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Inventory of plant parasitic nematodes of cultivated solanaceae in Côte D'ivoire: case of tomato (*Solanum lycopersicum* L.)

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Abstract

Vegetable crops in Côte d'Ivoire, particularly tomato crops, are attacked by several pests, especially nematodes that cause yield losses. This study's objectivewas to uncover the plant-parasitic nematodes of tomato that remain unknown to Ivorian market gardeners. Therefore, a study on the diversity of the tomato-associated nematode population was conducted in six of the seven agro-ecological zones of Côte d'Ivoire as defined by Halle and Bruzon. Fifty-seven soil and tomato root samples were taken from fields in the surveyed localities at 10 to 30 cm depths. Nematodes in the soil samples were extracted using the modified Baerman method. Those contained in the root samples were extracted by the centrifugal flotation method. After extraction, the nematodes were counted and identified. The analysis of diversity revealed five genera of parasitic nematodes associated withtomato plants: *Meloidogyne, Radopholus, Pratylenchus*, which are endoparasites, and *Rotylenchulus* and *Helicotylenchus*, which are ectoparasites.The genus *Meloidogyne* was the most frequent and most abundant of all these, given its prevalence in all the surveyed localities. The localities that recorded the highest nematode densities were Yamoussoukro (YAK), Seguela (SEG), Songon (SON), and M'Batto (MBA). This study points to the need for effective and sustainable control strategies to prevent significant yield declines in tomato crops.

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Introduction

In Côte d'Ivoire, vegetable crops occupy a negligible proportion (3.3%) of all agricultural households (Coulibaly *et al.*, 2002). However, tomato is proving to be an important market garden crop, given its impact on the market gardeners' economy. It is one of the most prized foodstuffs by Ivorian households (Soro *et al.*, 2015).

Indeed, the annual need for tomatoes is estimated at more than 10,000 tons of fresh fruit. It is the second most important fruit vegetable after the local variety of eggplant "N'drowa" (*Solanum aethiopicum* L.) among the vegetables in Ivorian markets (Ildefonse, 1995). However, tomato crop, like almost all market garden crops, is the target of many pests and pathogens (Youdeowei, 2004). Among these pathogens are nematodes. Nematodes have remained unknown to growers whointerpreted their effects in terms of "reduced soil fertility."Nematodes cause considerable damage and yield reductions on a large number of crops (Potter *et al.*, 1993; Koenning *et al.,* 1999). The damages depend on the soil nematode population density, which varies according to climatic and soil conditions and cropping practices (Scotto la Massese, 1986). The thresholds of nematode damage on different crops in various parts of the world and Côte d'Ivoire are mostly unknown. The estimation of danger associated with the presence of nematodes remains approximate. Some of these pathogens are also vectors for viruses (Fauquet *et al.,* 1987). The high genetic and pathogenic diversity of nematode strains makes the control of these pathogens more complex. The chemical-related resistance in nematode developed depends on the environmental conditions and the strains present in each culture area (Opena *et al.,* 1989).

The present study's objective is to make an inventory of the plant-parasitic nematodes associated with tomato cultivation and to evaluate their densities, diversities, and structures through surveys in the different market gardening zones in Côte d'Ivoire. These surveys will help understand the risks related to their introduction or spread to protect against them by setting up effective and sustainable control methods.

Materials and methods

Study site

Surveys were carried out in six of the seven agro-ecological zones of Côte d'Ivoire (Table 1) described by Halle and Bruzon (2006).

Technical equipment: Sampling tools consisted of a hand trowel, scissors, knives, pruning shears, polyethylene bags, permanent markers, labels, and a GARMIN GPS device.

Sampling

Soil sampling was carried out in the planting lines of the surveyed sites in the rhizosphere from the 10 to 30 cm layer of the soil according to the method of Tabarant (2013). From a surveyed field, 10 samples were randomly collected at various points to form a composite sample. Ten tomato plants were taken from each field for root collection during the surveys, and at least two fields were visited in each locality. 39 root samples were collected from 27 localities, and 18 soil samples were collected from 15 localities.

The total number of samples collected in the different localities was 57.Once all 57 samples were tagged with the date and location of collection and transferred to the laboratory for analysis.

Nematode extraction

The modified Baerman method (1971) was used to extract nematodes from soil. Soil samples were homogenized in a tank and crumbled by hand stirring. Aggregated soil particles were crushed and ground for complete homogenization. Then, the various samples were cleaned of plant debris and stones. Finally, 50 g of soil was taken per sample for nematode extraction.

