International Journal of Biology Research
ISSN: 2455-6548
www.biotechjournals.com
Volume 1; Issue 1; March 2016; Page No. 19-22

Extraction and Identification of Plant Parasitic Nematode from Some Vegetable Crops Cultivated by Local Farmers in Abakaliki, Nigeria

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Abstract
Plant parasitic nematodes remain a major challenge to crop production that has hitherto received minimum research attention in Sub-Saharan Africa. The study of the sources of plant parasitic nematode on vegetable crops fertilized with organic manure at Presco Campus was conducted between the month of August to November, 2014 to determine the presence of plant parasitic nematode using centrifuge sedimentation methods and microscopy. The study revealed that out of the three vegetable crops examined, 30% death observed on watermelon (*Citrullus lanatus*) were as a result of the heavy load of the two nematodes of the *Tylenchoid* and *Rotylenchulus* where other vegetable crops assayed showed the presence of *meloidogyne* and *Heterodera incognita, pratylenchus* and *Helicotylenchus* at their various life cycle stages. Plant parasitic nematode can be controlled by adding agro-based fertilizer and not organic manures which can be seen to constitute the nematode pathogens responsible for plant damage and deaths.

Keywords: Nematode**,** Parasite**,** *Abelmoschus esculentus, Cucumis sativus, Citrullus lanatus.*

1. Introduction
Crops are plants grown in the field with hope of harvesting them at a particular time for food or feed and as a source of income to the farmers. These crops when planted in the field feel a lot of stress because of pests and diseases, which include weeds, bacteria, insects, fungi, nematodes, viruses etc., all these hinder the production of adequate food for the population.

Plant parasitic nematodes such as root knot nematode (*Meloidogyne* spp); lesion nematodes (*Pratylenchus* spp); *remiform* nematodes (*Rotylenchulus remiformis*) and cyst nematodes (*Heterodera* spp) have been reported to be probable limiting factors in crop production especially in the tropics [1]. The root knot nematodes are known to be the most important in all the above mentioned plant parasitic nematodes which when infest the crop form galls that prevent proper growth of the root. Due to the microscopic nature of these organisms, farmers do notice its attack late to control them and therefore it is regarded as the farmer secret enemy [2]. The damage from these nematodes prevent normal uptake of water and nutrients to support high production of the plant.

Plant parasitic nematodes have a number of hosts which were studied in this work. They include; Okra (*Abelmoschus esculentus* L.) an important vegetable grown for its green tender fruits which are used in a variety of ways. It can be fried and cooked; the tender fruits can be cut into small pieces, boiled and served with soup. It is also a source of vitamin [3].

Cucumber (*Cucumis sativus* L.) aside from being tasty and good source of vitamin in the body, it is an important vegetable crop that gives cash to the farmers.

Watermelon (*Citrullus lanatus* V.) is an important staple food crop in Nigeria and the seeds are majorly for making soup. It has a smooth exterior ring and a juicy, sweet, usually red interior flesh. The aim of the study was to determine the prevalence of soil nematode in vegetable crops grown with organic manure.

2. Materials and Method
2.1 Study Area
The experiment was conducted at an experimental field of vegetable crops belonging to Applied Biology Department, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, South East region of Nigeria.

2.2 Source of Plant Sample and dentification
The plants were collected from the Department of Crop Science and Management, Ebonyi State University CAS Campus Abakaliki Nigeria. They were identified by plant taxonomist Prof JC Okafor from Applied Biology Department Ebonyi State University Abakaliki, this was before planting. The vegetation type of the area where the crops were planted was in the tropical rainforest and rainfall was between 1,150mm to 2,000mm in the Southeast zone of Nigeria. The crops were also planted out in the farm in Presco Campus using organic manure in three ridges of 2 x 2 ft each, crops planted include: Okra (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*) and watermelon (*Citrullus anatus*). At tenth week after planting, soil samples were collected from the ridges where the plants were grown for extraction of plant parasitic nematodes. The tagged crops were randomly selected; samples and soils were collected from the rhizosphere.

