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POPULATIONS OF PHYTO-PARASITIC NEMATODES ON GROUNDNUT (*ARACHIS HYPOGEAL)* CULTIVATED FIELDS IN EGBOLOM, ABUA/ODUAL LOCAL GOVERNMENT AREA OF RIVERS STATE, NIGERIA

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Abstract
This study was aimed at assaying soil and roots to determine the populations of phyto-parasitic nematodes associated with groundnut in Egbolom. Three groundnut fields monoculture designated site F1-F3 were randomly selected for the study. The soil was collected between 6-7:30 a.m. each day once a week in four mouths. Twenty-five soil and Twenty-five roots were collected from each groundnut field making a total of one hundred and fifty samples. The soil was collected using a hand trowel while a kitchen knife was used to collect roots. The sieve plats technique was employed for nematode extraction, and nematodes were identified by the use of a pictorial key. A total of 650 nematodes representing 11 genera were recovered from soil and root samples. *Meloidogyne* species 73 (16.3%) had the highest prevalence followed by *Helicotylenchus* species 65(14.5%) while *Tylenchus* species 2 (0.4%) showed the least prevalence in soil. However, *Pratylenchus* species was reported more often than any other species in roots with a total affluence of 51 (25.2%) followed by *Meloidogyne* species with 44 (21.8%) while *Tylenchus* species occur less than all other species with 10 (5.0%); an observation which is ascribed to a nematodes survival strategy. The result shows that soil nematodes are important pest of groundnut in Egbolom and should be considered in pest control programs.

Key words: *Arachis hypogeal*, Egbolom, Fields, Groundnut, Incidence, Parasitic Nematodes

Introduction

Groundnut is a significant vegetable crop grown locally for human consumption. Groundnut seeds are highly cherished worldwide because of their health significance to the human body. Jiang et al. (2002) and Kane et al. (2010) reported that groundnut seeds contain important nutrient which is required in small quantity for normal body functioning and development. The people of Egbolom grow groundnut because of its economic value. The economic value of the crop could be compared to that of palm trees. It constitutes a significant raw material for a variety of industries. Residues from groundnut extracted oil can be processed into peanut flour which in many localities could serve as feed for husbandry. The nut from groundnut is often used as a supplement for hunger in developing countries where deficiency is enormous (Wu et al., 2007; Kane et al., 2010). Groundnut is a valuable food that if properly packaged, could help in solving the problem of global food insecurity.

Groundnut farming in Nigeria has faced a variety of obstacles, the most significantly mentioned being infections by soil-dwelling pathogenic nematodes. Soil inhabiting phyto-parasitic nematodes are versatile, occurring in every cultivated field worldwide (McSorley, 2003; Abdel-Momen & Starr, 2004; Imafidor & Ekine, 2016; Ekine et al., 2018) at all conditions making their existence possible in every season of the year (Ekine et al., 2020). There is no doubt that about 35% of crop loss in Nigeria and West Africa could out-rightly be attributed to the actions of plant pathogenic nematodes (Imafidor & Ekine, 2016).

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The availability of nematodes in soil constitutes a threat to the farmer relying on soil produce for food. This is because nematodes never think in their assignment of causing waste to the farmer. They are efficient in degrading the immune system of plants hence paving the way for infections by related microbes which ordinarily would not have been able to parasitize the crop (Imafidor & Nzeako, 2010; Lambert & Bakal, 2009 Ekine et al., 2020).

Important species of these soil organisms have been reported in groundnut fields in West Africa with a species of notable effect being the root-knot nematode. (Imafidor, 2008; Hooks et al., 2010). Groundnut injuries due to infestation by Meloidogyne species have demonstrated more destructive potency than any other nematode species on the crop (Luc et al., 2012; Imafidor & Ekine, 2016) yet Radopholus species occurred more abundantly in groundnut field globally but the rate at which it could cause damage is not clear (Abdel-Momen & Starr, 2004). Nevertheless, a clear view of the nematode pattern of abundance under groundnut fields is necessary for developing a good cultural management technique for rural farmers. Therefore, this study is aimed at assaying the population assemblage of phyto-parasitic nematodes of groundnut (Arachis hypoea).

