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Population distribution of *Helicotylenchus species* on *Parkia biglobosa* (Jacq) Benth and its association with some horticultural crops in Southern Guinea Savanna Ecological Zone of Nigeria

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Abstract

The African locust bean (*Parkia biglobosa* Benth) is a perennial, deciduous fruit tree that is important for its myriad medicinal and nutritional benefits. The association of the spiral nematodes of the genus *Helicotylenchus* spp. with *P. biglobosa* has not been previously reported in Nigeria. Three *P. biglobasa* trees from University of Ilorin in the Guinea Savanna Ecological zone of Nigeria were purposively selected for nematode sampling for a period of five months (May to September). Eighteen field and horticultural crops were also surveyed to assess the population density of *Helicotylenchus* spp on selected agricultural crops in the local environment. Soil samples were collected monthly from the rhizosphere of *P. biglobosa* trees and also from the field crops to a depth of about 15 cm and within a 25 cm radius from the base of the plants. Vermiform nematodes were extracted from 250 g each of the composite samples using a modified Baermann extraction tray set-up. The spiral nematodes were frequently encountered in association with all the crops. Higher soil population of *Helicotylenchus* spp was recorded on *Celosia argentea*, *Colocasia esculenta* and *Azadirachta indica* at relative densities of 55.33, 42.11 and 25.6, respectively. The African locust bean trees also supported population build-up of *Helicotylenchus* spp which were found at a frequency rating of 100% in all the soil samples. Higher soil population of *Helicotylenchus* spp were recorded in June and September, coinciding with the two rainfall peaks while lower nematode population was recorded in August, at the lowest ambient temperature**.** The study indicated spiral nematodes as abundant and often associated with many agricultural crops at University of Ilorin, Guinea savanna of Nigeria. *P. biglobosa* was a suitable host for *Helicotylenchus* spp while the rainfall pattern and temperature changes influenced the population distribution of soil nematodes in the local environment.

Key words: *Helicotylenchus* spp, *Parkia biglobosa*, population distribution, rainfall pattern, temperature changes, horticultural crops, *Celosia argentea*, *Colocasia esculentum*, *Azadirachta indica*, frequency rating, nematodes, Nigeria

Introduction

Nematodes are among the most successful group of invertebrates that can live freely in fresh or salt water and also in the soil, feeding parasitically on plant roots. They remain one of the most important groups of organisms limiting production and causing economic losses to horticultural and field crops in the developing countries (Stirling and Pattison, 2008: Ravichandra, 2008).

Parkia biglobosa (Jacq) Benth is an economically important fruit tree belonging to the plant family Fabaceae - Mimosoideae. It is popularly known as the African Locust Bean tree which is an important source of a local food condiment (Dadawa) that is indispensable in many African dishes (Egwim *et al.,* 2013). It is also a traditional medicinal plant, having being found useful for the treatment of ailments and diseases such as diabetes, malaria, pneumonia, bronchitis, stomachaches, severe cough, diarrhoea, piles, and arterial hypertension (Millogo-Kone *et al*., 2008; Builders *et al*., 2012). Extracts from the plant also possess anti-plasmodial, antibacterial, antimicrobial, anti-venom and nematicidal properties (Ajayeoba, 2002; Udobi *et al.,* 2008).

The African locust bean tree is widely distributed all over the grass land of Northern Nigeria and has been found to occur in the Guinea Savanna region of the country as well (Oyenuga, 1968; Simeonyan, 2012). Large quantities of the *P. biglobosa* seeds are also produced in the Savanna region of Oyo and Kwara states of Nigeria (Odunfa and Adewuyi, 1985; Farayola *et al*., 2012). Despite the health and nutritional benefits obtainable from the African locust bean tree, *P. biglobosa* is host and susceptible to many pests and diseases including plant-parasitic nematodes.

Microscopic in size but destructive in action, plant-parasitic nematodes are tiny but mighty enemies of horticultural crops. Estimated overall average annual yield loss recorded on important horticultural crops worldwide due to damage by these nematodes has been put at about 13.54 % (Reddy, 2008). Also the monetary loses when all crops were considered, exceeded a staggering amount of $100 billion annually (Sasser and Freckman, 1987; Ravichandra, 2008). Spiral nematodes of the genus *Helicotylenchus* are among the most ubiquitous plant-parasitic nematodes associated with agricultural crops worldwide (Crow, 2012) and have been identified as one of the top ten economically important genera of phytonematodes associated with horticultural crops (Ravichandra, 2008).

