explain in the method

Comment [F21]: add photos of nematodes found

Comment [F22]: ???
how many soil samples were observed, please explain in the method

Comment [F23]: what species are not in other locations

Comment [F24]: what does it mean if a lot is found *Meloidogyne spp*

Xiphinema spp. are also abundant in the soils of the study area. They are the most abundant in site B. They tend to occur more in vegetable crops [25] as they are the major crops grown there. This can also be ascertained by the ability of these nematodes to withstand harsh conditions in the soil [26].

Trichodorus spp. and *Longidorus spp.* are also present in the soil. These species tend to infest flowers and could be less in the soil samples [27]. *Rotylenchus spp.* are less in abundance in the soil. Their low densities could be ascertained by their slow rate of reproduction and their high sensitivity to disturbances [28].

Heterodera spp. was found in the two of the sampling sites but absent in the other and are found to be less in abundance. The rationale behind these was due to the fact that these nematode species tend to have narrow range of hosts due to crop rotation as confirmed by [29].

Pratylenchus spp. was least in population in the sampling sites detected as they are migratory endoparasites with all stages found in the root cortex. Low soil populations can be associated with high root populations. The nematodes feed mainly on cortex cells and form cavities [30]; [31]; [32].

According to [33] the ten most economically damaging nematode genera are; *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Ditylenchus*, *Globodera*, *Tylenchulus*, *Xiphinema*, *Rotylenchus* and *Helicotylenchus*.

The result also showed a wide diversity of nematodes inhabiting the soil of the farmlands of the study area with diversity index of 1.77. The data according to statistical analysis shows there is no significant difference in nematode species occurrence between the three locations due to ANOVA calculated value (0.756) is less than the tabulated value (3.56) at $p < 0.05$. This also supports the findings of [22] that, there was no significant difference in nematode species occurrence between Salanta and Nomansland farmlands.

Conclusion

Soils of farmlands around KwareLake harbor nematodes. Nematodes are widely distributed within the study area and are most abundant in the Southern location of the lake (56 nematodes/400g of soil). The detected nematodes were *Xiphinema spp.*, *Heterodera spp.*,

Comment [F25]: what does diversity index of 1.77 mean?

Trichodorus spp., *Meloidogyne spp.*, *Rotylenchus spp.*, *Longidorus spp.*, and *Pratylenchus spp.*

The most common nematode species present are the *Meloidogyne spp.* while *Pratylenchus spp.* are the least in abundance. The presence of the detected plant parasitic nematodes across all stations of the lake highlights the extent of risk the exposed cultivated crops are in, which might in turn affect crops yield and productivity.

Recommendations

Farmers should adopt method of crop rotation which will narrow the host range of parasitic nematodes. Planting of resistant crop varieties is also recommended for the control of nematodes.

REFERENCES

- [1] Warwick, R.M., Dexter, D.M., and Kuperman, B.. Free living nematodes from the Salton Sea. *Hydrobiologia*, 2002: **473**: 121-128.
- [2] Blaxter, M.L., De Ley, P., Garey, J.R., Liu, X. and Frisse, L.M.A molecular evolutionary framework for the phylum Nematoda. *Nature*, 1998: **392**: 71-75
- [3] Wang, D.Y., Kumar, S., and Hedges, B.S. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society of London*, 1999: **266**: 163-171.
- [4] Poinar, G.O. *Nematodes for Biological Control of Insects*. CRC Press, Boca Raton, Florida. 2001
- [5] Lavelle, P., and Spain, A.V. *Soil ecology*, Kluwer Academic Publishers, Boston, MA. 2001: Pp.17-21.
- [6] Wharton, D.A. *A functional Biology of Nematodes*. The Hopkins University Press, Baltimore, MD. 2006.
- [7] Freckman, D.W. Bacterivorous nematodes and organic-matter decomposition. *Agricult. Ecosyst. Environ.* 1988: **24**: 195–217
- [8] Certini, G. Effects of fire on properties of forest soils. *Oecologia*, 2005: **143**(1): 1-10.

