



ISOLATION AND IDENTIFICATION OF PLANT PARASITIC NEMATODES AFFECTING TOMATO (*SOLANUM LYCOPERSICUM*, LINN.) IN GIWA LOCAL GOVERNMENT AREA, KADUNA, NIGERIA

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ABSTRACT

Plant-parasitic nematodes are the major biotic stressor in crop cultivation. They are recognized as one of the greatest threats to crops worldwide. The study evaluated nematodes that affect tomato in Giwa Local Government area, Kaduna State, Nigeria. Samples were collected from two selected farms each from seven locations. The tomato samples were classified as diseased and healthy based on the appearance of the plants. In each farm, four samples were taken during the growing season; two from diseased plants and two from apparently healthy looking plants. Similarly, soil samples from diseased and apparently healthy soil were collected. The soil and tomato samples were extracted using Cobb-Sieving and Decanting method. Descriptive statistics, students t-test and species diversity were used to analyze the data. Nineteen (19) genera of plant parasitic nematodes were isolated and identified, with 18 genera each in diseased soil and root samples, 12 and 9 genera from apparently healthy soil and roots respectively. *Scutellonema* spp. (1121) had the highest number of nematodes genera while *Tetylenchus* (20) had the lowest, in diseased soil samples. In diseased root samples, *Meloidogyne* (415) had the highest nematodes while *Tetylenchus* (10) had the lowest number of nematodes. In apparently healthy soil samples, *Scutellonema* (522) had the highest number of collection, while *Tylenchorynchus* (20) had the least. In apparently healthy root samples, *Pratylenchus* (415) had the highest and *Hoplolaimus* (10) had the lowest number of collection across all the locations. There was no significant difference ($p > 0.05$) in the presence of nematodes in the soil and tomato samples. The results show the widespread distribution of plant parasitic nematodes against *Solanum lycopersicum* L. plants in the study areas. The parasitic nematode populations built up, could results to great reduction of the crop yield.

Keywords: Nematode, *Meloidogyne*, Giwa, Uprooted, Identification, Extraction

INTRODUCTION

Nematodes are microscopic worms that are serve as plants parasite which cause billions of dollars in crop losses annually, and all crops in the world are susceptible to at least one species of nematode parasites (Bozbuga et al., 2018). The economic consequences of crop losses caused by nematodes come in many variations and are associated with a decrease in the crop quality and yield. In the presence of plant-parasitic nematodes, the drop in the yield of vegetables can sometimes reach 29% for susceptible genotypes (Sabeh et al., 2019). Nematodes are among the most important and abundant animals in the animal kingdom and are able to survive in any environment (Aleuy and Kutz, 2020). Plant-parasitic nematodes are the major biotic stressor in crop cultivation (Treonis et al., 2004).

The most damaging plant-parasitic nematodes are considered to be the root-knot nematodes (RKNs), *Meloidogyne* sp. (Almohithet et al., 2018), which are responsible for losses in vegetable crops throughout the world and determine the common use of chemical pesticides (Sikandar et al., 2020). Nematode infection in soil can lead to secondary infection with fungal and bacterial pathogens and even to the transmission of plant-infecting viruses, which negatively affect the yield (Gullino et al., 2019).

Plant parasitic nematode usually attack the roots, stems, leaves, flowers and even bulbs causing galling, lesion,

stunting, poor development of the leaves and fruits, yellowing of the leaves, decrease in yield and increased susceptibility to pathogens and sometimes plant death. These nematodes cause billions of dollars in losses of tomato crop annually (Banora, 2023). The destruction of tomato plant caused by plant parasitic nematode is common throughout Nigeria including Kaduna State, resulting in severe losses of tomato yields (Wonang and Akueshi, 1997). There is limited information on plant parasitic nematode in Giwa Local Government Area. Therefore, this study aimed to isolate and identify plant-parasitic nematode in tomatoes growing fields in Giwa Local Government area.

MATERIALS AND METHODS

Study Area

The study was conducted in Giwa Local Government area of Kaduna State, Nigeria. The coordinates ranges between latitudes 11° 20' 0" N - 10° 50' 0" N and longitudes 7° 40' 0" E - 7° 10' 0" E (Fig. 1). The local government had an estimated population of about 292,384 (NPC, 2006). The average rainfall is about 1100mm spanning from late April to late October, whereas mean temperature varies from 27-35°C. The people are mainly peasant farmers, while some are engaged in livestock production.

