AGRO PRODUCTIVIDAD

In vitro effectiveness of plant extracts on the mortality of root-knot nematodes

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ABSTRACT

Objective: To identify plants with nematicidal potential against *Meloidogyne enterolobii* and *Nacobbus aberrans* for potential use in sustainable agricultural practices.

Design/methodology/approach: Six aqueous extracts (AEs) from the leaves of *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* were evaluated for the control of second instar (J2) juveniles of *Meloidogyne enterolobii* and *Nacobbus aberrans*. The solution obtained was designated as standard (100%) and was diluted with distilled water to concentrations of 25%, 50% and 100%. Sterile distilled water was included as a test sample. Each concentration was repeated four times, using 400 J2/concentration. Mortality observations were performed at 6, 24 and 48 h. A nonparametric factorial analysis was performed using aligned ranks in R.

Results: The factorial analysis of variance for the main effects —P (Plant) and T (Time)— on the mortality of *M. enterolobii* (Me) and *N. aberrans* (Na) J2 showed highly significant differences ($p \leq 0.05$), as well as a significant P*T interaction effect. All aqueous extracts at 100% concentration caused the highest J2 mortality for *M. enterolobii* at 24 hours and for *N. aberrans* at 12 hours using the 25% concentration.

Limitations on study/implications: This study serves as a foundational step for future research and represents the first attempt to propose the use of these plant species for the control of root-knot nematodes through nematicidal activity.

Findings/conclusions: Our results demonstrate, for the first time, the in vitro nematicidal activity of plant extracts of *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare* and *Thymus vulgaris* against the nematodes *M. enterolobii* and *N. aberrans*. These findings support the need for further research under soil conditions and metabolomic studies to identify the active compounds responsible for this effect.

Keywords: Aqueous extracts, *Meloidogyne enterolobii*, *Nacobbus aberrans*.

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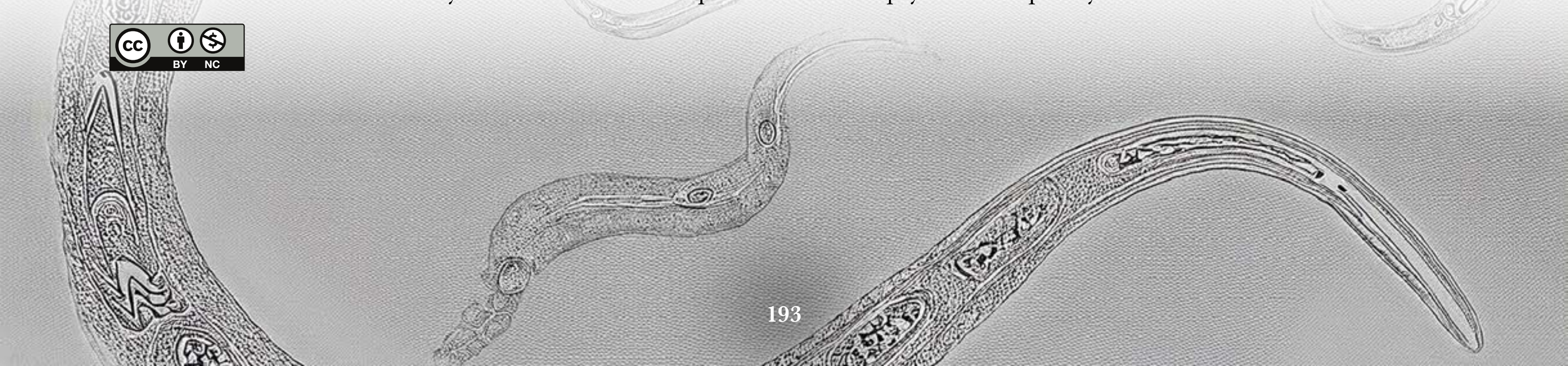
INTRODUCTION

Root-knot nematodes are considered among the most important soil-borne phytoparasites. They are responsible for economic losses in agricultural crop productivity exceeding 170 billion USD annually worldwide (Bernard *et al.*, 2017). Their polyphagous capacity and considerable phenotypic plasticity have allowed them to establish in various geographical areas, where they can achieve higher rates of infection, development, and reproduction (Mbow *et al.*, 2019). This, in turn, poses significant challenges for agricultural systems due to their impact on both crop yield and quality.



