Extracts and semi-purified fractions of \textit{Tagetes patula} flowers in the control of root-knot nematodes

Extratos e frações semipurificadas de flores de \textit{Tagetes patula} no controle do nematoide-das-galhas

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Abstract

The genus \textit{Tagetes} constitutes a group of antagonistic plant species that are cytotoxic against plant pathogenic nematodes, with \textit{T. patula} being particularly efficient. The aim of this study was to evaluate the in vitro effect of extracts and semi-purified fractions of \textit{T. patula} flowers on eggs and second-stage juveniles (\textit{J}_2) of \textit{Meloidogyne incognita}, \textit{M. javanica}, and \textit{M. paranaensis}, as well as to verify the nematicidal effect of the flavonoids present in \textit{T. patula} flowers. Extracts and semi-purified fractions were obtained from dried \textit{T. patula} flowers after maceration and liquidification, yielding crude aqueous (CAE) and crude ethanol-water (CEWE) extracts. Dried flowers were also treated with \textit{n}-hexane to obtain a crude defatted aqueous extract (CDAE) and a crude ethanol-water defatted extract (CEWDE). Then, the CEWE was fractionated, and the ethyl acetate (EAF), ethanol (EF), methanol (MF), and ethanol:water (EWF) fractions were obtained. CAE, CEWE, CDAE, and CEWDE were tested to evaluate their effects on hatching, mobility, and mortality of \textit{J}_2 of \textit{M. incognita}, \textit{M. javanica}, and \textit{M. paranaensis}. EAF, EF, MF, and EWF fractions were tested on the same variables of \textit{M. incognita}. All extracts significantly reduced \textit{J}_2 hatching of \textit{M. incognita}, \textit{M. javanica}, and \textit{M. paranaensis} when compared to water and water + DMSO. CEWE had nematicidal effects on the three evaluated species, whereas CEWDE demonstrated nematicidal effects against \textit{M. incognita} and \textit{M. javanica}, and nematostatic effects on \textit{M. paranaensis}. This toxic effect showed by CEWE may be related to the high content of quercetin, a major substance present in this sample. It was also observed that EAF accentuated the nematicidal response on \textit{Meloidogyne} spp., suggesting that other medium polarity (methoxylated) flavonoids act as nematotoxic substances. Thus, these results suggest that quercetin contributes significantly to the nematicidal activity of CEWE and EAF.

**Key words:** Chemical fractionation. Flavonoids. \textit{Meloidogyne}. Nematicidal activity.

Resumo

Entre as espécies de plantas antagonistas, as do gênero \textit{Tagetes} apresentam citotoxicidade contra fitonematoides, sendo que \textit{T. patula} mostra-se eficiente. Assim, objetivou-se avaliar o efeito in vitro de...
extratos e frações semipurificadas de flores de *T. patula* sobre ovos e juvenis de segundo estádio (*J₂*) de *Meloidogyne incognita*, *M. javanica* e *M. paranaensis*, e a capacidade nematicida dos flavonoides presentes nas flores de *T. patula*. Os extratos e frações semipurificadas foram preparados a partir de flores secas de *T. patula*, que após maceração foram submetidas à turbólise, obtendo-se os extratos bruto aquoso (EBA) e bruto etanol-água (EBAE). Também houve tratamento de flores secas com *n*-hexano para a obtenção do extrato bruto aquoso desengordurado (EBAD) e do extrato bruto etanol-água desengordurado (EBEAD). Em seguida, o EBAE foi fracionado, sendo obtidas as frações acetato de etila (FAE), etanólica (FE), metanólica (FM) e etanol:água (FEA). O efeito dos extratos EBA, EBAE, EBAD e EBEAD foram avaliados sobre a eclosão, mobilidade e mortalidade de *J₂* de *M. incognita*, *M. javanica* e *M. paranaensis*. As frações FAE, FE, FM e FEA foram avaliadas frente a *M. incognita* usando as mesmas variáveis. Os extratos reduziram significativamente a taxa de eclosão de *J₂* de *M. incognita*, *M. javanica* e *M. paranaensis*, quando comparados com água e água + DMSO. Quanto ao efeito dos extratos, observou-se que EBAE apresentou efeito nematicida para as três espécies avaliadas, e EBEAD demonstrou capacidade nematicida contra *M. incognita* e *M. javanica*, e sendo nematostático para *M. paranaensis*. Tal capacidade pode estar relacionada com o alto teor de quercetina (substância majoritária no EBAE). Observou-se, também, que o fracionamento químico e a obtenção da FAE acentuaram a resposta nematicida sobre *Meloidogyne* spp., sugerindo que outros flavonoides de média polaridade (metoxilados) atuam como substâncias nematotóxicas. Desta forma, os resultados sugerem que a quercetina contribui significativamente para a atividade nematicida do EBAE e da FAE.