Fifty (50) g of each soil sample were placed on a 1 mm diameter sieve, previously covered with filter paper. The sieve-soil assembly was then placed in a tray into which tap water was carefully poured until the soil sample was moistened. This allowed the nematodes to migrate to the bottom of the tank as they were able to leave their usual site and move downwards when placed in an aqueous medium. The soil-containing sieve was then placed in the dark (at room temperature) for 48 h to maintain the nematodes in the culture conditions. The sieve was then removed, and the water from the tray was transferred to a beaker, left to rest for 2h so that the nematodes would settle to the bottom of the tray. Finally, the supernatant was carefully removed, and the resulting suspension was examined under an optical microscope.

Nematode extraction from the roots was then carried out using the centrifugal-flotation method described by Sarah and Boisseau (2008) using a 10 g of previously washed and blended roots from a composite sample of 10 plants for each sampled locality.

Nematode enumeration and identification

2 ml of nematode suspension was collected and placed in a counting slide and examined under a light microscope. The number of nematodes obtained was standardized by the nematode suspension's initial volume and the soil volume. For each nematode genus, each locality's root infestation rate is expressed as the number of individuals per 100 g of fresh roots.

The identification and enumeration of individuals of each nematode genus were carried out under an AMSCOPE optical microscope equipped with a camera according to morphological and morphometric criteria based on the identification key of Siddiqi (2000) by Hunt *et al.* (2005) and Mekete *et al.* (2012).

Statistical analyses

Analysis of variance from statistica Version 7.1softwarewas computed on nematode density, abundance, and occurrence. DUNCAN test at the 5% significance level followed in specifying the different significant groups of nematode genera based on the same parameters.

Results

Inventory of the nematodes extracted from the surveyed localities

After extraction, the plant-parasitic nematodes' densities and composition present in the sampled localities were determined. Analysis of roots and soil samples revealed five genera of plant-parasitic nematodes belonging to the same order, namely Rhabditida. The five genera are *Meloidogyne*, *Radopholus*, *Pratylenchus*, *Rotylenchulus,* and *Helicotylenchus*. Of these collected plant-parasitic nematodes, three are endoparasitic, and two are ectoparasitic (Table 2).

Table 1. Prospected localities and their different characteristics adapted from Halle and Bruzon (2006).

AEZ	Locality	Characteristics	Altitude (m)	Average rainfall (mm)	Annual temperature in °Celsius (SD)
I	Azaguié, Adiaké Gagnoa, Agnibilékro, Bonoua, Azodpé, Dabou, , Hiré, Agou, songon, Divo, N'zikro	Dense humid forest zone in the south	0-200	1400-2500	29 (5,6)
II	Fresco, Sassandra, Méagui, Bouaflé, Zatta, Issia, Gabiadji, Soubré, Tabou	Dense humid forest zone in the west	~1000	1300-1750	23,5 (13,4)
III	Bangolo, Guiglo	Semi-mountainous forested area in the west	> 1000	1300-2300	24,5 (7,7)
IV	Yamoussoukro, Kotobi, Bongouanou, Dimbokro, Arrah, Prikro, Daoukro, M'batto, Tanda	Area of semi-deciduous dense humid forest	0-200	1300-1750	23,5 (1,.4)
V	Bouaké, Seguéla	Transition forest zone	300-600	1300-1750	23,5 (13,4)
VI	Boundiali, Katiola, Niakara, Ferkessédougou, Nassian,	Humid tropical savannah zone	300- 500	1150-1350	26,7 (1,1)
VII	-	Dry tropical savannah zone	300-500	1150-1350	26,7 (1,1)

The density of plant-parasitic nematodes in roots and soils

Densities (p< 0.001) varied strongly and significantly from one locality to another at the root level. Indeed, Yamoussoukro (YAK) and Séguéla (SEG) localities recorded the highest densities with averages of 277,083.3 and 258,266.7 individuals, respectively. The lowest density was observed in the locality of Adiaké (ADI) with 1,466.7 individuals. Intermediate densities ranged from 13,333.33 to 39,000 individuals (Fig. 2). A significant difference (p<0.000001) was observed between densities in Odiénné (ODI), (7,200 individuals) and Songon (SON), (93,333.5 individuals) for soil-extracted plant-parasitic nematodes. Localities such as M'Batto (MBA), (39,000 individuals) and N'Zikro (NZI), (31,500individuals) showed the highest densities. Intermediate densities values were also recorded and ranged from 7,733.33 individuals for Prikro (PRI) to 26,133.33 individuals for Agboville (AGB) (Fig. 3).

Table 2. Composition of the plant-parasitic nematodes identified in the surveyed localities.

