Plant-parasitic nematodes associated with vegetables in Northern Minas Gerais, Brazil

Fitonematoides associados a hortaliças no Norte de Minas Gerais, Brasil

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ABSTRACT
Plant-parasitic nematodes are frequently associated with vegetables causing yield losses. There is little information on the occurrence of these pathogens in the state of Minas Gerais, Brazil. The objective of this work was to identify plant-parasitic nematodes associated with vegetable crops, focusing on the detection of Meloidogyne spp. in commercial production areas located in the

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municipality of Montes Claros, Northern Minas Gerais, Brazil. Soil and roots samples were collected from 15 farms including 19 vegetable crops. Nematodes were extracted from soil and identified to genus level, and the roots were assessed for gall index. In soil samples, the genera *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp., *Criconemoides* spp., *Pratylenchus* spp. and *Tylenchus* spp. were observed in a percentage of 46.1; 31.8; 11.8; 7.4; 6.4 and 3.6%, respectively. The average gall index in vegetable crops were: squash (4.5), lettuce (2.2), eggplant (9.3), beetroot (2.4), broccoli (1.9), carrot (0.3), coriander (2.6), cabage (0.3), cauliflower (1.2), spinach (2.0), beans (2.8), scarlet eggplant (3.3), gherkin (1.9), cucumber (3.0), pepper (3.3), okra (2.3), arugula (0.7), parsley (3.7) and tomato (3.3).

**Keywords:** *Meloidogyne* spp., occurrence, infectivity, olericulture.

**RESUMO**
Os fitonematoides estão comumente associados a hortaliças causando perdas. No estado de Minas Gerais existe carência de informações sobre a ocorrência desses patógenos. O objetivo deste trabalho foi identificar fitonematoides associados a hortaliças, com ênfase na detecção de *Meloidogyne* spp. no município de Montes Claros, norte de Minas Gerais. Foram coletadas amostras de solo e raízes em 15 propriedades, envolvendo 19 espécies de hortaliças. As amostras de solo foram processadas e os fitonematoides identificados em nível de gênero e as raízes foram avaliadas com relação ao índice de galhas. Nas amostras de solo, os gêneros *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp., *Criconemoides* spp., *Pratylenchus* spp. e *Tylenchus* spp. foram observados em uma porcentagem de 46.1; 31.8; 11.8; 7.4; 6.4 e 3.6%, respectivamente. As culturas amostradas apresentaram os seguintes índices médio de galhas: Abóbora (4.5), Alface (2.2), Beterraba (2.4), Brócolis (1.9), Cenoura (0.3), Coentro (2.6), Couve (0.3), Couve Flor (1.2), Espinafre (2.0), Feijão (2.8), Jiló (3.3), Maxixe (1.9), Pepino (3.0), Pimentão (3.3), Quiabo (2.3), Rúcula (0.7), Salsa (3.7) e Tomate (3.3).

**Palavras-chave:** *Meloidogyne* spp., ocorrência, infectividade, olericultura.

**1 INTRODUCTION**

Vegetable crops are an important source of food plenty in protein, vitamins, minerals and fiber. In 2018, Brazil accounted for approximately 3 million tons of vegetables freshness (FAOSTAT, 2020). However, despite its importance, vegetable production is affected by several pathogens, including plant-parasitic nematodes which represent an important limiting factor to these crops. Of particular importance are the root-knot nematodes *Meloidogyne* spp., followed by others such as the root-lesion nematodes *Pratylenchus* spp., stem and bulb nematode *Ditylenchus dipsaci* (Kuhn) Filipjev, reniform nematode *Rotylenchus reniformis* Linford & Oliveira, dry rot of tubers *Scutellonema bradys* (Steiner & LeHew) Andrassy and the spiral nematode (*Helicotylenchus dihystera* (Cobb) Sher (Pinheiro, 2017).