Materials and Methods

This study was carried out in three groundnut-cultivated fields designated as fields F1, F2 and F3 in the Egbolom community in Abua/Odual Local Government Area of Rivers State, Nigeria. In each field, the duration of fallow and type of previous crop cultivated was noted after having an interview with the farmers from each farm site. Site F1 is located at Latitude $4^{o}47^{l}21^{ll}$ N, Longitude $6^{o}40^{l}52^{l}$E, site F2 is located at Latitude $4^{o}49^{l}52^{ll}$ N, Longitude $6^{o}39^{l}15$ E and site F3 is located at Latitude $4^{o}50^{l}35$N, Longitude $6^{o}39^{l}11$E. The inhabitants of the Egbolom community are predominantly farmers and most of them produce groundnut as a source of income. They are third to Aminiboko and Owerewere in groundnut production in Abua. The area experiences an annual average rainfall range of 2000 – 2500mm and a temperature range of 25ºC – 32ºC which encourages rainforest vegetation type. Ethical approval for the collection of samples from the selected farms was sought from the farm owners. The groundnut stands uprooted were paid for. The Uwema of the Egbolom community was consulted and permitted bush entry. Soil samples were collected randomly from the rhizosphere of the groundnut in each field with the aid of a hand trowel. A total of fifty soil samples were collected from each groundnut cultivated field. Soil samples were collected from the crop rhizosphere at depths 0-15 cm in the early hours (6:30-7:30 am) once weekly for four months. The soil samples were packed into properly labelled waterproof bags and were transported to the laboratory for nematode extraction. In each field, ten groundnut stands were uprooted, and the roots were taken at the same time from the same location as for soil with the aid of a kitchen knife. The samples were placed into well-labelled polyethene bags and were transported to the laboratory for nematode extraction.

Nematodes were extracted using the sieve plate method (Imafidor & Ekine, 2016). The soil samples in each sample bag were poured into a 5-10cm plate and were thoroughly mixed. A measure of 10g of soil was taken. The 10g of soil was spread evenly on a circle of tissue paper supported on a plastic sieve standing on a plastic plate. Water was added to the extraction plate gently until the soil became wet but not immersed. The extraction set-ups were left undisturbed for 48 hours. After which the soil was removed and the supernatant discarded. The nematodes aliquot were emptied into clean grease-free specimen bottles and allowed to sediment fixed with 5 % formalin and stored for microscope view, 0.1ml of the nematodes aliquot were taken with a pipette placed on glass slides and observed using x4 and x10 objectives of light microscope.

The groundnut roots from each sample bag were thoroughly washed in tap water to remove soil particles and cut into 2cm segments before removing a 5g fresh mass sub-sample. The 5g sub-sample of the root was macerated in an electric blender for 10-20 seconds at low speed. Each macerated sub-sample of the root was spread evenly on a piece of tissue paper supported on a plastic sieve standing on a plastic plate. Water was added to the plate until the samples were wet but not immersed. The set-up was left undisturbed for 48 hours. The root samples were removed, and the nematode suspension was poured into clean specimen bottles fixed with 5% formalin and stored. Nematodes aliquot 0.1ml was taken with a pipette placed on a glass slide and examined for nematode species using the x4 and x10 objectives of a light microscope. Nematodes were identified using the light microscope of x4 and x10 objectives, and identification was done using a pictorial key according to Siddiqi (2000) and Mekete et al. (2012).

The analysis of data was done using analysis of variance (ANOVA) in SPSS and Shannon Weiner's index (H= pi x lnpi, $E = H/H_{maxi}$).