Order	Family	Genus	Trophic type
Rhabditida	Meloidogynidae	*Meloidogyne*	Endoparasite
	Pratylenchidae	*Radopholus*	
		Pratylenchus	
	Hoplolaimidae	*Rotylenchulus*	Ectoparasite
		Helicotylenchus	

Population structure of tomato plant-parasitic nematodes

Concerning the population structure of tomato plant-parasitic nematodes, 5 genera of nematodes were analyzed in terms of frequency and relative abundance. The genera revealed after nematological analysis of the roots and soil of the different localities are *Meloidogyne, Radopholus, Pratylenchus, Rotylenchulus,*and *Helicotylenchus*.

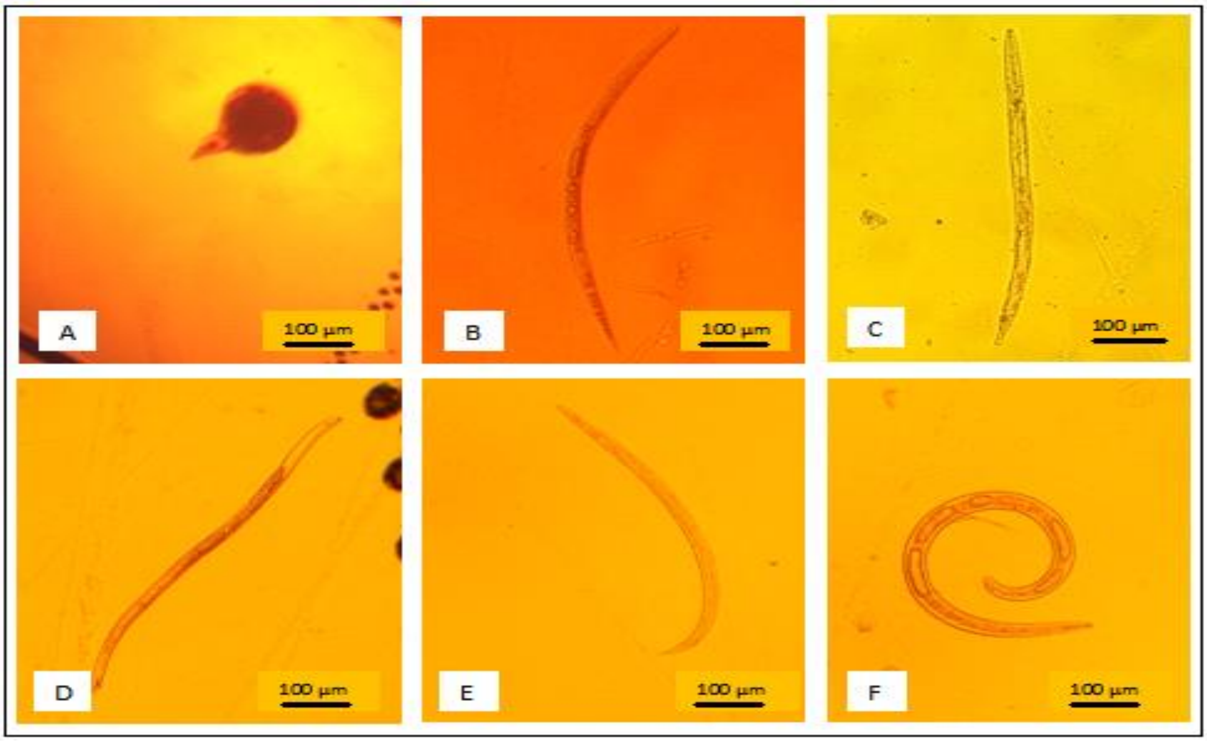

Fig. 1. Nematodes belonging to the genera *Meloidogyne*, *Radopholus*, *Pratylenchus*, *Rotylenchulus* and *Helicotylenchus*.

A: Meloidogyne (female individual); B: Meloidogyne; C: Radopholus; D: Pratylenchus; E: Rotylenchulus; F: Helicotylenchus.

In the collected roots, the genus *Meloidogyne* was the most frequent with an abundance of 100% in almost all localities. Genera such as *Pratylenchus*, *Rotylenchulus*, and *Helicotylenchus* were almost absent or infrequent in some localities such as Gabiadji (GAB), Issia (ISS), Bongouanou (BON), and Niakara (NIA) (Fig. 4).

