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# First report of *Meloidogyne javanica* on *Celosia argentea* in the Northeast of Brazil

# Primeiro relato de *Meloidogyne javanica* em *Celosia argentea* no Nordeste do Brasil

**SOUZA JUNIOR, Francisco Jorge Carlos[1]; ASSUNÇÃO, Mayara Castro[2]**

[1] 0000-0003-2086-9857; Secretaria da Educação do Estado do Ceará (SEDUC-CE). Fortaleza, CE, Brasil. E-mail: jorgeldp2011@gmail.com.
[2] 0000-0002-3127-7817; Universidade Federal do Oeste da Bahia (UFOB). Barra, BA, Brasil. E-mail: mayara.assuncao@ufob.edu.br.

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## A B S T R A C T

*Celosia argentea* is a plant well adapted to the conditions of tropical and subtropical climates, being widely used for medicinal purposes and having high economic value for some regions. Infections caused by *Meloidogyne* spp. stand out as one of the main phytosanitary problems of *C. argentea*, with this, the work aimed to identify *Meloidogyne* species associated with *Celosia argentea* in areas in the municipality of Guaramiranga, CE, Brazil. The collections were made from roots with galls. Subsequently, the species were characterized through the morphology, esterase phenotypes and molecular analysis of the ITS and 28S rDNA regions. Plants of *C. argentea* were kept under greenhouse conditions, inoculated with the population of *M. javanica* (Ms01). The control treatment was not inoculated. The inoculated plants showed root gall symptoms, while the non-inoculated plants showed no symptoms. The species *M. javanica* was identified. This is the first detection of *Meloidogyne javanica* in *Celosia argentea* in Northeast Brazil.

## R E S U M O

*Celosia argentea* é uma planta bem adaptada às condições de climas tropicais e subtropicais, sendo amplamente utilizada para fins medicinais e possuindo alto valor econômico para algumas regiões. Infecções causadas por *Meloidogyne* spp. destacam-se como um dos principais problemas fitossanitários de *C. argentea*, com isso, este trabalho teve como objetivo identificar espécies de *Meloidogyne* associadas a *Celosia argentea* em áreas do município de Guaramiranga, CE, Brasil. As coletas foram feitas a partir de raízes com galhas. Posteriormente, as espécies foram caracterizadas através da morfologia, fenótipos de esterase e análise molecular das regiões ITS e 28S DNAr. Plantas de *C. argentea* foram mantidas em casa de vegetação, inoculadas com a população de *M. javanica* (Ms01). O tratamento controle não foi inoculado. As plantas inoculadas apresentaram sintomas de galhas nas raízes, enquanto as plantas não inoculadas não apresentaram sintomas. A espécie *M. javanica* foi identificada. Este é o primeiro relato de *Meloidogyne javanica* em *Celosia argentea* no Nordeste do Brasil.

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**Introduction**

*Celosia argentea* popularly known as cocks comb, it a herbaceous plant of the genus *Celosia* L. of the Amaranthaceae family, originating in Africa and Asia, it is propagated by seed and well adapted to the conditions of tropical and subtropical climates (Gilman & Howe, 1999; Surse et al., 2017). Due to its adaptability, it is widely used as an ornamental plant and for medicinal purposes, in addition to having high economic value for some regions (Akinfasoye et al., 2008; Singh et al., 2020).

This plant has a high content of proteins and vitamins and is a good source of calcium, iron, carbohydrates and phosphorus (Ayodele & Olajide, 2011), however, despite its nutritional popularity in tropical regions, *C. argentea* is also considered an invasive plant (Council, 2006). Thus, it is distributed irregularly in the areas of agricultural crops, being considered one of the main obstacles in production, since it contributes to the reduction of productivity and loss of product quality, as they compete for sunlight, water, nutrients and space with the cultures (Sausen et al., 2020).