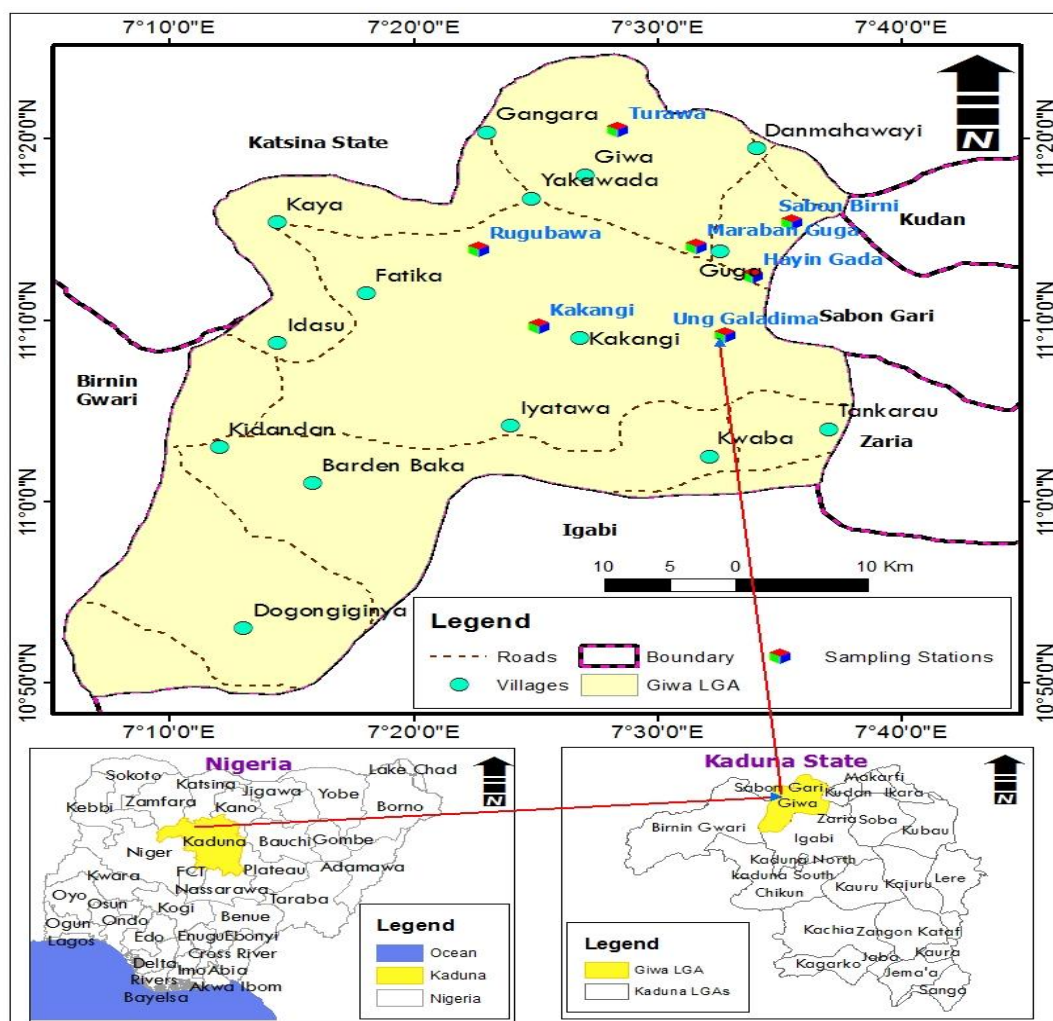


Figure 1: Giwa Local Government with Study Area

Source: GIS Lab Department of Geography and Environmental Management ABU, Zaria

Study Design

The study was conducted in Giwa LGA of Kaduna State where seven tomato farming locations were selected for this study. The soil and tomato samples were collected from diseased and apparently healthy tomato plants.

Soil and Root Samples Collection

Soil and root samples were collected from seven (7) different locations namely: Hayin Gada, Unguwan Galadima, Sabon Birmi, Mararaban Guga, Turawa, Rugubawa and Kakangi. Each location has tomatoes plants grown under irrigation condition. Soil and whole tomato plant samples were collected in March, 2020. Two farms were randomly selected from each location, making a total of fourteen (14) farms. These farms were tagged as farm 1 and farm 2 in each of the study location. Four samples were taken from each farm 1 and 2 respectively, making a total of 56 samples from all the locations. Consequently, all the samples of farm 1 and farm 2 of the seven (7) locations were pooled together and analyzed as a single sample respectively. Thereafter, and compared. Moreover, two stands of tomato crop showing one or all of the following disease symptoms were randomly selected: chlorosis, wilting, stunted growth, leaf spot, stem rot, and vascular discoloration (Coyne et al., 2007). Also, two healthy stands of the tomato were sampled. The plant was carefully uprooted using hoe. Rhizosphere soils from uprooted plants were transferred into polyethylene bag for

onward transportation to the laboratory. Information regarding the location, date of collection, number of samples collected, apparent symptoms of disease were noted. The Collected samples were taken to the Department of Crop Protection Laboratory in Faculty of Agriculture, Ahmadu Bello University, Zaria for processing.

Extraction of Nematodes from The Soil

Extraction of nematodes from the soil was carried out using sieving and decanting method of Cobb's (1918) as modified by Coyne et al. (2007). The dissolved soil sample was allowed to settle and decant the suspension that was screened through decantation, isolation and extraction.

