



Distribution and Occurrences of Parasitic Nematodes Associated with Beans (*Phaseolus vulgaris* L.) in the Sotuba Market Gardening Area in Mali

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A nematological faunistic study was carried out on common bean plots in Bamako between June and September 2023. It took place on the experimental plots of the fruit and vegetable program of the Sotuba Agricultural Research Center. The objective of the study was to identify the occurrence of nematode genera associated with beans. Two varieties of beans were concerned: Blanc de Kati and GLP. The trials were set up on two 400 m² plots and ten samples were taken using a systematic method using an auger. In the laboratory, two extraction techniques were used: sieving

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and the modified Baermann technique. Plant-parasitic nematodes were observed and identified under an optical microscope at x400 magnification. The observations revealed the presence of 8 genera of nematodes in the soil and 6 in the roots. The genera found in the soil are: *Scutellonema*, *Tylenchorhynchus*, *Paratylenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Criconebella* and *Hoplolaimus*). Those found in the roots were *Scutellonema*, *Paratylenchus*, *Helicotylenchus*, *Meloidogyne*, *Heterodera* and *Pratylenchus*). The analysis of the results shows that the genera *Pratylenchus*, *Tylenchorhynchus* and *Helicotylenchus* are the most important with 69% of the nematodes identified. Among the nematodes identified were species dangerous for crops in general, including *Meloidogyne*. Analysis of physicochemical parameters indicates a trend of variation in the distribution of nematodes depending on the soil structure. This study demonstrates the need to monitor pest thresholds to prevent extreme outbreaks that can cause significant yield declines.

Keywords: Occurrence; parasitic nematodes; *Phaseolus vulgaris*; densities; Sotuba.

1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a legume of the Fabaceae family. It is native to Latin America. The genus *Phaseolus* includes approximately 80 cultivated and wild species, but *P. vulgaris* is the most widely cultivated species (Romero-Astudillo, 2024).

In Africa, common beans are cultivated on an area estimated at approximately four million hectares, primarily by women. Its legume status makes it a fertilizer, a source of food, and income for producer households. Beans represent an important source of income for rural farmers. Annual sales in Africa amounted to more than \$580 million in 2005. Beans, with a protein content of 22%, represent a source of protein for nearly one hundred million people. In addition to protein, they also contain iron, zinc, fiber, and complex carbohydrates. East Africa holds the

world record for bean consumption per capita, at approximately 60 kg per year (Coulibaly, 2024).

In Mali, bean production is estimated at 4,252 tonnes (Delhove, 2023). The Bamako district and the Koulikoro region are the two main green bean production regions in 2019, with local consumption of around 3,300 tonnes, or around 97% of national production (Delhove *et al.*, 2023).

Despite this good production and all the nutritional importance linked to the consumption of beans, its production remains subject to numerous constraints such as climate change, low soil fertility, etc. In addition to these abiotic constraints, there are parasites such as fungi, insects and especially nematodes. The latter occupy an important place among the biotic constraints. Nematodes of the genus *Meloidogyne* and *Pratylenchus* are important



Fig. 1. *Meloidogyne* spp. galls on beans (Chabrier, 2014)

pests of beans. Their attacks induce the formation of root galls (Fig. 1) and necrosis respectively (Delhove *et al.*, 2023). The yield losses they cause in tropical and subtropical countries are estimated at 14.6% in developed countries (Nicol *et al.*, 2013). In terms of money, these crop losses were estimated at nearly 150 billion US dollars per year (Coyne *et al.*, 2018).

2. MATERIALS AND METHODS

2.1 Location and Description of the Study Site

From June to September 2023, during the rainy season, this faunal study was carried out on two common bean plots, each measuring 400 m². It took place on two experimental plots at the Sotuba Agronomic Research Center in Bamako, Mali (Table 1). Two bean varieties related to the two plots were involved: Blanc de Kati (12°39'45"N; 7°55'27"W) and GLP (12°39'34"N; 7°55'13"W).

