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Determination of the genus *Meloidogyne* species and study of their impact on the market gardening in the area of Bamako, Mali

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The development of market gardening is faced with many problems among which there are the nematodes of the genus *Meloidogyne*. The aim of this study is twofold: (a) determining the *Meloidogyne* species that are crowned by the perineal plates of female and (b) studying the impact of the multiplication of these nematodes on the tomato cv Roma (*Lycopersicon esculentum* L). Root samples were collected at five sites around Bamako, Mali. In a trial, nematode larvae were extracted from the roots of culture and then inoculated on tomato plants. A second tomato plants non-inoculated was used as control. Using a maceration-filtration method, three *Meloidogyne* spp. were identified: *Meloidogyne arenaria, Meloidogyne javanica* and *Meloidogyne incognita*. All of the inoculated plants reacted by the presence of galls that were not observed on the non-inoculated plants.

Key words: Market gardening, genus *Meloidogyne*, galls, *Lycopersicon esculentum*, perineal plate, roots of culture, root samples.

INTRODUCTION

Market gardening for several reasons occupies a place of choice among irrigated crops. Among these reasons is their appreciable contribution to food self-sufficiency, the increase of the income of the farmers and especially of the women and young people who maintain them. Vegetable production in Mali has increased considerably in recent years to 1 900 173 tons on an area of 173 110 ha (Ministry of Agriculture of Mali, 2018). This production allows the farmers to diversify as well as to improve their diet thanks to the contribution of vitamins and mineral salts, it ensures besides an increase of the monetary income of the actors. According to the results of a survey of the Planning and Statistics Unit of the Rural Development Sector of the Ministry of Agriculture of Mali (2016), the city of Bamako consumes about 22,932 tons of vegetables per year.

The development of vegetable crops is faced with many problems among which there is the scarcity of water and parasites. Of the parasites, nematodes are the most important group after insects. They cause a lot of

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damage resulting in lower yields (Nadine, 2015). Mokrini and Sbaghi (2017) reports that in nematode populations, species of the genus *Meloidogyne* are best known for their pathogenicity in vegetable crops. They are by far the most formidable, polyphagous that attack the majority of crops resulting in a significant decline in yield. This crop loss has been estimated globally at 14% per year (Groover and Lawrance, 2018), 15-25% or even 75% in some cases according to Jiang et al. (2018) great power of multiplication allowing them to quickly invade the roots of the plants on which they cause galls. In terms of money, Phani et al. (2017) have estimated the damage caused by these nematodes globally to 173 million Dollars by year. These parameters are currently posing serious problems for the market garden sites visited in Bamako and Ségou (Touré, 2017). The most sensitive crops are Solanaceae (tomatoes, eggplant, potato), Cucurbitaceae (melon, cucumber), Legumes (beans), Umbellifera (carrots, celery ...), Compounds (lettuce). In the present study, on one hand, *Meloidogyne* species on vegetable crops were characterized by perineal plaques and on the other hand their pathogenicity on tomato variety Roma (*Lycopersicon esculentum*) evaluated.

MATERIALS AND METHODS

Sites and sampling

Five permanent market gardening sites located near the Niger River were chosen for the collection of root samples: Samanko (12°31'419N; 08°04'921W), Sotuba (12°39'721N; 07°56'726W), Tiébani (12°32'807N; 08°02'347W), Daoudabougou (12°36'878N; 07°58'598W), and Baguinéda (12°37'975N; 07°47'503W). These sites were selected to be representative as possible of those used around the Bamako city, Mali (Figure 1). A systematic sampling was performed using a spreader. The collection of root samples took place on vegetable crops (tomato, eggplant, okra, lettuce, onion...). The roots collected were immediately placed in plastic bags marked with the site and crop names, and the date. They were then placed in a cooler to protect them from solar rays that can kill nematodes very quickly (Coyne et al., 2007). In the laboratory, the samples were kept in a refrigerator for analysis within two weeks at the latest.

Extraction and observation of the *Meloidogyne*

Extraction of larvae

Nematodes were extracted using the maceration-filtration method (Coyne et al., 2007). In brief, the roots were first gently washed under a trickle of tap water to clear the soil and much of the saprophagous nematodes. They are then cut into small pieces using a secateurs or a knife. The root pieces are then disinfected by soaking in a solution of bleach (1% active chlorine) for 4 to 5 min to lighten them.