In Nigeria, eighty-two species of plant parasitic nematodes have being reported (Caveness, 1967) with large population of *Helicotylenchus* species found in association with some tree and horticultural crops, among which are: cocoa (*Theobroma* *cacao*), oil palm (*Elaeis guinensis),* coconut (*Cocos nucifera*), rubber (*Hevea brasiliensis*), kola (*Cola nitida*), pawpaw (*Carica papaya*), teak (*Tectona grandis*), mango (*Mangifera indica*), banana (Musa sp), citrus (*Citrus sp*), pineapple (*Ananas comosus*), date palm (*Phoenix dactylifera*), and breadfruit tree (*Artocarpus communis*). More recent reports have revealed that plant parasitic nematodes impose losses of up to 70% on plantains and cooking bananas in Africa with about 31-50% reduction in crop yield due to root and plant damage caused by *Helicotylenchus dihystera* and *H. multincintus* in association with *Meloidogyne* spp, *Pratylenchus coffeae* and *Radopholus similis* (Speijer *et al.,* 2001; Rotimi *et al*., 2004; Tripathi *et al*., 2015).

Although the population dynamics and seasonal fluctuations of phytonematodes associated with some crop plants have been reported (Nath *et al*., 1988; Adekunle, 2009; Sayed *et al*., 2014), very little or no information is available on the association of *Helicotylenchus* species with the African locust bean tree in Nigeria. A good knowledge of nematode-host relationship and an understanding of the population dynamics of specific nematodes on plants is crucial for planning nematode control programmes. The present study seeks to provide an update on the association of *Helicotylenchus* species on some important field and horticultural crops on typical guinea savanna ecology in Nigeria and also to explore the population distribution of the nematodes on *P. biglobosa* in the local environment over a period of five months.

Materials and methods

Site Description: The studies on the population distribution of *Helicotylenchus* species on the African locust bean trees (*P. biglobosa*) and its association with some agricultural crops were carried out on the main campus of The University of Ilorin, (Longitude 4°35'E, Latitude 8°29'N) at an approximate altitude of 306 m a.s.l. Ilorin is about 500 km South-West of Abuja, the Federal Capital City of Nigeria, and is located in the Southern Guinea Savannah ecological zone of Nigeria. The city experiences two seasons: the dry (November to February) and the rainy season (March to October) with a mean annual rainfall of about 1222 mm. The mean monthly temperature ranges between (25 and 29 °C).

Nematode survey of agricultural crops in the local environment: Soil samples were collected from eighteen field and horticultural crops. They include; mango (*Mangifera indica*), coconut (*Cocos nucifera*), cashew (*Anarcardium occidentale*), and guava (*Psidium guajava*) which were collected from an orchard at the Catholic Chapel on the University; tomato (*Solanum lycopersicum*) and pepper (*Capsicum fructescens*) from a farmland behind the chapel; banana (*Musa* sp.), cocoyam (*Colocasia esculentum*) and amarantus (*Amarantus* sp.) were collected from a farmland behind the University Health Centre; pawpaw (*Carica papaya*), and cassava (*Manihot esculenta*) from a farmland behind the Post Graduate School; shea butter (*Butyrosperimum parkii*) and neem (*Azadirachta sapientum*) from the front of the statistical department while the soil sample under oil palm (*Elaeis guinensis*) was collected from the Faculty of Agriculture.

The soil samples were collected with the aid of a hand trowel after digging to a depth of about 5-10 cm and were kept in black polythene bags, which were tied loosely to promote aeration. The soil samples were labeled according to the crop type, date and site of collection and preserved at room temperature.

Collection of soil samples from the rhizosphere of *Parkia biglobosa*: Three African locust bean trees, located in the University of Ilorin main campus were purposively selected for this study. Soil samples were collected monthly from the rhizosphere of three locust bean trees for a period of five months (May to September). Digging to the root zones of the trees, the soil samples were collected in black polythene bags with the aid of a hand trowel, labeled appropriately and preserved in loosely tied polythene bags at room temperature.