2.1.1 Plant material

The plant material covered in this study consists of two varieties of common bean (*Phaseolus vulgaris*): the first is called Blanc de Kati, a variety known to be adapted to poor soils, and the second is called GLP, a variety resistant to several diseases (Fig. 2). These are both large-grained varieties with a yield of two tons per

hectare (Ministry of Agriculture of Mali. 2024). For all these reasons, these two varieties are highly valued by producers and have been the subject of numerous studies aimed at improving food security, income, and the health of producers on the African continent.

2.2 Samples Collection

2.2.1 Soil samples

Soil samples were collected using a systematic method, i.e., one that took into account the plot as a whole and the aggregate distribution of nematodes. Soil samples were taken at a depth of 30 cm using a soil auger. For each of the 400 m² plots, 10 samples were taken on the diagonals and mixed to form a composite sample. 250 g of this sample was placed in a plastic bag, labeled with the date, crop, site and plot number, then placed in a cooler for transport to the laboratory.

2.2.2 Root collection

To collect the roots, randomly uprooted bean plants using a trowel. After gently shaking off most of the adhering soil, the roots were cut with scissors. Ten bean plants were collected from each plot. These roots, along with the soil, were placed in plastic bags, labeled, and then placed in a cooler for transport to the laboratory.

Table 1. Geographic coordinates of the experimental plots

Plots	<i>Phaseolus</i> Variety	Plot Coordinates
Plot 1	Blanc de Kati	12°39'45"N ; 7°55'27"W
Plot 1	GLP	12°39'34"N ; 7°55'13"W



Fig. 2. Grains of the two varieties of *P. vulgaris*: Blanc de Kati on the left and GLP on the right
Soil sample collection

2.3 Nematological Analyses

2.3.1 Extraction of nematodes from the soil using the modified Baermann technique

Using a beaker, 100 ml of soil from the plots were collected, the clods of soil were broken up, and large debris such as stones, twigs, roots, etc. were removed. The soil was placed on a Kleenex-type filter, then on a 1 mm mesh sieve, and the entire sample was placed in a plate. Water was then added to the sample so that it was completely moistened but not completely submerged (Fig. 3). This device was covered with another labeled plate and left for 48 hours. After these two days, all the water in the plate was filtered through a 38 µm sieve. The resulting filtrate was placed in a 100 ml beaker for decantation and pipetting. This decantation lasts at least two hours. Following this decantation, the nematode suspension is reduced to the pellet by carefully reducing the amount of water in the surface part using a Pasteur pipette with a bulb. This pipetting is done in such a way as to maintain a suspension of approximately 25 ml (V). From this suspension, an aliquot of 5 ml (v) was taken and then placed in a counting box for observation under a microscope.

2.3.2 Extraction of nematodes from the roots

The roots were gently cleaned under a running tap to remove soil and several saprophytic nematodes. The roots were cut into small pieces and disinfected by soaking in a bleach solution (1% active chlorine) for 4 minutes to brighten them, then rinsed with tap water for 15 minutes.

A 20g subsample was taken from the composite sample and ground with water using a kitchen blender. The roots were ground for three 30-second periods, separated by a brief settling period. The analysis process was conducted according to the Baermann technique described above.

2.3.3 Nematode identification

Nematode Fixation for Counting: The nematodes were fixed by immersion in a boiling FA solution (10 ml formalin; 1 ml glacial acetic acid; 89 ml distilled water) (Coyne *et al.*, 2018). The technique consisted of adding the heated FA solution to the nematode suspension in equal volumes (v/v) in a small, stoppered bottle. This fixation allows the nematodes to be preserved for at least one month.