Results

Percentage abundance of soil nematodes in groundnut

Plant Parasitic nematodes were reported in all the samples viewed in this study with a total of 448 nematodes from 11 species and genera. The nematode species encountered in this study were *Gracilachus* spp., *Meloidogyne* spp., *Heterodera* spp., *Radopholus* spp., *Helicotylenchus* spp., *Criconema* spp., *Pratylenchus* spp., *Rotylenchus* spp., *Scutellonema* spp., *Tylenchus* spp., and *Ditylenchus* spp. Among the 448 nematodes recovered, 140 (31.25%) were encountered in Farm site F1 while Farm site F2 reported 91 (20.3%) and 217 (48.4%) were extracted in Farm site F3. The individual nematode recovered in farm site F1 were *Gracilachus* species 26 (18.6%), *Meloidogyne* species 27 (19.3%), *Heterodera* species 24 (17.4%), *Radopholus* species 8 (5.7%), *Helicotylenchus* species 21 (15.0%), *Criconema* species 6 (4.3%), *Pratylenchus* species 16 (11.4%) and *Rotylenchus* species 12 (8.6%). Site F2 recorded *Heterodera* species as the most frequently encountered nematode with 26 (28.6%) followed by *Ditylenchus* species with 15 (16.5%) while *Tylenchus* species were the least in occurrence with 2 (2.2%). Other species also recorded in F2 were *Gracilachus* species 11 (12.0%), *Pratylenchus* species 11 (12.0), *Meloidogyne* species 10 (10.9%), *Helicotylenchus* species 10 (10.9%) and *Scutellonema* species 6 (6.6%). In site F3, 217 (48.4%) were extracted among which *Meloidogyne* species show the highest occurrence with 36 (16.6%) closely followed by *Helicotylenchus* species with 34 (15.6%) while *Ditylenchus* species had 30 (13.8%) and *Scutellonema* species show the least population with 12 (5.5%). Also reported in farm site F3 were *Radopholus* spp., *Gracilachus* spp, *Rotylenchus* spp, *Criconema* spp, *Pratylenchus* spp and *Heterodera* species which had 24 (11.1%), 20 (9.2%), 19 (8.8%), 16 (7.4%), 13 (5.9%) and 13 (5.9%) respectively.

Table 1: Population of plant nematode of groundnut in Egbolom

Nematode species	Farms			Total		
	F1 (%)	F2 (%)	F3 (%)		F	Sig
					3.773	.035
Gracilachus spp	26 (18.6)	11 (12.0)	20 (9.2)	57 (12.7)		
Meloidogyne spp	27 (19.3)	10 (10.9)	36 (16.6)	73 (16.3)		
Heterodera spp	24 (17.4)	26 (28.6)	13 (5.9)	63 (14.1)		
Radopholus spp	8 (5.7)	0	24 (11.1)	32(7.1)		
Helicotylenchus spp	21 (15.0)	10 (10.9)	34 (15.6)	65 (14.5)		
Criconema spp	6 (4.3)	0	16 (7.4)	22 (4.9)		
Pratylenchus spp	16 (11.4)	11 (12.0)	13 (5.9)	40 (8.9)		
Rotylenchus spp	12 (8.6)	0	19 (8.8)	31 (6.9)		
Scutellonema spp	0	6 (6.6)	12 (5.5)	18 (4.0)		
Tylenchus spp	0	2 (2.2)	0	2 (0.4)		
Ditylenchus spp	0	15 (16.5)	30 (13.8)	45 (10.0)		
Total	**140 (31.25)**	**91 (20.3)**	**217 (48.4)**	**448 (75.1)**		

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Root nematodes of Groundnut

The roots of groundnut reported nematodes across the farm sites where samples were collected for bioassay. A total of 202 nematodes from 6 species and genera were found occurring in this study. Among these 49 (24.3%) were extracted in site F1 while farm site F2 showed 65 (32.2%) nematodes and 88 (43.6) species were recovered from site F3. The nematode species reported were *Pratylenchus* species with the highest species abundance of 51 (25.2%) closely followed by *Meloidogyne* species with 44 (21.8%) while *Helicotylenchs* species, *Radopholus* species, *Ditylenchus* species, Rotylenchus species and *Tylenchus* species were 30 (14.9%), 24 (11.9%), 22 (10.8%), 21 (10.4%) and 10 (5.2%) respectively.

