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Identification of plant-parasitic nematodes associated with *Citrus sinensis* L. Osebeck (sweet orange) in Benue State, Nigeria

§, [1]Adamu Maryam Yahaya, [1]Ochapa Charles, [1]Philip Peter and [2]Eche Christopher Oche

[1]Department of Crop Protection, Modibbo Adama University Yola, Adamawa State, Nigeria
[2]Center for Innovation in Procurement, Environmental and Social Standards,
Joseph Sarwuan Tarka University, Makurdi, Nigeria

[§]**Corresponding author:** Adamu Maryam Yahaya**. Email:** Maryamyayhyaadamu@gmail.com

Abstract

Plant-parasitic nematode (PPN) genera associated with rhizospheres of citrus trees was conducted in Ado and Gboko Local Government Areas of Benue State during May/June 2022 farming season. A total of sixty (60) rhizosphere soil samples each from the two (2) local government areas were collected at a depth of 10-15 cm using auger. The soil samples were then transported to Crop Protection Department Laboratory, Modibbo Adama University, Yola for extraction and identification at the generic level using the standard identification keys. Results showed a total of ten and eight genera of PPNs were identified from the soil samples collected from around the root of citrus trees in Ado and Gboko local government areas respectively. These genera, in a general descending order of occurrence frequency (FO %) were: Tylenchulus (59.00), Helicotylenchus (28.00), Pratylenchus (41.00), Rotylenchulus (25.00), Trichodorus (20.00), Melodogyne (19.00), Radopholus (14.00), Longidorus (11.00) Xiphinema (8.00) and Scutellonema. (6.00). Among the plant-parasitic nematodes identified, *Tylenchulus* spp. appears to be the most abundant in occurrence and population density on the citrus orchards surveyed in the two surveyed location with a frequency of occurrence of 59.00 and 52.00 and a population density of 924% and 532% in Ado and Gboko respectively. While Scutellonema and Rotylenchulu are the least frequently occurring nematodes in Ado and Gboko local government areas respectively. Based on the obtained result, it was concluded that the occurrence of PPN is probably because the local environmental and soil conditions are more suitable for their growth.

Keywords: Parasites, Nematodes, Citrus, Sweet orange, Benue State, Nigeria

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INTRODUCTION

Sweet orange (*Citrus sinensis* L. *Osbeck*) belongs to the family Rutaceae and sub-family Aurantioideae. It is regarded as the most widely grown, and most popular citrus species worldwide (Agnes *et al.,* 2019). It is widely cultivated in tropical and sub-tropical Africa and originated from South-East Asia. It was cultivated in China in 2500 B.C, where it was referred to as Chinese apple (Ehler, 2011). It was introduced in Europe. Citrus was introduced in Nigeria by the federal department of Agriculture colonial administration missionaries in 1930s, and spread in few counties, rated as the most widely planted fruit tree and rank as 9th major citrus producing country globally (Mebna *et al.,* 2017). In Mbah *et al*., (2018). Sweet orange ranked 3^{yd} position among tropical and subtropical fruits and second largest in terms of production (Mohammed*,* 2013). In Nigeria sweet orange is cultivated in sixteen (16) states namely, Benue, Cross River, Imo, Anambra, Osun, Ondo, Lagos, Ogun, Kwara, Oyo, Abia, Plateau, Kogi, Kaduna, Enugu and Bauchi state (*Mbah et al., 2018).* However, Benue State having the highest annual production of sweet orange according to (Avan and Uza, 2002).

Sweet oranges grow best on well-drained sandy loamy soils, with pH 6.5 – 7.5 at the temperature of 15 – 25^{O}C, and a rainfall of 50 – 75 cm, they are sensitive to waterlogging and require a considerable amount of sunshine. It is now grown almost all over the country and the world. Sweet orange is an important fruit in Nigeria, which can be eaten in fresh state or processed for its fresh squeeze juice, marmalade and/or fragment peel (Orwa *et al*., 2009). Sweet orange has high nutritional benefits, and is a major source of vitamin C, thiamin, niacin, folacin and minerals, magnesium and potassium. It also has medicinal value because it contains biologically active compounds such as liminoids, synephrine. These compounds have protective action against arteriosclerosis, cancer, kidney stones, and stomach ulcers and have been shown to reduce the cholesterol level. (Etebu and Nwauzoma, 2014).