Extraction of Nematodes from The Root System

Two methods were employed for the extraction of nematodes from the root system; namely direct examination and maceration-filtration technique by Stermerding (1964).

Direct examination: Plants were examined for visible symptoms of disease such as necrosis, canker, stunted growth, wilt, leaf spot, blight, chlorosis, insects, stem rot and vascular discoloration was rated according to the level of damage (Stermerding, 1964). Washed roots were examined for root galls, loss in root hairs and root rot. Roots were rated for galls. **Maceration-filtration technique:** This was employed to extract nematodes from the roots. The roots were thoroughly washed under tap water to get rid of adhering soil. Roots were

chopped into small pieces of about 1-2cm length and 5g weighed. These pieces were placed in the jar of a warring blender and covered with water. They were macerated for 5seconds at 1000 rpm. The suspension thus obtained was poured over a filter paper in a Baermann's extraction dish. Then 100mls of water was poured into the dish and left 24hours. Chopped roots were examined under the dissecting microscope for galls, adult females and nematode egg masses/cyst. The nematodes were identified under a dissecting microscope.

Counting of Nematode Population

The suspension of nematodes was made up to 100ml volume by the addition of water for both soil and plant tissues extracted. Air was passed into the suspension using an aquarium pump. The mobility of nematodes was increased by thoroughly homogenizing it. Ten millilitres of the bubbled suspension was pipette into the Doncaster's counting dish and the nematode population were counted and identified under a stereoscopic dissecting microscope. Counting and identification was done twice for each sample, and the average recorded.

Data Analyses

Descriptive statistics was used to determine the nematodes in rhizosphere soils and tomato roots obtained from the seven study locations. T-test was used to compare the occurrence of plant parasitic nematode in the diseased and apparently healthy soils and roots and tested at 0.05 alpha level. Shannon-Wiener index (H') was used to calculate the diversity of nematode in soil and root samples.

RESULTS AND DISCUSSION

Genera of plant parasitic nematode identified from diseased soil samples

A total of nineteen (19) genera of plant parasitic nematode (PPN) were isolated and identified from the collected diseased soil and root samples; and apparently healthy soil and root samples of tomato across the 7 selected growers field in Giwa LGA. These nematodes consisted of Aphelenchoides spp., Aphelenchus spp., Criconomoides spp., Helicotylenchus spp., Hemicyclophora spp., Heterodera spp., Hoplolaimus spp., Longidorus spp., Meloidogyne spp., Paratylenchus spp., Pratylenchus spp., Rotylenchus spp., Scutellonema spp., Tetylenchus spp., Tylenchorynchus spp., Tylenchulus spp., Tylenchus spp., Xiphinema spp., and Trichodorus spp. However, diseased soil and root samples recorded eighteen (18) of the identified taxa of the nematodes each while twelve (12) and nine (9) genera were recorded in apparently healthy soil and root samples respectively.

Population of nematodes in diseased and apparently healthy soil rhizosphere samples

Results for the population of plant parasitic nematode in the soil are presented in Figure 1 and 2. The results obtained in Farms 1 of all the seven (7) locations, showed Scutellonema spp as the highest (403) and (290) in both diseased and healthy soils, respectively while Criconomoides spp., Tylenchorynchusspp., and Longidorus spp., had the lowest

(20) collection in the diseased soil. In addition, Tylenchorynchus were the least (40) in terms of occurrence in healthy soil while Criconomoides and Longidorus were not found in the healthy soil samples (Figure 1). There was a significant difference ($p < 0.05$) in the occurrence of Meloidogyne in the diseased and apparently healthy soils in Farm1 of all the seven (7) locations. While Farm 2, in all the seven (7) locations, had Scutellonema as the highest (721) and (232) in both the diseased and healthy soils respectively, while Tetylenchus and Longidorus had the lower frequency (10) in the diseased soil but absent in the healthy soil (Figure 2). However, there was no significant difference ($p > 0.05$) in the density of plant parasitic nematodes in the diseased and healthy soils in Farm 2 of all the seven (7) locations.

Population of nematodes in diseased and apparently healthy roots samples

The results obtained, from the population of nematode in the roots studied in farm 1 of the 7 locations, indicated Meloidogyne spp as the highest (190) and (125) in both diseased and healthy roots respectively, while Criconomoides spp. and Longidorus spp., were the lowest (10) in the diseased root and were not found in the healthy root samples (Figure 3). Thus, there was no significant difference ($p > 0.05$) in the occurrence of plant parasitic nematode in the diseased and healthy soils of farm1.

In farm 2, Scutellonema had the highest (194) and (50) occurrence in both the diseased and healthy roots respectively while Tylenchus was the lowest (10) and (10) In occurrence. Tetylenchus and Tylenchulus were not found in both the diseased and healthy roots in Farm 2 (Figure 4). There was no significant difference ($p > 0.05$) in the occurrence of plant parasitic nematode in the diseased and healthy roots in farm 2.