The state of Minas Gerais is the second largest vegetable producer in Brazil. It has a planted area of around 120 thousand ha and an estimated production of 3.5 million tons of more than 50 species of vegetables annually. It is believed that cultivation of vegetables in the state generates
about 120 thousand direct and indirect jobs and that the gross value of production is of US$ 1 billion (EMATER, 2017). In the state of Minas Gerais, vegetables are mainly produced in the regions of the Triângulo Mineiro/Alto Paranaíba and municipalities in the metropolitan region of Belo Horizonte, while the northern of Minas Gerais stands out on the state horticultural map. In the municipality of Montes Claros, northern region of Minas Gerais, the green belt for vegetable production is located mainly in the counties of Planalto Rural, followed by Lagoinha and Nova Esperança, which are still expanding and targeting the domestic market. However, despite their economic importance, there is little information on current nematode prevalence in the vegetable farming areas within the state. Such information is required in order to implement adequate strategies for nematode management to reduce yield losses. In view of this, the objective of this work was to identify plant-parasitic nematodes associated with vegetable crops, focusing on the detection of Meloidogyne spp. in commercial planting areas located in the municipality of Montes Claros-MG, Brazil.

2 MATERIAL AND METHODS

The survey was carried out from April to September 2018, in 15 sites located in the Planalto Rural in the municipality of Montes Claros, Northern Minas Gerais, Brazil. The climate of the region is characterized as Aw-tropical savanna with dry winter and rainy summer according to Köppen classification (ALVARES et al., 2013). The survey included the following vegetable crops: squash (Cucurbita pepo L.), cucumber (Cucumis sativus L.), gherkin (Cucumis anguria L.) (Cucurbitaceae); lettuce (Lactuca sativa L.) (Asteraceae), beetroot (Beta vulgaris L.) (Chenopodiaceae), carrot (Daucus carota L.), coriander (Coriandrum sativum L.), parsley (Petroselinum crispum Hoff) (Apiaceae); cabagge (Brassica oleracea L. var. acephala DC.), cauliflower (Brassica oleracea var. botrytis L), broccoli (Brassica oleracea L. var. italica Plenk), arugula (Eruca sativa L.) (Brassicaceae), spinach (Spinacia oleracea L.) (Amaranthaceae), beans (Phaseolus vulgaris L.) (Fabaceae), okra (Abelmoschus esculentus L. Moench) (Malvaceae); eggplant (Solanum melogena L.), scarlet eggplant (Solanum gilo Raddi), pepper (Capsicum annuum L.), and tomato (Solanum lycopersicum L.) (Solanaceae).

Following the systematic zigzag pattern, soil samples were collected in the root zone to a depth of 15 to 20 cm. For root sampling, three plants from each vegetable crop were removed from the soil, and a portion of the root system was detached. Approximately 60 g of soil and 10 g of roots were taken. Subsequently, the subsamples were mixed and composite samples of approximately 80 g of roots and 500 g of soil were packed in plastic bag, labeled and taken to laboratory for processing.
Soil samples were also used for analyses of physical and chemical characteristics, according to EMBRAPA (1997). Moreover, in each area, sampling points were georeferenced and the data were submitted to Google Earth computational program, for spatial visualization of the infested and non-infested fields.

Nematodes were extracted in triplicate 100 cm³ of soil by the method of fluctuation-sieving-centrifugation (Jenkins, 1964). The soil was stirred in 600 ml of water for 60 s, left to rest for 20 s, then passed through a 325-mesh sieve. Then, the nematode suspensions were centrifuged at 141g for 5 min, and subsequently the supernatants were discarded, and their sediments were added with 1M sucrose solution for further centrifugation for 60 s. Nematodes from the supernatants were collected by a 325-mesh sieve and washed. After extraction, nematodes were counted on a Peters slide under light microscope. All nematodes were identified to the genus level (Mai and Mullin, 1996; Ferraz, 2016).

