Study of the Diversity of Nematodes in Vegetable Crops in the Koulikoro Region (Mali)

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Abstract
The market gardening sector is confronted with numerous constraints which weaken and hinder its development. Among these constraints is the importance of plant parasitic nematodes. For a sustainable management of these worms, a diagnostic evaluation was carried out in two permanent market gardening of Sébéninkoro (Kati) and Koulikoro town in order to determine their diversity. 54 soil samples of 500 g were taken from 3 plots, ie 27 samples per site. Analysis of these samples revealed the presence of 8 genera of nematodes. Among these nematodes there are nematodes of the genus Meloidogyne very harmful to crops and Tylenchorhynchus. They are common and abundant throughout both sites.

Keywords: Market gardening; Phytoparasitic nematodes; Diagnostic; Crops; Koulikoro.

1. Introduction
In Mali, market gardening occupies a prominent place among irrigated crops for several reasons. Among these reasons is their important contribution to food security, and also the increase in the monetary income of actors [1]. In Mali, revenue directly linked to this activity is estimated at CFAF 27 billion [2]. Vegetable production in Mali has increased significantly in recent years to reach a value of 1 900 173 tonnes over an area of 173 110 ha [3]. According to the same statistics in terms of production, shallot is the most important crop with 401.285 tonnes. Next comes okra 254 545 tonnes and tomato 175 577 tonnes.

The World Health Organization (WHO) recommends consuming at least 400g of vegetables per day per capita, or five 80g servings to take full advantage of their beneficial effects on health and nutrition [4]. In Mali consumption is estimated at 103 kg per person per year [5]. According to the planning and statistics unit of the rural development sector [6], the city of Bamako alone consumes around 22 932 tonnes of vegetables per year. Onions and tomatoes are the most consumed with 34% and 25.5% respectively. Next come okra (12.5%) and cabbage (9%).

Yield losses caused by plant parasitic nematodes are estimated at 14.6% in tropical countries and 8.8% in developed countries [7]. Globally, this drop in yield is of the order of 12 to 20% [8]. In terms of money, the related crop losses were estimated at 80 to 150 billion US dollars per year [9]. In Mali, nematological analyzes have shown that these nematodes pose serious problems on the maliain market gardening sites visited in Bamako and Segou [10]. The most sensitive crops are Solanaceae (tomatoes, eggplant, potato), Cucurbitaceae (melon, cucumber), Legumes (beans), Umbelliferae (carrot, celery, etc.), Compositae (lettuce).

Despite the important role that market gardening plays in production, economic growth and food security, the sector remains subject to constraints that weaken and hinder its development.

Among these constraints, we can cite insect pests and especially nematodes. The objective of this study is to determine the diversity of parasitic nematodes associated with vegetable crops in the Koulikoro region.

2. Materials and Methods
2.1. Study Sites
Two permanent market gardening sites in the Koulikoro region (Koulikoro town and Sébéninkoro) were chosen for the collection of soil samples (Table 1). The samples were taken using a systematic method using a trowel.
Table-1. Location of study sites

| Market gardening sites     | Geographical coordinates     |
|---------------------------|------------------------------|
| Koulikoro region          | Sébéninkoro (Kati)           |
|                           | 12°31'419N ; 08°04'921W     |
| Koulikoro town            | 12°39'721N ; 07°56'726W     |

2.2. Plant material

Several vegetable crops that are exploited on these market garden sites have been selected for soil sampling: tomato, eggplant, lettuce and cabbage.

2.3. Nematological Data

2.3.1. Sampling

The samples were collected in the rhizosphere of vegetable crops along the two diagonals and two medians of the plots. The sample was taken during the off-season in February that is to say towards the end of the cold season by means of a trowel, at a depth of 25 cm.

Twenty-seven silty-sandy soil samples per crop plot, ie 54 soil samples of 500g, were taken from 3 plots. These soils are characterized by their light, airy and permeable nature, therefore favorable to the development of nematodes. Each sample is placed in plastic bags labeled with the name of the site, a sample number, the name of the crop, all well sealed with a thread. The samples were then collected in a cooler and transported to the lab for extraction.

2.3.2. Extraction of Nematodes

In the laboratory, a sub-sample of 100 cm$^3$ is taken from each of the samples from the rhizosphere of the cultures to be analyzed. The remaining soil is kept moist for analysis in the event of accidents. The soil sub-sample is well homogenized by hand and cleared of all large debris. It then passed through a 2 mm mesh sieve before proceeding to nematology analysis.

