Nematicidal activity of essential oils and organic amendments from Asteraceae against root-knot nematodes

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The essential oil of \textit{Chrysanthemum coronarium} flowerheads showed strong nematicidal activity \textit{in vitro} and in growth-chamber experiments. Essential oil concentrations of 2, 4, 8 and 16 μL mL\textsuperscript{-1}, significantly reduced hatch, \(J_2\) survival (determined by final value and area under curves of cumulative percentage hatch or mortality) and reproduction rate of \textit{Meloidogyne artiellia} \textit{in vitro}, with the lowest values occurring at 16 μL mL\textsuperscript{-1}. In pot trials with chickpea cv. PV 61, essential oil concentrations of 10–40 μL per 500 cm\textsuperscript{3} soil, applied on sterile cotton pellets, also significantly reduced the nematode’s reproduction rate. The biological processes of mortality and hatching/reproduction were adequately described by the monomolecular and expanded negative exponential models, respectively. Effectiveness of soil amendment with either flowers, leaves, roots or seeds of \textit{C. coronarium}, and flowers from several species of Asteraceae (\textit{Chrysanthemum segetum}, \textit{Calendula maritima}, \textit{Calendula officinalis} and \textit{Calendula suffruticosa}) at 5 g per 500 cm\textsuperscript{3} soil was tested for suppression of \textit{M. artiellia} and growth of chickpea cv. PV 61 under growth-chamber conditions. In these tests, flowers of all five Asteraceae species and various parts of \textit{C. coronarium} significantly reduced reproduction rates of \textit{M. artiellia}, by 83·0–95·9%, with the minimum rates occurring in infected chickpea plants amended with flowers of \textit{C. officinalis} and \textit{C. suffruticosa}. The \textit{in vitro} and \textit{in planta} results suggest that the essential oil of \textit{C. coronarium} and organic amendments from Asteraceae species may serve as nematicides.

Keywords: \textit{Calendula maritima}, \textit{Calendula officinalis}, \textit{Calendula suffruticosa}, \textit{Chrysanthemum coronarium}, \textit{Chrysanthemum segetum}, \textit{Meloidogyne artiellia}, nematicide

Introduction

Plant-parasitic nematodes are an economically important group of soilborne pathogens that may be controlled by cultural practices, chemical nematicides and the use of resistant cultivars. However, nematicides do not provide long-term suppression of nematodes, and environmental and human health concerns are resulting in increased restrictions on their use. Some safe procedures for nematode control have been developed based on biological control agents and organic amendments; however, there is still a need for alternative, environmentally friendly measures or compounds for effective nematode control to be developed (Noling & Becker, 1994). One way of searching for such nematicidal compounds is to screen naturally occurring compounds in plants.

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Accepted 10 February 2003

Plants are an important source of naturally occurring pesticides. Many compounds with nematicidal activity have been found in plants, including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thiényls (Gommers, 1981; Chitwood, 2002). Many compounds with nematicidal activity have been isolated from species in the family Asteraceae (Gommers, 1981; Chitwood, 2002). Essential oils of some plants and/or their components have been tested for nematicidal activity \textit{in vitro} and in soil (Chatterjee \textit{et al.}, 1982; Soler-Serratosa \textit{et al.}, 1996; Oka \textit{et al.}, 2000). Recently, the antifungal and insecticidal activity of the essential oil of \textit{Chrysanthemum coronarium} flowerheads has been reported (Pérez & Pascual-Villalobos, 1999; Alvarez-Castellanos \textit{et al.}, 2001). However, there is no information about the activity of these compounds against nematodes.

The cereal root-knot nematode \textit{Meloidogyne artiellia} has been found to be the causal agent of severe yield losses of wheat and barley in Greece (Sikora, 1987), and especially of chickpea in the Mediterranean Basin (Tobar...
Jiménez, 1973; Greco, 1984; Di Vito & Greco, 1988), where 10–12% of stunted chickpeas were infected with the nematode. In addition, investigations on the host range of this root-nematode indicated that, with the exception of oat, corn and lentil, M. artiellia reproduces well on cereals, cruciferous plants and legumes (Di Vito et al., 1985). These crops are grown extensively in the Mediterranean Basin and severe damage can be expected in infested fields. The objectives of this work were: (i) to evaluate the effect of the essential oil of C. coronarium flowerheads on hatching of M. artiellia eggs and viability of second-stage juveniles; and (ii) to determine the nematicidal activity of organic amendments from several plant parts and species of the family Asteraceae against M. artiellia.