Diversity of Nematode in Soil and Root Samples

Results of the nematode diversity in the soil and root samples in Farm 1 and Farm 2 from the seven locations in the 14 farms are presented in (Tables 1 and 2). This diversity was estimated based on three indices: the dominance, Shannon-Wiener index (H') and Evenness (EH). Higher diversity (2.374) of nematodes species were observed in diseased soil samples in farm 1 while lower diversity was recorded in apparently healthy root samples Table. Also, nematodes were found to be evenly distributed (0.913) in apparently healthy soil sample in farm 2 (Table 1).

Shannon index showed higher diversity (2.505) of nematodes in the diseased soil samples in farm 1 while lower diversity (1.090) was observed in the apparently healthy root samples. In addition, nematodes species were found to be evenly distributed (0.992) in apparently healthy root samples in farm 2 (Table 2).

Plates of Some identified parasitic nematode in collected plant samples

Plate I: Shows the Photomicrographs of Scutellonema, Plate II: shows the Photomicrographs of Meloidogyne and plate III shows the Photomicrographs of Rotylenchus.

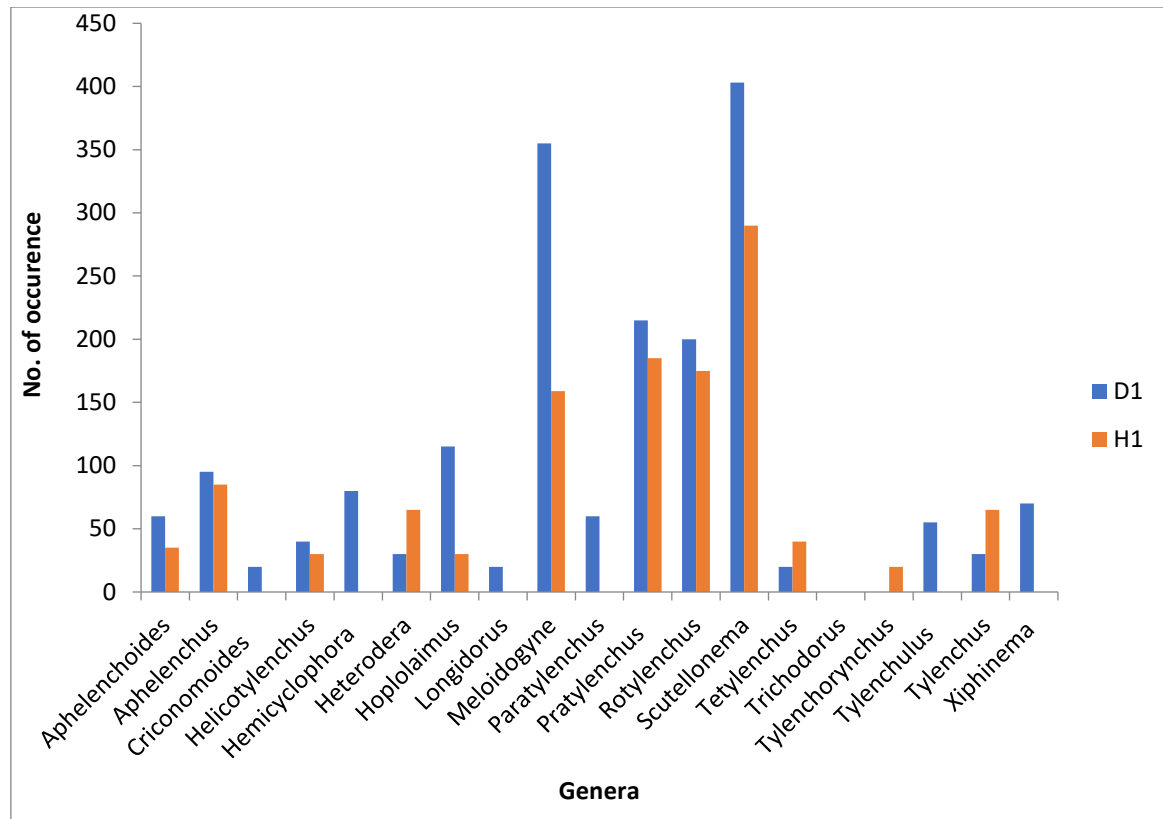


Figure 1: Occurrence of plant parasitic nematode in the diseased and apparently healthy soil in farm 1
Key: D1 = diseased soil and H1 = healthy soil.