They are then rinsed with tap water for 15 min, followed by maceration in a kitchen blender. The ground material is passed through a series of three sieves having holes diameter 150, 75 and 38 µm, respectively. This last sieve is used alone and tilted at about 45 degrees to maximize the retention of nematodes. The filtrate collected by the last sieve should contain larvae and nematode eggs. In addition to this maceration-filtration technique, the Baermann technique (Tessier, 2010) was also used to extract root nematodes. The macerated roots were placed on small Baermann type sieves. The extraction of the eggs is done under stereoscope. In brief, the roots were disinfected with 1% bleach for 1 min and placed in a petri dish. Using a scalpel and a lanceolate needle, the gall was opened superficially. The eggs in mucus at the posterior end of the female under the bark is then torn off and placed on an optical microscope slide for observation.

Identification of *Meloidogyne* spp.

The fixation of the nematodes was made on roots carrying galls in good condition, by the method of sodium hypochlorite-fuchsin acid (Phani et al., 2017 ; Fayzia et al., 2018). The roots were cut into 2 cm fragments. These pieces of roots were then lightened with bleach for 4 min and then rinsed with tap water for 15 min to remove the after-effects of the bleach. They must be boiled for 30 s in 30 ml of distilled water plus 1 ml of a stock solution of fuchsin acid (0.35 g fuchsin acid, 25 ml acetic acid, 75 ml distilled water) and then refreshed for 30 min at room temperature. Then the roots were discolored in an acidified glycerol solution by adding 6 drops of nitric acid and boiled. The discolored roots will be freed of glycerol and placed in a petri dish containing lactophenol for temporary storage.

Female were dissected under stereoscope on a slide. The female nematodes were fixed with fuchsin acid (extraction) and were deposited on an object slide in a drop of distilled water. Then with entomological spines the female is pierced and emptied of its contents by slightly pressing on the nematode.

This identification was made from the perineal plates of nematodes (Rusinque et al., 2018). A drop of glycerol or lactophenol is then added. The set was covered with a coverslip object. The air bubbles were eliminated by slightly blazing the blade. The coverslip is then sealed with nail polish for a medium shelf life. These preparations are used for microscopic observation (400x). For a specific determination of nematodes, these perineal figures were compared to those published by Boros et al. (2018).

Study of the *Meloidogyne* pathogenicity on the cv Roma tomato

Nematode rearing was done in plastic, drilled at the bottom using a fire-heated needle. These pots were filled with pasteurized soil heat and were arranged on wooden boards. A 4 weeks old Roma tomato plant (*L. esculentum L.*) was transplanted into each pot. The objective was to study the impact of the multiplication of nematodes of the genus *Meloidogyne* on the development of tomato *cv* Roma VF which is known to be resistant to *Verticilium* and *Fusarium*. The Roma VF tomato variety is known to be vigorous and productive, but susceptible to nematodes.

The seed was disinfected by soaking for 3 min in 1% active chlorine sodium hypochlorite solution, rinsed twice with sterile distilled water and dried, treated with Apron star: a fungicide and insecticide (20% Thiamethoxam, 20% Metalaxyl and 20% Difenoconazole) and then dried. The transplanting pots were carefully washed with soap before filling them with pasteurized soil in the fire. Seeding was done in wooden trays filled with previously pasteurized potting soil. The substrate used was formed by a mixture of field compost (three volumes), fluvial sand (one volume); rice hull (5 kg); fertilizers (5 kg of compost, 120 g of NPK 6-10-20); water in adequate quantity. This substrate was used both in the germinating tanks and in the containers of the trial.

Four weeks after emergence, the young tomato plants are transplanted into 24 pots of 10 dm^3 volume filled with pasteurized soil with 1 plant per pot. The trial design was two treatments