Table 2: Population of root nematode of groundnut in Egbolom

Nematode species	**Sampled Farms**			**Total**	**F**	**sig**
	F1 (%)	**F2 (%)**	**F3 (%)**		**.710**	**.505**
Meloidogyne spp	15 (30.6)	7 (10.8)	22 (25.6)	44 (21.8)		
Ditylenchus spp	8 (16.3)	2 (3.1)	12 (1.4)	22 (10.8)		
Pratylenchus spp	13 (26.5)	32 (49.2)	6 (6.8)	51 (25.2)		
Rotylenchus spp	7 (14.3)	14 (21.5)	0	21 (10.4)		
Tylenchus spp	0	2 (3.1)	8 (9.1)	10 (5.0)		
Radopholus spp	0	0	24 (27.3)	24 (11.9)		
Helicotylenchus spp	6 (12.2)	8 (12.3)	16 (18.2)	30 (14.9)		
Total	**49 (24.3)**	**65 (32.2)**	**88 (43.6)**	**202(24.9)**		

The actual incidence of phyto parasitic nematodes of groundnut in Egbolom

In this study, the sum of 650 nematodes was reported to occur in the root rhizosphere and root tissues of groundnuts. Four hundred and eight nematodes representing 75.1% were reported in soil (root rhizosphere) among which 189 (29.1%) were found in site F1 while156 (24.0%) nematode species were extracted from site F2 and site F3 yielded a total of 305 (46.9%) species. However, 202 (24.9%) nematodes were recovered from the root tissues of groundnuts across the farm sites where samples were collected in the study with 24.3%, 32.2% and 43.6% representing percentage extraction from sites F1, F2 and F3 respectively.

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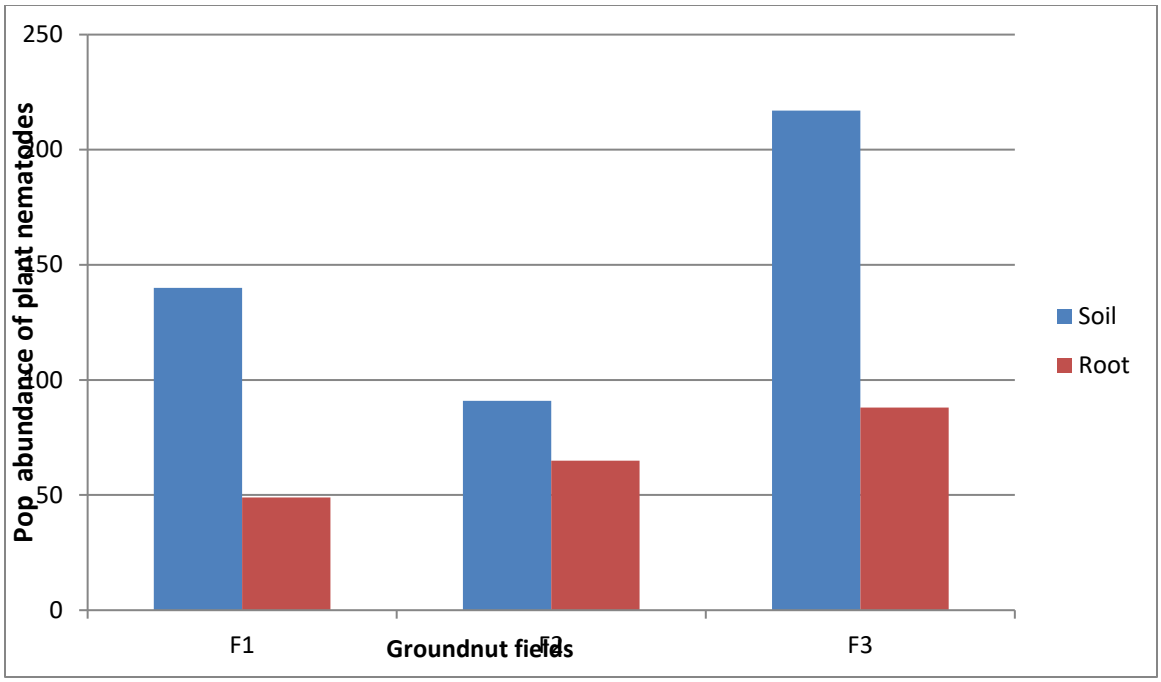