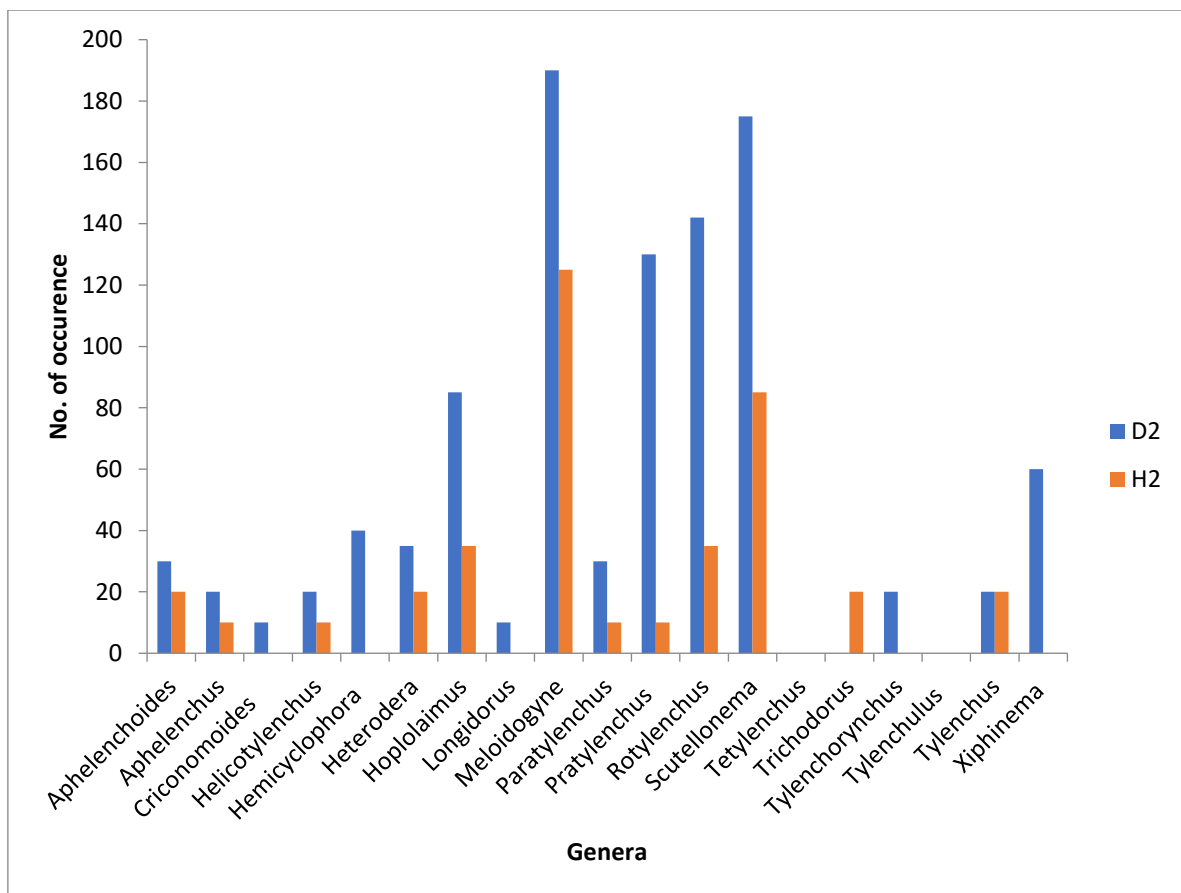


Figure 2: Occurrence of plant parasitic nematode in the diseased and apparently healthy soil in farm 2
Key: D2 = diseased soil and H2 = healthy soil