Extraction of nematode from soil samples: The modified tray method of Whitehead and Hemming (1965) was used for the extraction of the nematodes. About 250 g of the soil sample was placed in a 10 litre plastic bucket which was half filled with water. The soil crumbs were carefully broken with hand and the mixture gently stirred to allow the soil particles to settle for about 60 seconds before pouring the supernatant with the nematode suspension into another 10 litre bucket. This process was repeated twice. Nematodes were extracted from the soil samples by pouring the supernatant over a bank of sieves which have been arranged according to their mesh sizes as described by Coyne *et al*. (2007). This nematode suspension was then poured over the modified Baerman Extraction set-up and collected free of debris after twenty-four hours.

Nematode preservation: The nematode suspension from the extraction tray was collected in a 250 mL beaker and allowed to settle for about four hours before decanting to reduce the volume and later transferred into a glass petri dish for observation under a stereoscopic microscope. The nematodes were killed slowly by gentle heat. Many nematodes were observed to straighten out as they die while the *Helicotylenchus* species take a characteristic spiral shape. The nematodes were then preserved in a formaldehyde solution of about 2-3 drops of 5% formalin contained in specimen bottles.

Nematode identification and counting: The nematode density in each specimen bottle was determined by pouring the nematode suspensions into a counting dish that was placed under a stereoscope microscope. With the aid of a tally counter, the population density of the total nematodes on each crop was determined. Also the number of the spiral nematodes from each sample was recorded.

Relative density and absolute frequency were calculated as follows:

$$\text{Relative Density} = \frac{\text{Number of individual of a specie in a sample}}{\text{Total number of individuals in a Sample}} \times 100$$

$$\text{Absolute Frequency} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

Statistical analysis: The data on nematode population was subjected to analysis of variance and the means partitioned by Duncan's multiple range tests at 5% level of probability.

Result

Population density of nematodes associated with some field and tree crops: High population of parasitic and non-parasitic nematodes was recorded on the selected field and horticultural crops on the main campus of University of Ilorin, Nigeria. The crops include: *Mangifera indica*, *Vigna unguiculata*, *Cocos nucifera*, *Capsicum fructescens*, *Solanum lycopersicum*, *Celosia argentea*., *Sorghum bicolor*, *Zea mays*, *Psidium guajava*, *Elaeis guinensis*, *Butyrospermum parkii*, *Carica papaya*, *Azadirachta indica*, *Amaranthus* sp., *Musa* sp., *Anarcardium occidentale*, *Manihot esculenta* and *Colocasia esculenta* (Table 1).

Helicotylenchus (Fig. 1) was recorded and occurred in large numbers in all the eighteen crops sampled. Higher nematode density was recorded on *Celosia argentea*, *Colocasia esculenta* and *Azadirachta indica* at relative population densities of 55.33, 42.11 and 25.6 respectively (Table 1). Lower population of the spiral nematode was observed on *Zea mays* (0.02%) on the University campus.

Population distribution of nematodes associated *Parkia biglobosa*: Total number of nematodes, including the parasitic and non parasitic ones that were recovered from the rhizosphere of *P. biglobosa* was highest in the month of August. Low nematode population was recorded in the month of May. There is, however, no significant difference in the total population of nematodes recovered from the trees during the five months of study (Table 2).

Population distribution of spiral nematodes associated with *Parkia biglobosa*: Population of the genus *Helicotylenchus* associated with *P. biglobosa* was significantly high in the month of September while low nematode population was observed in August (Table 3). There is however no significant difference in the population of nematodes recorded in August and May.

Population of distribution of other associated nematodes on *Parkia biglobosa*: There was a significant increase in the population of other associated nematodes (apart from the spiral nematodes) that were recorded on the African locust bean trees in the month of August (Table 4). Significantly lower nematode population was recorded in May, however no significant difference was observed in the nematode population for June and July.

Frequency of occurrence and relative density of the spiral nematodes associated with *Parkia biglobosa*: The frequency rating and population density of the spiral nematodes associated with the African locust bean trees is given in Table 5. The nematodes recorded in the soil samples at a frequency rating of 100% during the period of study. Low nematode density of the spiral nematodes (3.20%) was recorded in August while higher density of 15.71% was observed in September.

