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MATHEUS MILANI PRETTO

AVALIAÇÃO DE ÓLEOS ESSENCIAIS E REAÇÃO DE PLANTAS AROMÁTICAS NO CONTROLE DE NEMATOIDES DO GÊNERO *Meloidogyne*

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Agronomia (Proteção de Plantas).

Orientadora: Profa. Dra. Silvia Renata Siciliano Wilcken

Coorientadoras: Dra. Adriana Aparecida Gabia; Dra. Carla Maria Nobre Maleita

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Aos meus pais e irmãos,

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RESUMO

Os nematoides parasitas de plantas (NPP) do gênero *Meloidogyne* (nematoides das galhas radiculares) causam danos significativos no sistema radicular de diversas culturas agrícolas, resultando em perdas de produtividade. O controle destes parasitas é essencial para garantir que se possa cultivar em áreas infestadas. Diante disso, os objetivos deste trabalho foram estudar a reação de diferentes espécies de plantas aromáticas a diferentes espécies de *Meloidogyne*, e avaliar a atividade nematicida de óleos essenciais. Desta forma, o trabalho foi separado em dois capítulos. No primeiro, é apresentada a reação das espécies de plantas aromáticas *Hypericum androsaemum*, *Lippia alba*, *Mentha spicata*, *Origanum majorana*, *O. vulgare* e *Thymus vulgaris* a *M. chitwoodi*, *M. enterolobii*, *M. incognita*, *M. javanica* e *M. luci*. Os resultados indicaram que, com exceção de *H. androsaemum* e do tomateiro (*Solanum lycopersicum;* incluído como controle), todas as espécies testadas foram resistentes às espécies de NPP, sendo promissoras para práticas de manejo integrado de pragas, como a rotação de culturas e o cultivo em consórcio. O segundo capítulo explorou o efeito de óleos essenciais das espécies *Aloysia citriodora*, *Corymbia citriodora*, *Cymbopogon citratus*, *C. winterianus*, *Elionurus muticus* e *L. alba* no controle *in vitro* de *M. chitwoodi* e *M. incognita*. Os resultados mostraram que os óleos essenciais, especialmente os de *C. citriodora* e *C. citratus*, apresentaram alta eficácia na mortalidade e na inibição da eclosão de jovens do segundo estágio (J2), evidenciando o potencial destas misturas de moléculas no desenvolvimento de novos nematicidas. Assim, ambos os capítulos fornecem estratégias sustentáveis e rentáveis para o manejo de diferentes espécies de *Meloidogyne*, utilizando tanto a resistência natural das plantas como também óleos essenciais, contribuindo para implementação de novas estratégias no controle e redução da população de nematoides das galhas radiculares em ambientes agrícolas.

Palavras-chave: nematoides das galhas radiculares; fator de reprodução; biocontrole; sustentabilidade; óleos essenciais.

ABSTRACT

Plant-parasitic nematodes (PPN) of the genus *Meloidogyne* (root-knot nematodes) cause significant damage to the root system of various agricultural crops, resulting in losses of productivity. The control of these parasites is essential to ensure that crops can be grown in infested areas. The aims of this work were to study the reaction of different aromatic plant species to different species of *Meloidogyne* and evaluate the nematicidal activity of different essential oils. The work was divided into two chapters. The first chapter present the reaction of the aromatic species *Hypericum androsaemum*, *Lippia alba*, *Mentha spicata*, *Origanum majorana*, *O. vulgare* and *Thymus vulgaris* to *M. chitwoodi*, *M. enterolobii*, *M. incognita*, *M. javanica* and *M. luci*. The results indicated that, except for *H. androsaemum* and the tomato plant (*Solanum lycopersicum*, included as control), all the species tested were resistant to the *Meloidogyne* species included in this work, making them as promising for integrated pest management practices, such as crop rotation and intercropping. The second chapter explore the effect of essential oils from the species *Aloysia citriodora*, *Corymbia citriodora*, *Cymbopogon citratus*, *C. winterianus*, *Elionurus muticus* and *L. alba* on the control *in vitro* of *M. chitwoodi* and *M. incognita*. The results showed that the essential oils, especially those of *C. citriodora* and *C. citratus*, were highly effective on second-stage juveniles (J2) mortality and hatching inhibition, demonstrating the potential of these mixtures of molecules to the development of new nematicides. Thus, both chapters provide sustainable and cost-effective strategies for the management of *Meloidogyne* species, using both natural plant resistance and essential oils, contributing to the implementation of new strategies for the control and reduction of the root-knot nematodes in agricultural environments.

Keywords: root-knot nematodes; reproduction factor; biocontrol; sustainability; essential oils.

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INTRODUÇÃO GERAL

Nematoides parasitas de plantas (NPP) são microrganismos pertencentes ao filo Nematoda, podendo ser considerados um dos principais agentes causadores de perdas econômicas na agricultura. Estes patógenos penetram as raízes ou outras partes das plantas, afetando significativamente a produtividade, de modo que se estimam perdas econômicas de cerca de 870 bilhões de reais, nos próximos 10 anos (Syngenta, 2022). Estes danos são substanciais em diversas culturas agrícolas quando parasitadas por espécies de NPP dos gêneros *Heterodera*, *Meloidogyne*, *Pratylenchus*, *Radopholus* e *Rotylenchulus.* Entre estes, os nematoides do gênero *Meloidogyne*, conhecidos vulgarmente por nematoides das galhas radiculares (NGR), ocupam a primeira posição no Top10 dos NPP com maior impacto econômico e científico (Jones *et al*., 2013; Campos, 2020). Até ao momento, encontram-se descritas cerca de 100 espécies, apresentando grande relevância mundial as espécies *M. chitwoodi, M. enterolobii, M. incognita, M. javanica,* e *M. luci* (Marquez *et al*., 2022; Fullana *et al*., 2024).

A gestão de NPP pode ser realizada através de diferentes técnicas/estratégias, sendo as principais o controle químico, biológico, resistência genética e rotação de culturas. A utilização de plantas resistentes a NPP constitui uma importante alternativa à aplicação de nematicidas de origem sintética, que representam um elevado impacto negativo no ambiente e na saúde humana, e garantem simultaneamente a produção e o retorno econômico para o agricultor.

As plantas aromáticas produzem compostos secundários, através do metabolismo especializado, e cuja produção é influenciada por fatores de estresse, sejam eles bióticos ou abióticos (Schmidt *et al*., 2024). Mundialmente, diversas espécies de plantas aromáticas, como por exemplo *Hypericum androsaemum* L. (Hypericaceae)*, Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae)*, Mentha spicata* L. (Lamiaceae)*, Origanum majorana* L. (Lamiaceae)*, O. vulgare* L. subs. *hirtum* (Lamiaceae) e *Thymus vulgaris* L. (Lamiaceae)*,* apresentam diferentes possibilidades de uso. A ornamentação de jardins; o uso na medicina tradicional devido às suas atividades antimicrobianas; atividade diurética como o *H. androsaemum* (Aguiar.; Costa, 2023); bem como o controle de pragas e doenças na agricultura, nomeadamente NPP, são algumas dessas possibilidades (Fontana *et al*., 2020; Demartelaere *et al*., 2021; Addabbo *et al*., 2024). Dentre as pragas e doenças

na agricultura, os óleos essenciais, oriundos do metabolismo especializado/secundário, podem ser utilizados no controle de diversas espécies de NPP: os óleos de *Chamaemelum nobile*, *Foeniculum vulgare*, *Laurus nobilis*, *Moringa oleifera*, *Rosmarinus officinalis*, *T. vulgaris* e *Verbena officinalis* no controle de *Tylenchulus semipenetrans* (Zoubi *et al*., 2023) e de *Schinus terebinthifolius* no controle de *Pratylenchus zeae* (Schwengber *et al*., 2017). Ferreira *et al*. (2025) relatam óleos essenciais de diferentes espécies no controle de *M. javanica, P. penetrans* e *Bursaphelenchus xylophilus,* concluindo que os óleos de *Cinnamomum burmanni* e *M. pulegium* são os mais promissores.

Áreas infestadas com NGR devem ser geridas adequadamente para garantia da produtividade das culturas. As plantas aromáticas podem ser uma alternativa de grande valia nos manejos culturais, visto que elas podem ser resistentes ou imunes ao ataque destes nematoides (Dias-Arieira *et al*., 2012; El-Mesalamy *et al*., 2020). *Allium fistulosum, A. tuberosum, Origanum vulgare, O. majorana, Satureja montana,* e *F. vulgare* foram consideradas resistentes (fator de reprodução < 1) a *M. javanica e M. incognita*, enquanto *Ocimum basilicum* foi considerado suscetível (fator de reprodução 2,35 e 4,75, respectivamente) (Dias-Arieira *et al*., 2012).

Além do manejo cultural, os NGR são controlados com recurso a produtos químicos e/ou biológicos. Alguns dos nematicidas sintéticos têm vindo a ser retirados do mercado devido à sua toxicidade para os humanos e para o meio ambiente (Pathak *et al*., 2022). Desta forma, tem-se intensificado a procura por nematicidas com menor impacto no ambiente, que possam controlar eficazmente os NGR, associados ou não, a outras estratégias de controle já existentes. Para suprir esta necessidade, multiplicam-se os trabalhos de investigação sobre a eficácia de óleos essenciais no controle de *Meloidogyne* spp. Alguns dos trabalhos têm demonstrado elevada eficácia dos óleos essenciais no controle de NGR, favorecido pela presença de uma ampla diversidade de moléculas num único óleo essencial (Raveau *et al*., 2020). Por exemplo, os óleos essenciais de *L. alba* e *Cymbopogon winterianus* (Poaceae) são eficientes no controle do NGR *M. incognita*, em campo (Moreira *et al*., 2015). Como também os óleos de *M. spicata* (Lamiaceae) e *O. basilicum* (Lamiaceae) preparados em nanoemulsões contribuiram para o controle de *M. incognita* (fator de reprodução de 0,87 e 1,01, respectivamente, enquanto a testemunha apresentou valor de 4,57), demonstrando a sua eficiência (Hammad.; El-Sagheer, 2023).

Além de serem eficientes no controle de pragas e doenças, os óleos essenciais são passiveis de serem aplicados na agricultura orgânica, conforme estabelecido na portaria do Ministério de Agricultura Agropecuária e Abastecimento (MAPA) Nº 52 de 15 de março de 2021 (Brasil, 2021). No mercado internacional e nacional, já é possível encontrar produtos à base de óleos essenciais e extratos vegetais. No Brasil, estão autorizados produtos com atividades inseticida, fungicida e bactericida, utilizando óleos essenciais ou extrato vegetais de *Argemone mexicana, Cinnamomum verum, Larrea tridentata*, *Melaleuca alternifolia, Rheum palmatum* and *Swinglea glutinosa,* e, particularmente para a atividade nematicida estão disponíveis produtos à base de *Allium sativum* e *Azadirachta indica* (Agrofit, 2024).

Diante do exposto, os objetivos principais deste trabalho foram estudar a interação entre nematoides do gênero *Meloidogyne* e plantas aromáticas, e avaliar a potencialidade de diferentes óleos essenciais no controle das espécies de NGR *M. chitwoodi* e *M. incognita*. Os estudos realizados incluíram a avaliação da reprodução de *M. chitwoodi*, *M. enterolobii*, *M. incognita*, *M. javanica*, e *M. luci* em seis espécies de plantas aromáticas (*Hypericum androsaemum*, *L. alba*, *M. spicata*, *Origanum majorana*, *O. vulgare* e *T. vulgaris*), em ensaio de vaso em condições controladas (Capítulo 1); e a avaliação do efeito, *in vitro*, dos óleos essenciais de *Aloysia citriodora*, *Corymbia citriodora*, *Cymbopogon citratus*, *C. winterianus*, *Elionurus muticus* e *L. alba* na mortalidade de jovens do 2º estádio de *M. chitwoodi* e *M. incognita* (Capítulo 2).

CHAPTER 1

HOST SUITABILITY OF AROMATIC SPECIES TO FIVE *Meloidogyne* SPECIES AND THEIR POTENTIAL USE FOR NEMATODE MANAGEMENT[1]

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Abstract

Plant-parasitic nematodes (PPN) of the genus *Meloidogyne* are one of the most important phytosanitary threats in cultivated areas, reducing the yield quality and quantity of different crops. Cultural strategies contribute to reduce the population density of PPN, however plant species/cultivars that are resistant to these parasites are required. The main aim of this study was to evaluate the host suitability of six aromatic plant species (*Hypericum androsaemum, Lippia alba, Mentha spicata, Origanum majorana, O. vulgare*, and *Thymus vulgaris*) to the PPN *M. chitwoodi, M. enterolobii, M. incognita, M. javanica*, and *M. luci.* A pot assay was conducted, in which twelve seedlings of each plant/species were transplanted and inoculated with 400 *Meloidogyne* spp. eggs. Tomato (*Solanum lycopersicum*) cv. Coração-de-Boi was included as control. Sixty days after inoculation, roots were assessed for gall index (GI), egg mass index (EMI), and reproduction factor (Rf). Fresh root and dry/fresh shoot weights, chlorophyll a, b and a+b were also recorded. Except for *H. androsaemum* and *S. lycopersicum*, all tested aromatic species were considered resistant to all *Meloidogyne* species tested and can be included in integrated pest management schemes, namely crop rotation and intercropping, providing income to farmers.