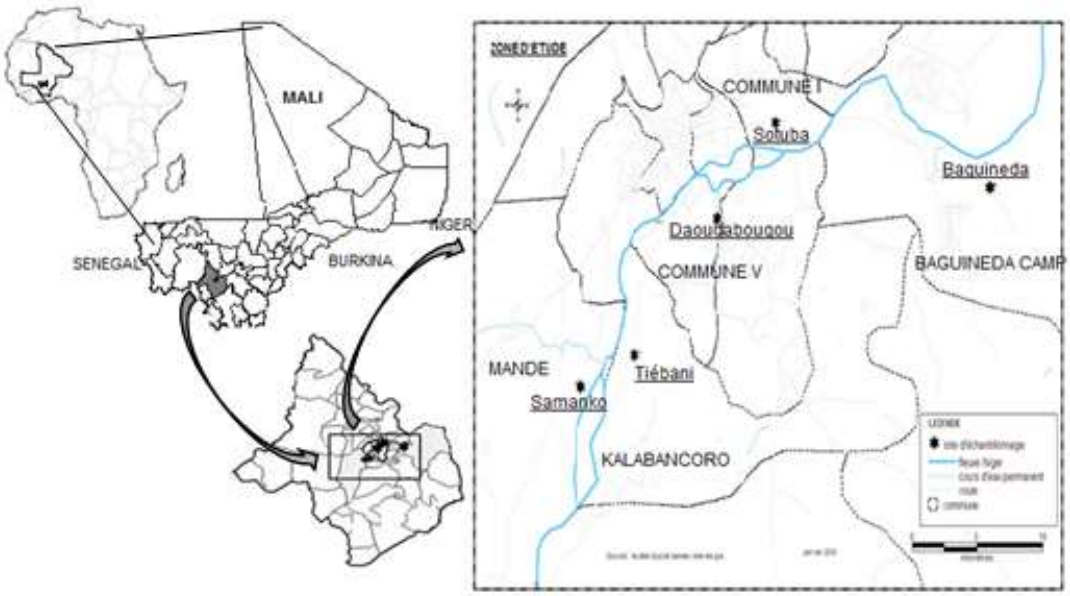

Figure 1. Localization of sites where samples were collected (Touré et al., 2019).

(inoculated and non-inoculated or control) and twelve pots per treatment. Three weeks after transplanting, the plants were inoculated with juveniles of the second stage (j2) nematodes by pouring a solution of 300 juvenile into a small gap dug around the roots.

The date of inoculation was marked on each pot. The inoculum rate was determined after counting on a box with a grid bottom. Other tomato plants were inoculated with the eggs obtained by dissection of the galls. The eggs carried in a tube with a plug or on a microscope slide was gently transferred to the roots of the tomato plants. The roots were then covered with soil. The success of the inoculation must be manifested by the formation of galls. The pots are watered once a day during the two months of the experiment. Phytosanitary treatments were applied one week after transplanting the tomato plants.

Two months after, the plants were removed and the roots were carefully washed, the rate of withered plants, the gall index of inoculated plants of nematodes, the dry weight of the plants cut at the neck were determined. The gall index was estimated using a scale of 0 to 5: 0 = no gall, 1 = traces of infection with some small galls, 2 = more than 25% of roots have galls, 3 = 25 to 50% of the roots have galls, 4 = 50 to 75% of the roots are galls; 5 = more than 75% of the roots have galls (Fayzia et al., 2018). The roots infested with galls are then ground in a kitchen blender and the ground material was filtered to extract *Meloidogyne* j2 confirming that the worms are at the origin of the galls.

RESULTS

Determination of nematodes

Microscopic observation of perineal plates revealed three types of tracks (Figure 2), which were compared to those published by Sasser et al. (1985). The first type corresponds to *Meloidogyne arenaria*, the second is to *Meloidogyne incognita* and the third to *Meloidogyne javanica*. *M. arenaria* is the most abundant 43.1% of the samples, followed by *M. incognita* 33.8% of the samples. *M. javanica* is the least abundant 23.1% of the samples observed (Figure 3).

Pathogenicity

One month after the symptoms expressed by root gall indices were significant on all inoculated plants. The degree of infestation varied from one plant to another. Of the 12 infested plants, one had the index 5 (more than 75% of the roots carry galls), three plants had the index 4, three others the index 3. The index 2 was the most represented with 5 plants. No infested plants of index 0 and 1 were noted. Thanks to phytosanitary treatments no withered plants were observed (Figure 4).

According to the severity formula of the gall index rate: 1 plant had the index 5 or a severity of 100%, 3 plants had 80% of severity (index 4), 3 others had severity 60% (index3) and 5 had severity 40% (index 2). Figure 4 shows the rate of gall index found on tomato roots.