2.3 Collection of Soil Samples
The soil samples was collected at 9am in the morning using a spade and poured into a sterile transparent (polyethylene) bag to ensure proper motility, and transported immediately to the

laboratory. These soil samples were collected at the roots of the vegetable crops grown with organic manure

2.4 Extraction of the Nematodes from Soil Samples

Materials used: Soil samples, sieve, pasture pipette, tissue paper, whatman paper, Bearmans funnel, beaker, microscope, microscope glass slide, spoon, Distilled water 250mls, masking tape, centrifuge, weighing balance, thermometer, pH metre and stop watch.

2.5 Procedure for the Extraction Plant Parasitic Nematodes

The soil sample was collected and sieved using a sieve of 0.05mm mesh size to obtain fine particles of the soil. The fine particle was weighed to obtain the individual weight 50-500 grams each using weighing balance. The temperature of each soil samples was determined to be 4 ^{0}C using mercuric thermometer and the pH value of the soil ranges from 1-5, showing that, the soil is acidic. The samples were poured into the three beakers of 250mls capacity while distilled water was added to sediment and allowed for 3 - 6hrs to settle before decanting to obtain the residue of the soil samples. The residue is then centrifuged for 5mins at 4000 r/mins. The supernatants of the centrifuged sample were decanted and they were smeared on the microscope slides and viewed on the microscope using x10 objective lens.

2.6 Soil Sample Analysis

Modified bearmans technique and centrifuge sedimentation was used to exam the soil samples for the extraction of the plant parasitic soil nematodes larvae and adult worm stages were implicated from the soil.

3. Results

Table 1: Nematode Threshold Numbers per Gram of Soil Samples

Threshold of Soil Sample	Nematodes Isolated
5-100g	*Pratylenchus* spp *Tylencheoidis* spp, *Helicotylencgus* spp
100-250g	*Rotylenchulus* spp and *Heterodera* spp
250-500g	*Heterodera* spp, *Meloidogyne* and *M. arenariao*

Table 1 show that various nematodes were isolated at different threshold of the soil samples collected from the organic manure fertilized farm of vegetable crops at Presco Campus.

Table 2: Isolation of Nematode in Vegetable Crop Assayed

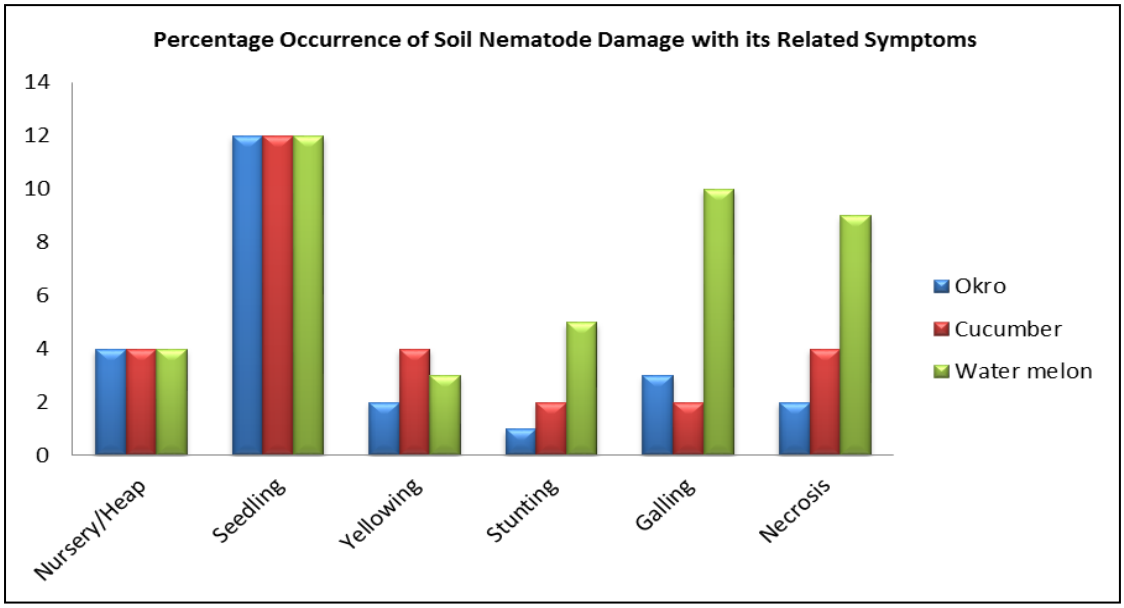
Plant Sample	Nematodes Present
Okra	Pratylenchus spp Rotylenchulus spp Tylencheoidis spp, Meloidogyne egg.
Cucumber	Helicotylencgus spp and Meloidogyne egg.
Water melon	Heterodera spp, Meloidogyne egg, Pratylenchus spp, Brachyurus spp and Aphelicochus spp.