Keywords: *Hypericum androsaemum*, *Lippia alba*, *Mentha spicata*, *Origanum* spp., pathogenicity, reproduction factor, root-knot nematodes, *Thymus vulgaris*

1.1 INTRODUCTION

Aromatic plants, as result of a secondary metabolism of flowers, buds, seeds, leaves, wood, fruits, roots, twigs and bark, produce a complex mixture of tens to hundreds of volatile, hydrophobic and low molecular weight chemicals with strong sensorial characteristics, designated as essential oils. Essential oils contain mainly phenolic, alcoholic, and terpenoids compounds, such as citral, limonene, eugenol, and several others, being the most common chemical group (Singh *et al*., 2024a); and their composition can vary according to the genotype and the edaphoclimatic conditions (Saba *et al*., 2024). Many aromatic species are commonly known as culinary herbs or spices, often used as seasonings or in teas, such as *Ocimum americanum* L. (American basil) and *Lippia alba* (Mill.) N. E. Br. ex Britton & P. Wilson (Yerba mate) (Rucinque *et al*., 2021). In addition to being used as fresh and dried leaves, these plants are processed to be used as raw materials for the extraction of essential oils and plant extracts to be used in the industry (Taghouti *et al*., 2022). The utilization of essential oils in cosmetic industry, food and beverages industry (flavorings, preservatives, and additives), in agro-industrial proposes (e.g., antifungal and insecticidal) and medical industry (for the reduction and prevention of diseases, such as inflammations, due to their antioxidant properties) has been significantly enhanced in recent years (Samarth *et al*., 2017; Rodrigues *et al*., 2023; Fontana, 2021).

The use of aromatic plants is highly diverse, requiring sufficient material to meet the demands of national and international markets. Studies on the cultivation of these species are of great importance to ensure a production of high-quality (Parvin *et al*., 2023). The cultivation of these species is predominantly carried out in monocultures; however, due to the release of volatile compounds, studies are being conducted to explore the intercropping of aromatic plants with other crops (Lima Neto, 2020). Carvalho *et al*. (2009) have investigated the intercropping of *Solanum lycopersicum* L. (tomato) with *Foeniculum vulgare* Mill. (fennel), *Mentha piperita* L. (peppermint), *O. basilicum* L. (common basil), and *Ruta graveolens* L. (rue) and observed that the tomato-rue intercropping increased tomato yield, while the tomato-fennel combination reduced the incidence of the borers *Neoleucinodes elegantalis, Spodoptera* sp. and *Trichoplusia* sp. These findings demonstrate the potential benefits of such interactions, highlighting the need for further studies with different plant species and pests.

Given that intercropping among aromatic species is also feasible, it becomes essential to study the interactions between aromatic plants and pests/diseases associated with agricultural crops. Soil-borne and aerial diseases such as *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp. have been reported in several aromatic species, such as *Cymbopogon* spp.*, Lavandula* spp., *Mentha* spp.*, Origanum* spp.*,* and *Salvia* spp. (Avan, 2021). Additionally, aromatic plants have been reported as susceptible to plant-parasitic nematodes, particularly root-knot nematodes (RKN), *Meloidogyne* spp., such as, *M. enterolobii* and *M. paranaensis* (Anjos, 2019).

Nematodes of the genus *Meloidogyne* establish their feeding sites in the root cells, causing reduced absorption of water and nutrients and deformed root system (galls), which compromise the plant's support and consequently its health crop productivity (Débia *et al*., 2020). They are responsible for high yield losses in agriculture, spreading to many cultivated species, as well as facilitating the entry of other plant pathogens, such as *Fusarium* spp. (Vashisth *et al*., 2024).

Several studies have shown the occurrence of RKN in some species of aromatic plants. Baida and colleagues (2011), when evaluated the reaction of 15 aromatic plant species to *M. incognita* and *M. javanica*, found that only *Matricaria recutita* was susceptible to *M. incognita*, being all the other resistant to these RKN species. Dias-Arieira and colleagues (2012) reported *O. basilicum* as susceptible to *M. incognita* and *M. javanica*, *Satureja montana* L. (winter savory) as susceptible to *M. incognita* and resistant to *M. javanica,* whilst *Allium fistulosum* L. (welsh onion), *A. tuberosum* Rottler ex Spreng. (garlic chives)*, Foeniculum vulgare, Origanum majorana* L. (sweet marjorum) and *O. vulgare* L. (oregano) were considered resistant to both RKN species. In turn, according to El-Mesalamy *et al*. (2020), *O. basilicum* and *Salvia rosmarinus* Spenn. (=*Rosmarinus officinalis* L.) (rosemary) were susceptible to *M. javanica*, while *Hibiscus sabdariffa* L. (roselle), *Mentha* x *piperita* L. (peppermint)*, M. viridis* (L.) L. (garden mint) and *O. sanctum* L. (holy basil) were immune.

The objectives of this research were to: 1) assess the pathogenicity of the aromatic plant species *Hypericum androsaemum* L. (Hypericaceae)*, Lippia alba* (Verbenaceae)*, Mentha spicata* L. (Lamiaceae)*, Origanum majorana* (Lamiaceae)*, O. vulgare* subsp. *hirtum* (Link) A. Terracc. (Lamiaceae) and *Thymus vulgaris* L. (Lamiaceae) to *M. chitwoodi*, *M. enterolobii, M. incognita, M. javanica* and *M. luci*; 2) evaluate the impact of RKN on plant growth parameters (fresh and dry weight of the

aerial part, fresh root weight and photosynthetic pigments); and 3) evaluate potential use of aromatic plant species, as a sustainable alternative, on integrated pest management programs.

1.2 MATERIAL AND METHODS

The experiments were conducted at the NEMATO-lab (CFE, Department of Life Sciences, University of Coimbra).

1.2.1 Root-knot nematodes inocula

Five isolates of the species *M. chitwoodi, M. enterolobii, M. incognita, M. javanica* and *M. luci* were selected from the RKN collection of the NEMATO-lab (Table 1). These isolates have been maintained on tomato cv. Coração-de-Boi, by transferring the culture to new seedlings (5 egg masses/pot), every two months, being the plants kept in a growth chamber with a 12 h photoperiod, ≈23°C and 50% humidity. To obtain the inoculum for the host status assay, RKN eggs were extracted from infected tomato roots using a 0.52% sodium hypochlorite (NaOCl) solution (Hussey & Barker, 1973). The species identification of each of the five isolates was confirmed by esterase phenotype analysis (Pais *et al*., 1986).

Table 1. Portuguese root-knot nematodes, *Meloidogyne* spp.*,* isolates included in this study, host plants, geographic origin and reference.

Meloidogyne **species (Isolate code)**	**Host plant**	**Geographic origin**	**Reference**
M. chitwoodi (PtCh)	*Solanum tuberosum*	Porto	Conceição *et al*., 2009
M. enterolobii (PtEn)	*Cereus hildmannianus*	Coimbra	Santos *et al*., 2019
M. incognita (PtI)	*Ipomoea batatas*	Belmonte	Maleita *et al*., 2022
M. javanica (PtJ)	*S. tuberosum*	Guarda	Maleita *et al*., 2012
M. luci (PtL1)	*S. tuberosum*	Coimbra	Maleita *et al*., 2018

1.2.2 PATHOGENICITY ASSAYS

The aromatic plant species *H. androsaemum* (Hypericaceae), *L. alba* (Verbenaceae), *M. spicata*, *O. majorana*, *O. vulgare* subsp. *hirtum* and *T. vulgaris* (Lamiaceae) were obtained from a nursery (Germiplanta —Viveiros de Plantas Lda., Portugal). Plants of tomato cv. Coração-de-Boi, grown from seeds, were included as positive control, to check the inoculum viability. Non-inoculated plants were included as negative controls.

The seedlings were transplanted into 0.25 dm^3 pots containing a mixture of sterilized (121°C; 1 h) sandy loam soil, sand and commercial substrate (NPK 9-2-2; Siro Germinação Bio, SIRO, Portugal) (1:1:1). Three days after transplantation, twelve plants of each species were inoculated with 400 eggs (initial population density, Pi), distributed in three holes, one cm deep, equidistant from the plant shoot. The plants were maintained in a growth chamber as referred before. During the experiment, the plants were fertilized at 20 and 40 days after planting with NUTREA 12-4-6 (Genyen, Crop Solutions), a liquid fertilizer containing 5% N, 8% P and 10% K.

Sixty days after inoculation, the root systems were washed carefully, and the egg masses stained with a 0.0015% Floxin B solution (Hartman, 1982). The number of galls (G) and egg masses (EM) per root system was recorded and gall (GI) and egg mass (EMI) indices assessed based on a scale from 0 to 5 (0 = no G or EM, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 > 100 G or EM) (Taylor & Sasser, 1978). The number of eggs per root system (final population density, Pf) were determined after extraction with a 1% NaOCl solution (Hussey & Barker, 1973). The reproduction factor (Rf = Pf/Pi) was calculated.

Plants with GI > 2 and Rf > 1 were considered susceptible, with GI > 2 and Rf ≤ 1 hypersusceptible, with GI ≤ 2 and Rf ≤ 1 resistant and with GI ≤ 2 and Rf > 1 tolerant (Sasser *et al*., 1984).

Plant fresh root and shoot weights, dry shoot weight and photosynthetic pigments were also recorded. The photosynthetic pigments, chlorophyll a (Chla) and b (Chlb) were extracted using the methodology proposed by Wellburn (1994). For *T. vulgaris*, due to the size of the leaves, 3 leaves/repetition were collected, and their area measured using ImageJ® software, while for the other species were used 2 mm discs. After collecting the discs for photosynthetic pigments, the fresh shoot weights were

determined. The shoot weights were obtained after dried in an air circulation oven at a maximum temperature of 40ºC until reached a constant weight.

1.2.3 STATISTICAL ANALYSIS

The experiment was conducted using a completely randomized design with 12 replicates/plant species, each plant corresponding to an experimental unit. All the data was checked for normal distribution and homogeneity of variance using the Shapiro-Wilk test in the SISVAR® software, with $p > 0.05$ considered normal, before the analysis of variance (ANOVA). Data that did not show a normal distribution were transformed using the formula $\sqrt{x + 0.5}$ and subjected to the Scott-Knott test. The plant species and their respective variables analyzed that did not show a normal distribution were: *L. alba* (Rf, GI, EMI, PDM and Chlb), *M. spicata* (Chlb), *O. majorana* (Chlb), *O. vulgare* (Rf, GI, EMI, FRM and Chlb), *T. vulgaris* (Rf, GI, EMI, FRM, Chla, Chlb and Chla+b) and *S. lycopersicum* (Rf, GI, EMI, FRM, Chla, Chlb and Chla+b).

For the Rf, a 5 x 7 factorial design was used, with 5 species of *Meloidogyne* (*M. chitwoodi*, *M. enterolobii*, *M. incognita*, *M. javanica*, or *M. luci*) and 7 plant species (*Hypericum androsaemum*, *Lippia alba*, *Mentha spicata*, *Origanum majorana*, *O. vulgare*, *Thymus vulgaris*, and *Solanum lycopersicum*), with 12 replications.

The means of the photosynthetic pigments and mass were compared using the Scott-Knott test at a 5% significance level using Agroestat® software. The same test was applied to assess the combined effect of the factors for Rf variable.

1.3 RESULTS

The six aromatic plant species when inoculated with *M. chitwoodi, M. enterolobii, M. incognita, M. javanica* or *M. luci* showed different reactions. The tomato cv. Coração-de-Boi used as control for inoculum viability was susceptible to all RKN species, demonstrating that the inoculum used was viable (Table 2). *Hypericum androsaemum* was also susceptible to all the *Meloidogyne* species, with *M. javanica* having the highest Rf (82.33), and *M. enterolobii* the lowest one (11.41), where no differences were found among the GI and EMI (Table 2). *Lippia alba*, *M. spicata, O.*

majorana, O. vulgare and *T. vulgaris* when inoculated with these *Meloidogyne* species were considered as resistant with a Rf ranging between 0.00 and 0.87 (Table 2).