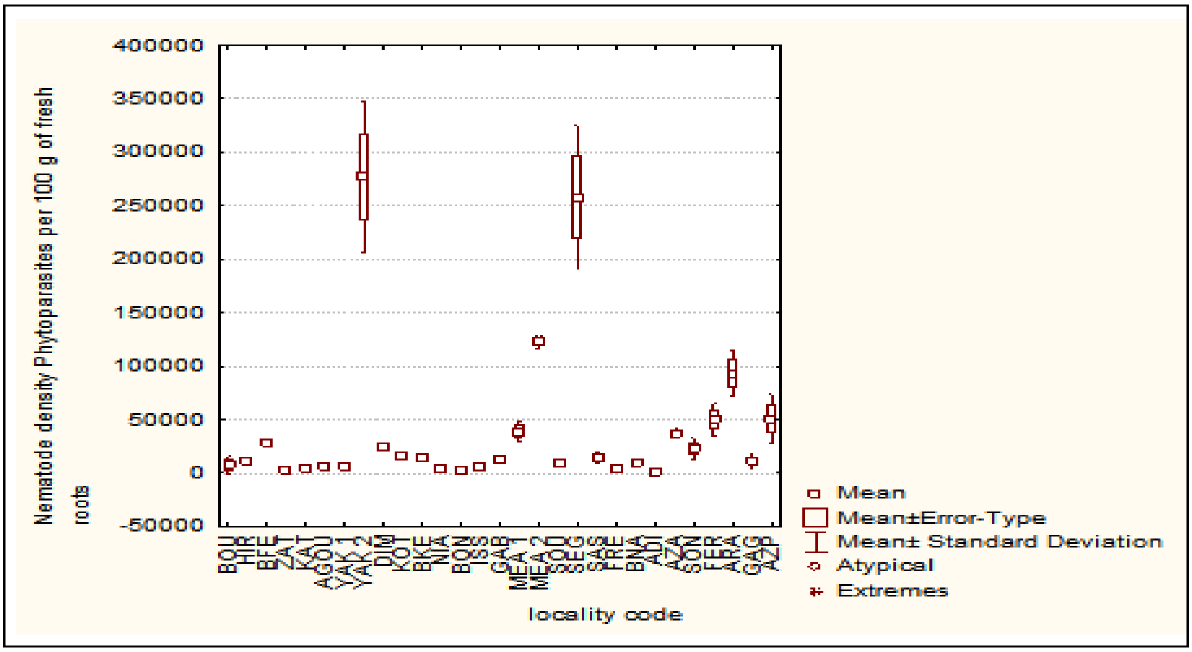

Fig. 2. Densities of plant-parasitic nematodes extracted from the roots of the plots in the surveyed localities.

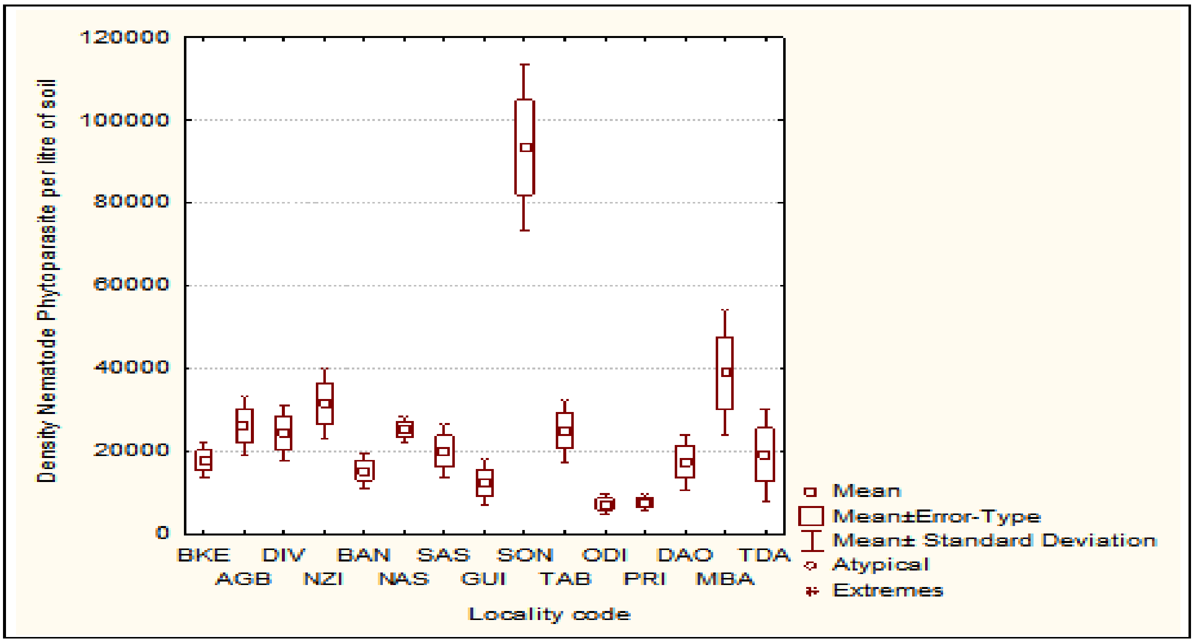

Fig. 3. Densities of plant-parasitic nematodes extracted from the soil in the plots of the surveyed localities.