**Introduction**

Root-knot nematodes, of the genus *Meloidogyne* Goeldi, are among the most harmful agricultural pathogens (MUKHTAR et al., 2013). They are distributed over a wide area and are obligate root parasites of hundreds of plant species, causing widespread agricultural damage (CASTRO et al., 2003; RADWAN et al., 2012). These pathogens account for a loss of up to 30% of worldwide crop yield (SIKORA; FERNANDEZ, 2005), with the exact local amount varying depending on the level of soil infestation, host susceptibility, soil and climatic conditions, and management strategies.

Phytonematodes, in general, are controlled by crop rotation with non-host species, the cultivation of resistant varieties, and the use of nematicides (BUENA et al., 2008). However, in this case, crop rotation is restricted by the wide host range of the root-knot nematode, whereas the availability of resistant genotypes is limited. Meanwhile, chemical nematicides, despite their effectiveness, have some drawbacks, such as environmental and microbial toxicity, environmental pollution, risks to human health, and high costs (BUENA et al., 2008; MUKHTAR et al., 2013).

For these reasons, alternative control methods have been studied, including the use of both antagonistic plants and their extracts (BALDIN et al., 2012; FERREIRA et al., 2013). Antagonistic plants that negatively affect phytonematode populations include trap crops, non-host plants, and those that contain nematicidal and/or nematostatic compounds (ZAMBOLOM et al., 2007). In particular, species of the genus *Tagetes* display cytotoxicity towards phytonematodes, with *Tagetes patula* L. having been shown as effective in managing these populations (FERRAZ; VALLE, 1997; PLOEG, 2002).

Previous studies have attributed the nematicidal activity of *T. patula* to the presence of thiophenes in its roots (KYO et al., 1990; MAROTTI et al., 2010; FAIZI et al., 2011). Moreover, Xu et al. (2005) found 126 substances in *Tagetes* spp.. Some of these groups of substances were predominant in certain plant-specific organs, with essential oils and flavonoids predominantly in leaves and flowers, carotenoids present only in petals, and thiophenes mainly found in roots (MAROTTI et al., 2010).

Important nematicidal activity has been attributed *in vitro* to the thiophene α-terthienyl and its derivatives (GOMMERS; BAKKER, 1988;
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Regardless, no study has assessed the nematicidal properties of T. patula flower extracts to date. Therefore, this study aims to determine the in vitro effects of extracts and semi-purified fractions of T. patula flowers on eggs and juveniles of Meloidogyne incognita, M. javanica, and M. paranaensis. As a consequence of the high concentration of flavonoids in the tested extracts, this study also has the goal of garnering more information on the nematicidal activity of this class of compounds.

Materials and Methods

Plant material

Tagetes patula L. (Asteraceae) seeds were provided by Syngenta Flowers, Brazil, and grown in the under greenhouse conditions at the State University of Londrina, Londrina, PR, Brazil. The flowers were collected in November, after which the material was identified by Dr. Jimi Naoki Nakagima of the Federal University of Uberlândia. Vouchers are on file for this paper, deposited as a taxonomic document in the State University Herbarium of Maringá as HUM 21.907.

The flowers were dried in a forced air circulation oven (Pardal), heated to 38 ± 2 °C, and comminuted in a hammer mill (Tigre ASN5).