Table 2. Reproduction factor (Rf), gall index (GI), egg mass index (EMI) and host status (HS) of aromatic plant species inoculated with the root-knot nematodes *Meloidogyne chitwoodi, M. enterolobii, M. incognita, M. javanica* and *M. luci*, 60 days after inoculation with 400 eggs/plant in a pot assay conducted in a growth chamber (≈23ºC, 12 h daily photoperiod, and ±50% relative humidity).[1]

Aromatic plant species	Parameters	Root-knot nematode species					F[2]	CV[3]
		M. chitwoodi	*M. enterolobii*	*M. incognita*	*M. javanica*	*M. luci*		
Hypericum androsaemum	Rf[4]	43.32 ± 22.45 bB	11.41 ±11.15 cB	50.38 ± 34.99 bB	82.33 ± 51.68 aA	34.99 ± 49.96 cA	7.87**	40.43
	GI[5]	3 ± 1 a	3 ± 1 a	4 ± 1 a	3 ± 1 a	4 ± 2 a	0.63 ns	19.76
	EMI[5]	4 ± 1 a	3 ± 1 a	4 ± 1 a	3 ± 1 a	3 ± 2 a	0.91 ns	20.19
	HS[6]	S	S	S	S	S	-	-
Lippia alba	Rf	0.00 ± 0.00 bC	0.04 ± 0.07 aC	0.00 ± 0.00 bC	0.00 ± 0.00 bB	0.00 ± 0.00 bB	3.65**	15.04
	GI	0 ± 0 b	0.25 ± 0.45 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	3.67**	36.77
	EMI	0 ± 0 b	0.45 ± 0.39 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	2.20 ns	36.64
	HS	R	R	R	R	R	-	-
Mentha spicata	Rf	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.00 ± 0.00 aB	0.00 ± 0.00 aB	0	0
	GI	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0	0
	EMI	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0	0
	HS	R	R	R	R	R	-	-
Origanum majorana	Rf	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.00 ± 0.00 aB	0.00 ± 0.00 aB	0	0
	GI	0 ± 0 a	0.08 ± 0.29 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	1.00 ns	24.99
	EMI	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0	0
	HS	R	R	R	R	R	-	-

[1] Tomato cv. Coração-de-Boi was included as positive control of inoculum viability. Data are means of 12 replicates±standard deviation. The means in each row followed by the same combination of lower-case letters do not differ significantly from each other at $p > 0.05$, according to the Scott-Knott test. Equal upper-case letters in the same column do not differ from each other, according to the same test ns= not significant, ** = statistical interaction.

[2] F = F test.

[3] CV = coefficient of variation (%).

[4] Rf (reproduction factor) = final population density/initial population density (400 eggs).

[5] GI (gall index) or EMI (egg masses index): 0 = no galls/egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 ≥ 100 galls or egg masses /root system.

[6] HS (Host status category): T = tolerant (GI ≤ 2 and Rf > 1), R = resistant (GI ≤ 2 and Rf ≤ 1), HS = hypersusceptible (GI > 2 and Rf ≤ 1), and S = susceptible (GI > 2 and Rf > 1.

Table 2 (*Continuation*). Reproduction factor (Rf), gall index (GI), egg mass index (EMI) and host status (HS) of aromatic plant species inoculated with the root-knot nematodes *Meloidogyne chitwoodi, M. enterolobii, M. incognita, M. javanica* and *M. luci*, 60 days after inoculation with 400 eggs/plant in a pot assay conducted in a growth chamber (≈23ºC, 12 h daily photoperiod, and ±50% relative humidity).[1]

Aromatic plant species	Parameters	Root-knot nematode species					F[2]	CV[3]
		M. chitwoodi	*M. enterolobii*	*M. incognita*	*M. javanica*	*M. luci*		
Origanum vulgare	Rf	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.00 ± 0.00 aC	0.03 ± 0.09 aB	0.00 ± 0.00 aB	1.00 ns	13.91
	GI	0 ± 0 a	0 ± 0 a	0 ± 0 a	0.08 ± 0.29 a	0 ± 0 a	1.00 ns	24.99
	EMI	0 ± 0 a	0 ± 0 a	0 ± 0 a	0.08 ± 0.29 a	0 ± 0 a	1.00 ns	24.99
	HS	R	R	R	R	R	-	-
Thymus vulgaris	Rf	0.00 ± 0.00 cC	0.87 ± 0.68 aC	0.29 ± 0.31 bC	0.00 ± 0.00 cB	0.54 ± 0.31 aB	22.69**	31.09
	GI	0 ± 0 c	2 ± 1 a	1 ± 1 b	0 ± 0 c	2 ± 1 a	40.83**	29.66
	EMI	0 ± 0 c	2 ± 1 a	1 ± 1 b	0 ± 0 c	2 ± 1 a	41.18**	29.99
	HS	R	R	R	R	R	-	-
Solanum lycopersicum	Rf	80.13 ± 30.62 aA	59.01 ± 19.08 aA	97.91 ± 45.89 aA	74.04 ± 39.51 aA	31.88 ± 12.97 bA	7.17**	23.61
	GI	5 ± 0 a	5 ± 0 a	5 ± 0 a	5 ± 1 a	5 ± 0 a	1.01 ns	2.83
	EMI	5 ± 0 a	5 ± 0 a	5 ± 1 b	5 ± 1 b	5 ± 0 a	2.87**	3.82
	HS	S	S	S	S	S	-	-

[1] Tomato cv. Coração-de-Boi was included as positive control of inoculum viability. Data are means of 12 replicates±standard deviation. The means in each row followed by the same combination of lower-case letters do not differ significantly from each other at $p > 0.05$, according to the Scott-Knott test. Equal upper-case letters in the same column do not differ from each other, according to the same test ns= not significant, ** = statistical interaction.

[2] F = F test.

[3] CV = coefficient of variation (%).

[4] Rf (reproduction factor) = final population density/initial population density (400 eggs).

[5] GI (gall index) or EMI (egg masses index): 0 = no galls/egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 ≥ 100 galls or egg masses /root system.

[6] HS (Host status category): T = tolerant (GI ≤ 2 and Rf > 1), R = resistant (GI ≤ 2 and Rf ≤ 1), HS = hypersusceptible (GI > 2 and Rf ≤ 1), and S = susceptible (GI > 2 and Rf > 1.

The fresh root weight (FRW) of *H. androsaemum* was higher when inoculated with *M. chitwoodi, M. incognita, M. javanica* and *M. luci,* when compared with *M. enterolobii* and control (Figure 1). Shoot weights were higher when inoculated with *M. enterolobii*, *M. javanica* and *M. luci* treatments, compared to control. *Lippia alba* had the highest FRW values when inoculated with *M. chitwoodi*, *M. incognita* and *M. javanica*, while fresh and dry shoot weights (FSW and DSW, respectively) showed no statistical differences (Figure 1). *Mentha spicata* displayed the highest FRW when inoculated with *M. incognita* (±6.5 g plant^{-1}), while FSW was higher when inoculated with *M. enterolobii, M. luci* and in the control treatment (Figure 1).

Origanum majorana exhibited the highest FRW in the control, and in *M. chitwoodi* and *M. javanica* treatments, while for FSW, when inoculated with *M. enterolobii*, *M. luci* and in the control. However, the greatest increase in dry matter (DSW) was found in the treatment without inoculation (control), indicating that the increase in dry matter is lower when inoculated with the RKN species (Figure 1). *Origanum vulgare* expressed greater FSW when inoculated with *M. incognita*, *M. javanica* and in the non-inoculated treatment, with the other variables having the highest values found in the non-inoculated treatment and when inoculated with *M. javanica* (Figure 1).

Thymus vulgaris was highly responsive when inoculated with the different nematode species, with *M. enterolobii* having the lowest FRW, *M. incognita* the lowest FSW, and both the lowest DSW values, indicating a lower increase in dry matter (Figure 1). *Solanum lycopersicum*, used as a control to confirm the viability of the inocula, only presented significance in the FSW variable, with the control and *M. chitwoodi* treatments having the lowest weights (Figure 1).

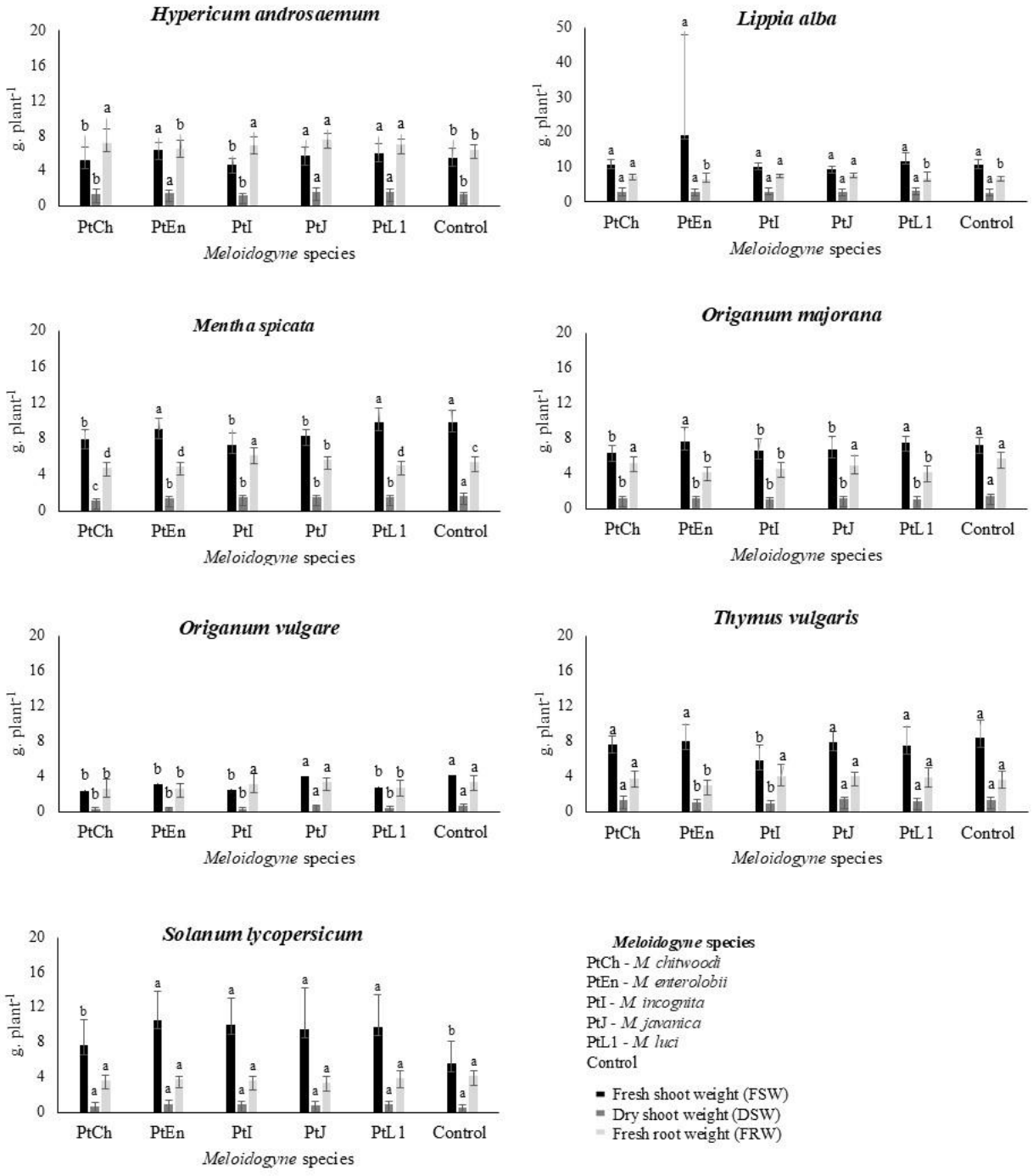


Figure 1. Effect of five *Meloidogyne* species on fresh and dry shoot weight and fresh root weight of *H. androsaemum, L. alba, M. spicata*, *O. majorana*, *O. vulgare, T. vulgaris* and *S. lycopersicum* cv. Coração-de-Boi (positive control). Bars represent means of 12 replicates±standard deviation. Means of each evaluated variable followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test.

Chlorophyll a, b and a+b are directly related to the photosynthetic activity of plants. *Hypericum androsaemum* showed higher levels of Chl a when inoculated with *M. enterolobii*, *M. incognita* and *M. javanica*, while for Chl b the highest values were found in the treatments with *M. enterolobii*, *M. incognita* and *M. luci*. *Lippia alba* also displayed higher Chl a values when inoculated with *M. enterolobii*, *M. incognita* and *M. javanica*, while for Chl b and Chl a+b no statistical differences were observed (Figure 2).

In *M. spicata*, only Chl b had a statistical difference, with the lowest concentration of the pigment being found in *M. javanica*. *Origanum majorana*, when inoculated with *M. incognita* and *M. javanica*, showed the highest Chl a, and b values, indicating that these species influence pigment production (Figure 2). *Origanum vulgare* exhibited a lower Chl a value when inoculated with *M. chitwoodi*, while Chl b was lower when the plants were inoculated with *M. chitwoodi* and *M. javanica*. This result complements that found for Chl a+b, where the lowest values were found when the plants were inoculated with *M. chitwoodi* and *M. javanica* (Figure 2). *Thymus vulgaris* revealed no statistical difference for the Chl a, b and a+b parameter (Figure 2). When evaluating chlorophyll in *S. lycopersicum*, it was observed that Chl a was not responsive to the treatments, while Chl b and Chl a+b were higher when the plants were inoculated with *M. incognita* and *M. luci* (Figure 2).