Observation: The nematode suspension obtained after decanting and pipetting was adjusted to 25 ml (V) by adding distilled water. From this volume (V), a 5 ml aliquot (v) was taken and placed in a counting dish for microscopic observation at 400X magnification. Nematode Identification:

The nematodes were identified using the key from (May and Mullin, 1996) and the discriminating morphological characteristics reported by (Mateille and Tavoillot, 2016): the length and shape of the stylet, the shape of the head and tail, the covering (i.e., the arrangement of the esophageal gland relative to the intestine), and the position of the vulva.

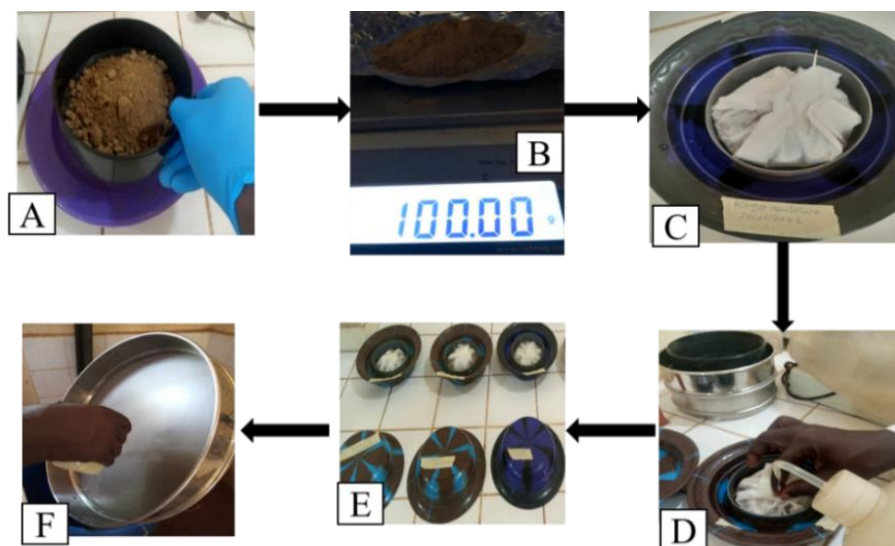


Fig. 3. Illustration of the modified Baermann technique. Key: A: Soil sieving; B: 100 ml of soil; C= Kleenex containing the soil; D and E: active passage and F: Filtration

Nematode Counting: This is done under a microscope by placing the solution in a transparent dish with a gridded bottom.

2.4 Soil Data

Soil samples from both plots were sent to the Soil Water Plant Laboratory (LABOSEP) in Sotuba, where a physicochemical analysis was performed to determine the pH, the different particle size fractions (sand, clay, and fine silt), and the carbon, nitrogen, potassium, and phosphorus contents. The texture triangle was used to determine the soil texture of the different sites.

2.5 Nematological Data

The data were entered into an Excel spreadsheet and analyzed. The importance of each nematode genus was determined by calculating the density and frequency of occurrence by genus and plot. An analysis of variance was performed to compare the average densities of nematodes living in the rhizosphere of the two bean varieties.

2.5.1 Nematode frequency

Occurrence provides information on the environmental preference of a given species. It consists of counting the number of times species x appears in the samples. This number is expressed as a percentage of the total number of records and provides information on the species frequently encountered in the environment. According to (Dajoz 1985) we distinguish: constant species ($F \geq 50\%$), accessory species ($25\% < F < 50\%$), accidental species ($F \leq 25\%$).

3. RESULTS

3.1 Nematological Analysis

3.1.1 Importance of soil nematodes

Analysis of soil samples from the two plots revealed the presence of eight genera of phytonematodes belonging to six families (Table 2): *Scutellonema*, *Tylenchorhynchus*, *Paratylenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Criconebella*, and *Hoplolaimus*. Among these nematodes, three genera accounted for 69% of the nematodes recorded: *Pratylenchus*, *Tylenchorhynchus*, and *Helicotylenchus*. The eight genera were found in the rhizosphere of the Blanc de Kati, compared to five around the GLP variety (Fig. 4). Average densities varied from one plot to another. The highest proliferation was observed for the genus *Pratylenchus* in the rhizosphere of the GLP variety, with a density of 233 individuals per 100g of soil. On the other hand, the lowest density was that of the genus *Scutellonema* around the GLP variety with 10 individuals/100g of soil. Statistical analysis of the average densities per variety showed a significant difference between the means (Anova, $p < 5\%$).