Materials and methods

Plant material and essential oils

The plants and plant parts used as organic amendments and for the extraction of essential oils are listed in Table 1. Plant material was obtained from plants grown in an experimental plot (Torreblanca Experimental Station, Campo de Cartagena, Murcia, Spain). Plants were sown in autumn (26 October 1995) and harvested in April of the following year. Voucher specimens (no. 207) were deposited in the Centro de Investigación y Desarrollo Agroalimentario Herbarium (CIDAHERB, Murcia, Spain). After harvest, plant-part samples were frozen at −40°C for 24 h and lyophilized. Lyophilized materials were crushed in a grinder to obtain particles 1 mm long.

Essential oil of C. coronarium flowerheads was obtained by the hydrodistillation method using a Clevenger-type apparatus. Before distillation, the flowerheads were lyophilized. The yield of oil was about 0-1% (v/w) and it had a blue colour and a disagreeable smell. The chemical composition of essential oils was determined by GC/MS (Alvarez-Castellanos et al., 2001). The main compounds identified were camphor (29.2%), α- and β-pinene (14-8 and 9.5%, respectively) and lycratil acetate (9-8%) (Alvarez-Castellanos et al., 2001).

Nematode inoculum

The nematode population used in the study was obtained from roots of chickpeas (cv. Ghab 1) collected in commercial fields at Monopoli, Italy. To establish and maintain cultures of M. artiellia, a single egg mass from a single gall containing a single female was removed from a root, surface-sterilized with 1% NaOCl for 4 min, and rinsed through four series of sterilized water. To increase nematode populations, a single egg mass was inoculated into a pot containing a 2–3-week-old chickpea plant (cv. UC 27) in sterilized soil and kept at 20–22°C for 3–4 months. More cultures were then raised in the same conditions to increase the nematode inoculum to the amount required for experiments. Egg inocula were prepared according to the NaOCl procedure (Hussey & Barker, 1973). Washed M. artiellia-infected chickpea roots were cut into small segments (1–2 cm long) and agitated for 3 min in 1% NaOCl. The suspension was passed through 75 and 5 μm sieves. The eggs and second-stage juveniles (J2) caught on the 5 μm sieve were washed several times with water, resuspended, and their concentration determined by dilution counts. For the hatching test, the J2s remaining in the egg suspension were removed by hand under a stereomicroscope. Nematodes [10 000 (eggs + J2s) per plant] were added in 10 mL suspension around the radicle of pregerminated seeds at sowing. Control plants were treated similarly with sterile distilled water.

In vitro experiments

**Influence of essential oils on hatch**

Hatching was monitored in chambers composed of 20 mm diameter microsieves (75 μm aperture) enclosed in Petri dishes containing sterile deionized distilled water (SDDW) covering the egg masses. Ten mature, uniformly sized egg masses, with mean viable egg contents of 225, were placed in each of six replicate hatching chambers for each essential oil concentration. Essential oil was added to the Petri dishes to obtain a final concentration. Essential oil solutions (10% ethanol, v/v) of C. coronarium were diluted with SDDW containing 0.3% Tween 20 (v/v) was added to the Petri dishes at final concentrations of 2, 4, 8 and 16 μL mL−1. The SDDW, with concentrations of ethanol and Tween 20 equivalent to those in treatments with essential oil, was used as the control. Petri dishes were maintained at 20°C (±1°C) in the dark for 4 weeks. The numbers of J2s that emerged were recorded at 2–3-day intervals for 4 weeks. After 4 weeks, egg masses from each hatching chamber were separated to estimate the number of unhatched eggs, and the number of hatched J2s.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Plant part</th>
<th>Seed origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Flowers</td>
<td>USDA-Pi586600, Korea</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Leaves</td>
<td>USDA-Pi586600, Korea</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Roots</td>
<td>USDA-Pi586600, Korea</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Seeds</td>
<td>USDA-Pi586600, Korea</td>
</tr>
<tr>
<td>Chrysanthemum segetum</td>
<td>Flowers</td>
<td>RBG, Kew-29795, UK</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>Flowers</td>
<td>University of Göttingen, Germany</td>
</tr>
<tr>
<td>Calendula maritima</td>
<td>Flowers</td>
<td>ETSIA, Madrid, Spain</td>
</tr>
<tr>
<td>Calendula suffruticosa ssp. algarbiensis</td>
<td>Flowers</td>
<td>ETSIA, Madrid, Spain</td>
</tr>
</tbody>
</table>

was expressed as a cumulative percentage of viable Jₜ. Experimental treatments were completely randomized in the incubator. The experiment was repeated once.