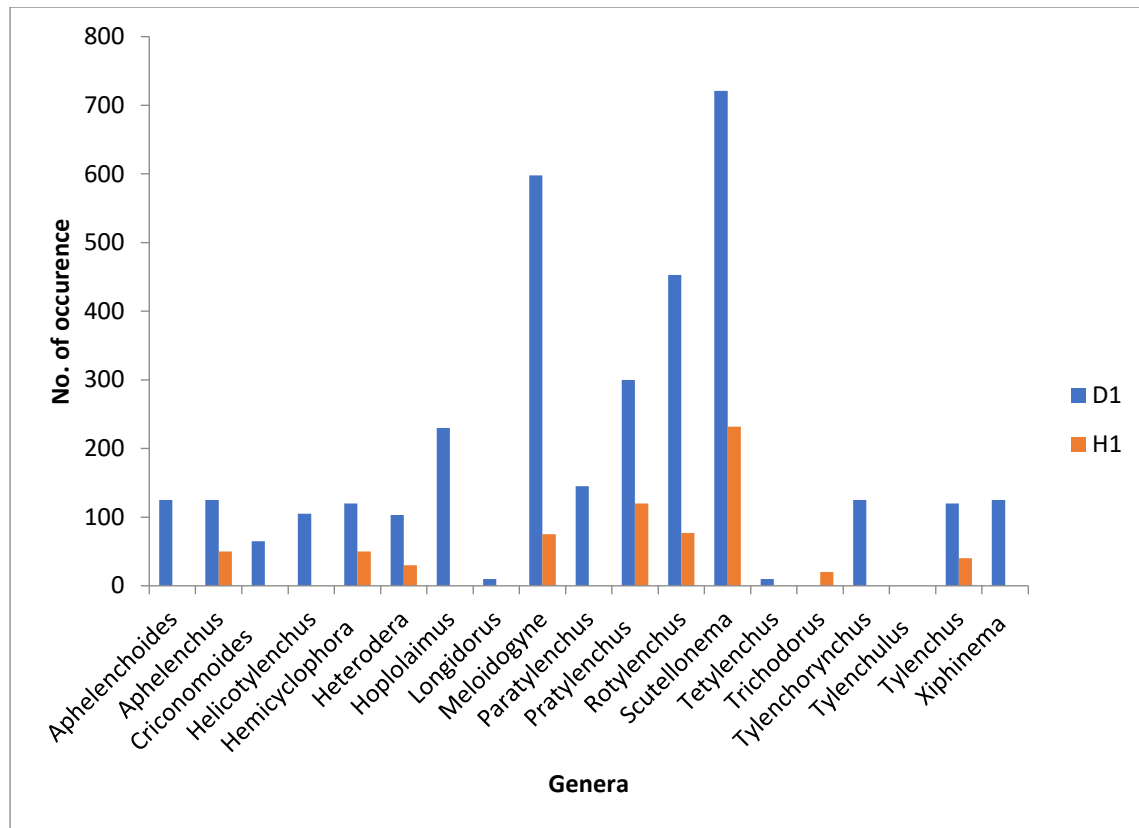


Figure 3: Occurrence of plant parasitic nematode in the diseased and apparently healthy root samples in farm 1
Key: D1 = diseased root and H1 = healthy root

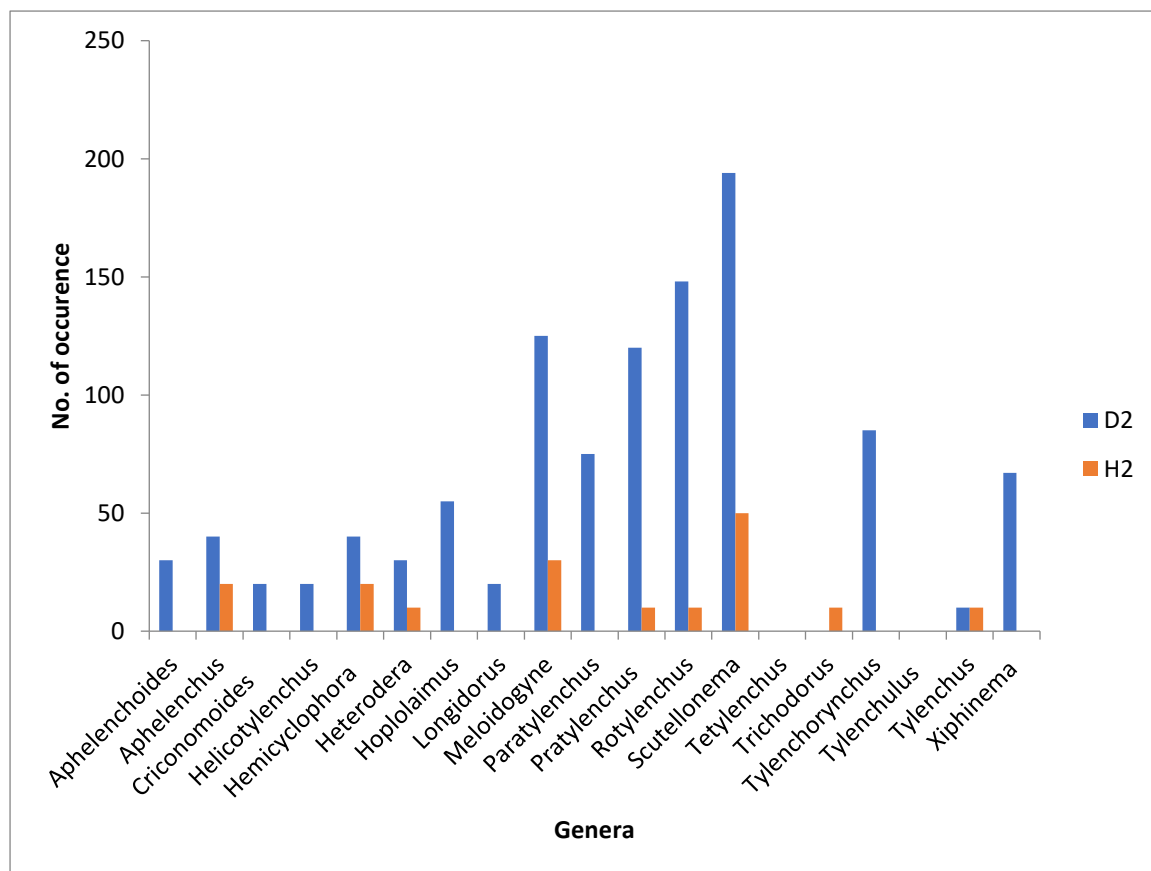


Figure 4: Occurrence of plant parasitic nematode in the diseased and apparently healthy root samples in farm 2
Key: D2 = diseased root and H2 = healthy root