3.1.2 Root nematodes

From the analysis of the roots of the two varieties, six genera of nematodes were observed. These were: *Scutellonema*, *Tylenchorhynchus*, *Paratylenchus*, *Helicotylenchus*, *Meloidogyne*, and *Pratylenchus*. Nematode densities varied by genera and by plot. Overall, the GLP variety showed a higher proliferation. The *Helicotylenchus* genus

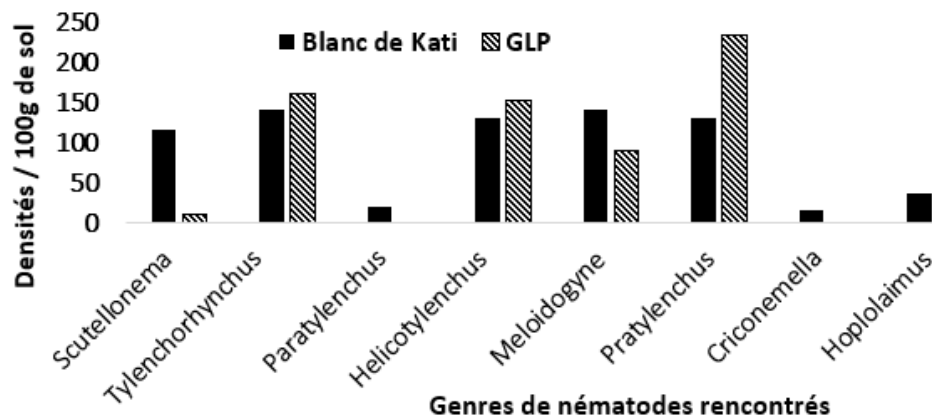


Fig. 4. Average nematode densities in the rhizosphere of the two bean varieties

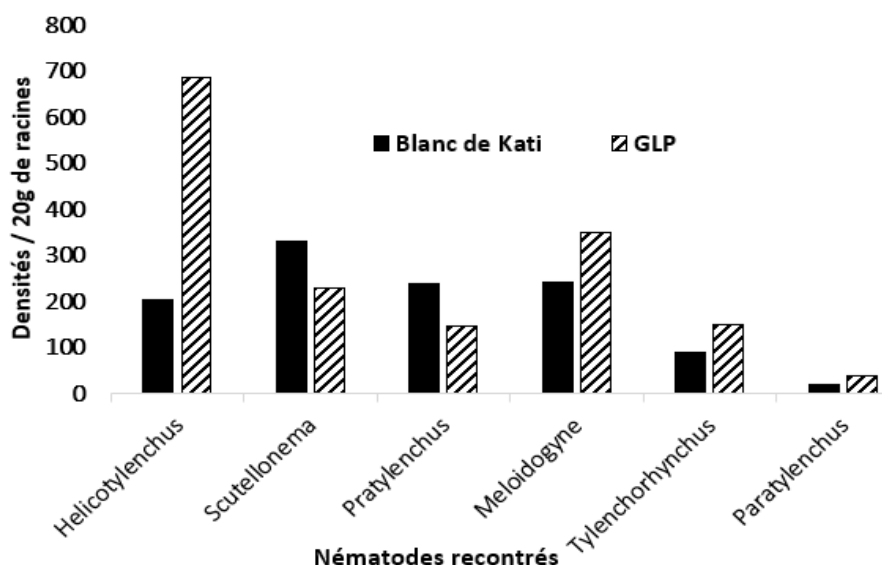


Fig. 5. Average nematode density in the roots of the two bean varieties

was the highest, with a density of 684 nematodes/20 g of fresh roots. The lowest density was noted for the *Paratylenchus* genus, with 37 nematodes/20 g of roots. For Kati white, the genus *Scutellonema* is the densest genus with 330 individuals/20 g of roots and the lowest density is that of *Paratylenchus* with 20 individuals/20 g (Fig. 5).

