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Identification Of Meloidogyne Species Associated With Tomato (Lycopersicon Esculentum L.) In The Bougouni Region (Mali)

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Abstract
Background*: The development of market gardening and particularly that of tomatoes is confronted with multiple problems among which there are nematodes of the genus Meloidogyne. The determination of these parasites is important to adopt a control strategy. The objective of this study is to identify Meloidogyne species associated with tomato (Lycopersicon esculentum L.) in the Bougouni region of Mali.*
Materials and Methods*: Root sample collection took place at two sites in Bougouni region. The roots were thinned with bleach 1%. The females were fixed on roots bearing galls in good condition, by the sodium hypochlorite-acid fuchsin method. These females were subsequently dissected and identified using the perineal plates.*
Results *: Microscopic observation of the perineal plates revealed the presence of three types of perineal plates corresponding to M. incognita, M. arenaria and M. javanica with respective rates of 52%, 27% and 21%. Root-knot nematodes are very important in the parasitic nematofauna in Mali, they were found everywhere regardless of the site considered.*
Conclusion *: These results confirm not only the omnipresence of these three genera in the intertropical zone but also the susceptibility of the Roma tomato to Meloidogyne.*
Keywords: *Meloidogyne, Lycopersicon esculentum, identification, dissection, fixation*

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I. Introduction

Market gardening occupies a very important place among irrigated crops. Among these reasons there is their substantial contribution to food self-sufficiency, the increase in the monetary income of farmers.

Vegetable production in Mali is estimated at 1,900,173 tonnes on an area of 173,110 ha [1]. A survey by the Planning and Statistics Unit of the Rural Development Sector of the [2] reported that the city of Bamako consumes approximately 22,932 tonnes of vegetables per year.

The development of market gardening is faced with multiple problems including drought and parasites. Among the parasites, nematodes constitute the most important group after insects. They cause a lot of damage leading to a drop in yields [3].

In Morocco, [3] reports that within nematode populations, species of the genus *Meloidogyne* are the best known for their pathogenicity on market garden crops. They are by far the most formidable, polyphagous which attack the majority of crops leading to a considerable drop in yield. This loss of harvest has been estimated at 14% per year worldwide [4].

In addition, they have a great power of multiplication allowing them to quickly invade the roots of the plants on which they cause galls (Figure 1). In terms of money, [5] estimated the damage caused by these nematodes worldwide at 173 million dollars per year.

Nematodes are currently posing serious problems on the market garden sites visited in Mali [6]. The most sensitive crops are Solanaceae, Cucurbitaceae, Umbelliferae, Compositae, etc. The objective of this study is to initiate the technique of identifying nematodes of the genus *Meloidogyne* on the tomato variety Roma (*Lycopersicon esculentum*).

More than 70 species identified, three of which are of agro-economic importance in intertropical environments: *Meloidogyne arenaria, Meloidogyne incognita* and *Meloidogyne javanica* [6]. *M. incognita* has a high, more or less square dorsal arch. The lateral fields are marked by breaks or junctions between the dorsal and ventral striae. The striae are smooth to wavy at the level of the lateral fields. The inner portion of the arch located above the anus is marked by broken striae forming zigzags [7].

The lateral field is indistinct. *M. javanica* is distinguished by a perineal section with a low to moderately high dorsal arch. The lateral fields are marked by a double incisure that breaks the continuity of the wrinkles between the dorsal arch and the ventral arch. The striae are smooth to slightly wavy. *M. arenaria* has a round, slightly oval perineal section. The dorsal arch is low. The striae are smooth and have some angular orientations at the lateral fields. The number of striae increases as one approaches the lateral lines [7]. It is the least pathogenic species of the above-mentioned nematodes.

Figure 1: Symptoms of attack on tomato roots by Meloidogyne spp [8]

II. Material And Methods

Plant material

The study was conducted on two varieties of tomato (*Lycopersicon esculentum* L.): Roma VF, vigorous, productive and ubiquitous in the market garden sites of the region, and Nainespomor, both susceptible to *Meloidogyne* species.