Fig 1: Actual incidence of phyto parasitic nematodes of groundnut in Egbolom

Diversity of phyto-parasitic nematodes of groundnut in Egbolom

Prominent phyto-parasitic nematodes recovered in this study were *Meloidogyne* species 117 (18.0%) i.e. 73 and 44 representing extraction from soil and roots respectively. Following *Meloidogyne* species was *Helicotylence* species 95 (14.6%), showing 65 in soil and 30 in roots. *Pratylenchus* had 40 and 51 (91 (14%) from soil and roots respectively. The least occurred nematode in this was *Tylenchus* species 12 (1.8%). Other Phyto-parasitic nematodes extracted were *Heterodera* species 63 (9.7%), *Gracilachus* species 57 (8.8%), *Radopholus* species had 56 (8.6%), *Rotylenchus* species were 52 (8.0%), *Ditylenchus* species 67 (10.3%), *Criconema* species were 22 (3.4%) and *Scutellonema* species had 18 (2.8%).

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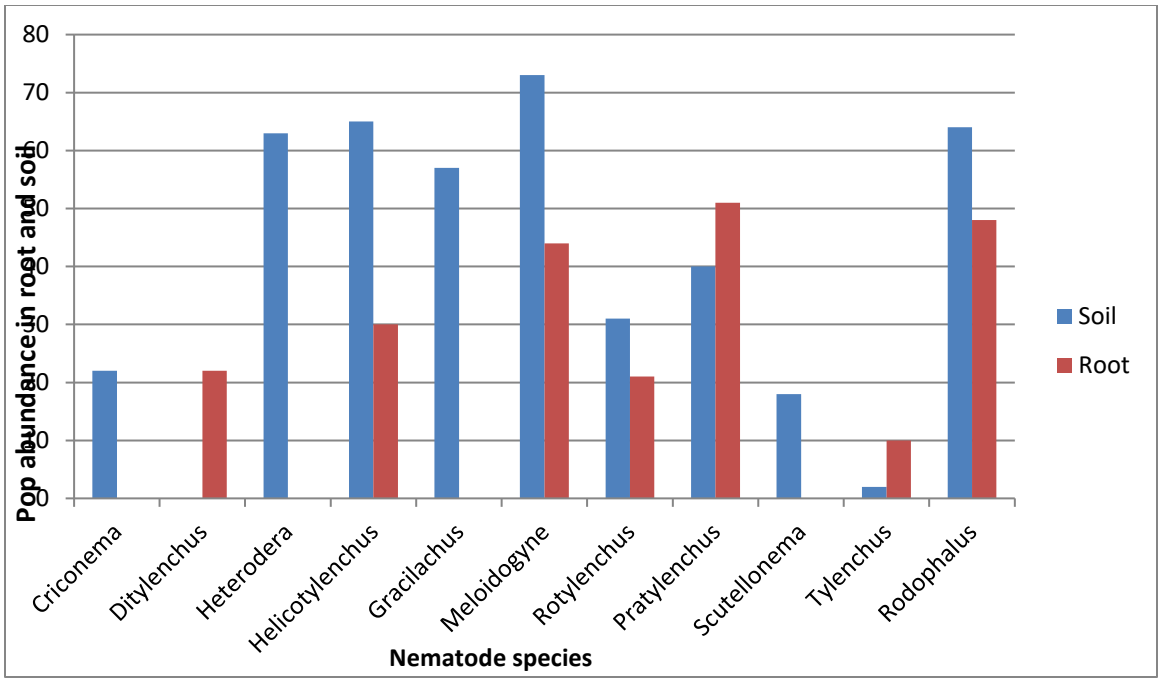

Fig 2: Diversity of phyto parasitic nematodes of groundnut in the study

Discussion

Eleven phyto-parasitic nematode genera were reported across the three groundnut fields sampled in Egbolom. These nematodes were unevenly distributed in terms of species' actual incidence and profusion, occurring in all the groundnut fields where soil and roots were collected and analysed. The nematodes so recovered in this study have been reported in many parts of the globe where groundnuts are cultivated including Nigeria (Barker et al., 2000; Chen et al. 2004). The occurrence of phyto-parasitic nematodes in all the sites in this study could be attributed to favourable environmental conditions experienced in Egbolom. This result suggests that phyto-parasitic nematodes are true pests of ground in Egbolom. Ekine et al. (2018) reported that nematodes are capable of surviving in every cultivated field with pleasant soil conditions most commonly in Africa.