The genus *Radopholus* was relatively frequent compared to the other 3 genera *Pratylenchus*, *Rotylenchulus,* and *Helicotylenchus*, with an occurrence varying from 25 to 100% from one locality to another (Fig. 5). Some localities such as Yamoussoukro, Azaguié (AZA), Séguéla (SEG), and Gagnoa (GAG) recorded all 5 genera of nematodes with always the genus *Meloidogyne* being the most frequent with 100% occurrence. The soil samples analysis also revealed 5 genera of plant-parasitic nematodes with a dominance of the genus *Meloidogyne* which recorded a Frequency of 100%. As for the genera *Pratylenchus*, *Rotylenchulus*, and *Helicotylenchus*, they were infrequent and absent in some localities such as Bouaké (BKE), Odienné (ODI), and Tanda (TDA) (Fig. 5). Also, localities such as SONGON (SON), Agboville (AGB), and N'Zikro (NZI) recorded all five genera of nematodes.

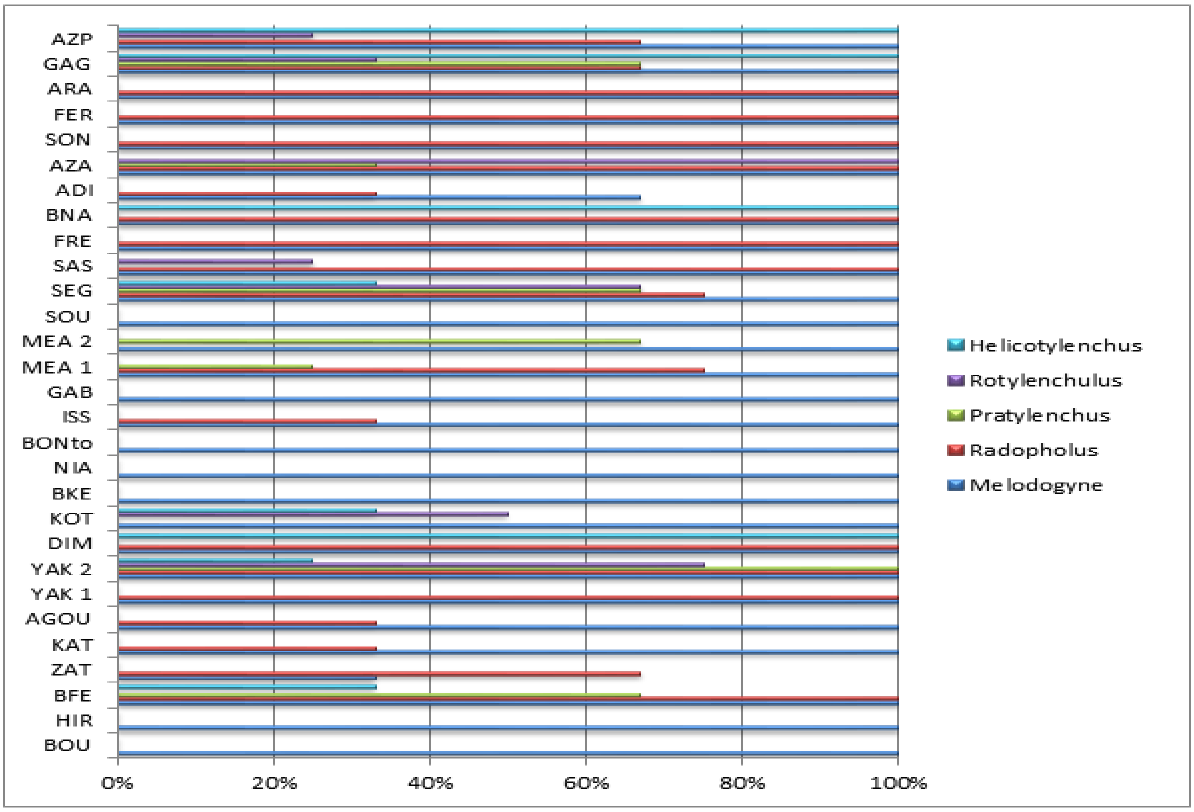

Fig. 4. Frequency of plant-parasitic nematodes extracted from the roots of surveyed localities.

Average relative abundance

The average relative abundance of the 5 genera of nematodes is represented with unevenly distributed prevalences in the root samples. Indeed, the genus Meloidogyne was the most abundant, with 89% in the surveyed localities. Radopholus reached 8% abundance, while Pratylenchus, Rotylenchulus, and Helicotylenchus obtained 1% (Fig. 6).