Table 1. Population density of *Helicotylenchus* sp. associated with some agricultural crops in Ilorin, Guinea Savanna ecological zone of Nigeria

Crop (Soil root)	Total nematode population /250 mL of soil	Spiral nematode population	Relative density of spiral nematode /250 mLof soil (%)
Mango (*Mangifera indica*)	1182	58	4.91
Cowpea (*Vigna sinensis*)	3154	421	13.35
Coconut (*Cocos nucifera*)	1354	31	2.30
Pepper (*Capsicum fructescens*)	813	69	8.24
Tomato (*Solanum lycopersicum*)	2076	473	22.78
Celosia (*Celosia sp.*)	3596	1987	55.33
Guinea corn (*Sorghum bicolor*)	1458	86	5.90
Maize (*Zea mays)*	450	8	0.02
Guava (*Psidium guajava*)	1106	99	8.95
Oil palm (*Elaeis guinensis*)	1139	28	2.46
Shea butter (*Butyrospermum parkii)*	723	23	3.18
Paw paw (*Carica papaya*)	761	137	18.01
Neem (*Azadirachta indica*)	617	158	25.6
Amaranthus (*Amaranthus* sp.)	1280	99	7.73
Banana (*Musa* sp.)	1103	89	8.07
Cashew (*Anarcardium occidentale*)	591	35	5.92
Cassava (*Manihot esculenta*)	756	96	12.70
Coco yam (*Colocasia esculenta*)	2299	968	42.11

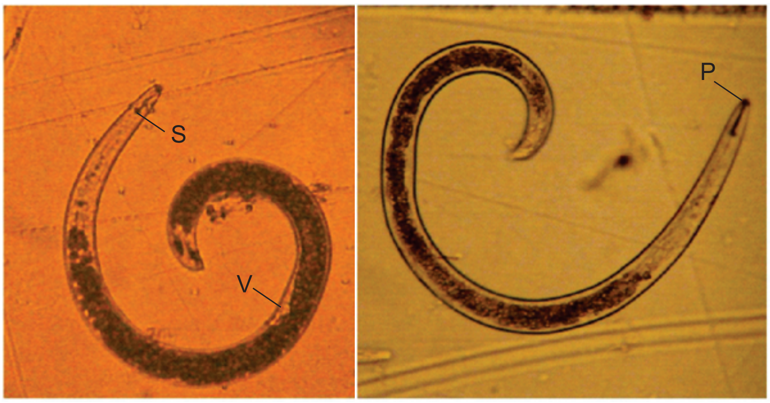

Fig. 1. Micrographs of *Helicotylenchus* sp. found in association with *Parkia biglobsa.* S = stylet, V = vulva, located around 60% of the body length from the anterior terminus, *P*= Projection on the ventral terminus

Effect of rainfall pattern and temperature changes on nematode population of *Parkia biglobosa* in the local environment: The effect of rainfall pattern and the changes in temperature on the soil population of nematodes recorded on *P. biglobosa* in Ilorin, Guinea savanna ecological

Table 2. Nematode population on the locust bean trees

Month	Nematode Population	Mean Nematode Population
May	1871	623.67[a]
June	2776	923.33 [a]
July	3137	1045.67 [a]
August	4534	1511.33 [a]
September	2711	903.67 [a]

*Means followed by the same alphabet in each column are not significantly different. [*P*=0.05]

Table 3. Mean population of spiral nematodes/250mL of soil under the locust bean trees

Month	Nematode population	Mean nematode population
May	197	65.7b
June	265	88.3[ab]
July	225	75.0[b]
August	145	48.3[b]
September	426	142[a]

*Means followed by the same alphabet in each column are not significantly different. [*P*<0.05]

Table 4. Mean population of other associated nematodes on the locust bean trees

Month	Nematode Population	Mean Nematode Population
May	1674	557.97^{b}
June	2511	837.03^{ab}
July	2912	970.67^{ab}
August	4389	1463.03^{a}
September	2285	761.67^{ab}

*Means Followed by the same alphabet in each column are not significantly different. [$P<0.05$]

Table 5. Monthly relative density, absolute frequency and prominence value of the spiral nematodes associated with *Parkia biglobosa* on University of Ilorin Main Campus

Month	Relative density (%)	Absolute frequency (%)	Prominence value
May	10.53	100	105.3
June	9.55	100	95.5
July	7.17	100	71.7
August	3.20	100	32.0
September	15.71	100	157.1

zone of Nigeria during the period of study is depicted in Fig. 2 and 3. Two rainfall peaks were recorded in June and September. The environmental temperature varied between 24.6 ^{o}C to 27.5 ^{o}C. The lowest temperature was also recorded in August.