Table 1: Diversity of Nematodes in the soil and root samples in Farm 1

Nematodes	SDS1	SDS2	RDS1	RDS2	SHS1	SHS2	RHS1	RHS2
Aphelenchoides	40	20	0	30	0	35	0	20
Aphelenchus	95	0	20	0	0	85	0	10
Criconomoides	0	20	0	10	0	0	0	0
Helicotylenchus	10	30	0	20	0	30	0	10
Hemicyclophora	50	30	10	30	0	0	0	0
Heterodera	10	20	0	35	45	20	0	20
Hoplolaimus	50	65	30	55	0	30	0	35
Longidorus	20	0	10	0	0	0	0	0
Meloidogyne	187	168	85	105	94	65	105	20
Paratylenchus	20	40	10	20	0	0	10	0
Pratylenchus	130	85	70	60	100	85	0	10
Rotylenchus	70	130	57	85	90	85	35	0
Scutellonema	208	195	40	135	155	135	85	0
Tetylenchus	10	10	0	0	40	0	0	0
Trichodorus	0	0	0	0	0	0	20	0
Tylenchorynchus	0	0	0	20	0	20	0	0
Tylenchulus	55	0	0	0	0	0	0	0
Tylenchus	30	0	20	0	65	0	20	0
Xiphinema	20	50	30	30	0	0	0	0
Dominance_D	0.120*	0.137*	0.136*	0.120*	0.170*	0.138*	0.269*	0.174*
Shannon_H'	2.374*	2.217*	2.160*	2.315*	1.855*	2.118*	1.495*	1.842*
Evenness_e^H/S	0.671*	0.706*	0.788*	0.779*	0.913*	0.831*	0.743*	0.902*

Key: * Mean value for soil and root samples. SDS= diseased soil sample; RDS = diseased root sample; SHS = apparently healthy soil sample; RHS= apparently healthy root sample.

Table 2: Diversity of Nematodes in the soil and root samples in Farm 2

Nematodes	SDS1	SDS2	RDS1	RDS2	SHS1	SHS2	RHS1	RHS2
Aphelenchoides	65	60	0	30	0	0	0	0
Aphelenchus	125	0	40	0	0	50	0	20
Criconomoides	45	20	0	20	0	0	0	0
Helicotylenchus	10	95	0	20	0	0	0	0
Hemicyclophora	120	90	0	40	0	0	0	0
Heterodera	35	68	0	30	20	10	10	0
Hoplolaimus	105	125	10	45	0	0	0	0
Longidorus	10	0	20	0	0	0	0	0
Meloidogyne	275	323	115	110	30	45	10	20
Paratylenchus	80	65	10	65	0	0	0	0
Pratylenchus	155	145	70	50	70	50	10	0
Rotylenchus	202	251	68	80	77	0	10	0
Scutellonema	332	386	112	82	135	97	35	15
Tetylenchus	10	0	0	0	0	0	0	0
Trichodorus	0	0	0	0	20	0	10	0
Tylenchorynchs	0	50	0	20	0	0	0	0
Tylenchulus	0	0	0	0	0	0	0	0
Tylenchus	120	0	10	0	40	0	10	0
Tylenchorynchs	65	0	65	0	0	0	0	0
Xiphinema	65	60	57	10	0	0	0	0
Dominance_D	0.099*	0.129*	0.135*	0.107*	0.211*	0.260*	0.202*	0.339*
Shannon_H'	2.505*	2.271*	2.135*	2.378*	1.728*	1.445*	1.790*	1.090*
Evenness_e^HS	0.720*	0.745*	0.769*	0.829*	0.804*	0.848*	0.855*	0.992*

Key: * Mean values for soil and root samples. SDS= diseased soil sample; RDS = diseased root sample; SHS = apparently healthy soil sample; RHS= apparently healthy root sample.