In the search for more sustainable and environmentally friendly alternatives to chemical pesticides for the control of various plant pathogens, several strategies are being developed that involve the use of phytochemicals. In recent years, there has been a significant increase in scientific and industrial interest in the development of new biopesticides, with plant extracts gaining prominence over the last decade as a sustainable and ecological alternative (Khursheed *et al.*, 2022). Interest in extracts obtained from various plants lies in their content of bioactive compounds —such as alkaloids, terpenoids, phenols, and saponins— that have demonstrated nematicidal properties by interfering with the physiological and metabolic processes of nematodes. These include motility, glycolysis, giant cell formation, neurotransmission, the urea cycle, polyamine synthesis, antioxidant activity, and nematode reproduction (Subramanian *et al.*, 2017; Naboulsi *et al.*, 2018). Metabolites such as kaempferol and its derivatives have been shown to affect root-knot nematode invasion, acting as defense compounds against these microorganisms by reducing gall formation in tomato plants (*Solanum lycopersicum* L.) and inhibiting egg hatching (Chin *et al.*, 2018; Zhao *et al.*, 2023). Other compounds —such as quercetin, myricetin, rutin, patuletin, and glyceollin— produced by various antagonistic plants have also been studied for their nematicidal properties (Faizi *et al.*, 2011; Hamaguchi *et al.*, 2019). Despite advances in this field of research, the number of plant species analyzed for such properties remains limited. Therefore, the objective of the present study was to evaluate *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* as potential plant species with nematicidal activity against *Meloidogyne enterolobii* and *Nacobbus aberrans*.

MATERIALS AND METHODS

Six aqueous extracts (AE) from the leaves of *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* were evaluated for the control of second-stage juveniles (J2) of *Meloidogyne enterolobii* and *Nacobbus aberrans*. A total of 50 g of plant leaves were macerated in 200 mL of distilled water for 48 hours. The resulting mixture was blended and filtered through Whatman No. 1 filter paper. The solution obtained was designated as the standard (100%) and subsequently diluted with distilled water to obtain concentrations of 25%, 50%, and 100%. Sterile distilled water was included as a control treatment. Each concentration was replicated four times, using 400 J2 per concentration. Mortality observations were recorded at 6, 24, and 48 hours.

For the efficacy tests, 140 μL of each concentration were placed into ELISA plates (Thermo Scientific, Nunclon™ Delta Surface). In each well, 100 J2 nematodes —obtained from tomato plants (*Solanum lycopersicum*) cultivated monoxenically under greenhouse conditions at the Colegio de Postgraduados, Montecillo Campus— were added. Each concentration was replicated four times, for a total of 400 J2 per concentration evaluated. The plates were incubated in a controlled growth chamber (APT.line™ KBWF model, E5.1) at 28±1°C (Pagamas and Nawata, 2007).

Since the assumption of normality in the distribution of residuals was not met in the factorial analysis of variance of the original data, a non-parametric factorial analysis based on the use of aligned ranks was employed. This methodology involves centering or aligning the data by subtracting the group mean from each observation. The aligned data, corresponding to the desired main and interaction effects, are then ranked, and parametric tests are applied to these aligned ranks (Wobbrock *et al.*, 2011). Following this analysis, pairwise mean comparisons were performed using Tukey's test on the adjusted group means. Three separate analyses were conducted according to the extract concentration used: 1) Factor 1 (six different plant extracts and a control consisting of distilled water), Factor 2 (three exposure times to the extract), at a 25% extract concentration; 2) the same two factors as the previous set, at a 50% extract concentration; 3) the same two factors as the first set, at a 100% extract concentration. All analyses were performed using the RStudio statistical software package. The experiments were conducted under a completely randomized design. To determine nematode mortality, individuals showing immobility were stimulated with a fine bristle near the cephalic region and then maintained in sterile water for 24 hours. Nematodes that did not move after this procedure were considered dead.

RESULTS AND DISCUSSION

The factorial analysis of variance of the main effects, P (Plants) and T (Time), for the variable mortality of J2 of *Meloidogyne enterolobii* (Me) and *Nacobbus aberrans* (Na) indicated highly significant differences ($p \leq 0.05$), as well as a significant interaction effect between P and T. All aqueous extracts evaluated at a 100% concentration achieved higher mortality in J2 of *M. enterolobii* after 24 hours of exposure (Table 1). Furthermore, it was observed that as both the concentration and exposure time increased, the mortality of J2 *M. enterolobii* with all evaluated extracts progressively increased.