Discussion

The results of this investigation provide an update on the association of *Helicotylenchus* species with diverse agricultural crops of importance in Nigeria. Caveness (1962) gave a cursory report on the association of *Helicotylenchus* species with some important agricultural crops from the North, East and Western regions of Nigeria. In the present study, the highest population of the spiral nematodes was recorded under *Celosia argentea*. This falls in line with the observations of Babatola and Oyedunmade (1992) where large numbers of *Helicotylenchus* sp were associated with *C. argentea* in University of Ilorin, Nigeria.

Large numbers of the spiral nematode was also recorded on *Celocasia esculentus*, *Vigna unguiculata* and *Solanum lycopersicum.* A similar report was observed by Sawadogo *et al.* (2009) where the genus *Helicotylenchus* was implicated as one of the important nematode genera having significant parasitic potential on *V. unguiculata* based on their frequency and abundance. Osei *et al.* (2012) had also noted that high population of *Helicotylenchus* spp are associated with *S. lycopersicum* in the Ashanti, Brong Ahafo and Upper East regions of Ghana. The result of this study also indicates that high numbers of *Helicotylenchus* species were associated with banana in the guinea savanna ecological zone. *H. dihystera* and *H. multincinctus* has been reported as a major pest of banana and plantain, causing significant reduction in crop yield in Nigeria and globally (Quénéhervé, 1989; Nath *et al.,* 1998; Sayed Abdul Rahman *et al.,* 2014; Olaniyi, 2014). Subbotin *et al.* (2011) has described the genus *Helicotylenchus* as globally distributed and associated with the root system of diverse groups of plants in

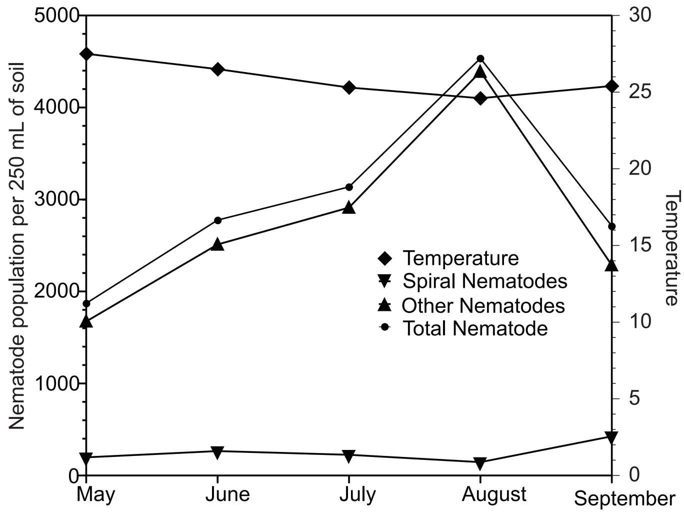

Fig. 2. Nematode population as affected by the ambient temperature in the local environment

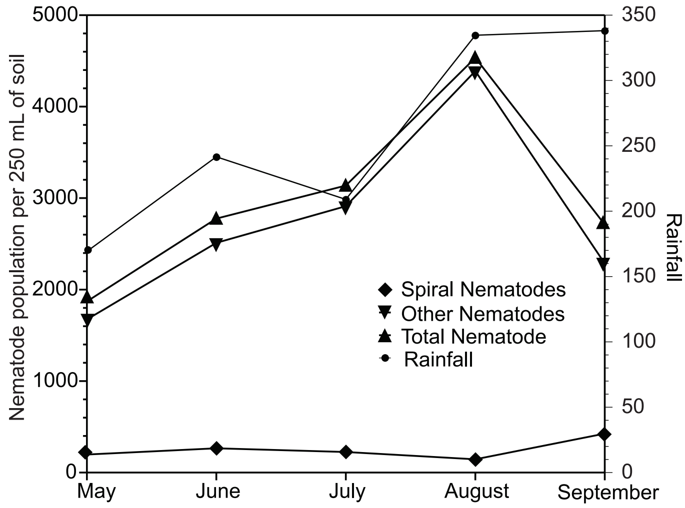

Fig. 3. Nematode population as affected by the amount of rainfall in the local environment

cultivated and uncultivated areas. Thus, there is every indication that these cosmopolitan nematodes are widespread, abundant and have a wide host range of crops in the Guinea savannah ecological zone of Nigeria.