The most prevalent species of photo-parasitic nematode encountered in this study was the *Meloidogyne* species (18.0%) followed by *Helicotylenchs* species (14.6%) while *the Tylenchus* species (1.8%) recorded the least in abundance (Fig 2). In a similar study, Cheryl et al. (1992) recorded higher populations of *Pratylenchus* species than any other nematode elsewhere in the world. This observed difference could be attributed to the location and season of research. The abundance of *Meloidogyne* in the present study could be ascribed to the versatile nature of the genus. This observation conform with that of Imafidor and Ekine (2016) who state that *Meliodogyne* is versatile in nature and could survive in every agricultural field not minding season. There were variations in the distribution of nematodes in soil and roots across the groundnut cultivated fields from site to site. More nematodes were reported from site F3, 217 (48.4%) followed by site F1 which recorded 140 (31.25%) species while 91 (20.3%) were found occurring in site F2. This result indicates that nematode actual incidence in soil is unpredictable and could be influenced by unapparent factors in soil. The variation in the distribution of nematodes could be attributed to nematode feeding patterns and survival strategies.

The distribution of root nematodes in this study followed a similar pattern as seen in soil such that site F3 showed the highest assemblage 88 (43.6%) while site F2 had 65 (32.2%) and 49 (24.3%) were reported from site F1. This observation was found to be statistically significant ($p< 0.05$). The uneven distribution of root nematodes in this study could be attributed to edaphic factors (Agbaje et al., 2002).

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Site F3 with the highest number of nematodes has a corresponding history of continuous monoculture of groundnut. This observation depicts that the cropping system is a significant factor contributing to the profusion of phyto-parasitic in a given field. The result also confirmed that continuous monoculture facilitates nematode buildup in soil and may increase nematode infection rate and damage extend to crops. Elsewhere Wu et al. (2007) reported a high prevalence of soil nematodes in groundnut monoculture. The assemblage of nematodes in site F2 with a record of ten years of fallow was relatively low (20.3 %) when compared with the occurrence in sites F1 and F3. This observation suggests that fallowing as a farming technique could help in bringing down nematode populations in fields and could enhance crop yield. It also depicts that frequent disturbance of soil by continuous cultivation does impact positively on nematode reproduction and abundance in soil. In separate studies, Agu (2008) reported relatively low populations of nematodes in fallowed sites.

The disparity in nematode affluence about the parameter sampled was noticed such that more nematodes were reported in soil 488 (68.9%) while 202 (31.1%) were reported in roots. This observation could be ascribed to nematode nutritional affiliation and survival strategy. This result disagrees with Cadet et al. (2003), Abdel-Momen and Starr (2004), Imafidor and Nzeako (2008) and Ekine et al. (2020). *Aphelenchiodes* species has always been reported as a major pest of groundnut in the tropics of Africa and Asia (Cheryl et al, 1992; Wu et al., 2007; Ingham & Dettling, 2012), but is missing in the present study; an observation which suggests that the inhabitation of plant parasitic nematodes in a place is dynamic and unpredictable. Shannon Weiner's index analysis showed a general possible relationship between the fields investigated and the occurrence of nematode species across the fields in the study areas. Nematodes diversity in this study were 2.853, 2.219 and 2.076 for sites F1, F2 and F3, and evenness was were1.239,0.925 and 0.901, for sites F1, F2 and F3 respectively.

Conclusion

The result of this study suggests that *Meloidogyne* species is a significant pest of groundnut pods in Egbolom. It also depicts that cultural systems such as bush fallowing impact negatively on nematode reproduction and profusion while continuous cropping systems encourage nematode buildup and enhance infection rate on groundnut.

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