By analysing the results, different responses were detected in the aromatic plants when they were inoculated with the RKN species *M. chitwoodi, M. enterolobii, M. incognita, M. javanica* and *M. luci* when compared to the non-inoculated plants.

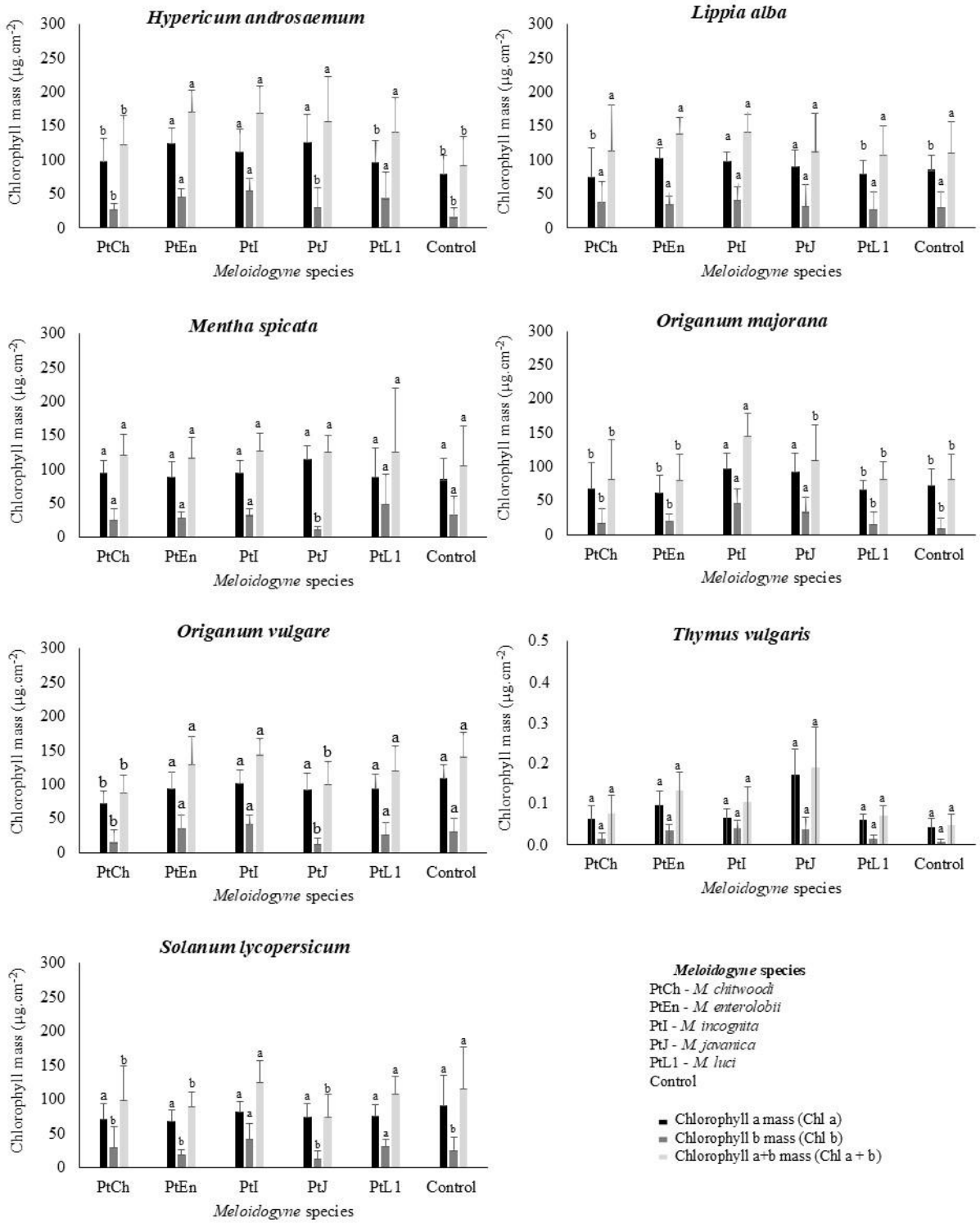


Figure 2. Effect of five *Meloidogyne* species on chlorophyll a (Chl a), chlorophyll b (Chl b) and chlorophyll a+b (Chl a+b) masses of *H. androsaemum, L. alba, M. spicata*, *O. majorana*, *O. vulgare, T. vulgaris* and *S. lycopersicum,* cv. Coração-de-Boi (positive control). Bars represent means of 12 replicates±standard deviation. Means of each evaluated variable followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test.

1.4 DISCUSSION

Aromatic plant species, when exposed to the selected five species of *Meloidogyne*, showed different responses, in terms of resistance or susceptibility, root and shoot weight and chlorophyll content. Root-knot nematodes can cause biotic stress in plants, and this stress can origin different responses, including a reduction in fresh and dry weight (Shakeel *et al*., 2023).

Solanum lycopersicum (positive control), used to verify the viability of the inocula of *M. chitwoodi, M. enterolobii, M. incognita, M. javanica* and *M. luci*, enabled a high RKN reproduction factor (31.88 ≤ Rf ≤ 97.91), confirming the viability of the inocula. The species *H. androsaemum* was susceptible to the five RKN species, and *M. javanica* had better reproduction in this aromatic plant than in the control (Rf = 82.33 and 74.04, respectively), corroborating the results found for *H. perforatum* when inoculated with *M. paranaensis* (Mendonça *et al*., 2017).

As observed in this study, *M. spicata* was resistant (Rf=0, EG/EMI=0) to the RKN species studied. Santos *et al*. (2022), using an isolate of *M. javanica* from Brazil, detected resistance in *M. spicata*, with Rf=0.03, that may be related to the pathogenicity of the isolate. This aromatic plant species can be used to reduce the population density in soils infested with these RKN species and, therefore, be included in pest integrated management programs.

Martins (2018), when studying the reaction of medicinal plants to RKN found that *O. majorana* was susceptible to *M. javanica*, whereas we observed a resistant response. As referred before, this could be related to differences in virulence between isolates. Also, Martins (2018), in accordance with our results, reported *O. vulgare* as resistant to *M. javanica*, and *T. vulgaris* as resistant to *M. incognita* and *M. javanica*. The results obtained for *L. alba* agree with Anjos (2019) and Moreira (2007), who observed that this aromatic species is resistant to *M. enterolobii* and *M. incognita* and immune to *M. paranaensis.*

Herbs and spices are marketed fresh and dehydrated. When they are made available on the market in dehydrated form, it is possible to increase shelf life and avoid economic losses (Palhares *et al*., 2021). Thus, the higher the dry matter production, the higher the productivity per area, which results in a higher income for the producer.

Lippia alba dry mass did not differ between treatments, indicating that the *Meloidogyne* species did not affect biomass production. On the other hand, *M. spicata*

and *O. majorana* showed greater dry mass in the control treatment (non-inoculated), which contrasts with the study by Noskov *et al*. (2024). According to Noskov *et al*. (2024), no significant differences in biomass were detected after inoculation of *M. chitwoodi*, *M. fallax, M. hapla* or *M. incognita* on *Mentha* sp. This may be because the plant directs its energy towards the production of secondary compounds, which act as natural repellents against pests and diseases (Mohamed & Alotaibi, 2023). *Origanum vulgare* had the highest dry mass when it was not inoculated with RKN and when it was inoculated with *M. javanica*. *Thymus vulgaris* had the lowest dry mass when inoculated with *M. enterolobii* and *M. incognita*. This can be explained by the fact that the plant is attacked by the nematode, even if only slightly, which ends up affecting its plant biomass (Singh *et al*., 2024b).

Infection by PPN, as referred, causes stress in the plant, as well as a decrease in the absorption of water and nutrients, which directly impacts the plant's growth and development, along with the process of photosynthesis, which starts with the absorption of light through photosynthetic pigments, such as chlorophylls, with chlorophyll a and b being the most abundant in the leaves (Taiz & Zeiger 2017).

Plants infected by nematodes tend to display a yellowish color or a low intensity of green, which is directly related to the amount of chlorophyll; so, a plant with a high chlorophyll content is more likely to grow and develop better (Silva *et al*., 2020). While *H. androsaemum* showed the lowest concentrations of chlorophyll a+b when inoculated with *M. chitwoodi*, non-inoculated plants did not have the highest masses in the same treatments. In turn, *L. alba* revealed the highest concentrations of chlorophyll a when inoculated with *M. enterolobii, M. incognita* and *M. javanica*, but no statistical differences detected to chlorophylls b and a+b, equaling the fresh and dry mass.

Mentha spicata presented the lowest concentration of chlorophyll b, when inoculated with *M. javanica*, while chlorophyll a and a+b did not differ statistically. Unlike, *O. majorana* showed the highest pigment values when inoculated with *M. incognita* and *M. javanica*, and *O. vulgare* displayed the lowest chlorophyll concentrations when inoculated with *M. chitwoodi* and *M. javanica*. These results can be explained by the fact that different cultivars or crops show different responses to PPN attacks. Within the same cultivar, the plant can show different responses to different RKN species, because it directs the energy produced by the plant to other

places, such as mass production, roots or specialized metabolism (Kalariya *et al*., 2024).

1.5 CONCLUSIONS

Five of the six aromatic plants, *L. alba, M. spicata, O. majorana, O. vulgare,* and *T. vulgaris*, can be recommended for cultivation in infested areas with the RKN *M. chitwoodi, M. enterolobii, M. incognita, M. javanica or M. luci*, being *H. androsaemum* susceptible to all *Meloidogyne* species included in this study.

Our findings are of great value as the aromatic plant species studied can contribute to reduce the population density of important RKN species, including quarantine species, in the soil and consequently generate incomes to the farmers, whether integrated into crop rotation schemes or in a consortium with other crops. Furthermore, considering that RKN infection can alter plant metabolism, future studies to evaluate the chemical changes (quantitative and qualitative) related to the production of plant extracts/essential oils are essential to understand whether these plants can not only act as an effective alternative to chemical control of RKN, but also whether they can be selected to produce specific compounds when exposed to these nematodes, offering new possibilities for valorization of these plant species in different agronomic contexts.

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CHAPTER 2

IDENTIFICATION OF ESSENTIAL OILS FOR MANANGING Meloidogyne chitwoodi AND M. incognita[2]

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Abstract

Plant-parasitic nematodes (PPN) are extremely important on agriculture worldwide, as they are responsible for significant crop losses. Among PPN, nematodes of the genus *Meloidogyne*, known as root-knot nematodes (RKN), rank first in the Top10 PPN with the greatest impact and few chances of effective control strategies available. The aim of this study was to evaluate the effects of essential oils from different plant species on mortality and hatching inhibition of the root-knot nematodes (RKN) *M. chitwoodi* and *M. incognita*. The essential oils of *Aloysia citriodora*, *Corymbia citriodora*, *Cymbopogon citratus*, *C. winterianus*, *Elionurus muticus* and *Lippia alba* were evaluated at 0.75, 1.5, 3.0, 6.0, 9.0, 12.0, 15.0 and 18.0 µL/mL, diluted in 1% Tween 80 (polysorbate). Tween 80 (1%) and sterilized distilled water were included as controls. Then, a single dose of 3 µl/mL was selected to assess the efficiency on second-stage juveniles (J2) mortality in soil and on *M. chitwoodi* and *M. incognita* hatching. The experiments were conducted twice, with eight replicates/treatment and data subjected to Probit analysis to determine the 50 and 90 % lethal concentration. The results show that the essential oils have nematicidal activity *in vitro*, with the essential oils of *Corymbia citriodora* and *Cymbopogon citratus* being the most effective against *M. chitwoodi* and *M. incognita* mortality, in soil. As a result, it can be concluded that the essential oils are effective in controlling these PPN, requiring further studies to develop a formulation for application in agricultural areas infested with RKN.

Keywords: *Aloysia citriodora,* biocontrol, biomolecules, *Corymbia citriodora, Cymbopogon* spp.*, Elionurus muticus, Lippia alba,* root-knot nematodes, sustainability.

2.1 INTRODUCTION

Plant-parasitic nematodes (PPN), phylum Nematoda, penetrates the roots or other parts of plants, causing yield losses and consequently significant negative economic impacts. The most important species of PPN belong to the genera *Meloidogyne, Globodera, Heterodera, Nacobbus, Pratylenchus, Radopholus, Rotylenchulus* and *Tylenchulus*. Among them, nematodes of the genus *Meloidogyne*, (root-knot nematodes, RKN), are an important threat to crucial crops worldwide due to their high degree of polyphagy (Jones *et al*., 2013).