Solvents and reagents

All solvents and reagents used were analytical grade. n-hexane, dichloromethane, ethyl acetate, and methanol were produced by Synth. Grain alcohol (Cerealcool), DMSO (Sigma), and silica gel 60 (Merck) were also employed.

Preparation of semi-purified extracts and fractions

Granulometric separation was not used in obtaining the crude extract. After maceration for 10 min, the dried and comminuted T. patula flowers were subjected to liquidification (Skymsen, LS-04) using both water and an ethanol:water mixture (1:1 v v⁻¹) as extracting liquids, at a ratio of 2.5% (m v⁻¹) each. This process occurred for 9 min at 10 min intervals, so that the temperature did not exceed 40 °C. Then, the crude aqueous extract (CAE) and the crude ethanol-water extract (CEWE) were vacuum-filtered, evaporated under reduced pressure (Büchi, R-200), and lyophilized (Christ, Alpha 1-4).

In an attempt to increase the yield of flavonoids in the crude extract, the plant material was also treated with n-hexane by dynamic maceration for 6 d. After drying, the defatted flowers were submitted to the procedure described above to obtain the crude defatted aqueous extract (CDAE) and the crude ethanol-water defatted extract (CEWDE).

After a preliminary evaluation of the nematicidal activity of the CAE, CEWE, CDAE, and CEWDE, we chose to fractionate the CEWE.

The CEWE (50 g) was homogenized in 40 g of silica gel 60 and subjected to vacuum liquid chromatography (VLC) with approximately 220 g of silica. The eluent system employed consisted of hexane (500 mL), dichloromethane (1 L), ethyl acetate (6 L), ethanol (1.5 L), methanol (2 L), and ethanol:water (1:1 v v⁻¹) (3 L). Four semi-purified fractions were obtained, namely the ethyl acetate fraction (EAF), ethanol fraction (EF), methanol fraction (MF), and ethanol:water fraction (EWF). All fractions obtained were evaluated for nematicidal activity.
Effect of semi-purified extracts and fractions on the hatching of second-stage juveniles (J$_2$)

*Meloidogyne incognita*, *M. javanica*, and *M. paranaensis* populations were obtained from roots of tomato cultivar Santa Cruz according to the procedure established by Boneti and Ferraz (1981). The inoculum suspensions were calibrated in a Peters counting chamber. To evaluate juvenile hatching, 1.0 mL of a suspension containing 500, 300, and 350 eggs of *M. incognita*, *M. javanica*, and *M. paranaensis*, respectively, were transferred to test tubes. Next, 1.0 mL of CAE, CEWE, CDAE, and CEWDE, at a concentration of 4 mg mL$^{-1}$, were individually added to their respective test tubes. The tubes were incubated in a biological oxygen demand (BOD) incubator at 26 ± 2 °C for 16 d.

The hatched J$_2$ were counted under a stereoscopic microscope for 16 d, at 48 h intervals. Water and water + DMSO were used as controls. The EAF, EF, MF, and EWF fractions were evaluated against *M. incognita* at concentrations (µg mL$^{-1}$) of 1000 (S1), 500 (S2), 250 (S3), 125 (S4), and 65 (S5), according to the protocol described above.

Data analysis

The treatments had four replications with a completely randomized design. The results were subjected to the Scott-Knott test at 1%. To prove the recovery of J$_2$, the balance of live J$_2$ (recovered) was calculated using the formula

\[ [(100 – J_2 \text{ dead}) – (100 – J_2 \text{ inactive})] = \text{balance} \]

When the balance was zero or negative, the extract was classified as having potential as a nematicide; in the case of a positive balance, the $t$-test at 5% was performed to assess whether this average was significantly greater than zero. Such a result would indicate recovery and only nematostatic potential.

Evaluation of the inactivation and death of J$_2$

To evaluate mobility and mortality, 1.0 mL aliquots of suspensions containing approximately 700, 400, and 500 J$_2$ individuals of *M. incognita*, *M. javanica*, and *M. paranaensis*, respectively, were transferred to test tubes containing 1.0 mL of CAE, CEWE, CDAE, and CEWDE at a concentration of 4 mg mL$^{-1}$, respectively. The tubes were then placed in a BOD incubator at 26 ± 2 °C for 24 h. After the first 24 h of incubation, the number of inactive J$_2$ (nematostatic activity) was measured; after another 24 h of incubation, and subsequent rinsing in distilled water, the number of dead J$_2$ (nematicidal activity) was then quantified. Water and water + DMSO were used as a control.