3.1.3 Nematode occurrence frequency

The frequency of occurrence allowed us to analyze the structure of the stands. The results showed that eight and five genera were found around the Blanc de Kati and GLP varieties, respectively (Table 2). All these genera were consistent, with an occurrence frequency $\geq 50\%$.

3.2 Soil Data

The soil data included physicochemical and particle size parameters. The results for the physicochemical parameters are shown below in the Fig. 6.

3.2.1 Physicochemical Parameters

Regarding Hydrogen Potential (pH), almost similar values were found in the different plots. These values were below the neutral threshold (pH > 7). A comparison of these values shows that they ranged from 5.7 in the GLP plot to 6.5 in the Kati White plot (Fig. 6). This acidic soil trend, highlighted by the low values in all plots, could be due to oxidation of organic matter. Regarding organic carbon, the levels did not vary greatly between plots; the percentages fluctuated slightly between 0.88 and 0.91 (Fig. 6). These lower levels are explained by the intensification of cropping at the experimental site. Indeed, intensive soil exploitation can reduce the organic matter content, leading to a drop in fertility. For organic nitrogen, the levels are low and perfectly similar. A rate of 0.02 was in both plots (Figure). As for available phosphorus, it is expressed in ppm; the highest values were recorded in the Kati white plot at 12.99 ppm compared to 12.52 ppm in the GLP plot (Fig. 6).

Table 2. Nematode occurrence frequencies in the two plots combined

Genres	Famillies	Frequencies Of occurrence %	Types of occurrence
<i>Scutellonema</i>	Hoplolaimidae	100	Constant
<i>Tylenchorhynchus</i>	Belonolaimidae	100	Constant
<i>Paratylenchus</i>	Paratylenchidae	100	Constant
<i>Helicotylenchus</i>	Hoplolaimidae	100	Constant
<i>Meloidogyne</i>	Heteroderidae	100	Constant
<i>Pratylenchus</i>	Pratylenchidae	100	Constant
<i>Criconemella</i>	Criconematidae	100	Constant
<i>Hoplolaimus</i>	Hoplolaimidae	50	Constant

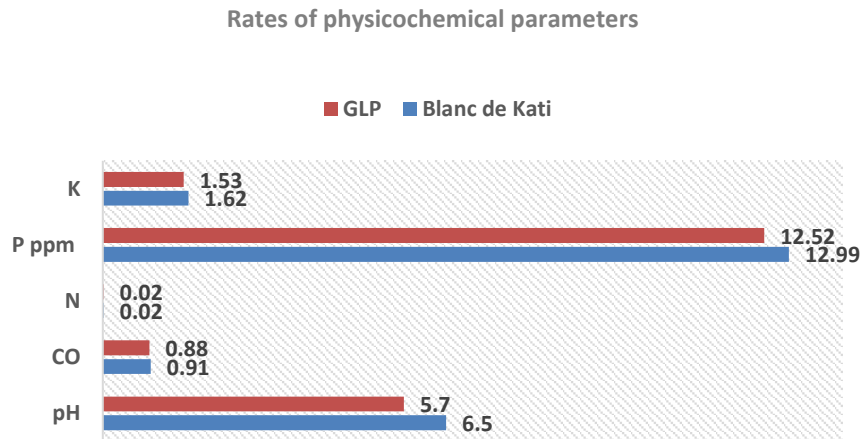


Fig. 6. Chemical parameters of the plots

3.2.2 Grain Size Parameters

Grain size encompasses three measured parameters: sand (Sb) refers to particles larger than 0.05 mm, fine silt (Lf) is formed by particles between 0.05 and 0.002 mm, and clay (Ag) by those smaller than 0.002 mm. Overall, the sand content was higher than that of the other grain size parameters, followed by silt (Figure), with a sand content of 65.5%, fine silt 27.5%, and clay 7%. The soil texture determined using the triangle shows a sandy loam soil type.