Another damage caused due to the constant presence of invasive plants in the areas of agricultural crops includes the increase in production management costs, from the preparation of the soil for planting as well as during the harvest, which requires an operational change for its elimination (Pitelli, 2015). In addition, the presence of these plants contributes to the hostility of plant pathogens, including nematodes, since several invasive species are hosts of these organisms, serving as a source of inoculum, helping in the dissemination and survival (Carvalho, 2013; Gould, 2017).

Among the plant parasitic nematodes, the genus *Meloidogyne* Goeldi (1887), also known as the root-knot nematode, stands out for parasitizing almost all plant species, many of which are of economic importance, and alternative hosts, having a wide geographical distribution. In the world ranking, it occupies the first position among nematodes harmful to agricultural crops, being also associated with alternative hosts, such as ornamental and invasive plants, which act as a source of inoculum, maintaining populations in the areas and contributing to productivity losses (Jones et al., 2013; Moens et al., 2009).

*Meloidogyne* specimens have a high rate of reproduction and aggressiveness, in addition to being mandatory parasites with a sedentary habit, which favors this group of nematodes to be the main responsible for damage and loss of productivity in several plants (Moens et al., 2009). The life cycle consists of six phenological stages: egg, four juvenile stages (J1, J2, J3 and J4) and adult; being in the J2 stage the infectious phase, in which the nematode penetrates the roots and induces the formation of galls due to the establishment of its feeding site - from the formation of giant cells - (Abad et al., 2009; Vidal et al., 2019). Due to this specialization and evolution of the genus, the damages caused are great and, in the majority, irreversible, therefore the importance of the detection of this organism in all types of plants.

In the Northeast of Brazil, this genus is often found associated with the roots of other plants, both agricultural crops, such as sugar cane, yams, fruit, vegetables, and invasive plants, commonly from the Amaranthaceae, Asteraceae, Convolvulaceae families, Euphorbiaceae, Poaceae and Solanaceae (Moura, 1996; Ramos et al., 2019). Nematode surveys taking place in alternative hosts are essential for the identification of species and population density, in addition to being an important instrument for the adoption of more effective management measures. With this, the work aimed to identify *Meloidogyne* species associated with *Celosia argentea* in areas in the municipality of Guaramiranga, CE, Brazil.

## Material and Methods

Root samples of *C. argentea* with symptoms of meloidoginosis, were collected in an agricultural cultivation area in February 2019 in the municipality of Guaramiranga (4° 14' 59"S 38° 56' 25"W), state of Ceará, Brazil. For the extraction of nematodes, the roots were processed according to the method of Coolen and D'Herde (1972). The identification of *Meloidogyne* was carried out by esterase phenotypes (n = 20) (Carneiro & Almeida, 2001), perineal patterns (n = 20) according to the methodology of Taylor and Netscher (1974), morphological measurement of second-stage juveniles (J2) (n = 20) and females (n = 20).

Molecular identification of *Meloidogyne* species was performed by amplifying and sequencing the D2-D3 regions of the 28S rDNA segment with the primers D2A (5´-ACAAGTACCGTGAGGGAAAGTTG-3´) and D3B (5´-TCGGAAGGAACCAGCTACTA-3´) (De Ley et al., 1999) and ITS primers with VRAIN2F (5´-CTTTGTACACACCGCCCGTCGCT-3´) and VRAIN2R (5´-TTTCACTCGCCGTTACTAAGGGAATC-3´) (Vrain et al., 1992).

Consensus sequences were assembled from forward and reverse sequences using the Staden package (Staden et al., 1998). The assembled consensus sequences were used for a Blastn search against the NCBI nucleotide database. The assembled consensus sequences were used for a Blastn search against the NCBI nucleotide database. Multiple sequence alignments were performed with the MAFFT 7 online version using the iterative refinement method L-INS-i (Katoh, 2013; Katoh & Toh, 2008) for each individual gene and region.