EAF, EF, MF, and EWF were evaluated against *M. incognita* at concentrations (µg mL$^{-1}$) of 1000 (S1), 500 (S2), 250 (S3), 125 (S4), and 65 (S5), according to the protocol described above.

Results and Discussion

All extracts significantly reduced the hatching rate of second-stage juveniles (J$_2$) of *M. incognita* (Table 1), *M. javanica* (Table 2), and *M. paranaensis* (Table 3) when compared with water and water + DMSO.
Table 1. Hatching rates and mobility and mortality values of second-stage juveniles (J$_2$) of *Meloidogyne incognita* treated with extracts of *Tagetes patula* flowers.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Hatching (%)</th>
<th>MOBILITY AND MORTALITY</th>
<th>Day 1 (%)</th>
<th>Day 2 (%)</th>
<th>Balance$^3$</th>
<th>Effect$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>J$_2$, Immobile</td>
<td>J$_2$, Dead</td>
<td>J$_2$, Alive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. H$_2$O</td>
<td>88$^{a2}$</td>
<td>5$^{b2}$</td>
<td>11$^{b2}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dist. H$_2$O + DMSO</td>
<td>84$^{a}$</td>
<td>2$^{b}$</td>
<td>0$^{b}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAE</td>
<td>14$^{b}$</td>
<td>100$^{a}$</td>
<td>34$^{b}$</td>
<td>0.001</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>CEWE</td>
<td>11$^{b}$</td>
<td>90$^{a}$</td>
<td>70$^{a}$</td>
<td>0.320</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>CDAE</td>
<td>17$^{b}$</td>
<td>99$^{a}$</td>
<td>17$^{b}$</td>
<td>0.005</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>CEWDE</td>
<td>10$^{b}$</td>
<td>98$^{a}$</td>
<td>76$^{a}$</td>
<td>0.258</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>CV (%)</td>
<td>30</td>
<td>10</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^1$Data are means of four replicates. $^2$Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% significance. $^3$Balance: [(100 – J$_2$, dead) – (100 – J$_2$, inactive)]. $^4$Effect: zero balance or negative nematicide effect – NC; positive balance (t-test at 5% to assess if this mean was significantly greater than zero), proving recovery and the nematostatic effect – NT. CAE: crude aqueous extract; CEWE: crude ethanol-water extract; CDAE: crude defatted aqueous extract; CEWDE: crude ethanol-water defatted extract.

Table 2. Hatching rates and mobility and mortality values of second-stage juveniles (J$_2$) of *Meloidogyne javanica* treated with extracts of *Tagetes patula* flowers.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Hatching (%)</th>
<th>MOBILITY AND MORTALITY</th>
<th>Day 1 (%)</th>
<th>Day 2 (%)</th>
<th>Balance$^3$</th>
<th>Effect$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>J$_2$, Immobile</td>
<td>J$_2$, Dead</td>
<td>J$_2$, Alive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. H$_2$O</td>
<td>81$^{a2}$</td>
<td>0$^{c2}$</td>
<td>6$^{b2}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dist. H$_2$O + DMSO</td>
<td>96$^{a}$</td>
<td>4$^{c}$</td>
<td>13$^{b}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAE</td>
<td>15$^{b}$</td>
<td>72$^{b}$</td>
<td>23$^{b}$</td>
<td>0.005</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>CEWE</td>
<td>10$^{b}$</td>
<td>72$^{b}$</td>
<td>71$^{a}$</td>
<td>0.973</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>CDAE</td>
<td>14$^{b}$</td>
<td>97$^{a}$</td>
<td>18$^{b}$</td>
<td>0.001</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td>CEWDE</td>
<td>9$^{b}$</td>
<td>67$^{b}$</td>
<td>80$^{a}$</td>
<td>-</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>CV (%)</td>
<td>35</td>
<td>17</td>
<td>51</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^1$Data are means of four replicates. $^2$Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% significance. $^3$Balance: [(100 – J$_2$, dead) – (100 – J$_2$, inactive)]. $^4$Effect: zero balance or negative nematicide effect – NC; positive balance (t-test at 5% to assess if this mean was significantly greater than zero), proving recovery and the nematostatic effect – NT. CAE: crude aqueous extract; CEWE: crude ethanol-water extract; CDAE: crude defatted aqueous extract; CEWDE: crude ethanol-water defatted extract.