During the first 6 hours of exposure, significant differences ($p \leq 0.05$) were observed among the plant species evaluated at a 25% concentration. The *Melissa officinalis* leaf extract achieved the highest mortality percentage (39%) compared to the control (4%). This was followed by extracts from *Foeniculum vulgare* and *Thymus vulgaris*, which reached mortality rates above 30% (Figure 1).

The remaining extracts ranged between 25% (*Dysphania ambrosioides*) and 30% (*Origanum vulgare*). Only the *Dracocephalum moldavica* leaf extract showed a lower mortality percentage (20%) compared to the other extracts evaluated ($p \leq 0.05$); however, its performance was still superior to the mortality observed in the control group. From 12 hours of exposure onward,

Table 1. Maximum mortality of *Meloidogyne enterolobii* J2 juveniles in relation to concentration and exposure time for each evaluated extract.

Extract	Concentration (%)	Exposure time (h)	Mortality (%)
Origanum vulgare	100	24	100
Foeniculum vulgare	100	24	100
Dracocephalum moldavica	100	24	100
Thymus vulgaris	100	24	100
Dysphania ambrosioides	100	24	100
Melissa officinalis	100	24	100

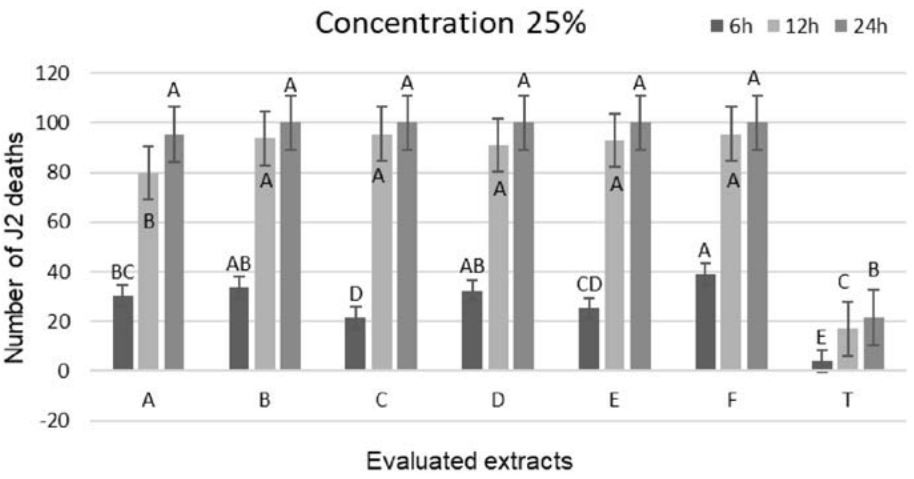


Figure 1. Effect of 25% aqueous plant extracts on the mortality of *M. enterolobii*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. Degrees of freedom (df)=6. Means sharing the same letter are not significantly different (p≤0.05).

mortality of *M. enterolobii* J2 juveniles was homogeneous, as all aqueous extracts evaluated achieved mortality rates higher than 80%. Specifically, the plant species *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* reached 100% mortality at 24 hours (Figure 1).

This behavior positively increased mortality as the concentration of the aqueous extract increased from 50% to 100% (Figures 2 and 3). Starting at 6 hours of exposure at a 50% concentration, leaf extracts of *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, and *Dysphania ambrosioides* achieved mortality rates of Me J2 exceeding 50% compared to the control (Figure 2). An exponential increase in Me J2 mortality was observed at 100% concentration from 6 hours of exposure in all extracts evaluated, reaching maximum

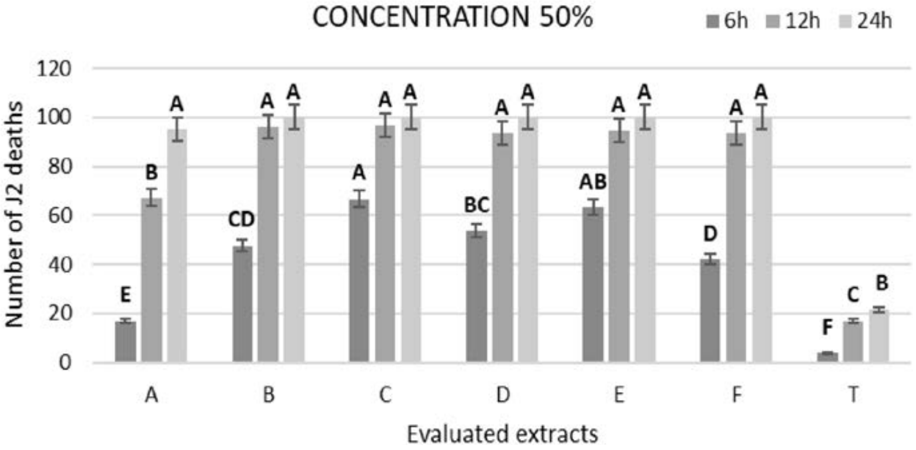


Figure 2. Effect of 50% aqueous plant extracts on *M. enterolobii* mortality. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing the same letter are not significantly different (p≤0.05).