The production of sweet orange is hampered by several pest and diseases. Among the various pest limiting successful production of sweet orange, is the plant parasitic nematodes (PPN). They are being regarded as the most damaging and destructive pests *(*Ferguson *et al.,* 2014*)* because of their wide host range. Plant parasitic nematode have been reported as a major constraint of Horticultural crop production in Nigeria as stated by Etebu and Nwauzoma *(*2014) and there is little and/or no study on the survey of plant parasitic nematode associated with sweet orange in Ado and Gboko of Benue State Nigeria. It is in that regard; this research work was conducted with the following objectives; first to determine the most prevalence Plant Parasitic Nematode of sweet orange (*Citrus sinensis L. Osbeck*) in the study area. And secondly to identify different plant parasitic nematodes genera associated with sweet orange (*Citrus sinensis L. Osbeck*).

MATERIALS AND METHODS

Study area

The survey has been carried out in Ado and Gboko local government areas of Benue State, (Plate V) exemplifies a tropical sub-humid climate, with two distinct seasons, wet and dry season. The wet season starts from April to October with annual rainfall in the range of 1120 to 1500mm. The dry season begins in November and ends in March (Alapa, 2019). Benue State lies within the lower river Benue trough in the middle belt region of Nigeria. Its geographic coordinates are longitude 7° 47’ and 10° 0' East. Latitude 6^{O} 25' and 8° 8' North; Temperatures range between 21 – 37^{O}c.

Method of sampling

Random sampling Technique was use for collecting soil sample from the various farms of each of the two local government areas, Ado and Gboko LGA.

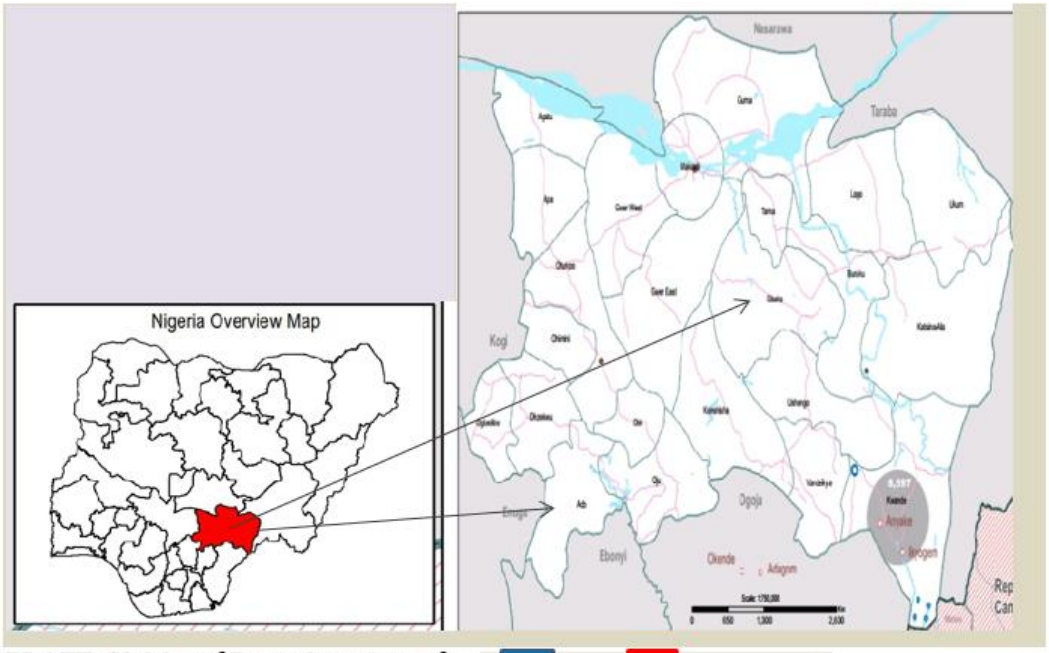

PLATE: V: Map of Benue State Area of the Study Ado and Gboko

SOURCE: Tier and LGA (2007)

Collection of soil samples for nematode extraction

Sixty (60) soil samples each from the two (2) local government areas was collected at a depth of 10 - 15cm using soil auger. Bulk together for each location to form composite soil sample. Each sample was placed in a polythene bag and properly labeled according to farm location. The soil (250cm) was then transported to Crop Protection Department laboratory, Modibbo Adama University Yola for microscopic studies.