A similar result was described by Ferreira et al. (2013), who reported that the aqueous extract of the aerial part of *T. patula* caused 91% inhibition of *M. incognita* J$_2$ hatching. However, the authors did not evaluate other species of *Meloidogyne*.

The tests to evaluate the mobility and mortality rates of *M. incognita* (Table 1) revealed that all extracts considerably reduced J$_2$ mobility on day 1. However, the highest mortality rates were obtained with CEWE and CEWDE, which presented values of 70% and 76%, respectively. With regard to *M. javanica* (Table 2), treatment with CDAE presented a higher percentage of immobile J$_2$ (97%) on day 1, followed by CAE (72%), CEWE (72%), and CEWDE (67%). Nonetheless, the highest mortality rates, measured on day 2, were observed with CEWE (71%) and CEWDE (80%). Regarding *M. paranaensis* (Table 3), all extracts reduced J$_2$ mobility on day 1, but the only treatment that resulted in higher mortality of J$_2$ on day 2 was CEWE (84%).
Table 3. Hatching rates and mobility and mortality values of second-stage juveniles (J2) of *Meloidogyne paranaensis* treated with flower extracts of *Tagetes patula*.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Hatching (%)</th>
<th>MOBILITY AND MORTALITY</th>
<th>Effect of the extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAY 1 (%)</td>
<td>DAY 2 (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J2 Immobile</td>
<td>J2 Dead</td>
</tr>
<tr>
<td>Dist. H2O</td>
<td>76a2</td>
<td>5b2</td>
<td>24b2</td>
</tr>
<tr>
<td>Dist. H2O + DMSO</td>
<td>90a</td>
<td>4b</td>
<td>9b</td>
</tr>
<tr>
<td>CAE</td>
<td>21b</td>
<td>48a</td>
<td>7b</td>
</tr>
<tr>
<td>CEWE</td>
<td>8b</td>
<td>70a</td>
<td>84a</td>
</tr>
<tr>
<td>CDAE</td>
<td>8b</td>
<td>57a</td>
<td>8b</td>
</tr>
<tr>
<td>CEWDE</td>
<td>8b</td>
<td>58a</td>
<td>25b</td>
</tr>
<tr>
<td>CV (%)</td>
<td>36</td>
<td>26</td>
<td>43</td>
</tr>
</tbody>
</table>

1Data are means of four replicates. 2Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% significance. 3Balance: [(100 – J2 dead) – (100 – J2 inactive)]. 4Effect: zero balance or negative nematicide effect – NC; positive balance (t-test at 5% to assess if this mean was significantly greater than zero), proving recovery and the nematostatic effect – NT. CAE: crude aqueous extract; CEWE: crude ethanol-water extract; CDAE: crude defatted aqueous extract; CEWDE: crude ethanol-water defatted extract.

As to the effect of the extracts, CEWE exhibited nematicidal activity for the three species evaluated (Tables 1, 2, and 3). Meanwhile, CEWDE showed nematicidal activity against *M. incognita* (Table 1) and *M. javanica* (Table 2), as well as nematostatic activity, meaning a positive balance of live J2, for *M. paranaensis* (Table 3). In accordance with Gardiano et al. (2009), nematostatic activity is characterized by the immobilization of the nematode, followed by recovery. This may even result in a reduction in the number of galls. However, nematicidal activity leads to a decrease in the nematode population due to mortality.