**Nematicidal activity of essential oils on Jₜ**

The nematicidal activity of the essential oil of *C. coronarium* was evaluated against Jₜ of *M. artiellia*. Mature egg masses of the nematode were placed in SDDW for 72 h in hatch chambers, as previously described. The Jₜ emerging were collected every 24 h, but those collected in the first 24 h were discarded. A 5 mL suspension of about 100 Jₜ was poured into six replicate 5 cm Petri dishes for each essential oil concentration. Essential oil solution (10% ethanol, v/v) was diluted with water containing 0-3% Tween 20 (v/v) and added to the Petri dishes to obtain final concentrations of 1, 2, 4, 8 and 16 μL mL⁻¹. The SDDW, with equivalent concentrations of ethanol and Tween 20 to those in the essential oil treatments, was used as a control. Petri dishes were maintained at 20°C (±1°C) in the dark for 10 days. The Jₜ were observed under a stereoscopic microscope after 1, 2, 3, 4, 6, 8 and 10 days, and percentage mortality was calculated from the number of Jₜ that did not move even after pricking the tail. The experiment was repeated once.

**In planta experiments**

**Effect of organic amendments**

This experiment was carried out under a controlled environment in a growth chamber adjusted to 22 ± 1°C, 60–90% relative humidity and a 14 h photoperiod of fluorescent light at 360±5 ± 24-7 μE m⁻² s⁻¹. These environmental conditions are favourable for development and reproduction of *M. artiellia* (Di Vito & Greco, 1988). Germinated chickpea seeds (cv. PV 61) were sown in clay pots (one per pot) containing 0-5 L of an autoclaved soil potting mixture (sand : clay loam, 2 : 1, v/v) which, for the experimental treatments, was amended with 5 g lyophilized and homogenized plant material (representing ≈1% of the soil substrate, v/v). Plant species and plant parts used as soil amendments are listed in Table 1. For the nematode inoculation, 10 mL sterile distilled water containing nematode inoculum [10 000 (eggs + Jₚ)] were added to soil at sowing. There were 10 treatment combinations (Table 2) arranged in a completely randomized design and replicated six times, each replicate consisting of a single potted plant. Plants were watered daily with 100 mL tap water and fertilized weekly with 100 mL 0-1%, 20-5-32 + micronutrients hydro-sol fertilizer (Haifa Chemicals Ltd, Haifa, Israel).

The experiment was terminated 62 days after inoculation, when plants were at the full-bloom to early podding stage. The shoot of each plant was cut off at soil level and the roots washed free of soil. The following variables were assessed: dry shoot and fresh root weight; number of nematodes in roots and soil; and nematode reproduction rate (Rᵢ = final population/initial population). Nematode population density was determined by extracting eggs and Jₜ from soil samples (Coolen, 1979) and from the entire root system after exposure to 1% NaCl for 5 min (Hussey & Barker, 1973). Severity of nematode galling of the root system was not assessed because no visible symptoms are caused by *M. artiellia* infection (Greco, 1984). The experiment was repeated once.

**Effect of essential oils**

This experiment was carried out under a controlled environment in a growth chamber adjusted to the conditions described above. Essential oil of *C. coronarium* flowerheads was added in four concentrations (10, 20, 30 and 40 μL per 500 cm³ soil) by applying measured quantities of stock solution to four sterile cotton pellets distributed

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**Table 2** Influence of organic amendments from several Asteraceae on the growth of chickpea cv. PV 61 and reproduction of *Meloidogyne artiellia*.