Plate 1: Scutellonema extracted from infected tomato plant



Plate 2: Meloidogyne extracted from infected tomato plant

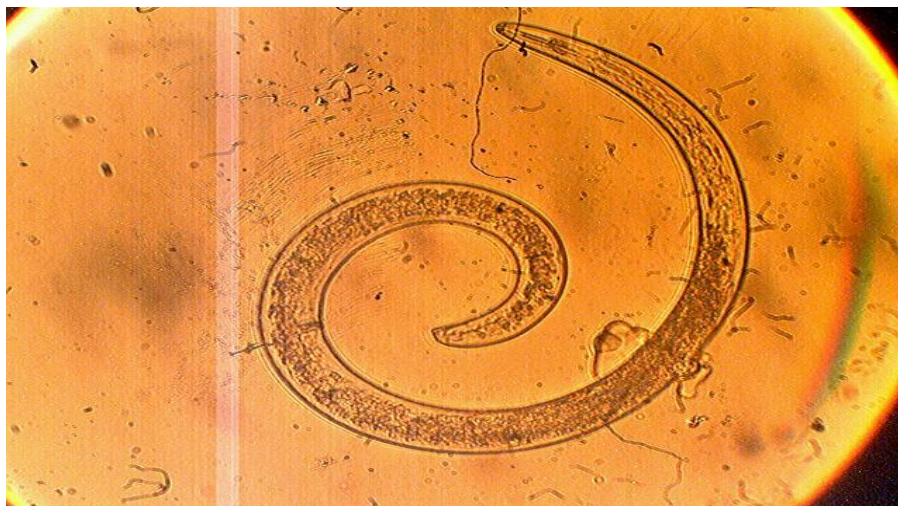


Plate 3: Rotylenchus extracted from infected tomato plant

Discussion

In this faunal study, a total of eighteen (18) genera of plant parasitic nematodes (PPN) were identified from both diseased soil and roots samples, twelve (12) from apparently healthy soil and nine (9) genera from apparently healthy root samples under dry season irrigated production. This contrast the work by Haougui et al. (2013), who reported eight (8) genera of plant parasitic nematodes associated with vegetables under rain fall condition. However, this study is similar to that of Bulus et al. (2016) and Zakari (2008) who reported twenty (20) and (13) genera of PPN associated with vegetables in Zaria. The variation observed in the number of PPN could be

due to the fact that the previous researchers sampled under sole cropping while this research was conducted based on prevailing farming system of mix cropping, as the usual practice of farmers in Giwa Local Government areas under irrigated cultivation.

The microscopic examination of the diseased root samples in this study revealed the presence of 19 genera of plant parasitic nematodes; among all, Meloidogyne and Scutellonema had the highest occurrence. This might be due to the less weather fluctuation, which might have favoured the active multiplication of these parasites. In addition, most of the *Solanum lycopersicum* sampled plants were found to exhibit

weak stemming with patches, yellowed leaves, and poor flowering. This could lead to poor yield and damage to the tomato crops. This is in line with Eisenback and Triantaphyllou (1991) who reported that tomato plants grown in warm climates experience severe losses from root knot nematodes (*Meloidogyne* spp). This could result in poor growth, decline in quality and yield of the crop and reduced resistance to other stresses leading to total crop loss.

The research reported the parasitic nematodes extracted from the roots and soil infested and apparently healthy tomato plants. *Meloidogyne* spp among others were found to possess a piercing needle structure in their mouth parts, which can penetrate the root cells of the plants. This structure might be responsible for sucking up cell contents of the tomato. The presence of these needle like structure reported in this study might serve as contributing factor that enable the nematode parasites to live mainly in soils and gain chance of hatching more eggs that may in turn contribute to attack and feeding on the roots of the tomato plants causing stunted growth, chlorosis and lesion. This work agrees with the work of Bawa et al. (2014) who reported that gradual increase of nematode eggs at their J2s in the tomato plants yield poor flowering set-up, leading to loose nutrients, with knots formed on the roots region. Tariq et al. (2007) reported approximately 75% reduction in tomato yield because of *Meloidogyne* and *scutellonema*.

The presence of *Aphelenchoides*, *Longidorus*, *Trichodorus* and *Xiphinema* was also reported. Perforation of leaf and fruits of the tomato was established in this study, the incidence of this parasites was investigated, but its pathogenicity was not fully determined. However, the parasitic nematodes examined and reported in this study might be responsible in facilitating the transmission of some plant viruses which cause some viral diseases thereby triggering the development of these parasitic nematodes. These nematodes feed on cell sap of infected plants causing damage to the plants (Leonetti et al., 2018).

The high population of *Scutellonema* in the diseased soil could be due to dry season (irrigation farming). In all cases, the population of nematodes in the soil samples was higher than that of root samples, probably, because most of the plant parasitic nematodes (PPN) are ectoparasites, they insert their stylet into the root, but do not go inside the roots, thereby increasing their abundance in the soil environment.

In this study, soil physico-chemical properties were not measured; however, it has been demonstrated that the abundance, trophic structure and population of soil nematodes depends on the soil properties such as organic matter content, temperature and salinity (Mills and Adl, 2011; Mateille et al., 2014). Based on our result, in all the sampling points the diversity of nematodes in the diseased soil rhizosphere was higher than that of the healthy soil, and this is consistent with the diversity of nematodes in the roots.

CONCLUSION

A total of eighteen (19) genera of plant parasitic nematode (PPN) were identified from the collected diseased samples of soil and root each. *Scutellonema*, *Meloidogyne*, and *Rotylenchus* had the highest population while *Criconomoides*, and *Longidorus* was the least occurred.

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