Although, damage resulting from the feeding activities of *Helicotylenchus* spp. on plant roots is often neglected by farmers and most times not reported (Nicol *et al*., 2002), in recent times, many nematode species which were previously considered unimportant and inconsequential are now being considered as pests and damaging on fruit and horticultural crops (Nicol, 2002; Ravichandra, 2008). Therefore, the association of *Helicotylenchus* sp. with important agricultural crops should be given more in depth focus and damage potentials be carefully evaluated.

The African locust bean tree is a perennial, deciduous tree that is hardy and can often survive long periods of drought without being easily affected by desiccation. It has a capacity to withstand drought conditions because of its deep tap root system and an ability to restrict transpiration (Orwa *et al*., 2009). It therefore holds great potential to function as a reservoir for many endo- and semi-endoparasitic nematodes that could have otherwise being negatively affected by unfavorable environmental and climatic conditions.

The association of *Helicotylenchus* spp. with *Parkia biglobosa* has not been previously reported in Nigeria. In the present investigation, the spiral nematodes occurred and were frequently found in association with African locust bean trees throughout the five months of study. This indicates that *P. biglobosa* is a suitable host of *Helicotylenchus* spp. and can support population build-up of the nematode. The present investigation also demonstrates the effects of temperature changes and rainfall pattern on the population distribution of plant parasitic nematodes on *P. biglobosa* over the study period of five months. This indicates that temperature and soil moisture are inseparable factors that co-influence the distribution of nematodes in the soil. The result of this study shows that the rainy season, during which the study was conducted, supported high population of soil nematodes and thus was favorable for the survival of the nematodes found in association with locust bean trees at the University of Ilorin.

The total nematode population was observed to increase progressively from May to August. The increase in soil moisture as the rain became more established which was accompanied by a decline in the ambient temperature could have contributed to the build up in the nematode population experienced in the first four months of the investigation. The decline in the soil population of the nematodes recorded in September however could have resulted from the increase in the ambient temperature for that month. A similar report by Rodriguez *et al.* (1986) showed that nematode population increases during the vegetative stage of crops and thereafter, declines during maturity stage. They further reported that nematode population increased at lower soil temperature and decreased at higher air and soil temperature. Avhad *et al.* (2014) also demonstrated that soil temperature and pH shows a negative correlation on nematode population. Inactivity of most nematodes often occurs at high temperatures, however increase in egg hatch and activity following quiescence is expected when rainfall follows a long dry spell as indicated in the present study.

The spiral nematodes were encountered in all the soil samples throughout the five months of investigation. Their population density on *P. biglobosa* however varied with changes in the amount of rainfall and temperature experienced in the months. Significantly higher population of *Helicotylenchus* spp was recorded in June and September which coincides with the rainfall peaks recorded during the period of study. A similar observation in the population fluctuation of *H. multicinctus* had been made by Quénéhervé (1989) who reported that fluctuations in number of *H. multicinctus* and soil showed several peaks coinciding with heavy rainfall periods.

Helicotylenchus species are semi-endoparasites that can pose a threat to *P. biglobosa* because of their slow debilitating effects which often go unnoticed but can eventually result in gradual decline of trees. Also their feeding effects can cause damage to plant roots, thus facilitating infection and creating disease complexes with other pathogens. Annual crop losses caused by plant-parasitic nematodes are estimated as 8.8-14.6% of total crop production and 100-157 billion USD worldwide (Sasser and Freckman, 1987; Koenning *et al*., 1999; Nicol *et al*., 2002; Singh *et al*., 2013) and actual losses may be higher due to unavailability of data from some countries.

The available information on the population distribution of spiral nematodes on *P. bigloba* from the present study is insufficient to establish a damage potential, but adequate enough to suggest that *P. biglobosa* is a suitable host that can support population build-up of *Helicotylenchus* spp over time. Thus, it is important to embrace a nematode management strategy for optimum production. Further research to investigate the population dynamics of *Helicotylenchus* on *P. biglobosa* for an extended period of time is essential to establish an effective management regime. It is also imperative to assess the actual level of damage and economic losses inflicted by plant parasitic nematodes on African locust bean trees.

The results of this investigation confirm the association of *Helicotylenchus* spp with *P. biglobosa* and some field and horticultural crops at University of Ilorin, Guinea Savanna ecological zone of Nigeria. The study also suggests that that the same environmental conditions that favor crop growth and development can also influence the soil population of nematodes associated with the plant roots, thus posing a threat to the development and overall productivity of agricultural crops.

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