Amongst the *Meloidogyne* species that cause significant damage to the main crops are *M. chitwoodi* and *M. incognita*. The Columbia RKN *M. chitwoodi* is considered a quarantine species in Brazil (A1 list) and in European and Mediterranean Plant Protection Organization region (A2 Quarantine pest) (Brasil, 2018; Gonzaga & Moura, 2019; EPPO, 2025). *Meloidogyne* spp. are widely distributed across the world, with some species being absent in some countries. *Meloidogyne chitwoodi* has already been reported in some countries in Europe, North America, South America, and southern Africa, but, as far as we know, it has not yet been reported in Brazil. *Meloidogyne incognita*, on the other hand, is one of the most common RKN with a widespread distribution in tropical and subtropical regions, which reveals its high adaptability and plasticity (Subedi *et al*., 2020).

Chemical management is a very important option, among the available strategies for PPN control, due to its efficiency and efficacy in reducing nematode infestation. Although they offer immediate advantages, synthetic nematicides present significant risks to the environment, due to their application methods and spread in soil and water, which can result in impacts on ecosystems and on health of living organisms, including humans (Pathak *et al*., 2022). For this reason, in several countries, many synthetic nematicides have been banned from the market, resulting in a limited supply of commercially available chemicals for the control of PPN (Catani *et al*., 2023).

To meet this need, there is a growing interest in research to find natural resources, through the use of plants and their natural chemical compounds to apply to NPP control, since they are organic molecules synthesised in plants and are rich in compounds, which reduces the chance of pathogens becoming resistant to the molecules (Barros *et al*., 2019; Swaraparani & Shanamugam, 2024).

Essential oils are an excellent example of plant derivatives used to control pests and diseases that can be applied to several agricultural crops (Wagner *et al*., 2021; Assadpour *et al*., 2024). Result of secondary metabolism, also known as specialized metabolism, they can be obtained by different extraction methods, the most widely used being steam distillation and hydrodistillation for aromatic plants (Kant & Kumar, 2022). The specialized metabolism has a protective function against pests and diseases, due to the large number of associated molecules, which enhances its effects (Toan *et al*., 2021). Essential oils feature prominently in bioproduct applications such as weed control (bioherbicides), insect control (bioinsecticides), fungus control

(biofungicides) and PPN control (bionematicides) (Gupta *et al*., 2023). Several studies have shown the effects of essential oils, and their bioactive compounds, isolated from different plant species, especially those from the Lamiaceae, on the control of *M. incognita*, *M. javanica*, *M. graminicola*, *M. hapla*, *M. ethiophica* and *M. enterolobii* (Sarri *et al*., 2024).

Additionally, the compounds can show different behaviors, such as synergism between molecules, which is often the differential in the use of crude essential oils (without separating the compounds), or antagonism (Ntalli *et al*., 2011). Ntalli *et al*. (2011) reported the greatest synergism between compounds mixed with *trans*-anethole and antagonism when mixed with carvone, where they found the best results of synergistic activity between *trans*-anethole/geraniol, followed by *trans*-anethole/eugenol, carvacrol/eugenol and geraniol/carvacrol in the control of *M. incognita*. Other studies have shown the reduction of *M. incognita* reproduction when exposed to *Lippia sidoides* and *Cymbopogon winterianus* essential oils (Moreira *et al*., 2015); and the nematicidal effect of *L. alba* and *Allium sativum* essential oils (Gonçalves *et al*., 2016; Jardim *et al*., 2020). A similar effect was reported on the control of *M. chitwoodi*, using the essential oils of *Lavandula* spp., *Rosmarinus officinalis*, *Sesamum indicum* and *Thymus vulgaris* (Uludamar, 2023). Faria *et al*. (2016), evaluating 56 essential oils, found the best results with the essential oils of *Dysphania ambrosioides*, *Filipendula ulmaria*, *Ruta graveolens*, *Satureja montana* (Sm1, Sm1O and Sm2) and *Thymbra capitata* (Tc and TcO). Currently, there are products available on the market based on plant extracts, namely from *Allium sativum*, which have proven to be effective in controlling PPN (Agrofit, 2025).

Worldwide, various measures have been established to prevent the introduction of new species of pathogens into regions where they do not occur, their dissemination, as well suppression and eradication measures. In Brazil, considering the potential introduction of *M. chitwoodi*, these measures include phytosanitary education; training of professionals; and preparation, coordination and execution of phytosanitary actions (Brazil, 2019). As a practical example, we can mention the inspection of seed potatoes, which requires a phytosanitary certificate staying that there are no pests in the propagation material (Brazil, 2012).

Given the problematic nature of the RKN species worldwide, research is being carried out to understand the effects of essential oils on the biology of RKN (direct toxicity or inhibition of hatching) in order to contribute to the development of more

sustainable agricultural management practices. The aims of this study were to evaluate the effects of essential oils from *Aloysia citriodora* (Palau) Kunth (Verbenaceae), *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson (Mytaceae), *Cymbopogon citratus* (DC.) Stapf (Poaceae), *C. winterianus* Jowitt ex Bor (Poaceae), *Elionurus muticus* (Spreng.) Kuntze (Poaceae) and *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae) against *M. chitwoodi* and *M. incognita* and evaluate their potentiality as alternatives to the use of synthetic nematicides.

2.2 MATERIAL AND METHODS

2.2.1 ESSENTIAL OILS

The study was conducted at the Nematology Laboratory NEMATO-lab (CFE, University of Coimbra, Portugal); however, the essential oils were obtained from the Aromatic Extractives Laboratory (LEA) at the Federal University of Santa Maria, Brazil.

The plants were collected from the LEA's aromatic plant garden. The essential oils of *A. citriodora* and *L. alba* were obtained through hydrodistillation with an extraction time of three and two hours, respectively. *Corymbia citriodora*, *Cymbopogon citratus*, *C. winterianus* and *E. muticus* essential oils were obtained by steam distillation with an extraction time of one hour for *C. citriodora* and four hours for the remaining. After extraction, the essential oils were stored in amber bottles at -20ºC until transport. Transport to Portugal was carried out in an expanded polystyrene box containing rigid reusable artificial ice, and after arrival they were stored at -20ºC until use. Essential oils were prepared in 1 % Tween 80 aqueous solution at 0.75, 1.5, 3.0, 6.0, 9.0, 12.0, 15.0 and 18.0 µL/mL.

2.2.2 CHEMICAL COMPOSITION OF ESSENTIAL OILS

The chemical composition of the essential oils was determined using gas chromatography with flame-ionization detection (GC-FID) and gas chromatography mass spectrometry (GC-MS), on an Agilent 7890A hyphenated system equipped with a 5975C series mass selective detector, according to Bianchini *et al*. (2022). An HP5-MS fused silica capillary column was used (Hewlett Packard, 5 % phenyl, 95 %

methylsiloxane, 30 m x 0.25 mm, film thickness: 0.25 mm). Analysis parameters were the following: ionization energy of 70 Ev; flow division 1:100; temperature program: 40 °C for 4 min; 40-320 ºC at 4 ºC min-1. Carrier gas: He, at 1 mL min^{-1}. Injector and detector temperature: 250 ºC. The GC-FID was injected with a flow split of 1:50; injector and detector temperature: 300 ºC. The compounds were identified by comparing their retention indices determined by of the N-alkanes injected under the same chromatographic conditions and compared with mass spectral profiles stored in the MS library NIST and the literature (Adams, 2009; NIST/EPA/NIH, 2010). 1 µL of essential oil was added to 999 µL of n-Hexane 95% for pesticide analysis and injected.

2.2.3 NEMATODE INOCULUM

Two RKN isolates of *M. chitwoodi* and *M. incognita* were obtained from the PPN collection at NEMATO-lab. The isolates were maintained in tomato cv. Coração-de-Boi in a growth chamber at 25±2 °C and 12 h of photoperiod. *Meloidogyne chitwoodi* (PtCh) was obtained from *Solanum tuberosum* L. roots collected in Porto (Portugal) and *M. incognita* (PtI) from *Ipomoea batatas* L. (Belmonte, Portugal) (Conceição *et al*., 2009; Maleita *et al*., 2022). For hatching assays, eggs were extracted from infected tomato roots using a 0.5 % sodium hypochlorite solution (Hussey & Barker, 1973). To obtain second-stage juveniles (J2) for the mortality assays, egg masses were handpicked from tomato infected roots and incubated in a hatching chamber, at 24 ºC. Second-stage juveniles from the first 24 h were discarded, and only J2 from the subsequent hours until a maximum of three days were used in the experiments.

2.2.4 HATCHING AND MOBILITY/MORTALITY ASSAYS - IN VITRO

The hatching and mortality experiments were carried out in 96-well plates. Two-hundred µL of a *M. incognita* or *M. chitwoodi* suspension containing 30 eggs or J2 were added to each well, along with 50 µL of each essential oil, to obtain final concentrations of 0.75, 1.5, 3.0, 6.0, 9.0, 12.0, 15.0 and 18.0 µL/mL. Sterilized distilled water and 1 % Tween 80 aqueous solution were included as controls. The plates were maintained at 24 ºC in the dark.

Second-stage juveniles mobility/mortality was assessed at 48 h of exposure, using an inverted stereoscopic microscope. Nematodes that were moving were considered “alive”, otherwise they were considered “immobile”. Hatching was

evaluated seven days after incubation, and it was assumed that the activity of the essential oils is conserved during the assay.

The trials were conducted in a completely randomized design, with a total of six essential oils, at nine concentrations/each and eight replicates per treatment. The experiments were conducted twice to confirm the results. The results were converted to hatching and mortality percentage:

$$Mortality/hatching\% = \left(\frac{no.of\ dead/hatched\ nematodes}{total\ no.of\ J2/eggs}\right) \times 100$$

Data on J2 hatching were corrected by Abbott's formula (Abbot,1925) with reference to Tween 80 control:

$$Corrected\ hatching = \left(1 - \frac{\%\ hatching\ in\ treatment}{\%\ hatching\ in\ control}\right) \times 100$$

2.2.5 MORTALITY ASSAY – *IN SOIL*

An experiment in soil was performed to validate the results obtained in mortality experiment *in vitro* following the methodology of Chelinho *et al*. (2017), with some modifications. An agricultural soil with no history of pesticide application or chemical fertilization, at least for the last two years, were collected in Coimbra region (Portugal). The soil was homogenized, sieved (0.5 cm) and defaunated through two freeze-thawing (F-T) cycles (48 h F: 48 h T: 48 h F). Soil moisture was adjusted to 40% of soil water holding capacity (considering the volume of solution added in the following steps) and the soil distributed among the glass vessels. Then, the soil was spiked with 2.5 mL of each essential oil at 3 µL/mL (selected according to the results of *in vitro* assays, due to their efficacy on RKN mortality) and inoculated with 150 *M. incognita* or *M. chitwoodi* J2. After five days of incubation, at 20-22 ºC, nematodes were recovered from soil using the Tray method (Whitehead & Hemming, 1965) and counted. Each treatment was replicated five times and water and 1 % Tween 80 aqueous solution included as controls. The experiment was repeated twice and the results expressed as the number of nematodes recovered.

2.2.6 STATISTICAL ANALYSIS

The effects of the essential oils and concentrations on J2 hatching and mortality were compared by analyses of variance (ANOVA) followed by Scott-Knott test. Statistical analyses of the data were performed using AgroEstat statistical software. Data on hatching and mortality derived from the 48 h and 7 days observations, respectively, were subjected to Probit analysis (Finney, 1971) using R software, and the lethal concentrations causing 50 % and 90 % hatching inhibition and mortality (LC_{50} and LC_{90}) were calculated.

2.3 RESULTS

The essential oils included in the study have a distinct chemical composition, varying in the number of compounds identified and relative proportions. The main compound identified in each essential oil was α-citral in *A. citriodora, C. citratus* and *E. muticus* (27.96 %, 45.02 % and 49.85 %, respectively), β-citronellal in *Corymbia citriodora* and *Cymbopogon winterianus* (85.04 % and 36.62%, respectively), and β-linalool at 70.22 % in *L. alba* (Table 1).

All the essential oils tested showed high nematicidal activity against *M. chitwoodi* and *M. incognita*, either by inhibiting hatching or inducing J2 mortality (Figures 1-4). For *M. chitwoodi* and *M. incognita*, in both experiments, in the control (sterilized distilled water and 1 % Tween 80 aqueous solution) treatments no J2 mortality was observed, and the maximum of hatching was ≈60 % (Figures 1-4).

Except for *E. muticus* and *L. alba*, the remaining essential oils induced 100 % inhibition of hatching of *M. chitwoodi* J2 from 3 µL/mL (Figure 1). For *E. muticus* and *L. alba*, 100 % of inhibition was only achieved for concentrations ≥ 6 µL/mL (Figure 1).

Table 1. Chemical composition of essentials oils from *Aloysia citriodora* (ALC), *Corymbia citriodora* (COC), *Cymbopogon citratus* (CYC), *C. winterianus* (CYW), *Elionurus muticus* (ELM) and *Lippia alba* (LIA) by gas chromatography mass spectrometry and included in bioassays.