Phylogenetic analyzes were performed using the maximum likelihood (ML) methods for individual genes, performed via RAxML-HCP2 v.8.2.8 (Stamatakis, 2014) and MrBayes v 3.2.1 (Ronquist et al., 2012) programs implemented in the CIPRES Portal v.2.0 (https://www.phylo.org/portal2/home.action) with 1.000 repetitions. The MrModeltest 2.3 program was used to find the best-fit model of nucleotide evolution according to BIC parameters (Nylander, 2004). The best-fit nucleotide substitution models were selected using BIC in jModelTest2 v. 2.1.6 (Darriba et al., 2012; Guindon & Gascuel, 2003).

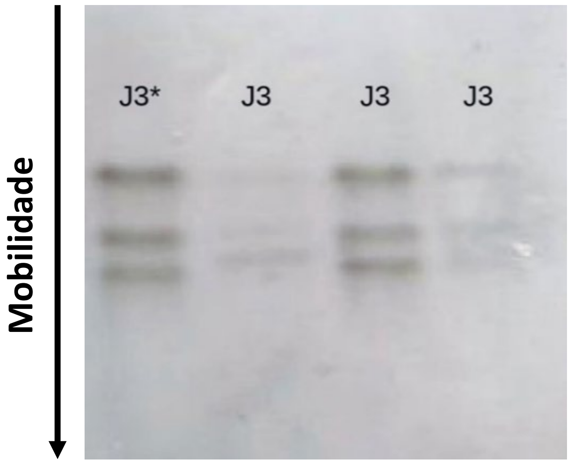
In greenhouse tests, plants of *C. argentea* were kept in 2 L pots containing previously sterilized soil, under greenhouse conditions with an average temperature of 25.5 ± 1 ° C. The inoculation of 5.000 eggs + J2 from the population of *M. javanica* (Ms01) was carried out with five replicates and the control treatment was not inoculated. After 60 days, the inoculated plants showed root gall symptoms similar to the plants observed in the field, while the non-inoculated plants showed no symptoms.

## Results and Discussion

In females, the body length was 590.0±40.5 (480.1-635.5)μm; the stylet measured 13.5±0.6 (12.8-14.5)μm in length; the dorsal esophageal gland orifice (DGO) was 3.8±0.4 (2.7-4.1)μm. For J2, the body length was 375.0±30.5 (320.3-434.5)μm; stylet length 10.8±1.1 (10.0-12.4)μm; DGO equal to 2.8±0.4 (2.7-3.6)μm; and tail measurements c= 8.9±0.5 (7.7-11.4)μm and c'= 5.1 ±0.4 (4,3-5.6)μm, showing tapering in the terminal region (MAI & MULLIN, 1996).

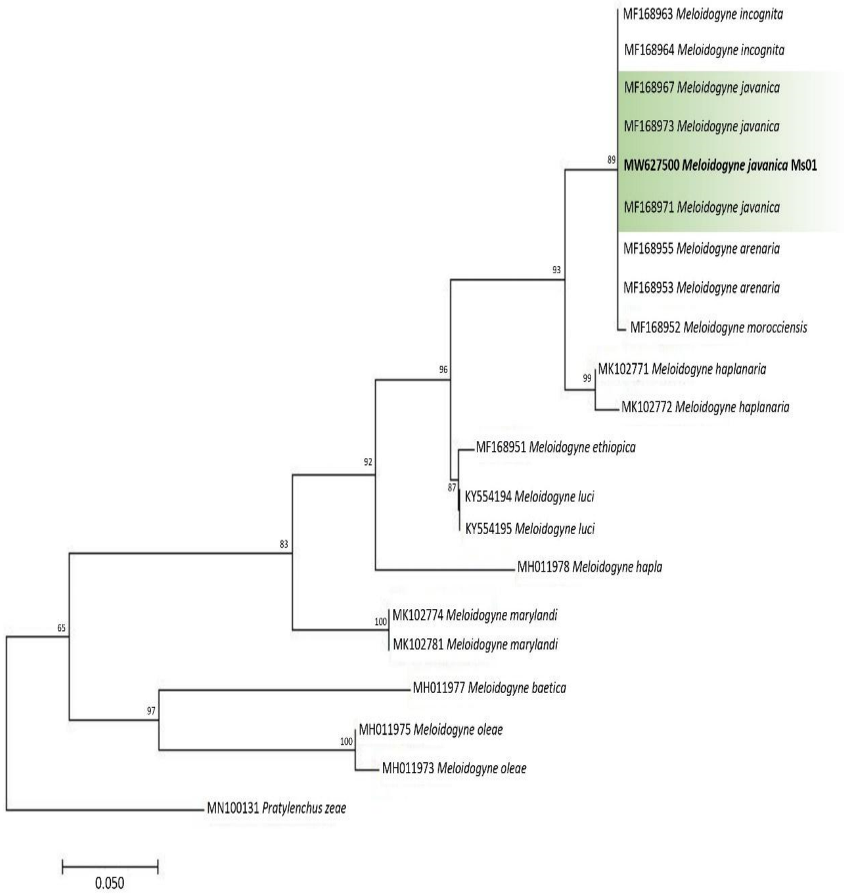
The polymorphisms of esterase bands by electrophoresis revealed the phenotype J3 (Rm = 1.00, 1.25, and 1.40) typical of *M. javanica* (Figure 1). The sequences of the studied rDNA regions were submitted to GenBank (ITS: MW627500 and D2-D3 28S: MW627475). Research in BLAST showed 99% identity with sequences of *M. javanica* isolates from Brazil, USA and China. Phylogenetic analyzes according to ML, placed the *Meloidogyne* (Ms01) population isolated from *C. argentea* in a clade with the *M. javanica* sequences from GenBank, confirming it as *M. javanica* (Figure 2 and Figure 3).