In the laboratory, roots were carefully washed in order to observe symptoms of galls. Roots were visually assessed using a gall index (0-10): 0 = no galls on roots and 10=all roots severely knotted; no root system; plant usually dead (Bridge and Page, 1980).

3 RESULTS AND DISCUSSION

Nematodes from the genus *Meloidogyne* spp., *Helicotylenchus* spp., *Rotylenchulus* spp., *Pratylenchus* spp., *Tylenchus* spp. and *Criconemoides* spp. were identified in soil samples. Of these plant-parasitic nematodes, *Meloidogyne* spp. was the most frequently genus, being observed in 82% of the samples, followed by *Helicotylenchus* - 61%, *Rotylenchulus* - 47%, *Tylenchus* - 37%, *Pratylenchus* - 34% and *Criconemoides* 7.4%.

The high frequency of the genus *Meloidogyne* may be due to the high level of polyphagia, which facilitates its survival for successive cycles in planting areas. Our data are in accordance with Gonçalves (2014) and Oliveira (2016) who also observed high frequency of *Meloidogyne* spp. in vegetable crops in the states of São Paulo and Goiás, Brazil, respectively. Other genera such as *Helicotylenchus*, *Rotylenchulus*, *Pratylenchus* were also found by these authors.

In this study gall index showed high variation among the evaluated crops. Eggplant, squash, parsley, scarlet eggplant, cucumber, pepper and tomato showed an average rating index of more than 3, indicative of susceptibility (Figure 1). Only cabbage, broccoli, cauliflower, snap bean, gherkin, okra and tomato showed symptomatic plants. However, in all cases, the number of symptomatic plants was higher, except for tomato where the number of plants with or without symptoms was equivalent (Figure 2). The severity of symptoms may be related to the spreading
behavior of *Meloidogyne* spp. due to the proximity between plantations (Figure 3A). In addition, the uniformity and distribution of the nematode in the cultivation area associated with crop succession/rotation and management with biological control, partly explains the low gall index for some vegetables and the form of dissemination in the green belt region of the Planalto Rural. On the other hand, regarding the plant-parasitic nematodes observed in soil samples, *Meloidogyne* spp. showed the highest frequency (46%) (Figure 3B), which can be attributed to the use of resistant cultivars by the growers. In fact, in tomato cultivation, producers have used the Woodstock and Volt tomato rootstocks, which are resistant to *M. incognita* (races 1, 2, 3, 4) and *M. javanica*, which may explain a reduction in infectivity and expression of symptoms in tomato. However, the main cultivars used by producers for growing squash (cvs. Adele, Alícia and Corona), eggplant (cv. Nápoli), cucumber (cvs. Racer and Diplomata), pepper (cvs. Dahra, Nataly and Magali), tomato (cvs. Protheus and Compack), parsley (cv. Chácara), lettuce (cv. Vera), cauliflower (cvs. Juliana, Sharon, Paloma and Sarah), broccoli (cvs. Hanapon and Logan) and coriander (cvs. Santo, Verdão and Aztec) are all susceptible to *Meloidogyne* spp., which partly explains the high percentage of infection severity expressed by gall index.
Figure 1. Mean index number of *Meloidogyne* spp. galls in vegetable crops sampled in the areas assessed. *Number of samples used to obtain the average.
Figure 2. Average number of vegetables sampled in 15 fields regarding the presence of Meloidogyne spp. symptoms in the root system.

| Vegetable crops       | Symptoms |
|-----------------------|----------|
| Tomato                | Presence |
| Parsley               | Absence  |
| Arugula               | Presence |
| Okra                  | Absence  |
| Pepper                | Presence |
| Cucumber              | Presence |
| Gherkin               | Presence |
| Scarlet eggplant      | Presence |
| Beans                 | Presence |
| Spinach               | Presence |
| Cauliflower           | Presence |
| Cabagge               | Presence |
| Coriander             | Presence |
| Carrot                | Presence |
| Broccoli              | Presence |
| Beetroot              | Presence |
| Eggplant              | Presence |
| Lettuce               | Presence |
| Squash                | Presence |