Concerning the soil samples, the Meloidogyne genus was the most abundant, with a percentage of 74%. However, this percentage of the genus Meloidogyne in the soil samples remained lower than that of the roots,which was 89%. The genus Radopholus recorded 15%, the genus Pratylenchus 7%, the genus Rotylenchulus 3%, and the genus Helicotylenchus 1% (Fig.7). Concerning soil samples, the relative abundance of the different genera of plant-parasitic nematodes decreased compared to the relative abundance of that observed in the roots collected in the different localities.

Discussion

The analysis of variance shows a significant difference in the densities of plant-parasitic nematodes in root and soil samples.Indeed, Yamoussoukro's localities, Séguéla (SEG) for the roots and Songon (SON), M'Batto (MBA) for the soil recorded high average nematode densities because the localities are important market gardening areas. In this regard, if these localities had high nematode densities, their soil's temperature and texture are conducive to developing these plant-parasitic nematodes (Halle and Bruzon, 2006). Also, it has been revealed that in these localities, the soil types encountered are moderately and weakly denatured ferralitic types with a sandy texture, except for the locality of Séguéla (SEG), which has a ferruginous type soil (Ettien, 2004). As for the locality of Songon (SON), the soil is of ferralitic type with sandy texture (Djidji *et al.,* 2010). This sandy texture could explain the proliferation of these nematodes in these localities. Indeed, Estioko and Reyes (1984) attest that the nematode species present in sandy soils are often different from those found in clay soils, as microporosity is a major factor affecting their movements, particularly. Also, Reddy (1983) points out that sandy soils are the most favorable for the developmentof plant-parasitic nematodes.

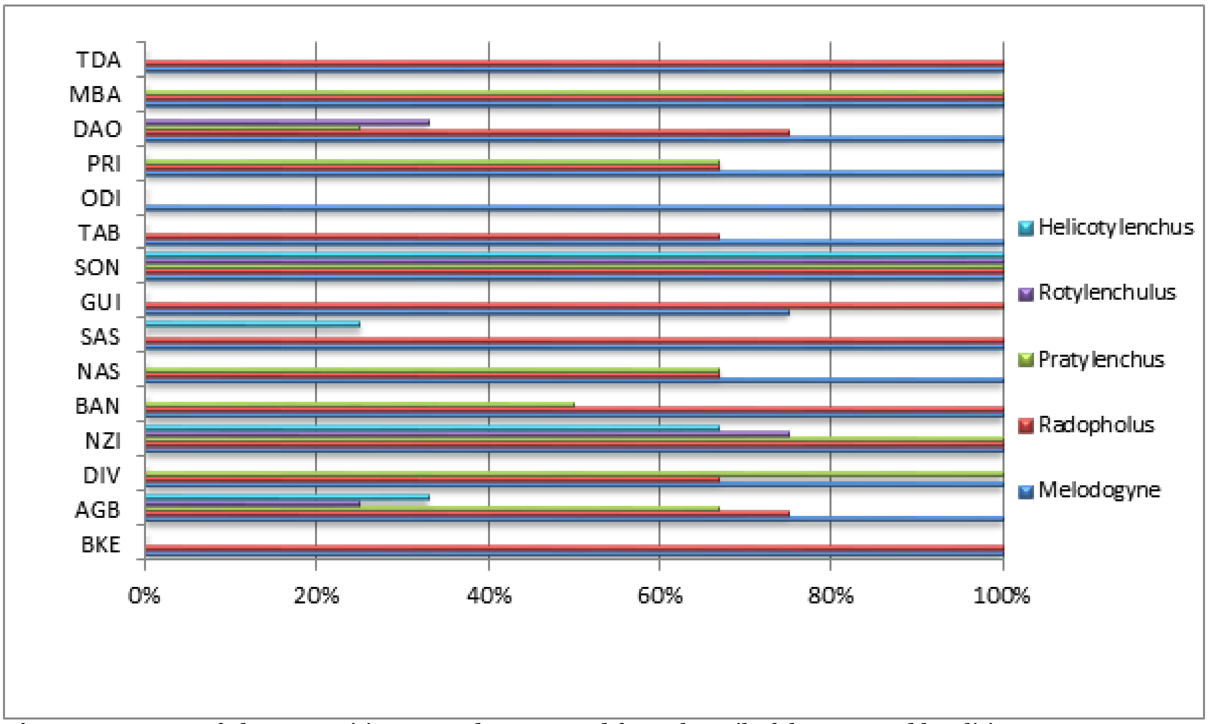

Fig. 5. Frequency of plant-parasitic nematodes extracted from the soil of the surveyed localities.