Nematode extraction and identification from soil samples

Nematodes was extracted from soil samples using the modified Whitehead and Hemming (1965) tray method. These involved the use of two plastic sieves of same diameter with a double-ply tissue paper sandwiched in between were setup. The setup was later placed in a plastic bowl of a bigger diameter. Soil samples were thoroughly mixed to obtain homogenous composite.

Each sample were distributed into the setup and left undisturbed for 48 hours. The extracted nematodes were identified using a pictorial key to genera as described by Mekete *et al.* (2012). and counting of nematode genera were done under the microscope using a counting dish as a guide during nematode identification (Mekete *et al., 2012*).

Data collection

Data was collected based on prevalence of Plant Parasitic Nematode, frequency of occurrence and Nematode Population Density.

Data analysis

The data collected for frequency of occurrence (FO) was analyzed and calculated using the formula described by Wickham, (2016) the formula below.

$FO = q/Q \times 100$

Where q = Number of times an individual nematode occurred in the sample, Q = Sample size

while the data for Plant Parasitic Nematode population density was calculated using percentage population density (PPD).

PPD = INN/TNS × 100, where INN = Individual number of nematodes; TNS = Total number of nematodes extracted in the samples

RESULTS

Nematode genera, frequency of occurrence and population density of plant parasitic nematode in Ado LGA (250cm^3 soil)

The result for nematode genera, frequency of occurrence, and population density in Ado LGA is presented in Table 1. A total of ten (10) PPN genera were identified from soil samples collected in Ado LGA. These genera are; Tylenchulus, Helicotylenchus, Pratylenchus, Rotylenchulus, Trichodorus, Meloidogyne, Radopholus, Longidorus, Xiphinema, and Scutellonema. The result further shows that Tylenchulus recorded the highest frequency of occurrence (FO) (25.54%) and a population density (PPD) of (25.5%). This is followed by Helicotylenchus, FO (17.75%) and PPD (17.8%), Pratylenchus with (12.12%) and (12.1%) frequency of occurrence, and a population density respectively and the least PPN frequency of occurrence and population density were recorded in Scutellonema with (2.60%) frequency of occurrence and a population density of (2.6%) respectively.

Nematode genera, frequency of occurrence of plant parasitic nematode in Gboko LGA (250cm^3 soil)

The result for nematode genera, frequency of occurrence and population density in Gboko LGA is presented in Table 2. A total of eight (8) PPN genera were identified from soil samples collected in Gboko LGA. These genera are; Tylenchulus, Helicotylenchus, Pratylenchus, Meloidogyne, Radopholus, Scutellonema, Xiphinema and Rotylenchulus. The result in Table 2 further shows that *Tylenchulus* recorded the highest frequency of occurrence (31.14%) and a population density of (31.1%), followed by *Helicotylenchus,* with frequency of occurrence of (25.75%) and a population density of (25.8%). The least PPN frequently occurrence and population density was recorded in *Rotylenchulus,* with (3.59%) and (3.6%) frequency of occurrence, and a population density respectively.