The dried biomass did not vary significantly between the two treatments (Student t-test, p-value = 0.3), although the average weight of non-nematode infested plants (8.69g) was slightly higher than that of the infested ones (7.36 g). The *Meloidogyne* populations have not reached the sufficient level to influence the development of the tomato plants. Phytosanitary treatments also contributed to good plant growth.

Figure 5 shows the variations in the biomass of the dried plants of the two treatments and the twelve pots. However, the observation of Figure 5 shows a trend of

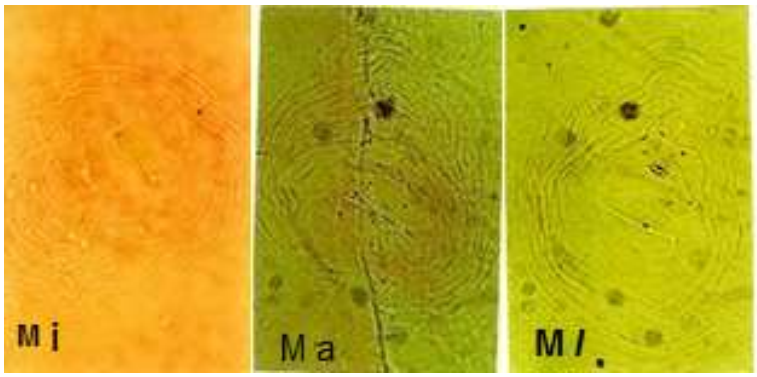

Figure 2. *Meloidogyne* female perineal patterns: Mj (*M. javanica*), Ma (*M. arenaria*), Mi (*M. incognita*).

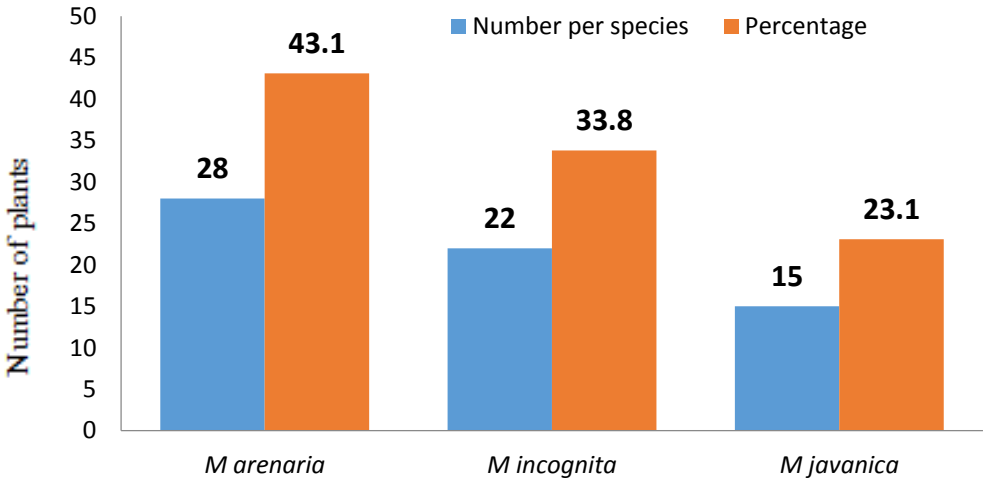

Figure 3. Number per species (blue) and percentage (orange) of *Meloidogyne* identified from female perineal plates.

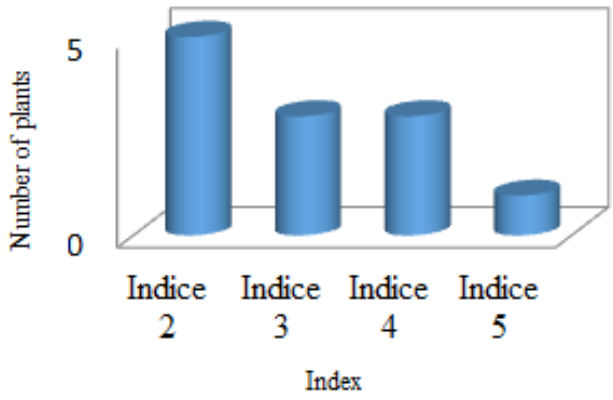

Figure 4. Gall indices observed on the tomato roots.

separation of the biomass values of the two treatments. This may be due to the fact that females of *Meloidogyne* by settling in the galls negatively influence the normal growth of tomato plants. Signs of infestation (galls) of tomato plants were observed to varying degrees in all plants. These indices show that *L. esculentum* responded