Figure 3. Aerial view of the location (A) and frequency of nematodes (B) observed in soil samples collected from 15 fields, located in Montes Claros, MG, Brazil.
The soil attributes, such as pH, soil organic matter, contents of phosphorus and calcium ranged from 6.0-7.0, 2.24-5.58 dag Kg⁻¹ e 11.87-1080 mg dm⁻³ e 2.94-5.35 cmolₖ dm⁻³, respectively and soil texture ranged from sandy to sandy loam (Table 1). According to Van Gundy (1985) *Meloidogyne* spp. survive, hatch and reproduce over a wide pH range, 4.0-8.0, and also these nematodes can occur on a wide range of soil types. Soil organic matter also can affect nematode population, however, nematode control efficacy is not always satisfactory (Oka, 2010). Regarding phosphorus its effect to control nematodes can vary according to the source used (Ferraz et al. 2010). According to Zambolim and Ventura (2012) the application of superphosphates may enhance protein synthesis and the cellular activity in plant tissues, improving plant resistance to nematodes. On the other hand, calcium plays a role as an essential element for the plant growth and development and also in cross-linking acidic pectin residues in the cell wall; low Ca⁺² increases the permeability in the plasma membrane (Hepler, 2005).

### Table 1. Chemical and Physical soil characteristics from sampled areas infested with Meloidogyne spp. in 15 fields, located in Montes Claros, MG, Brazil.

| Fields | Meloidogyne spp. populations (100 cm⁻³) | Soil texture | pH  | SOM (dag kg⁻¹) | Phosphorus (mg dm⁻³) | Calcium (cmolₖ dm⁻³) |
|--------|----------------------------------------|--------------|-----|----------------|----------------------|----------------------|
| 1      | 706                                    | SL           | 7   | 3.23           | 1080                 | 4.2                  |
| 2      | 1478                                   | S            | 6.4 | 2.78           | 1080                 | 2.94                 |
| 3      | 129                                     | SL           | 6.2 | 4.41           | 490                  | 4                    |
| 4      | 520                                     | S            | 7   | 3.39           | 460                  | 3.5                  |
| 5      | 0                                      | SL           | 7   | 4.06           | 510                  | 5.35                 |
| 6      | 41                                      | S            | 6   | 5.58           | 11.87                | 4.8                  |
| 7      | 234                                     | SL           | 6.9 | 4.79           | 1080                 | 5                    |
| 8      | 526                                     | S            | 7   | 4.06           | 540                  | 3.8                  |
| 9      | 85                                      | SL           | 6.7 | 2.50           | 860                  | 3                    |
| 10     | 408                                     | SL           | 6.5 | 3.71           | 840                  | 4.26                 |
| 11     | 140                                     | SL           | 6.6 | 3.23           | 1080                 | 4.1                  |
| 12     | 1764                                    | SL           | 6.3 | 2.37           | 480                  | 3                    |
| 13     | 530                                     | S            | 6.6 | 2.64           | 580                  | 3                    |
| 14     | 255                                     | SL           | 6.8 | 3.39           | 460                  | 3.43                 |
| 15     | 33                                      | S            | 6.8 | 2.24           | 1020                 | 4                    |

SOM = soil organic matter; SL = Sandy loam; S = Sandy.

Therefore, based on these soil characteristics it is not possible to explain the high *Meloidogyne* population densities in some of the sampled areas, once under field conditions it is difficult to separate the interacting effects of soil and environmental factors. In addition, the absence of crop rotation and the soil movement by farm equipment observed in the present study should have contribute to increase nematode population densities in the areas. Based on galls index, the
vegetable crops eggplant, squash, parsley, scarlet eggplant, cucumber, pepper and tomato were the most affected by *Meloidogyne* spp.

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