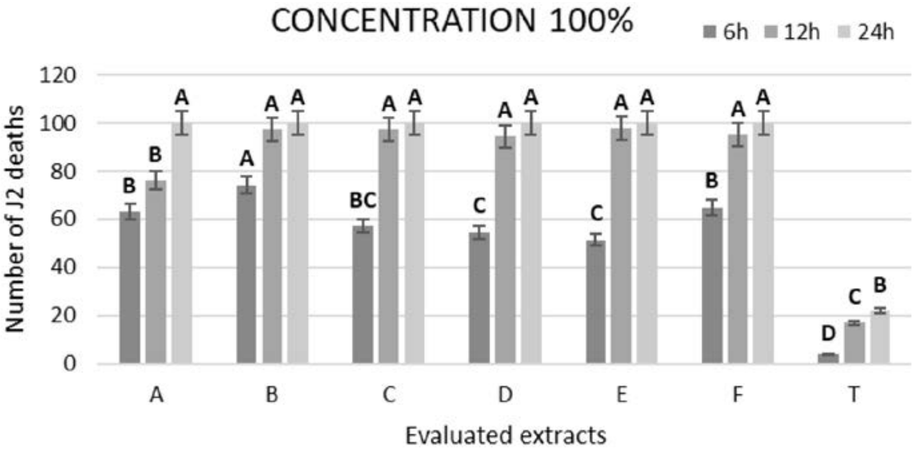


Figure 3. Effect of 100% aqueous plant extracts on *M. enterolobii* mortality. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing the same letter are not significantly different ($p \leq 0.05$).

mortality (100%) of Me J2 after 24 hours of exposure, with significant differences ($p \leq 0.05$) compared to the control (Figure 3).

In turn, the mortality observed in J2 of *Nacobbus aberrans* (Na) with all the aqueous extracts evaluated at a 25% concentration showed greater effectiveness after 12 hours of exposure (Table 2).

Similarly, it was observed that as the concentration and exposure time increased (12 and 24 h), the mortality of Na in all evaluated extracts exceeded 95% compared to the control, with highly significant differences ($p \leq 0.05$). However, unlike Me, *Nacobbus aberrans* reached 100% mortality after 12 hours of exposure at the lowest concentration of most evaluated extracts compared to the control (Figure 4). Similar to Me, the leaf extracts of *Melissa officinalis* and *Thymus vulgaris* at the lowest concentration (25%) showed the highest mortality rates (27% and 22%, respectively) within the first 6 hours of exposure, compared to the control and with significant differences from the other evaluated extracts (Figure 4). These were followed by *Foeniculum vulgare* (17%) and *Origanum vulgare* (15%). Although the extracts of *Dracocephalum moldavica* and *Dysphania ambrosioides* recorded the lowest J2 mortality rates of *N. aberrans* among the evaluated extracts ($p \leq 0.05$), their effectiveness remained higher than that of the control.

Table 2. Maximum mortality of J2 juveniles of *Nacobbus aberrans* in relation to the concentration and exposure time for each evaluated extract.

Extracts	Concentration (%)	Exposure time (h)	Mortality (%)
Origanum vulgare	25	24	100
Foeniculum vulgare	25	12	100
Dracocephalum moldavica	25	12	100
Thymus vulgaris	25	12	100
Dysphania ambrosioides	25	12	100
Melissa officinalis	25	12	100