Concerning the frequency of occurrence and relative abundance related to the nematodes observed in different localities in root and soil samples, 5 genera of plant-parasitic nematodes were detected, including 3 endoparasitic and 2 ectoparasitic genera. The endoparasitic nematodes are the genera *Meloidogyne*, *Radopholus,* and Pratylenchus, followed by the ectoparasitic genera *Rotylenchulus* and *Helicotylenchus*. The first mentioned genus, *Meloidogyne* spp. was very frequent in all the localities prospected whatever the type of sample. It is the most abundant genus.

Indeed, the genus *Meloidogyne* spp. is known for its polyphagia and ubiquity (Gnonhouri and Adiko, 2005; Nandjui *et al.,* 2007). It is a very important nematode that parasitizes almost all field crops and is most affectionate to market gardeners, particularly tomato cultivation (Djian-Caporalino, 2008). The preponderance of the genus Meloidogyne in the localities of Yamoussoukro, Séguéla (SEG), Songon (SON), and M'Batto (MBA) is explained by the fact that these localities are areas where vegetable crops are highly prized for consumption and marketing (CNRA, 2004). The high relative abundance of the genus Meloidogyne spp. could be explained by its polyphagy and also by the nematode's life cycle that develops within the root, creating feeding sites (Netscher and Sikora, 1990). This fact also illustrates the high abundance of the genus *Meloidogyne* in root samples compared to soil samples. Numerous studies on this genus of nematode have reported its abundance in sampling stations throughout the world. Our results corroborate those of Diongue (1996) and Sawadogo *et al.* (1993), who recorded high frequency and abundance of the genus *Meloidogyne* on vegetable crops in Niger and Burkina Faso, respectively.

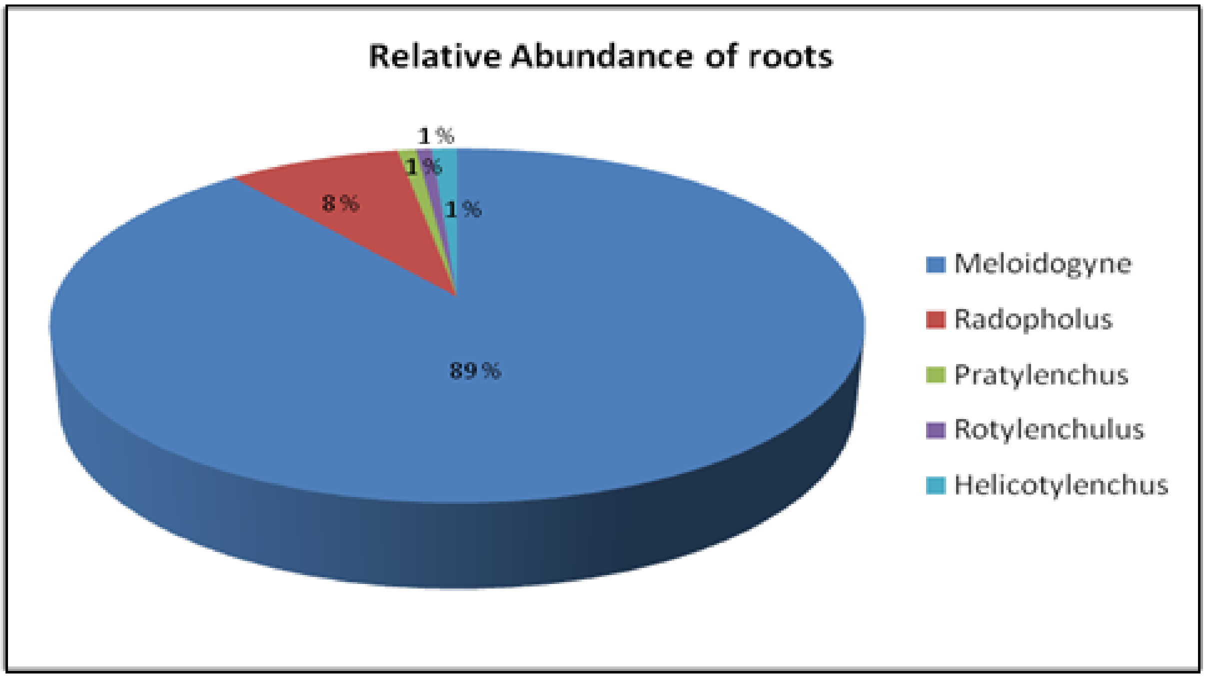

Fig. 6. The relative abundance of the different genera of plant-parasitic nematodes extracted from the roots of the plots in the surveyed localities.