<table>
<thead>
<tr>
<th>Treatment or plant species</th>
<th>Plant part</th>
<th>Nematode inoculum (eggs + Jₚ per cm³ soil)</th>
<th>Plant growth (g)</th>
<th>Rᵢ (R/P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated control</td>
<td>–</td>
<td>0</td>
<td>1.18 a</td>
<td>12.76 a</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>–</td>
<td>20</td>
<td>1.18 a</td>
<td>13.45 a</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Flowers</td>
<td>20</td>
<td>1.39 a</td>
<td>13.40 a</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Leaves</td>
<td>20</td>
<td>1.43 a</td>
<td>15.06 a</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Roots</td>
<td>20</td>
<td>1.35 a</td>
<td>12.31 a</td>
</tr>
<tr>
<td>Chrysanthemum coronarium</td>
<td>Seeds</td>
<td>20</td>
<td>1.53 a</td>
<td>11.93 a</td>
</tr>
<tr>
<td>Chrysanthemum segetum</td>
<td>Flowers</td>
<td>20</td>
<td>1.25 a</td>
<td>12.80 a</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>Flowers</td>
<td>20</td>
<td>1.30 a</td>
<td>10.88 a</td>
</tr>
<tr>
<td>Calendula maritima</td>
<td>Flowers</td>
<td>20</td>
<td>1.04 a</td>
<td>10.93 a</td>
</tr>
<tr>
<td>Calendula suffruticosa ssp. algarbiensis</td>
<td>Flowers</td>
<td>20</td>
<td>1.16 a</td>
<td>8.15 a</td>
</tr>
</tbody>
</table>

*Data are the averages of two experiments with five replicate plants per treatment combination in each experiment. Means followed by the same letter do not differ significantly (P > 0.05) according to Fisher’s protected LSD test.

²Rᵢ (nematode reproduction rate) = final nematode density per plant/initial nematode population density per plant. Data were transformed to log (X + 1) for analysis.
regularly around the chickpea radicle at sowing. Control plants were treated similarly with sterile distilled water. Nematode inoculum and inoculation method were as previously described. There were six replicate plants for each essential oil concentration in a completely randomized design, each replicate consisting of a single potted plant. The experiment was repeated once.

**Statistical analysis**

Data were subjected to ANOVA using STATISTIX (NH Analytical Software, Roseville, MN, USA). Similarity between experimental runs, tested by preliminary ANOVAs using experimental runs as blocks, allowed the data to be combined for ANOVAs. In the hatching and mortality of J2 test, the area under cumulative percentage hatch (AUCPH) and the area under cumulative percentage mortality (AUCPM) curves values were estimated by the trapezoidal integration method for each essential oil concentration (Campbell & Madden, 1990). Treatment means of AUCPH, AUCPM and final cumulative egg hatch and mortality of J2 at each essential oil concentration, as well as treatment means of in planta experiments, were compared using Fisher’s protected least significant difference test (LSD) at \( P = 0.05 \). In the in planta experiments, all data on nematode population density \((X)\) were transformed into \( \log_{10} (X + 1) \) before analysis (Gomez & Gomez, 1984).

To describe the effects of essential oil concentrations (OC) on cumulative percentage hatch, AUCPH and reproduction rate, the expanded negative exponential model:

\[
C \exp[-r \log(OC)] + K
\]

was fitted to the data, where \( C \) is a constant, \( r \) is the rate of decrease over OC, and \( K \) is the asymptote. Similarly, to describe the effects of OC on cumulative percentage J2 mortality and AUCPM, the monomolecular model:

\[
K \{1 - B \exp[-r \log(OC)]\}
\]

was fitted to the data, where \( K \) is the asymptote, \( B \) is a constant, and \( r \) is the rate of increase over OC (Campbell & Madden, 1990). Regression analyses were conducted by the least-squares program for nonlinear models of the Statistical Analysis System 6-08 (SAS Institute Inc., Cary, NC, USA). The coefficient of determination (\( R^2 \)), the mean square error, the asymptotic standard error associated with the estimated parameter, and the pattern of the standardized residuals plotted against either predicted values or the independent variable were used to evaluate the appropriateness of a model to describe the data (Campbell & Madden, 1990).

**Results**

**In vitro experiments**

**Influence of essential oils on hatch**

Percentage hatch of \( M. \text{artiiellia} \) eggs was significantly influenced \((P < 0.05)\) by the concentration of essential oil from \( C. \text{coronarium} \) flowerheads throughout the experiment (Fig. 1a). Final percentage hatch, as well as AUCPH, was significantly reduced \((P < 0.05)\) in egg masses incubated at any of the essential oil concentrations tested (Fig. 1a). AUCPH of curves from each treatment combination or final hatch followed by the same upper-case or lower-case letters, respectively, do not differ \((P > 0.05)\) according to Fisher’s protected LSD test.