Root sample collection:

Root samples were collected from two market gardening sites in the Bougouni region of Mali. These are Massablacoura where one plot was visited and Dialanicoro with two plots (Table 1). Samples were collected randomly using a trowel. Roots with galls in good condition were immediately placed in plastic bags marked with the name of the site, the crop and the date. They were placed in a cooler to protect them from sunlight which can kill nematodes very quickly and transported to the laboratory. In the laboratory, the samples were kept moist for analysis in two weeks at the latest.

Table 1. Geographic coordinates of the study sites

Sites	Latitudes	Longitudes
Massablacoura	11.4231374	7.4737616
Dialanicoro	11.393039	7.4793363

Identification of root-knot nematodes

Female attachment

Nematode attachment was performed on gall-bearing roots using the sodium hypochlorite-acid fuchsin method [9]. Roots were first thinned with bleach for 4 minutes and then rinsed with tap water for 15 min to remove bleach residue. They were then boiled for 30 seconds in 30 ml distilled water plus 1 ml of a stock solution of acid fuchsin (0.35 g acid fuchsin; 25 ml acetic acid; 75 ml distilled water) and then cooled for 30 min at room temperature.

Root decolorization

Then the roots were decolorized in an acidified glycerol solution by adding 6 drops of nitric acid and boiled. The decolorized roots will be freed from glycerol and then placed in a petri dish containing lactophenol for temporary storage. A root segment observed under the microscope shows the red-stained nematodes and the colorless root tissues (Figure 1). This facilitates the dissection of the females and the preparation of the perineal plates.

Preparation of perineal plates

The preparation of perineal plates was done according to the technique of [10]. 155 females were collected from tomato roots, including 65 in Massablacoura and 90 on the two Dialanicoro plots. The dissection of the fixed nematodes was done under a stereoscope (Figure 2.2.). The females fixed with fuchsin acid (Figure 2.1.) are fished and placed on a slide in a drop of distilled water, then using entomologist's spines it is pierced and emptied of its contents by pressing lightly on the nematode (Figure 2.4).

For a complete cleaning of the preparation, excess water was aspirated using blotting paper. A drop of glycerol or lactophenol was then added. The whole was covered with a coverslip. Air bubbles were eliminated by lightly flaming the slide. The coverslip is then sealed with nail polish for medium-term conservation. These preparations are used for microscopic observation of the perineal plates of *Meloidogyne* for identification. The photos of the observed perineal plates will be taken using a PARALUX microscope with inverted gallows (400x). These perineal figures were compared to those published by [11].

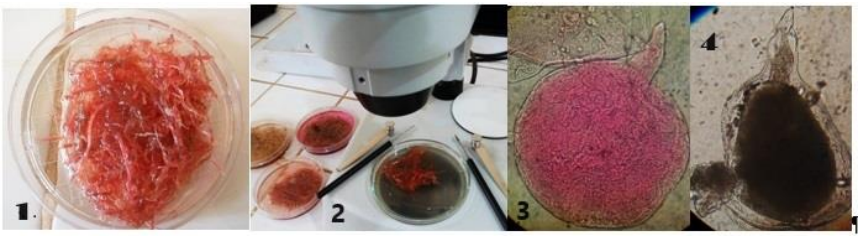

Figure 2. Steps of dissection of female *Meloidogyne*: 1= Fixation; 2= Dissection under stereoscope, 3= Female removed, 4= Dissection

III. Results

Identification of nematodes by perineal plates

Microscopic observation of perineal plaques revealed three types of plaques, which were compared to those published by [11]. The first type in terms of abundance corresponds to *M. incognita*, the second is *M. arenaria*, and the third to *M. javanica*. *M. incognita* represents the most abundant species 52% of the samples, followed by *M. arenaria* 27% of the samples *M. javanica* is the least abundant 21% of the samples observed (Figure 3). Statistical analysis of means by species of *Meloidogyne* shows that there is no significant difference (Anova, p>5%) between species.