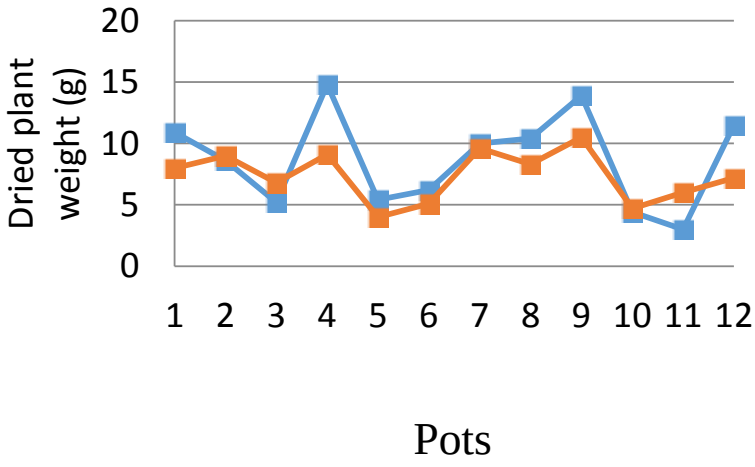

Figure 5. Dried biomass variation for inoculated (orange) and control (bleu) plants.

well to the attack of *Meloidogyne* larvae. The Roma tomato can therefore be considered as a host plant for nematodes of the genus *Meloidogyne*. The absence of significant variation may be due not only to the rate of inoculum (300 days per plant) which seems small compared to the threshold of 1000 j2/kg of soil (De Guiran, 1983), but also to the short vegetative period, 4 to 5 weeks, after transplanting.

DISCUSSION

The results obtained were compared to those for Mali neighbour countries. In this study, three of the major species of *Meloidogyne* were determined. These species are by far the most common in the inter-tropical zone (Powers et al., 2018; Rusinque et al., 2018). The present results show a difference with those of Alabama where Groover and Lawrance (2018) who found two species: *M. arenaria* with 3% of the observed samples and *M. incognita* 96%. The present results would also be different from those associated with tobacco in China reported by Zeng et al. (2018). There, the species *M. arenaria* occupies 61.9%; *M. javanica* 28.5% and *M. incognita* 9.5%. In these two countries, *M. javanica* is the most common species, 80% of the samples. The same species were reported by Mokrini and Sbaghi (2017) in Morocco in the orchard gardens confirm their presence around the world. The differences between the results obtained and those carried out elsewhere may lie in the fact that the samples were collected in a restricted area around the city of Bamako.

Perineal plates are most often variable within the same species, so they alone do not represent a sufficient method for a correct identification of the species of nematode galls. They do not distinguish *M. incognita* and *Meloidogyne mayaguensis* (Janete et al., 2002). It would therefore be desirable to continue this work to elucidate the problem of the existence of other nematode species in Mali. Other techniques such as the differential host pathogenicity test (cotton, chili, melon, tomato) and morphometric measurements will of interest. Examination of the perineal plates is only a first step to achieve. In addition, in the process of identifying root-knot nematodes, it gives an idea of the variability of the *Meloidogyne* spp. population around Bamako.

This study also showed that tomato variety cv Roma is susceptible to nematodes of the genus *Meloidogyne* despite a low inoculum rate of 300 larvae per plant. Statistical analysis of the dry weight of the treatments did not show a significant difference (Student's t-test, $p = 0.3$). This result may be due to the low inoculum rate of 300 larvae. This rate is below the threshold of 1000 eggs reported by da Silva Rabelo et al. (2018), from Wubie and Temesgen (2019) on Solanaceae. The biomass values of the treatments tend towards those reported by Prabhu et al. (2018) in India who noted a gradual decrease in growth and yield of turmeric (*Cucurma longa* L) based on inoculum rate (0, 100, 500, 1000, and 10000).

Conclusion

The species encountered are mainly *M. arenaria, M. incognita* and *M. javanica*. These species are known in most African countries (Mokrini and Sbaghi, 2017). 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 rough sketch of the identification of root-knot nematodes. On the basis of this preliminary work, new research could be undertaken to confirm identification by more precise techniques such as differential hosts,

esterase electrophoresis and DNA-based molecular methods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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