Table 2 shows the isolation of various nematode from each of the vegetable crops assayed, it is observed that nematode isolated form watermelon (*Citrullus lanatus*) were higher than others.

Table 3: Percentage Occurrence of Soil Nematode Damage with its Related Symptoms

Plant Sample	Nursery/ Heaps	Seedling	Yellowing	Stunting	Galling	Necrosis
Okra	4	12	2	1	3	2
Cucumber	4	12	4	2	2	4
Water melon	4	12	3	5	10	9

Table 3 above shows different damages mated at different parts of the vegetable crops as observed from the time of germination to the time of harvesting. It was seen that Okra and Cucumber had little resistance than watermelon which has high damages and this proved that the soil type was not suitable for its propagation because of the presence of nematode.



4. Discussion

Nematodes depend much on their success in soil environment and their ability to survive in the absence of host plant. The study on the plant parasitic nematode in vegetable crops grown with organic manure at Presco Campus revealed the presence of various nematodes and the site of damage on the crops. The result on nematode threshold in soil (Table 1) revealed that the volume of soil samples examined at a time determines the nematode concentration (Mol/d^3). It is also observed that a particular nematode species was more abundant than the others isolated and this was not in line with the findings of [4, 5, 6] who reported that the root gall on the roots were as a result of the infective second stage larvae of the nematode which infested the roots of the crop especially Okra crop. [7, 8] observed that the feeding on the root cells by nematode larvae of the *Meloidogune* spp led to the formation of giant cells and development of gall on infested roots. The presence of root gall on these crops probably indicates the presence of *Meloidogyne* spp in the field. It was also observed that the development of plant parasitic nematode in the vegetable crop planted was enhanced by the low temperature of the soil and then closeness or nearness to sewage/water body. Plant parasitic nematode thrived well when there was low temperature of the soil and their replication and distribution occurred rapidly.

Therefore, low temperature of the soil encourages the growth of soil nematodes. It was also observed that, humidity of the field was also another factor that enhanced the development and distribution of soil nematode in the field, and when there was much humidity or average humidity, soil nematode will have more chances to infested or attack crops in the field, 9 out of the 12 seeds planted had necrosis and some showed stunted growth while 3 of the watermelon showed yellowing of the leaves which lead to the death of the crop. It was also observed that organic manure may have contributed to the establishment of the nematode which was isolated in the vegetable crop examined as seen in Table I. The result in figure I showed that all the crop in the field were infested by plant parasitic nematode which may be as a result of the fact that organic manure applied to the crops were already infected by nematode. Although some of the nematodes may not be pathogenic or harmful to the plants and this is not in line with the work of [9, 10, 11, 12]. Table 3 showed the extraction of nematode in vegetable crops, nematode infestation were recorded much on *Citrullus lanatus* followed by *Cucumis sataivus* and lastly on *Abelmoschus esculentus L.* Extraction from the root of the crop showed varied number of plant parasitic nematodes, which was probably due to sandy loam texture of the experimental field, this is in agreement with the findings of [13], [14], [15] and [16] which reports that soil type was primarily an edaphic factor that influences the population of nematode. Extraction of nematode from soil sample did not vary for these crops because of their different nematode infestation level [15].

This study was carried out during rainy season of the year and this may have influenced the number of nematodes extracted from the soil. This result corresponds with the findings of [18] and [19] which reported that climate of the region where these crops were grown influence the population of different plant parasitic nematodes.

The three crops used in the work showed varied nematode number with watermelon having the highest number of occurrence. This implies that these crops showed certain degree of susceptibility to nematode infestation as a result of the organic manure and season. Therefore, farmers should be discouraged on the use of infested organic manure. Farmers should look out for those crops that are host to nematode and eliminate them on time to avoid buildup of the nematodes which could lead total to loss of the crops in the field and soil test should be conducted to determine the presence of soil/plant parasitic pathogens that influences the wellbeing of crops.

5. Reference

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