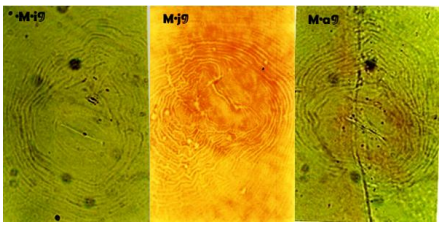

Figure 3. Perineal prints of female *Meloidogyne*: *Mj=M javanica; Ma= M. arenaria Mi = M. incognita*

With respect to the sites, the three species were found in the two villages with different values. The highest abundance was noted in Dialanicoro for *M. incognita* with 52 individuals. The lowest value is that of *M. arenaria* in the same locality with a value of 11 females. The species *M javanica* was found to be more abundant in Massablacoura with 22 individuals (Figure 4). Statistical analysis of means by site visited shows that there is no significant difference (Anova, p>5%) between sites.

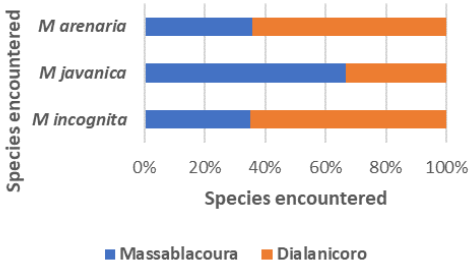

Figure 4. Frequencies of *Meloidogyne spp* species identified from perineal plates of females

IV. Discussion

This study identified three species of *Meloidogyne* in the Bougouni region of Mali during the cold season. The most important corresponds to M. incognita with 80 females identified on the two sites, it is followed by *M. arenaria* with 42 females, and the third to *M. javanica* with 33 individuals. *Meloidogyne incognita* represents the most abundant species 52% of the samples, it is followed by *M. arenaria* 27% of the samples *M. javanica* is the least abundant 21% of the samples observed. These results agree in terms of diversity with those of a study on nematodes of market garden crops in the peri-urban area of Bamako where the species *M arenaria* was denser with 43% [6]. This similarity in diversity could be due to the fact that both studies took place in southern Mali around Bamako and Bougouni 200 km to the south. Other studies have shown fairly similar results. On market garden crops under shelter in Turkey, a study revealed in addition to the presence of the three species mentioned above a fourth species *M ethiopica*. There too *M. arenaria* is more abundant with a rate of 42.2% [7]. In Iraq [12] reported the presence of two species, the most frequent of which is *M javanica* 73.33% followed by *M incognita* 20%. Less diversity was found in the Babylon region of Iraq on eggplant, another solanaceous plant on which characterization of *Meloidogyne* species revealed only two genera: *M javanica* 73.33% and *M incognita* 20% [12].

V. Conclusion

Root-knot nematodes (*Meloidogyne spp*) are very important parasitic nematofauna in Mali, they have been found everywhere regardless of the site considered. They are widespread and cause a lot of damage to market garden crops for which they constitute a limiting factor.

This could be linked not only to the climatic conditions (temperature and humidity) favorable to the development of nematodes but also to the cultivation techniques. The species encountered are mainly *M. arenaria, M. incognita, M. javanica*. Other species could probably extend this list if research continues on other sites and with more precise identification techniques. The results of this study are only a draft of the identification of root-knot nematodes because time and resources have not allowed for further research.

Perineal plates are most often variable within the same species so they alone do not represent a sufficient method for correct identification of root-knot nematode species. Other techniques such as pathogenicity testing on differential hosts (cotton, pepper, melon, tomato) and morphometric measurements are necessary, but time and resources have not allowed for all these additional works.

Examination of perineal plates is only a first step to be carried out. In addition, in the process of root-knot nematode identification, it gives an idea of the variability of the *Meloidogyne spp* population in Mali.

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