The genus Radopholus was found to be fairly frequent and not very abundant in the different localities surveyed. This genus of nematode's behavior is because it is more parasitic on banana crops (Koffi *et al.,* 2012). It is a migratory endoparasitic nematode that crosses plant tissues from cell to cell or leaves plant tissues searching for new food sources, unlike the genus *Meloidogyne* (Vilardebo, 1984). However, as this genus has been encountered as often as the genus *Meloidogyne* in different localities, it would be important to monitor its population dynamics.

It could adapt and be as severe in tomato plantations as *Meloidogyne* (Cadet, 1987).

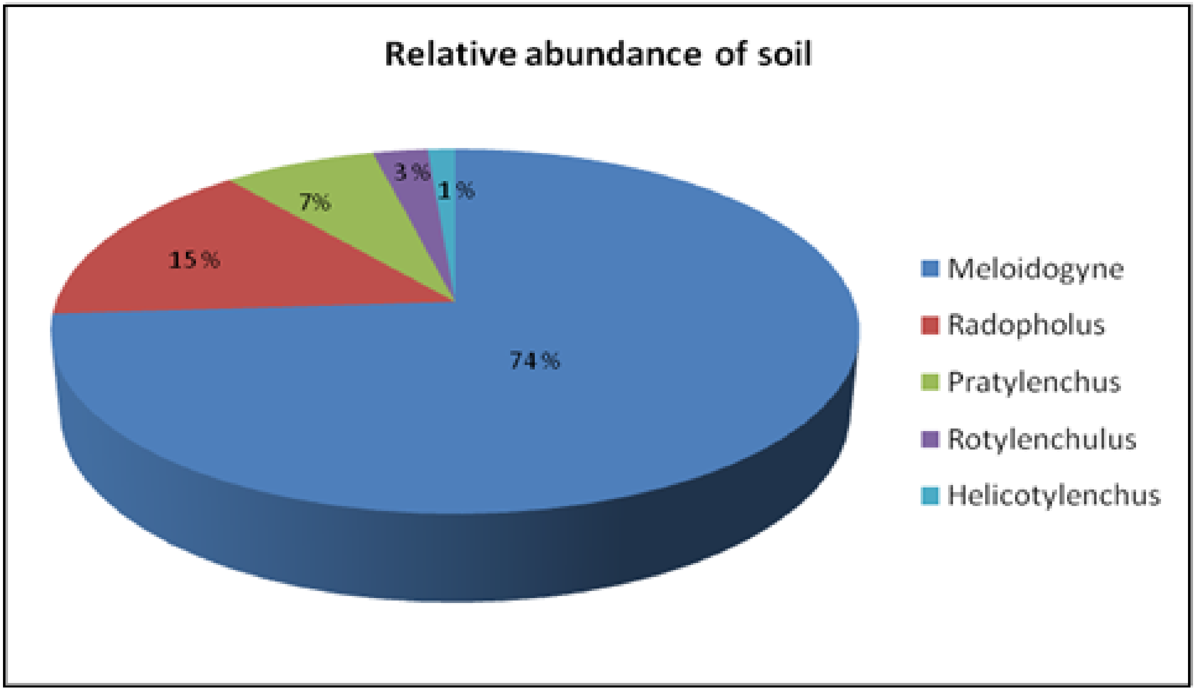

Fig. 7. The relative abundance of the different types of plant-parasitic nematodes.

The other three genera, *Pratylenchus*, *Rotylenchulus*, and *Helicotylenchus*, have been observed infrequently and in low abundance whatever the type of sample being analyzed. Indeed, these three genera of nematodes have not been identified as major pests specific to tomato cropping in Côte d'Ivoire. However, the genera Pratylenchus and Helicotylenchus are also polyphagous genera in Côte d'Ivoire (Gnonhouri and Adiko, 2005). The genera *Pratylenchus* and *Helicotylenchus* are predators of banana and sugar cane (Cadet, 1985; Quénéhervé *et al.*, 1986; Cadet and Debouzie, 1990; Gnonhouri and Adiko, 2005). Their polyphagy and different agricultural associations could explain the presence of these five nematode genera in certain localities.

Conclusion

The inventory of nematodes revealed various species of plant-parasitic nematodes associated with tomato cultivation in Côte d'Ivoire. The observed nematode density varied from one locality to another according to the type of sample. On the other hand, the most widespread genus was Meloidogyne in both root and soil samples. The most affected localities were Yamoussoukro, Séguéla (SEG), Songon (SON), and M'Batto (MBA).Plant-parasitic nematodes' analysis of diversity points to the genus Meloidogyne as a harmful nematode for market gardeners, particularly for tomato production in Côte d'Ivoire.

These results highlighted the geographical areas and soil types conducive to the development of nematodes, particularly Meloidogyne spp. Therefore, it is important to implement effective and sustainable control strategies against this nematode to offset any yield loss.

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