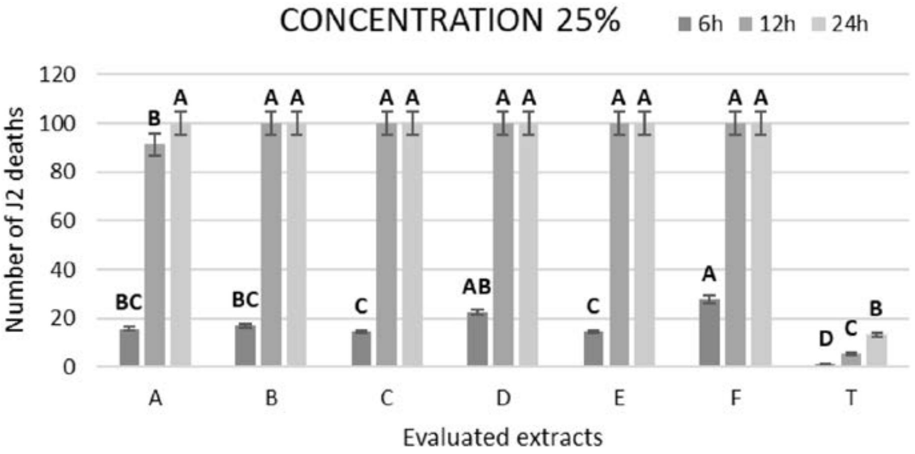


Figure 4. Effect of aqueous plant extracts at 25% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing a letter are not significantly different ($p \leq 0.05$).

The mortality observed with the *Melissa officinalis* extract remained the highest (43%) at the same exposure time (6 hours) and at a 50% concentration compared to the other evaluated extracts (Figure 5), followed by *Foeniculum vulgare* (41%). Extracts based on *Origanum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, and *Dysphania ambrosioides* showed *N. aberrans* J2 mortality ranging between 12% and 22%. Although the mortality recorded with these extracts was lower than that of the two aforementioned plant species, they still had a significant effect ($p \leq 0.05$) on Na mortality compared to the control. As the concentration of the evaluated extracts increased to 100% (Figure 6), J2 control was similar to that observed in *M. enterolobii*. However, Na proved to be more susceptible to these

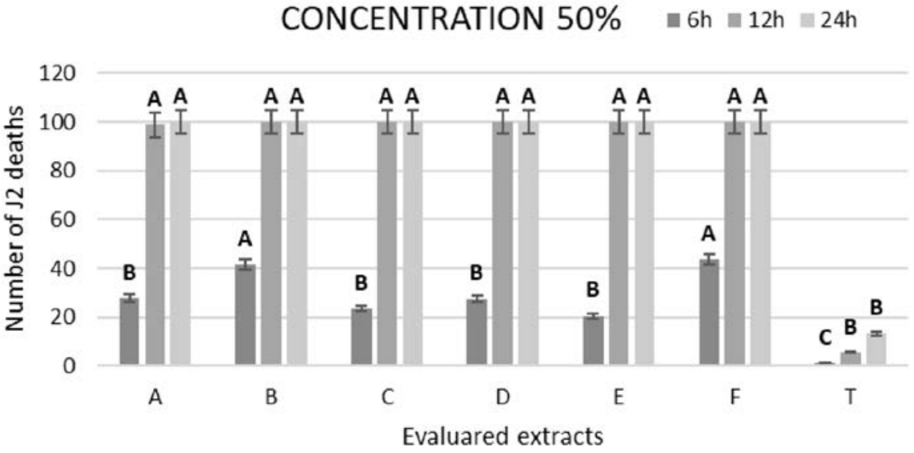


Figure 5. Effect of aqueous plant extracts at 50% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means with the same letter are not significantly different ($p \leq 0.05$).

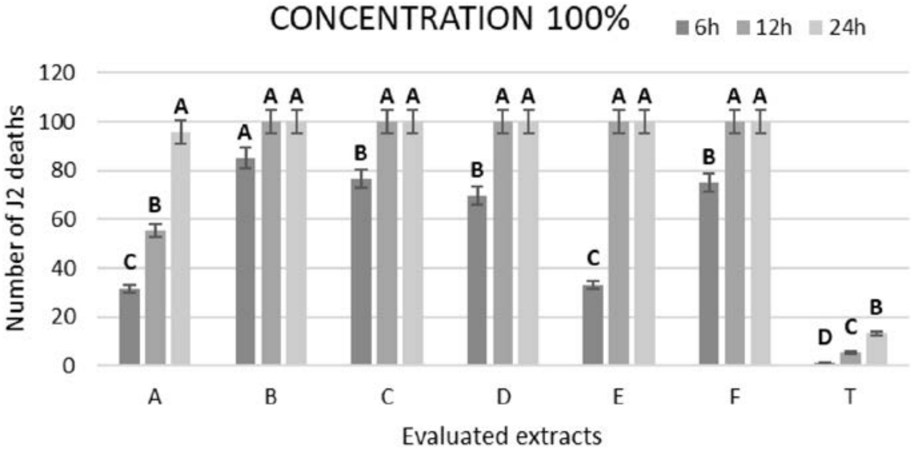


Figure 6. Effect of aqueous plant extracts at 100% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means with the same letter are not significantly different ($p \leq 0.05$).

extracts, as control rates greater than 80% were observed from the lowest concentration starting at 12 hours of exposure, with all evaluated species showing effectiveness.