Compound	RT[1]	LRI_{calc}[2]	LRI_{lit}[3]	Plant Species					
				ALC	COC	CYC	CYW	ELM	LIA
4(10)-Thujene	10.860	971	970						0.75
Sulcatone	11.571	989	988					0.70	
β-Pinene	11.666	991	991			15.46		0.51	
Eucalyptol	13.095	1028	1027						4.59
D-Limonene	13.045	1027	1026	20.52			1.97		
β-trans-Ocimene	13.543	1039	1039			0.40		0.61	
β-cis-Ocimene	13.919	1049	1049						0.67
β-Ocimene	13.926	1049	1046			0.24		0.41	
Linalool	15.903	1100	1095	0.80				3.24	70.22
Isopulegol	17.503	1143	1145		2.52				
β-Citronellal	17.942	1155	1155		85.04		36.62		
cis-Geraniol	20.602	1229	1229	0.92					
β-Citronellol	20.653	1231	1230		5.73		7.14		
neral	21.074	1243	1242	19.97		31.02		32.35	
trans-Geraniol	21.557	1257	1256	0.90					
cis-Geraniol	21.563	1257	1254			1.94	18.88	1.67	
geranial	22.142	1274	1270	27.96		45.02		49.85	
Citronellol acetate	24.843	1355	1355				2.20		
Nerol acetate	25.839	1386	1381	2.65				4.64	
β-Elemene	26.018	1391	1391				2.21		1.14
β-Caryophyllene	26.844	1418	1417	3.37					2.81
Germacrene D	28.776	1480	1480				2.19		4.48
α-Curcumene	28.859	1483	1482	3.91					
Cadina-1(10),4-diene	29.810	1515	1515				2.46		0.67
Elemol	30.833	1550	1549				8.86		
Germacrene B	31.017	1556	1556						3.00
Spathulenol	31.621	1577	1576	3.11					
Caryophyllene oxide	31.758	1582	1581	9.22				1.76	
γ-Eudesmol	33.162	1631	1631				1.78		
tau-Cadinol	33.803	1655	1651	1.7			4.11		
Percentage total				95.03	93.29	94.08	88.42	95.74	88.33
Nº Peaks				26	12	17	24	19	28
Identified peaks				12	3	6	11	10	9

[1] Retention time (minutes)

[2] LRIcal - Calculated linear retention index

[3] LRIlit - Linear retention index from literature

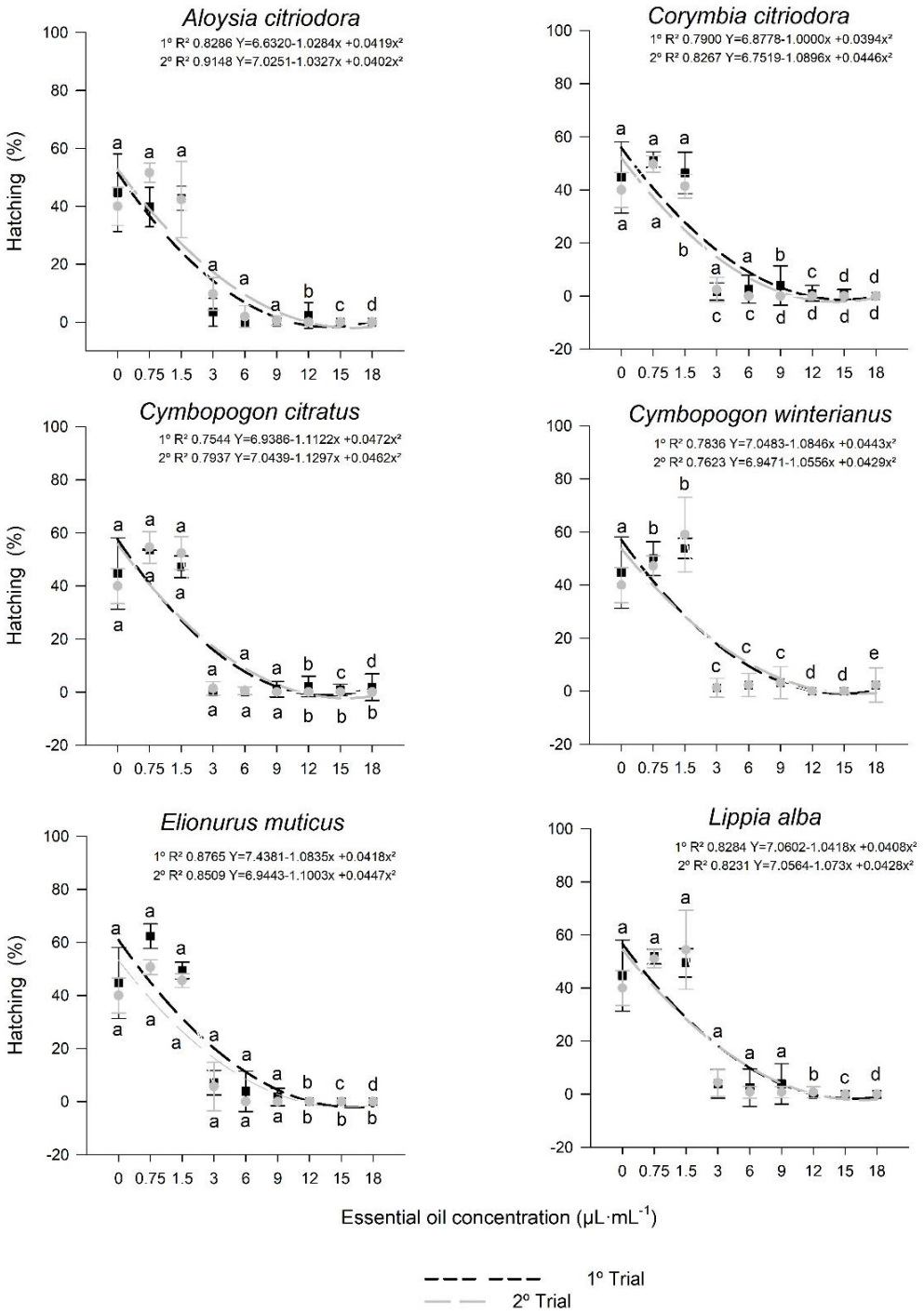


Figure 1. Second-stage juveniles hatching (%) of *Meloidogyne chitwoodi* when exposed during 7 days to essential oils of *A. citriodora, C. citriodora, C. citratus, C. winterianus, E. muticus* and *L. alba.* Data are means of eight replicates±standard deviation. 0 µL/mL corresponds to 1 % Tween 80 aqueous solution (control). Averages followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test. Graphs with one set of letters showed that the two experiments had the same statistical results, while in the others statistical results of both trials are presented (1st in the top; 2nd in the bottom).

A variation in hatching was observed between experiments, when *M. incognita* eggs were subjected to the essential oils (Figure 2). *Cymbopogon citratus* induced 100 % of hatching inhibition for concentrations ≥ 3 µL/mL; *A. citriodora* and *C. winterianus* caused 100 % inhibition for ≥ 6 µL/mL; *C. citriodora* and *E. muticus* caused 100 % inhibition for ≥ 9 µL/mL; and *L. alba* for concentrations ≥ 12 µL/mL (Figure 2).

Regarding the nematicidal effect of essential oils on *M. chitwoodi* J2, different responses were obtained. *Aloysia citriodora, C. citratus, E. muticus* and *L. alba* essential oils caused 100 % mortality at 3 µL/mL (Figure 3). *Corymbia citriodora* showed different responses between the two trials carried out, and only at 15 µL/mL dose 100 % mortality was achieved (Figure 3). *Cymbopogon winterianus* was less effective against *M. chitwoodi* J2, causing around 60 % mortality at 18 µL/mL (Figure 3).

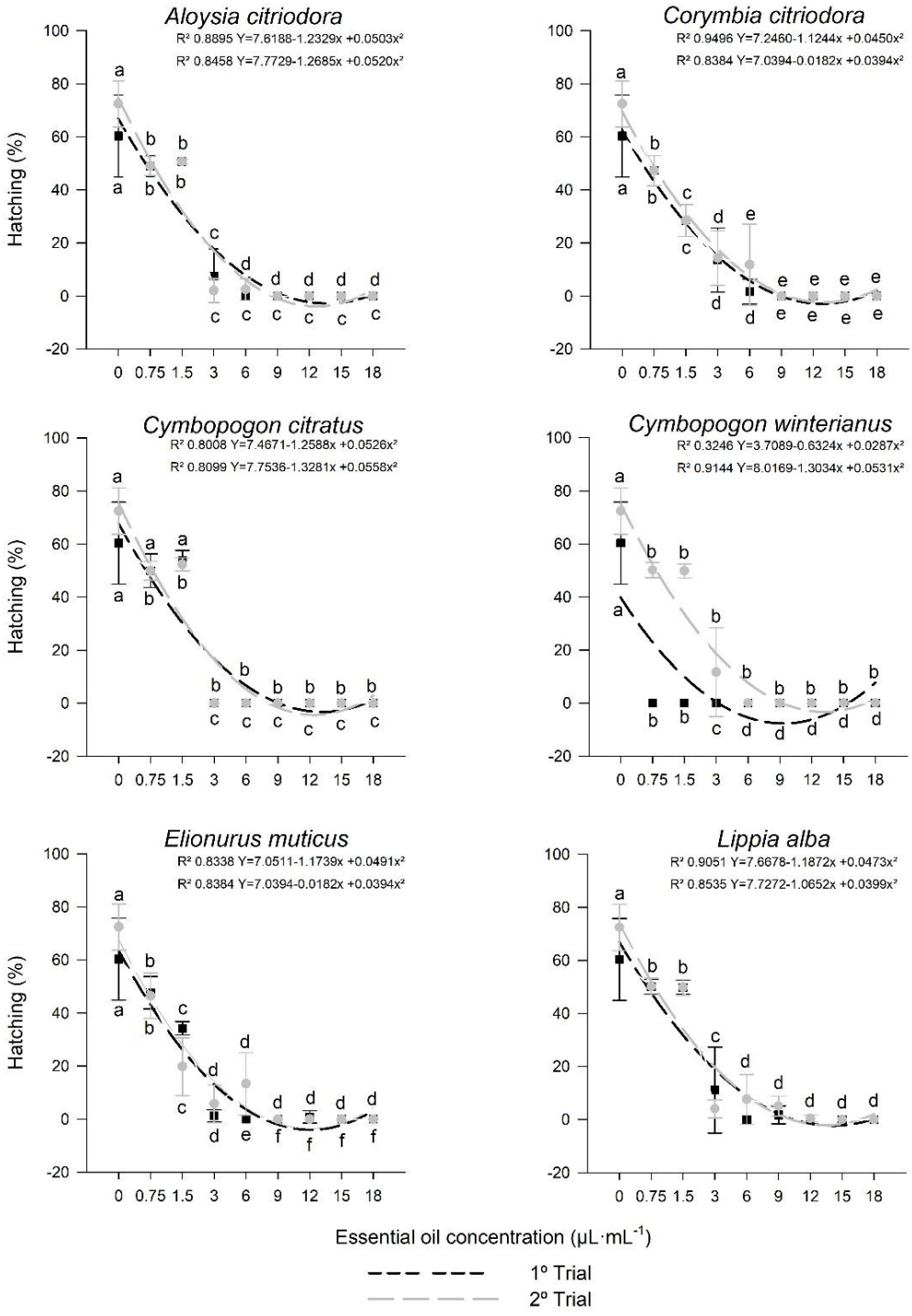


Figure 2. Second-stage juveniles hatching (%) of *Meloidogyne incognita* when exposed during 7 days to essential oils of *A. citriodora, C. citriodora, C. citratus, C. winterianus, E. muticus* and *L. alba.* Data are means of eight replicates±standard deviation. 0 µL/mL corresponds to 1 % Tween 80 aqueous solution (control). Averages followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test. Statistical results of both trials are presented: 1st in the top; 2nd in the bottom.