**Figure 1.** Esterase phenotypes of *Meloidogyne javanica* detected in *Celosia argentea* (J3: *M. javanica*; J3*: *M. javanica* reference isolate).



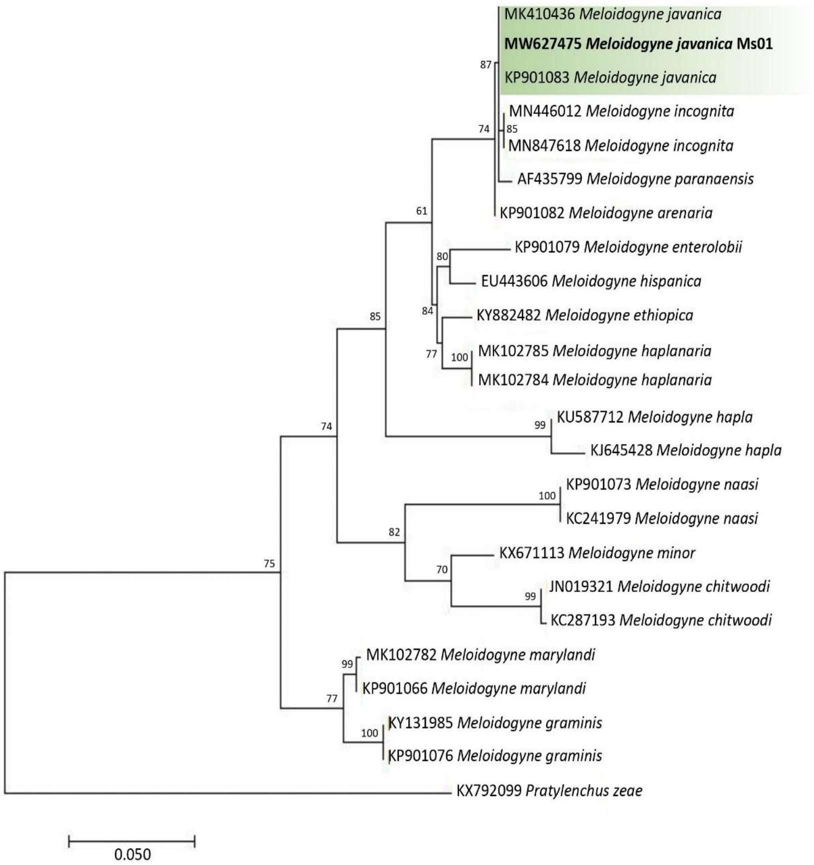

Source: Research data

**Figure 2.** Phylogenetic relationship of *Meloidogyne javanica* from Ceará, Brazil, based on alignment of the sequence of the ITS rDNA. The phylogenetic tree was estimated by Maximum likelihood. *Pratylenchus zeae* was used an outgroup.