- [9]Neher, D.A. Role of nematodes in soil health and their use as indicators.*J. Nematol.*2001:**33**:161–68.
- [10]Rohrbach, K.G., and Apt, W.J. Nematode and disease problems of Pineapple.*Plant Disease*,2007:**70**: 81-87.
- [11]Poinar, G.O., Acra, A., and Acra, F. Earliest fossil nematode (Mermithidae) in Cretaceous Lebanese amber.*Fundamental and Applied Nematology*,1994:**17**: 475- 477.
- [12]Barker, K. R., and Koenning, S. R. Developing sustainable systems for nematode management.*Annual review of phytopathology*,1998:**36**: 165-205.
- [13]Wyss, U., and Zunke, U. Observations on the behavior of second stage juveniles of *Heterodera schachtii* inside host roots.*Rev Nematol.*1986:**9**: 153-166.
- [14]Siddiqi, M.R. *Tylenchida: Parasites of plant and insects*. Wallingford. Oxon, UK.2000.
- [15]Stirling, G.R., and Pattison, A.B. Biological control of plant-parasitic nematodes.*J. Nematol.*2008:**37**: 253-254.
- [16]Hussey, R.S and Janssen, G.J.W. Root-knot nematode: *Meloidogynes*species. In: Starr JL, Cook R, Bridge J, editors. Plant Resistance to Parasitic Nematodes. Wallingford, UK: CAB International; 2002. Pp.43-70.
- [17]Rich, J.R., Brito, J.A., Kaur, A., and Ferrell, J.A. Weed species as hosts of *Meloidogyne*: A review. *Nematropica*, 2008:**39**: 157-185.
- [18]Decker, H., and Sveshnikova, N. M. *Plant nematodes and their control(Phytonematology)*.Brill Publishers, Leiden, Netherlands.1989:Pp 540-560.
- [19]Cobb, N.A. Estimating the nema population of the soil. Agric. Tech. Circ. Bur. Pl. Ind. U.S. Dep. Agric. 1981: Pp. 1-48.
- [20]Mekete, T. Dadabat, A. Sekora, N. Akyazi, F. and Abebe, E. Identification key for Agriculturally important plant-parasitic nematodes. International Nematode Diagnosis and Identification Course, Eskisehir, Turkey. 2012.
- [21]Singh, R. Plantdisease.Eight editions.Oxford and IBH Publishing Co. New Delhi.2005:Pp 100-106

- [22]Imam, T.S., and Garba, K.S. Survey of nematodes inhabiting rhizosphere soils in two selected irrigated farmlands in Kano metropolis, Nigeria. *Biological and Environmental sciences Journal for the Tropics*.2013:**10**(1): 13 – 18
- [23]Tom, K. *Identification of soil nematodes in Ngere Tea catchment Area of Muranga Country, Kenya*.M. Sc. Dissertation in Biotechnology.University of Agriculture and Technology.2013:Pp 30-40.
- [24]Becker, O. Root-Knot Nematode-destroying microorganism for home and land scape use.Final report to the Elvenia, J. Slosson Endowment fund.Slosson.ucdavis.edu /newsletter / Becker-329006. Pdf. 2013
- [25] Yu, Q., Badiss A., Zhang Z., and Ye, W. First report and morphological, molecular characterization of *Xiphinemachambersi* Thorne, 1939 (Nematoda, Longidoridae) in Canada. *ZooKeys*.2010:**49**:13–22
- [26]Oliveira C.M.G. and Neilson R.Taxonomy of Longidorid nematodes and dichotomous keys for the identification of *Xiphinema* and *Xiphidorus* species recorded in Brazil.*Arq.Inst. Biol*.2006:**73**:131-141.
- [27]Crow, W.T. Parasitism and pathogenicity of *Trichodorusproximus* to plant.*Plant Disease*.2004:**49**: 259 – 262.
- [28]Bonger, T. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*.1990:**83**:14–19
- [29]Handoo, Z. A. Plant nematodes and their control in the New East region.*Journal of Nematology*.1998:**22**: 10 – 15.
- [30]Handoo Z.A., Carta L.K. and Skantar A.M. Morphological and molecular characterisation of *Pratylenchusarlingtoni* n. sp., *P. convallariae*and *P. fallax*(Nematoda: Pratylenchidae). *Nematology*2001:**3**(6):607-618.
- [31]Kimenju, J.W. Muiro, D.M. Karanja, N. Nyogesa, W.M. Miano, D.W. and Mutua, G.K. Assessing the role of organic soil amendments in management of root not nematode on common bean, *Phaseolus vulgaris* L. *Tropic Microbiology and Biotechnology*. 2004: **3**:14-23.

[32]Luc, M., Sikora, R.A., and Bridge, J. *Plant parasitic nematodes in subtropical and tropical Agriculture*. 2nd Ed. CAB International, Wallingford. 2005: Pp. 1-61.

[33]Sasser, J.N. and Freckman, D.W.A world perspective on nematology, the role of the society.In VEECH, J.A. & DICKSON, D.W. (Eds.).*Vistas on Nematology*. Hyattsville: Society of Nematologists.1987:7-14p.

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