The nematicidal activity of the evaluated extracts can be related to the high content of flavonoids in the plant. In a previous study, Munhoz et al. (2013) reported flavonoid levels of 5.24% in *T. patula* flowers. This was confirmed by high performance vacuum liquid chromatography, which determined that quercetin was the major compound in CEWE.

Given the CEWE results, VLC fractionation was chosen in order to obtain semi-purified fractions, which presented a more efficient dose response. The chemical fractionation of CEWE resulted in the following fractions The chemical fractionation of EBEA resulted in the following fractions with their respective yields: EAF (4.78%), EF (14.78%), MF (35.49%), and EWF (34.40%). Of these, EAF displayed promising nematicidal activity, as illustrated in Figure 1, while FE, FM and FEA fractions were not promising (data not shown).

This result is similar to the one found by Adekunle and Aderogba (2008), who isolated the flavonoid quercetin from the ethyl acetate fraction obtained from the crude methanol extract of *L. leucocephala* leaves. As such, the results suggest that quercetin contributes significantly to the nematicidal activity of CEWE and EAF. In addition, chemical fractionation and obtaining EAF increased the nematicidal response (Figure 2), suggesting that other medium polarity (methoxylated) flavonoids act as nematotoxic substances.

The effect of the *Tagetes* species on nematodes has been widely studied (Buena et al., 2008; Marotti et al., 2010; Faizi et al., 2011), first having been reported over 70 years ago (Steiner, 1941). In total, 14 genera of *Tagetes*-sensitive nematodes are known, with the most affected being *Pratylenchus* and *Meloidogyne*. The substances that are most strongly related to nematicidal activity are thiophenes (Marotti et al., 2010; Faizi et al., 2011). Adekunle and Aderogba (2008) isolated the flavonoid quercetin from *L. leucocephala* leaves and evaluated their nematicidal activity. The tested
concentration (0.8%) inhibited the hatching of *M. incognita* juveniles by 75%. These data corroborate both the work of Osman and Viglierchio (1988), in which quercetin (400 ppm) was able to inhibit the reproduction of *M. javanica* in an *in vivo* study, and of Chitwood (2002), which describes flavonoids as nematotoxic substances.

**Figure 1.** Effect of ethyl acetate fraction (EAF) on hatching, mobility, and mortality of second-stage juveniles (*J_2*) of *Meloidogyne incognita*, at different concentrations.

The flowers and leaves of *Tagetes* spp. contain terpenes (PRAKASH et al., 2012) and flavonoids (GUINOT et al., 2008; FAIZI et al., 2011). Free and/or methoxylated aglycones, such as kaempferol and quercetin (IVANCHEVA; ZDRAVKOVA, 1993), patuletin (GUINOT et al., 2008), quercetagetin (TARPO, 1969), and luteolin and quercetagetin 5-methyl ether (PICCAGLIA et al., 1998) are among the flavonoids that have been isolated and identified, indicating a predominance of this class of compounds.

In this context, water and the ethanol:water mixture (1:1 *v*:*v*) were selected as the most optimized liquids for flavonoid extraction from this plant. Several studies have reported that the extraction efficiency of phenolic compounds, including flavonoids, is enhanced by the use of a mixture of water and organic solvents such as acetone, methanol, and ethanol (GONG et al., 2012; MENESES et al., 2013). In addition, Malwade et al. (2013) propose that quercetin is most soluble, in decreasing order, in the organic solvents acetone, ethanol, methanol, and acetonitrile. Therefore, this study determines that water and ethanol:water are the best solvents for obtaining these extracts, due to the safety and efficiency of these solvents in the extraction of flavonoids.
Figure 2. Comparison of the mortality rate of second-stage juveniles (J₂) of *Meloidogyne incognita* due to the effect of the crude ethanol-water (CEWE) and ethyl-acetate (EAF) extracts at different concentrations.

Given these results, it can be concluded that the extracts CEWE and CEWDE presented nematicidal activity against *M. incognita* and *M. javanica*, as well as nematostatic activity against *M. paranaensis*. The ethyl acetate fraction from CEWE led to high mortality rates for *M. incognita* at doses above 250 µg mL⁻¹.

References


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