Source: Research data

**Figure 3.** Phylogenetic relationship of *Meloidogyne javanica* from Ceará, Brazil, based on alignment of the sequence of the D2/D3 expansion fragments of 28S rDNA. The phylogenetic tree was estimated by Maximum likelihood. *Pratylenchus zeae* was used an outgroup.




Source: Research data

Associated with this plant species, *Meloidogyne* spp. in the United States (Gill et al., 2007; Hagan, 2005), *M. incognita* in Nigeria (Daramola et al., 2015) and Pakistan, (Anwar et al., 2009) e *M. javanica* in Pakistan (Maqbool et al., 1986). In Brazil, *M. javanica* has already been identified in *C. argentea* in São Paulo state, Southeast of Brazil (Paeadela Filho et al., 1971), but this is the first report of *M. javanica* parasitizing *C. argentea* in Northeast Brazil, updating the information on this parasitism and indicating this plant as a potential host for this nematode species.

The species *M. javanica* has also been described as parasitizing roots of other ornamental plants in Brazil, such as anthurium (*Anthurium digitatum*), chrysanthemum (*Chrysanthemum* sp.), daisy (*Crysanthemum maximum*), iresine (*Iresine herbstii*), little finger (*Sedum rubrotinctum*) (Costa et al., 2003) and buttercup (Silva et al., 2016). In addition to the host plants cited as susceptible to *M. javanica* parasitism in Brazil, Oliveira and Kubo (2006) also report *Ajuga reptans*, *Begonia rex*, *Calendula officinalis*, *Cassia* spp., *Exacum* sp., *Gladiolus* sp., *Grevillea robusta*, *Helianthus annuus*, *Helichrysum bracteatum*, *Hibiscus* sp., *Holocalix balansae*, *Impatiens balsamina*, *Opuntia* spp., *Pachystachys lutea*, *Primula officinalis*, *Rosa* sp. and *Tabebuia alba*.

Worldwide, *M. javanica* was reported during a field survey in Pakistan on the fire-extinguishing plant species (*Alternanthera tenella*), considered an ornamental plant in this country (Anwar-Ul-Haq et al., 2012). In a study carried out by Solano-González et al. (2015) in Costa Rica, evaluating the parasitism of the root-knot nematode in ornamental plants, *M. javanica* was described in *Gardenia* sp. and *Calatea* sp.

Another species of the genus observed in ornamental plants is *M. incognita*, which in Brazil has already been identified in the species centaurea (*Centaurea gymnocarpa*), daisy (*Chrysanthemum leucatemum*), crassula (*Crassula multicava*), phantom plant (*Graptopetalum paraguayensi*), ivy (*Hedera helix*), immortele (*Helicrysum petiolatum*), beijinho (*Impatiens walleriana*), four-leaf clover (*Oxalis adenopsis*), brilliantine (*Pilea microphylla*), eleven o'clock (*Portulaca grandiflora*), trade (*Zebrina pendula*) and celosia (*C. argentea*) (Costa et al., 2003; Silva et al., 2016; Souza Junior & Assunção, 2020).

Since there is no material resistant to root-knot nematodes and *Celosia* sp. are considered moderately to highly susceptible, identification of the *Meloidogyne* species present in the area is important, as it will direct more effective management strategies in order to keep nematode populations below economic limits (Daramola et al., 2015; Hagan, 2005).

## Conclusions

In view of the first report of *M. javanica* parasitizing roots of *C. argentea* in Northeastern Brazil, this work highlights the importance of this nematode as a damage-inducing agent for this plant species, as well as the need for further studies to evaluate this pathosystem in order to have a greater understanding of parasitic nematodes in agricultural plants in ornamental crops.

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