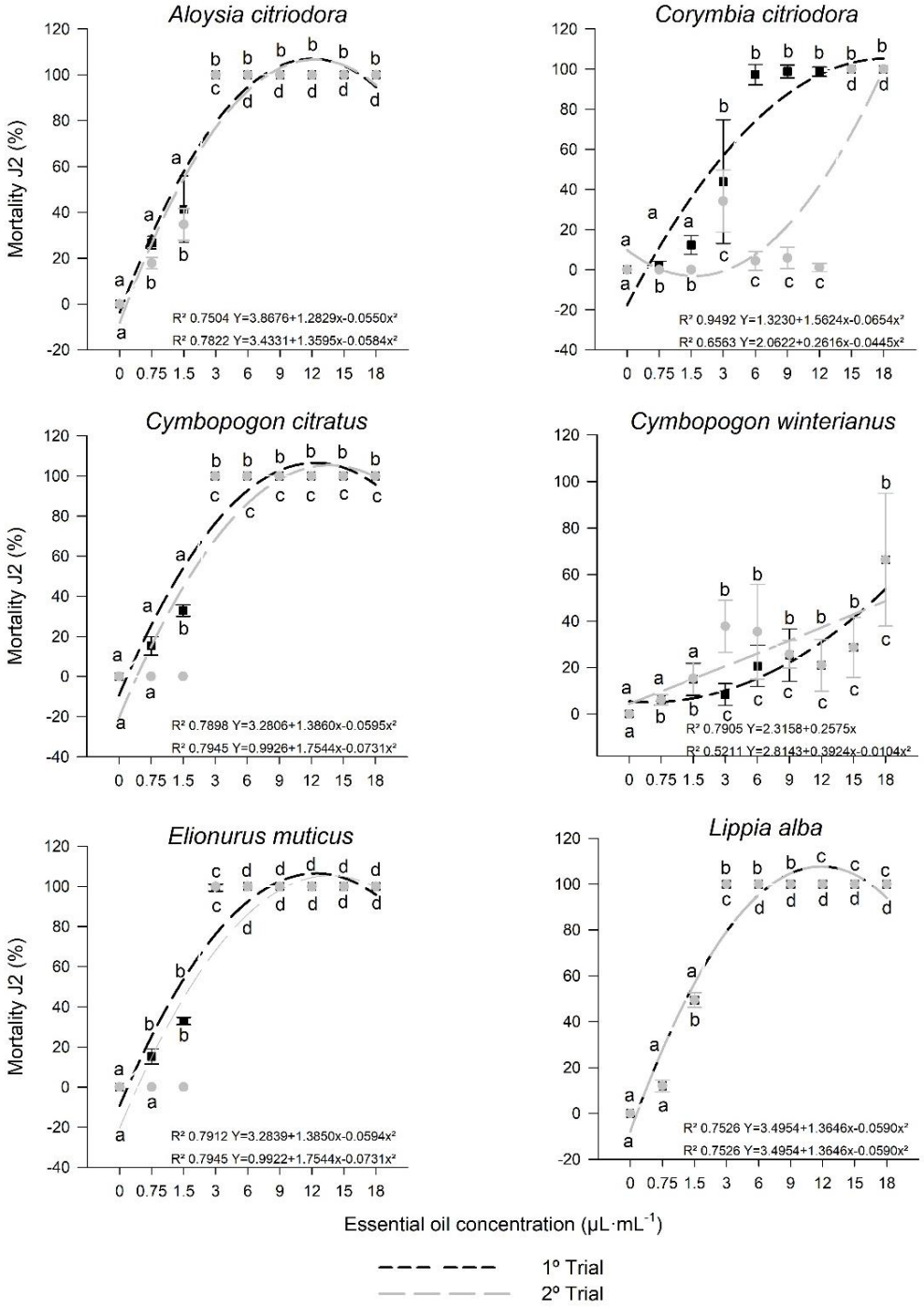


Figure 3. Second-stage juveniles mortality (%) of *Meloidogyne chitwoodi* when exposed, during 48 h, to essential oils of *A. citriodora, C. citriodora, C. citratus, C. winterianus, E. muticus* and *L. alba.* Data are means of eight replicates±standard deviation. 0 µL/mL corresponds to 1 % Tween 80 aqueous solution (control). Averages followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test. Statistical results of both trials are presented:1st in the top; 2nd in the bottom.

The results obtained for *M. incognita* mortality were variable when compared to *M. chitwoodi* (Figures 3 and 4). *Aloysia citriodora* caused > 90 % *M. incognita* J2 mortality at 9 µL/mL (Figure 4); for *M. chitwoodi*, a similar result was obtained for concentrations of 3 µL/mL (Figure 3). *Cymbopogon citratus* essential oil caused 100 % mortality at ≥ 6 and 3 µL/mL, for *M. incognita* and *M. chitwoodi*, respectively; and *E. muticus* essential oil was the most effective, inducing 100 % mortality at ≥ 3 µL/mL in both RKN species (Figures 3 and 4). *Cymbopogon winterianus* is the least effective essential oil compared to the remaining (Figures 3 and 4).

Summarizing, all the essential oils contributed to the control of *M. chitwoodi* and *M. incognita*, acting on hatching and causing J2 mortality (Figures 1 - 4). However, the increase in mortality and hatching inhibition was expressed differently between the two RKN species. Less than 3 µL/mL of *A. citriodora*, *C. citratus*, *E. muticus* and *L. alba* essential oils were required for 100 % mortality of *M. chitwoodi*. A similar result, for *M. incognita,* was only observed with *E. muticus* essential oil. Considering the effect on hatching, the best essential oils for *M. chitwoodi* control were *A. citriodora, C. citriodora, C. citratus* and *E. muticus* (3 µL/mL)*;* while for *M. incognita* only *E. muticus* essential oil presented a similar effect. Thus, the best results were found with the essential oils of *C. citratus* and *E. muticus*, requiring between 3 and 9 µL/mL to inhibit 100 % of *M. chitwoodi* and *M. incognita* hatching and cause 100 % J2 mortality (Figures 1 - 4).

As 100 % hatching was not observed in the controls, for *M. chitwoodi* and *M. incognita*, the data were corrected, using 1 % Tween 80 treatment as reference (Table 2), confirming the results observed in Figures 1 to 4. Estimated values of LC_{50} and LC_{90} for *M. chitwoodi* and *M. incognita* mortality and hatching inhibition are presented in Table 3.

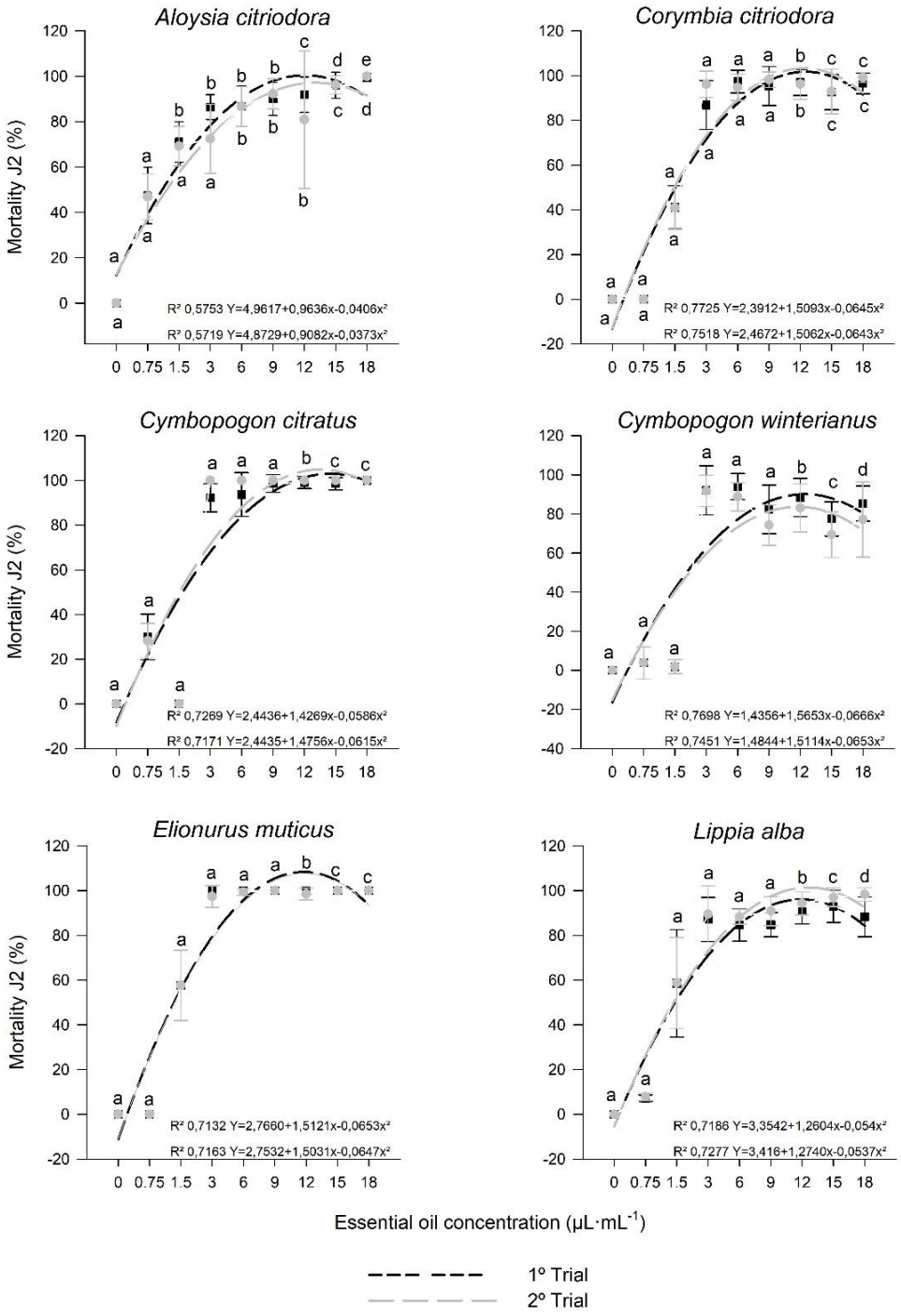


Figure 4. Second-stage juveniles mortality (%) of *Meloidogyne incognita* when exposed, during 48 h, to essential oils of *A. citriodora, C. citriodora, C. citratus, C. winterianus, E. muticus* and *L. alba.* Data are means of eight replicates±standard deviation. 0 µL/mL corresponds to 1 % Tween 80 aqueous solution (control). Averages followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test. Graphs with one set of letters showed that the two experiments had the same statistical results, while in the others statistical results of both trials are presented (1st in the top; 2nd in the bottom).

Table 2. Corrected percentage of *Meloidogyne chitwoodi* and *M. incognita* second-stage juveniles hatching, 7 days after exposure to six essential oils, with reference to the solvent used (1 % Tween 80 aqueous solution - control).

Essential oil	Concentration ($\mu L.mL^{-1}$)	*M. incognita*		*M. chitwoodi*	
		1º Trial	2º Trial	1º Trial	2º Trial
Control	0.0	63.3	67.3	44.7	39.9
Aloysia citriodora	0.75	22.6	27.1	11.1	-
	1.50	19.9	24.8	4.2	-
	3.0	77.2	96.9	92.4	75.7
	6.0	95.9	90.5	100.0	95.2
	9.0	100.0	100.0	100.0	98.6
	12.0	100.0	100.0	94.7	100.0
	15.0	100.0	100.0	100.0	100.0
	18.0	100.0	100.0	100.0	100.0
Corymbia citriodora	0.75	25.3	29.8	-	-
	1.50	55.0	57.7	-	-
	3.0	78.5	78.6	96.2	94.0
	6.0	97.4	78.7	94.1	100.0
	9.0	100.0	100.0	91.0	100.0
	12.0	100.0	100.0	97.7	100.0
	15.0	100.0	100.0	98.5	100.0
	18.0	100.0	100.0	100.0	100.0
Cymbopogon citratus	0.75	24.7	30.9	-	-
	1.50	45.8	70.6	-	-
	3.0	98.0	91.4	84.0	85.8
	6.0	100.0	80.2	91.3	100.0
	9.0	100.0	100.0	96.0	100.0
	12.0	98.7	100.0	100.0	100.0
	15.0	100.0	100.0	100.0	100.0
	18.0	100.0	100.0	100.0	100.0
C. winterianus	0.75	100.0	25.6	-	-
	1.50	100.0	26.0	-	-
	3.0	100.0	82.7	97.2	96.9
	6.0	100.0	100.0	94.9	94.3
	9.0	100.0	100.0	92.9	92.1
	12.0	100.0	100.0	100.0	100.0
	15.0	100.0	100.0	100.0	100.0
	18.0	100.0	100.0	94.9	94.3

Table 2 (*Continuation*). Corrected percentage of *Meloidogyne chitwoodi* and *M. incognita* second-stage juveniles hatching, 7 days after exposure to six essential oils, with reference to the solvent used (1 % Tween 80 aqueous solution - control).

Essential oil	Concentration ($\mu L.mL^{-1}$)	*M. incognita*		*M. chitwoodi*	
		1º Trial	2º Trial	1º Trial	2º Trial
Control	0.0	63.3	67.3	44.7	39.9
Elionorus muticus	0.75	21.0	25.8	-	-
	1.50	14.9	22.2	-	-
	3.0	100.0	100.0	100.0	96.7
	6.0	100.0	100.0	100.0	98.7
	9.0	100.0	100.0	97.7	100.0
	12.0	100.0	100.0	95.3	100.0
	15.0	100.0	100.0	98.3	100.0
	18.0	100.0	100.0	96.0	100.0
Lippia alba	0.75	20.8	25.6	-	-
	1.50	21.3	26.1	-	-
	3.0	82.4	94.0	91.3	89.1
	6.0	100.0	88.7	94.4	98.0
	9.0	97.2	92.5	91.3	98.2
	12.0	100.0	99.3	100.0	98.2
	15.0	100.0	100.0	100.0	100.0
	18.0	100.0	100.0	100.0	100.0

Table 3. Estimated values of lethal concentration (µL/mL) necessary to result in 50 % (LC_{50}) and 90 % (LC_{90}) *Meloidogyne chitwoodi* and *M. incognita* hatching inhibition and second-stage juveniles mortality, at 7 days and 48 h after exposure to essential oils, respectively.