To extract the nematodes we used the modified Baermann technique and a series of three sieves of 150 µm, 37 µm and 38 µm. Each sample was placed on a “kleenex” type paper, then placed on a PVC sieve with a 1 mm mesh. The whole thing is placed on a plate, water is then added to moisten it completely. 48 hours later, all of the water in the container is passed through the sieves so as to maintain a suspension of about 25 ml. A 5 ml aliquot solution of this suspension is observed under a microscope at 40x magnification. The nematodes were fixed by immersion in a boiling solution of FA composed of 10 ml formalin; glacial acetic acid 1 ml; distilled water 89 ml. The nematode count was done using a stereoscope at 40x magnification.

2.3.3. Identification of Parasitic Nematodes

The generic determination of phytoparasitic nematodes was made using a determination key [11] and the discriminating morphological characters reported by Mateille and Tavoillot [12]. The count of nematodes was carried out with an inverted pole microscope of the PARALUX type at 40X magnification.

2.3.4. Data Analyses

From a suspension adjusted to 25 ml (V), take a 5 ml aliquot (v) which is placed in a counting box with a grid bottom for counting. After enumeration, the population size was expressed in number of nematodes / dm$^3$ of soil (N / dm$^3$) according to the formula:

$$F = \frac{Pn}{Pt} \times 100$$

where $F$ is the frequency of occurrence of the species.

The importance of each nematode genus was determined by calculating the average density per site, frequency of occurrence and relative abundance. An analysis of variance was performed in order to compare the means by site and by gender.

Frequency of occurrence (F) of a species is the ratio expressed as a percentage of the number of samples where this species is noted to the total number of samples taken:

$$F = \frac{Pn}{Pt} \times 100$$

According to Dajoz [13] we distinguish: constant species ($F \geq 50\%$), accessory species ($25\% < F < 50\%$), accidental species ($F \leq 25\%$). The relative abundance (A) of a species corresponds to the ratio of the number of individuals of the same species to the total number of individuals, all species combined.

3. Results

The nematodes encountered: 54 samples from the two permanent market gardening sites were analyzed and the phytoparasitic nematodes extracted. 8 genera have been identified (Table 2), they are: Pratylenchus, Meloidogyne, Scutelonema, Tylenchorhynchus, Heterodera, Helicotylenchus, Criconemella, Hemicycliophora. The frequency of occurrence, abundance and density provide information on the biology and ecology of the nematofauna found. Four of them are ectoparasites (Criconemella, Scutelonema, Tylenchorhynchus, and Helicotylenchus). Endoparasites are distributed in sedentary (Meloidogyne), migratory (Pratylenchus).
3.1. Importance of Nematodes

Total nematode densities per kg of soil varied from site to site. Analysis of the results shows a variation of 790 nematodes per kg of soil and 878 nematodes per kg of soil respectively in Sébéninkoro and Koulikoro (Figure 1). Statistical analysis of the data shows that there is no significant difference between these densities (Anova, P = 0.76). Regarding generic densities, a variation was noted. The largest outbreaks were noted for *Meloidogyne* in Sébéninkoro and *Tylenchorhynchus* in Koulikoro, respectively 490 and 199 individuals per kg of soil. Statistical analysis of generic densities shows that there is no significant difference between the different genera (Anova, P = 0.54).

### Table 3. The phytoparasitic nematodes identified

| Families                  | Genus                        |
|---------------------------|------------------------------|
| Heteroderidae             | *Meloidogyne* : *Heterodera*  |
| Belonolaimidae            | *Tylenchorhynchus* : *Helicotylenchus* |
| Hoplolaimidae             | *Scutellonema*               |
| Pratylenchidae            | *Pratylenchus*               |
| Criconematidae            | *Criconemella*               |
| Hemicycliophoridae        | *Hemicycliophora*            |