The observed behavior can be explained by the specific biochemical characteristics of the evaluated plant species, as well as by the effect of the undiluted pure extract concentration. In aqueous extracts, it is more likely to obtain secondary metabolites with hydrophilic or moderately polar properties, such as certain phenols, tannins, and triterpenes, which could influence the results obtained in terms of the observed mortality. Within these groups, compounds such as kaempferol can be found. This metabolite and its derivatives have shown the ability to restrict the motility of Meloidogyne incognita and exhibit repellent effects on this species by inhibiting its penetration rate in tomato (*Solanum lycopersicum* L.) roots, resulting in a lower gall index due to reduced penetration (Zhao *et al.*, 2023). A similar effect has been observed in the extract of false arnica (*Heterotheca inuloides*), which inhibits egg hatching and J2 motility of *N. aberrans* (Rodríguez-Chávez *et al.*, 2019). This plant species has been reported to possess pesticidal properties associated with its high content of quercetin- and kaempferol-type flavonoids (Rodríguez-Chávez *et al.*, 2017). These metabolites, like the flavonol myricetin, have been shown not to require large doses to exert a nematicidal effect on *Meloidogyne* species, as reported by Wuyts *et al.* (2006). They observed that concentrations of 46 μg ml^{-1} of kaempferol, quercetin, and myricetin repel and limit the mobility of *M. incognita* juveniles. Similarly, in this study, despite using a small volume (140 μl) of the aqueous extracts evaluated, all showed a positive effect on the mortality of the root-knot nematodes studied.

The aqueous leaf extracts of *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare*, and *Thymus vulgaris*, and their effect on the mortality of *Nacobbus aberrans* and *Meloidogyne enterolobii*, may be related to the metabolites present in these plants. All of these species have been reported to contain compounds such as quercetin and kaempferol (Barros *et al.*, 2013; Petrison *et al.*, 2022; Shamkant *et al.*, 2014; Komaki *et al.*, 2015). However, the heterogeneous susceptibility observed between the two nematodes to these extracts may likely be explained by differences in chemosensory receptors, the binding affinities of flavonoid receptors, cellular signaling, and the permeability of metabolites through the cuticle in the different nematode species, or by gene expression prior to nematode infection —although this has not yet been studied. For example, the concentration of lauric acid has been shown to influence the expression of the Mi-flp-18 gene (Dong *et al.*, 2016). It was demonstrated that low concentrations (0.5-2.0 mM) of lauric acid from the root exudates of crown daisy (*Chrysanthemum coronarium*) attract *Meloidogyne incognita*, whereas a higher concentration (4.0 mM) repels the nematode. However, there is a lack of studies on the factors that regulate this gene expression. While some investigations show that root exudates regulate the expression of genes such as flp, which encode FMRFamide-like peptides —a diverse group of neuropeptides involved in nematode feeding, reproduction, and mobility, playing a key role in nematode chemotaxis (Peymen *et al.*, 2014)— it remains unknown which specific components of the exudates are involved in genetic regulation and what factors trigger that signaling.

Although research is increasingly focused on understanding the dynamics between metabolomics and interactions with pathogens, our knowledge of the molecular responses triggered by attractants, repellents, and toxins within the nematode's body —and their impact on host crops under greenhouse or field conditions, including their effectiveness in controlling root-knot nematodes, potential phytotoxicity on the host plant, or their effect on the microbial loop— remains limited.

CONCLUSIONS

To the best of our knowledge, our results demonstrate for the first time the *in vitro* nematicidal activity of plant extracts from *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare*, and *Thymus vulgaris* against the root-knot nematodes *Nacobbus aberrans* and *Meloidogyne enterolobii*. These plant species are proposed as subjects for future research aimed at identifying additional compounds responsible for their nematicidal activity and at providing non-toxic alternative chemical products for crop protection.

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