4. DISCUSSION

4.1 Fauna Study

This study provides information on the diversity and structure of nematodes associated with beans in the experimental plots of the Sotuba Agronomic Research Center in Mali. Eight genera belonging to 6 families were encountered, namely: *Scutellonema*, *Tylenchorhynchus*, *Paratylenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Criconebella* and *Hoplolaimus*. Previous studies in other countries have revealed the presence of several genera of plant-parasitic nematodes associated with beans that reduce yields and cause significant economic losses. For example, in Ghana, a study on nematodes associated with common beans reported the presence of 5 genera such as *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, *Helicotylenchus* and *Trypadorus* (Adomako et al., 2022). In Burkina Faso, a greater diversity of phytoparasitic nematodes was reported by (Traoré et al., 2014) on another Fabaceae (*Vigna unguiculata*). There, seven

genera were found with a higher rate of *Meloidogyne*.

Greater diversity was found in Ivory Coast on vegetable crops where nine genera were found: *Meloidogyne*, *Helicotylenchus*, *Radopholus*, *Pratylenchus*, *Hemicycliophora*, *Rotylenchulus*, *Hoplolaimus*, *Xiphinema* and *Heterodera* (Sandrine et al., 2024). All these studies report the frequency of the genus *Meloidogyne* spp on vegetable crops such as Solanaceae, Cucurbitaceae and Compositae (Bui et al., 2022). Thus in Egypt El-Nuby et al., 2019 report that the genus *Meloidogyne* was common on eggplants (55.8%) and watermelons (61.9%). It was also common in rice fields (49%) in India (Nisa et al., 2022). Delhove et al., 2023 report that *Meloidogyne*, *Pratylenchus* are very polyphagous with preferences for Poaceae, Solanaceae (tomato, potato), Cucurbitaceae, *Arachis hypogaeae*, *Zea mays*, tobacco, pineapple, sweet potato etc. This sedentary endoparasite is recognized as the most economically damaging in many countries on several crops, because it has a wide host range (Isgouhi and Teixeir, 2019; Sandrine et al. 2024). Its development leads, among other things, to poor plant growth, a drop in crop yield, etc.

4.2 Relationships between Soil Properties and Nematode Densities

Root analysis of both varieties revealed that nematode distribution could be linked to the higher sand (69%) and clay (9%) levels around the GLP variety. Indeed, 59% of nematodes were found around this variety, compared to 41% associated with Kati White. This result

demonstrates the importance of soil structure in nematode distribution. For example, in the USA, (Escalante Ortiz and Brye, 2023), during a study on soybeans, they reported that physicochemical parameters influence nematode population densities and indicated that a good understanding of the variation in these soil properties could provide a solution for nematode control.

5. CONCLUSION

This study revealed the presence of eight genera of nematodes in the rhizosphere of common beans. These nematodes belong to six families. The most significant proliferation of populations was observed around the variety known as: Blanc de Kati. Specifically, the most important genus in terms of density is *Pratylenchus*. Physicochemical analyses of the soil studied gave very similar values. Hence the distribution of nematodes around common bean varieties probably does not depend on soil structure. The difference in diversity could be due to a preference of nematodes for the Blanc de Kati variety. The constant type of frequency of occurrence for all genera encountered shows the susceptibility of the bean to phytoparasitic nematodes. High frequencies greater than or equal to 50% deserve special attention in research programs on crop protection in the Republic of Mali because among these nematodes there is a genus particularly damaging to crops: *Meloidogyne* spp. Future studies could be oriented towards research on the severity of attacks, population dynamics during the year to better control nematodes and ensure healthy vegetable production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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