Species	Hatching				Mortality			
	M. chitwoodi		*M. incognita*		*M. chitwoodi*		*M. incognita*	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Aloysia citriodora	-	4.2	0.9	4.0	1.5	2.4	1.3	9.7
Corymbia citriodora	-	4.4	0.5	4.4	8.0	14.8	3.1	8.8
Cymbopogon citratus	2.0	6.4	0.3	3.9	6.2	15.3	1.6	2.4
C. winterianus	-	5.9	0.3	2.3	17.5	33.6	5.6	16.0
Elionurus muticus	-	4.9	0.9	2.7	1.9	2.6	2.5	5.0
Lippia alba	0.2	4.5	0.8	4.9	1.5	2.2	2.9	10.7

When the essential oils were applied to the soil, it was possible to verify that the number of J2 recovered decreased significantly when compared to the control (1 % Tween 80 aqueous solution); and in the control, only ≈17 % of the inoculated J2 were recovered (Figure 5). The higher effect on RKN mortality was observed with *Corymbia citriodora* and *Cymbopogon citratus* essential oils for both RKN species. In soil, *Cymbopogon winterianus* and *L. alba* were the least effective against *M. chitwoodi* and *M. incognita*, respectively (Figure 5). However, the nematode population on the soil decreases by about 40 and 50 %, respectively, when compared with the control (Figure 5).

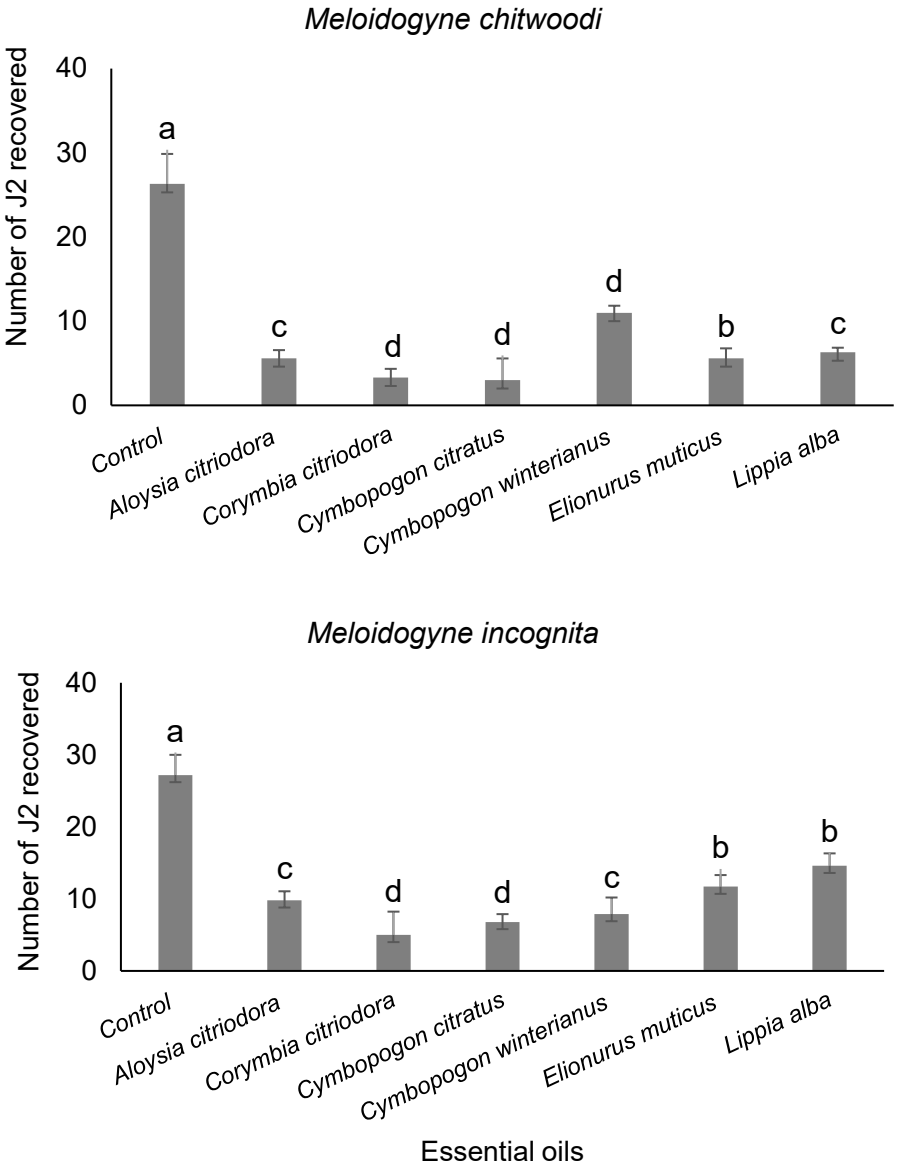


Figure 5. Number of *Meloidogyne chitwoodi* and *M. incognita* second-stage juveniles (J2) recovered from soil after 5 days of exposure to essential oils of *A. citriodora, C. citriodora, C. citratus, C. winterianus, E. muticus* and *L. alba* at 3 µL/mL. Data are means of five replicates±standard deviation; control corresponds to 1 % Tween 80 aqueous solution treatment. Means followed by the same combination of letters do not differ significantly at $p > 0.05$, according to the Scott-Knott test.

2.4 DISCUSSION

Essential oils are organic molecules obtained from different plant species, which chemical composition vary within the same plant species, mainly due to abiotic factors

(Rocha *et al*., 2022). Thus, the chemical characterization of the essential oil and knowledge of the plant access used are essential, to understand their impact on *Meloidogyne* spp. control (D' Addabbo *et al*., 2020).

Aloysia citriodora, C. citriodora and *L. alba* have their chemical composition described in the literature (Osório *et al*., 2023), which is similar to that found in this study. Essential oils of these aromatic plant species obtained from the same source were already reported to show nematicidal activity against sheep gastrointestinal nematodes (Osório *et al*., 2023). *Cymbopogon citratus* showed the same majority compounds when compared to another access of the same species, namely β-citral and α-citral (Rhimi *et al*., 2022). It is known that the chemical composition of essential oils can vary depending on where they were collected, how they were extracted, cultivation conditions and biotic and abiotic factors (Boaro *et al*., 2019), so only the majority compounds are considered for comparison purposes.

Few studies were performed on the control of *M. chitwoodi* using essential oils. In the present study, *A. citriodora, Corymbia citriodora, Cymbopogon citratus* and *C. winterianus* essential oils inhibited significantly *M. chitwoodi* hatching, at doses ≥ 3 µL/mL. However, Faria *et al*. (2016) reported a high reduction in hatching at a dose of 2 µL/mL for *C. citratus* essential oil. Aqueous plant extracts of *Ficus carica, Nerium oleander, Laurus nobilis, Asphodelus aestivus, Anethum graveolens, Melia azedarach, Eucalyptus camaldulensis, Juglans regia, Zingiber officinale* and *Eruca sativa* also showed nematicidal activity, *in vitro* and *in vivo*, against *M. chitwoodi* (Uludamar, 2024). Using the essential oil of *Thymus vulgaris* at 125 ppm, 100 % of *M. chitwoodi* mortality was achieved (Uludamar, 2023).

Different essential oils have already been studied for the control of *Meloidogyne* spp. For example, *Cuminum cyminum* seed oil, whose main compounds are γ-terpinen-7-al (34.9 %) and cumin aldehyde (26.5 %) caused *M. incognita* and *M. javanica* mortality over 90 % at doses of 500 µL/L, while a similar effect on hatching was only observed to a concentration of 2000 µL/L (Pardavella *et al*., 2020). This difference could be explained by the presence of a lipid layer in nematode eggs that provides a chemical barrier where substances cannot penetrate (Gaugler & Bilgrami, 2004), protecting the J2 from nematicidal compounds. *Artemisia judaica* and *Origanum vulgare*, at a concentration of 1000 mg/L, significantly reduced the number of *M. incognita* galls, by 63 % when compared to the control (*Solanum melongena* cv. Spanish patra) (El-Habashy *et al*., 2022).

The mode of action of essential oils in nematode control is not yet well understood. Their classification is in group N-UNE: botanical/animal derivatives, with this group listed as unknown or undetermined mode of action (IRAC, 2022). However, Sarri *et al*. (2024), after reviewing the literature, found that essential oils act by penetrating gelatinous masses, disrupting the central nervous system and the permeability of the plasma membrane, and altering cellular processes in females and J2. Knowing the mode of action of these molecules is extremely important and is necessary to define the application mode of the products in the field.

Questions have been raised about the biological activity of pure essential oils and the need, or not, to isolate their main compounds. Limonene when isolated showed a percentage of *M. incognita* hatching inhibition close to 80 %, compared to 50 % inhibition achieved with the essential oil of *Citrus reticulata*, demonstrating that it was not essential to use the pure compound, reducing costs of extraction/purification (Goyal *et al*., 2021). Other pure compounds, such as cineole, comunaldehyde, linalool and carvone showed nematicidal activity against *M. incognita* (Elsharkawy *et al*., 2022). This data is of particular importance, because it will help in the selection of essential oils according to their major compounds.

Terpenes are the compounds most commonly found in essential oils, with the highest levels of mono- and sesquiterpenes. Assessing the effect of the pure main compounds, such as borneal, carveol, citral, geraniol and α-terpineol, against *M. incognita*, it was possible to observe an inhibition of J2 hatching and mortality, at concentrations ≥ 250 mg/L (Echeverrigaray *et al*., 2010). This corroborates the data found in this study, considering that the compounds mentioned are present in the essential oils of *Cymbopogon citratus. A. citriodora* and *C. winterianus.*

The complexity of the chemical composition of essential oils is extremely positive when used to control microorganisms, as the increase in the number of molecules reduces the likelihood of microorganisms resisting these molecules (Oliveira *et al*., 2021). However, the complexity of the essential oils difficult to guarantee the homogeneity of the chemical composition of a product (bionematicide).

2.5 CONCLUSIONS

The results obtained in this study showed that the essential oils of *A. citriodora*, *C. citriodora*, *C. citratus*, *C. winterianus*, *E. muticus* and *L. alba* have nematicidal activity *in vitro,* with effects on hatching and mortality of *M. chitwoodi* and *M. incognita.*

The essential oils of *C. winterianus* and *E. muticus* showed the best results in inhibiting the hatching of *M. incognita*, while *C. citratus* essential oil showed the best results in J2 mortality. As for *M. chitwoodi*, essential oils of *A. citriodora* and *L. alba* showed the best results in terms of J2 mortality and hatching inhibition. When applying the essential oils to the soil, it was possible to observe that, for both RKN species, the essential oils of *C. citriodora* and *C. citratus* had the highest effects. Further studies should be carried out to verify/confirm the effects of *C. citriodora* and *C. citratus* essential oils on *M. chitwoodi* and *M. incognita* reproduction by performing pot assays, to then develop potential formulations, using techniques, such as nanotechnology, to guarantee the homogeneity and stability of the products developed, as well as defining the method of application of these products on the plant or in the soil.

2.6 ACKNOWLEDGMENTS

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CONSIDERAÇÕES FINAIS

O presente trabalho contribui significativamente para o entendimento e manejo de nematoides das galhas radiculares, um problema agronômico à escala mundial responsável por perdas econômicas consideráveis. A partir dos resultados obtidos, ficou evidente que as plantas aromáticas podem desempenhar um papel fundamental no manejo sustentável de nematoides do gênero *Meloidogyne*, sendo uma estratégia viável para redução da densidade populacional no solo, além de promover alternativas econômicas aos produtores rurais. Entre as sete espécies de plantas aromáticas estudadas, as espécies *Lippia alba*, *Mentha spicata*, *Origanum majorana*, *O. vulgare* e *Thymus vulgaris* demonstraram resistência às espécies *M. chitwoodi*, *M. enterolobii*, *M. incognita*, *M. javanica* e *M. luci*, podendo ser cultivadas em áreas infestadas por esses patógenos. Além disso, o estudo do uso de óleos essenciais revelou um potencial promissor no controle de *M. chitwoodi* e *M. incognita*, com destaque para os óleos de *Corymbia citriodora* e *Cymbopogon citratus*. Estes óleos demonstraram alta eficiência na mortalidade e na inibição da eclosão, apresentando-se como uma alternativa interessante ao uso de nematicidas sintéticos, além de oferecerem um enfoque mais sustentável.

Em conjunto, os resultados obtidos nas duas abordagens, o cultivo de plantas aromáticas e o uso de óleos essenciais, proporcionam novas perspectivas para o controle de nematoides das galhas radiculares, representando avanços tanto para a teoria quanto para a prática do manejo integrado de nematoides. O emprego destas estratégias pode ser integrado em programas de manejo sustentável, visando reduzir a dependência de produtos químicos e mitigar os impactos ambientais e econômicos causados por estes fitoparasitas.

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