### Table 3. Frequency of occurrence, Abundance and densities of nematodes encountered

| Genera of nematodes | Sébéninkoro | Koulikoro | Means |
|---------------------|-------------|-----------|-------|
|                      | F%  | A%  | D  | F%  | A%  | D  | F%  | Types F%  |
| *Criconemella*      | 33  | 1   | 7  | 50  | 18  | 155 | 83  | constante  |
| *Meloidogyne*       | 100 | 62  | 490| 100 | 18  | 152 | 100 | constante  |
| *Tylenchorhynchus*  | 100 | 25  | 200| 100 | 23  | 199 | 100 | constante  |
| *Heterodera*        | 100 | 8   | 67 | 88  | 18  | 161 | 94  | constante  |
| *Pratylenchus*      | 100 | 3   | 23 | 100 | 6   | 55  | 100 | constante  |
| *Scutellonema*      | 0   | 0   | 0  | 88  | 8   | 69  | 44  | Accessoire |
| *Heliocotylenchus*  | 0   | 0   | 0  | 33  | 5   | 44  | 17  | accidentelle|
| *Hemicycliophora*   | 33  | 0   | 3  | 50  | 5   | 45  | 42  | Accessoire |

Legend: F%: frequencies of occurrence, A%: relative abundances, D: average densities over 1 kg of soil.

3.2. Structure of Nematode Communities

Eight genera of phytoparasitic nematodes were identified on all two market gardening sites, including 6 in Sébéninkoro and 8 in Koulikoro. These nematodes are distributed among five families of the order Tylenchida.

The frequency of occurrence and relative abundance allowed us to analyze stand structure. The results obtained showed that 6 and 8 genera were found respectively in Sébéninkoro and Koulikoro (Table 3). Among these genera 5 are constant with a frequency of occurrence greater than 50%. These are: *Criconemella*, *Meloidogyne*, *Tylenchorhynchus*, *Heterodera*, and *Pratylenchus*. 2 are accessory: *Scutellonema* and *Hemicycliophora*. The genus *Heliocotylenchus* is the only one that is of the accidental type.

In Sébéninkoro, nematodes of the genus *Meloidogyne* present an abundance of 62.03% with an average density of 490 individuals per kg of soil. On the other hand in Koulikoro the genus *Tylenchorhynchus* is less abundant with 22.61% and a higher density of 199 nematodes per kg of soil (Table 3).

4. Discussions

This study provides information on the diversity and abundance of nematodes associated with vegetable crops in the Koulikoro region. Of the 8 genera encountered *Meloidogyne* and *Tylenchorhynchus* are the densest. Studies in other countries have revealed the presence of several genera of plant parasitic nematodes associated with vegetable crops that reduce yield by causing economically significant losses. In Niger, research on nematodes associated with Solanaceae shows similar results [14].

On the other hand, in Morocco, such studies have revealed a greater diversity. There, 12 genera were found [15]. Root-knot nematodes of the genus *Meloidogyne* and the genus *Tylenchorhynchus* were detected in 100% of the sites surveyed. All these authors are unanimous that *Meloidogyne* constitutes the most dangerous genus for vegetable crops. They can cause losses of up to 60% of tomato production [14].

The structure of the nematode population was analyzed from the frequency of occurrence and relative abundance. Thus 5 genera were reported on all two sites with a frequency of more than 50%.

Two have been described as incidental, meaning that their frequency of occurrence is between 25% and 50%. Only the genus *Heliocotylenchus* is considered accidental with F > 25%. This importance of the populations of *Meloidogyne*, *Tylenchorhynchus*; *Criconemella*, *Heterodera*, and *Pratylenchus* follows the permanent cultivation practice and the dominance of Solanaceae such as tomato, pepper, eggplant. The results found in Burkina show that this variation in distribution could be partly due to the type of soil [16].
5. Conclusion
The results obtained from this study confirm that there is a significant diversity of parasitic nematodes on crops. These results confirm that the nematodes of the genus Meloidogyne and Tylenchorhynchus are the most frequent on vegetable crops in the region. The 8 genera identified in this study seem to be the most abundant and the most common on crops. They are characterized by a very wide host range which includes vegetable crops, cereals and legumes.

Regarding ectoparasitic nematodes, the most important are Tylenchorhynchus and Helicotylenchus. These data provide useful information to guide prevention and control programs against these pests of vegetable crops in Mali.

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Figure 1. Distribution of plant parasitic nematodes by site.

Average densities of nematodes per kg of soil

- *C. reniformis*
- *M. elongata*
- *T. coffeae*
- *H. avenae*
- *P. penetrans*
- *S. pepsis*
- *H. rostochiensis*
- *H. cepa*

Sebeninkoro
Koulikoro