Table 1: Frequency of occurrence and nematode population in Ado LGA

S/N	Nematode genera	Frequency of occurrence (%)	Nematode population (%) (250cm^3)
1	*Tylenchulus* spp	25.54	25.5
2	*Helicotylenchus*	17.75	17.8
3	*Pratylenchus*	12.12	12.1
4	*Rotylenchulus*	10.82	10.8
5	*Trichodorus*	8.66	8.7
6	*Meloidogyne*	8.23	8.2
7	*Radopholus*	6.06	6.1
8	*Longidorus*	4.76	4.8
9	*Xiphinema*	3.46	3.5
10	*Scutellonema*	2.60	2.6

Table 2: Frequency of occurrence and nematode population in Gboko LGA

S/N	Nematode genera	Frequency of occurrence (%)	Nematode population (%) (250cm^3)
1	*Tylenchulus* spp	31.14	31.1
2	*Helicotylenchus*	25.75	25.8
3	*Pratylenchus*	12.57	12.8
4	*Meloidogyne*	8.98	9.0
5	*Radopholus*	7.18	7.2
6	*Scutellonema*	5.98	6.0
7	*Xiphinema*	4.79	4.8
8	*Rotylenchulus*	3.59	3.6

DISCUSION

Plant Parasitic nematode genera, frequency of occurrence and population density in Ado LGA.

The result of this study shows the presence of ten (10) genera namely, Tylenchulus, *Helicotylenchus, Pratylenchus, Rotylenchulus, Trichodorus, Meloidogyne, Radopholus, Longidorus, Xiphinema, and Scutellonema* genera of PPN. These nematodes are known to have a very wide range of host distribution as they are known to reduce root weight, which translates into significant yield losses, (Korayem *et al.* 2015). Also, Taha (2018) showed the association of the nematode genera Meloidogyne, *Helicotylenchus, Pratylenchus, Rotylenchulus, Tylenchorhynchus, Paratylenchus*, and *Tylenchus* with vegetables including pepper in Shoubra El-Kheima, El-Qalioubia governorate, Egypt.

Tylenchulus appears to be the most abundant in occurrence and population density on the citrus orchards surveyed in Ado LGA of Benue State with a frequency of occurrence of 25.54% and 25.5% and a population density respectively. This report is in line with the work of Mokrini *et al.* (2018) whose survey work revealed the presence of *Tylenchulus semipenetrans* in citrus growing areas in Morocco. The following most prevalent PPN genera in Ado is recorded *Helicotylenchus* with (FO) of 17.75 and PPD of 17.8.

Plant parasitic nematode genera, frequency of occurrence and population density in Gboko LGA of Benue State

The results in Gboko LGA shows the presence of eight (8) PPN genera namely *Tylenchulus*, *Helicotylenchus, Pratylenchus, Meloidogyne, Radopholus, Scutellonema, Xiphinema* and *Rotylenchulus. Tylenchulus* recorded the highest frequency of occurrence and population density 31.14% and 31.1% respectively. These genera PPN association with citrus, but also for the ability of citrus trees to support very high populations before vigor declines and symptoms appear (Shanmugam *et al.*, 2021). The wide distribution of *Tylenchulus,* could be attributed to many factors including infected seedlings, contaminated plant material, organic fertilizers, irrigation, Temperature and machinery (Abd-Elgawad *et al.*, 2016).

In addition, the high variability of PPN genera observed in this study can be attributed to the variation in ecological and edaphic factors between and within the different locations studied (Nisa *et al.*, 2021). The second most prevalent plant-parasitic nematode was *Helicotylenchus,* recorded with 25.75 of (FO) and 25.8 (PPD) respectively in Gboko. (AS De and De Santos ,2002), and the least were recorded by Rotylenchulus with 3.57 frequency of occurrence and 3.6 population density. These nematodes have been reported to be one of the main pests limiting growth and yield production in Morocco as significantly reduce root weight, which translated into significant yield losses (Mokrin *et al.,* 2019).

CONCLUSION AND RECOMMENDATIONS

It can be concluded that ten genera of plant parasitic nematodes are found associated with sweet orange in Ado and Gboko LGAs of Benue State, Nigeria. The genera *Tylenchulus* was the most abundant nematode identified in the soil. These nematodes within the soils of citrus-growing areas, which helps to reduce the growth and yield of sweet orange due

to favorable or unfavorable environmental conditions such as temperature, soil texture, moisture content and relative humidity on the geographical study areas. There is need to educate farmers from these study areas on the dangers PPN posed on Agricultural crops (citrus). The study also suggests that serious management practices should be put in place to avert the future occurrence of these nematode on Ado and Gboko study locations.

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