**Nematicidal activity of essential oils on J2s**

The viability of \( J_2 \)s of \( M. \text{artiiellia} \) was significantly influenced \((P < 0.05)\) by the concentration of \( C. \text{coronarium} \) essential oil throughout the experiment (Fig. 1b). Final \( J_2 \) mortality, as well as AUCPM, was significantly lower \((P < 0.05)\) in \( J_2 \)s incubated in SDDW than in those incubated at any essential oil concentration tested (Fig. 1a). Both cumulative percentage hatch and AUCPH decreased exponentially with increasing in essential oil concentration (Fig. 2).
mortality and AUCPM were significantly higher \((P < 0.05)\) in \(J_2\)s incubated in essential oil at 16 \(\mu\)L mL\(^{-1}\), the highest concentration tested, than at any other concentration (Fig. 1b). Cumulative percentage \(J_2\) mortality and AUCPM showed a monomolecular type of increase with increasing essential oil concentration (Fig. 3).

**In planta experiments**

**Effect of organic amendments**

Growth of chickpea cv. PV 61 plants, determined by dry shoot and fresh root weights, was not affected by infection with *M. artiellia* in the absence of organic amendments (Table 2). Similarly, growth of chickpea plants infected by *M. artiellia* grown in soil with organic amendments from several Asteraceae was not significantly affected \((P = 0.05)\) by nematode infection, irrespective of plant part or species evaluated (Table 2). The incidence of *M. artiellia* infection of chickpea plants was not affected by any of the organic amendments, but the reproduction rate of *M. artiellia* was significantly reduced \((P < 0.05)\) by all the organic amendments, irrespective of plant species or part, when compared with the nonamended treatment (Table 2). The reproduction rate of *M. artiellia* was significantly less \((P < 0.05)\) in chickpea plants amended with *Calendula officinalis* and *Calendula suffruticosa* than in plants amended with leaves of *Chrysanthemum coronarium*. Minor differences were observed among the other treatments, which gave intermediate reproduction rates (Table 2).

**Effect of essential oils**

Essential oil concentrations of *C. coronarium* did not influence \((P > 0.05)\) the growth of chickpea cv. PV 61, determined by dry shoot and fresh root weights (Table 3). However, the reproduction rate of *M. artiellia* on chickpea cv. PV 61 was significantly \((P < 0.05)\) reduced by application of all concentrations of essential oil compared with untreated plants. The percentage reduction ranged from 38.0 to 54.1\% of the reproduction rate reached in control plants. However, reproduction rate was not significantly \((P > 0.05)\) different between the concentrations of essential oil applied in this experiment.
Discussion

The suppressive effects of some phytochemical compounds on nematode populations has been well documented in several pathosystems (Chitwood, 2002). These compounds could be developed for use as nematicides themselves, or could serve as model compounds for the development of environmentally friendly synthetic derivatives. This study was designed to evaluate the nematicidal activity of the essential oil of *C. coronarium* flowerheads or organic amendments from several species of the family Asteraceae on the root-knot nematode *M. artiellia*. *In vitro* and *in planta* experiments clearly demonstrated that egg hatch, $J_2$ survival and reproduction rate of the nematode were significantly reduced by the essential oil of *C. coronarium* flowerheads. Similarly, treatment with each of several plant species and plant parts (flowers, leaves, roots and seeds of *C. coronarium*, and flowers of *Chrysanthemum segetum, Calendula officinalis, Calendula maritima* and *Calendula suffruticosa*) consistently reduced the reproduction rate of *M. artiellia* on chickpea compared to the nonamended treatment. The data for the nematicidal activity of Asteraceae agree with results of other researchers, who found that the population density of *Meloidogyne* spp. was reduced when host plants were grown in soil amended with *Chrysanthemum* spp. (Hackney & Dickerson, 1975; Tiyagi *et al.*, 1988; Bélair & Benoit, 1996) or *Calendula* spp. (Tiyagi *et al.*, 1988). However, the results of the present study regarding the effects of nematode parasitism on plant growth under artificial conditions are not in agreement with the results of other researchers under field conditions (Di Vito & Greco, 1988), who found a significant reduction in plant growth and seed yield. Differences in the susceptibility of the chickpea cultivars or differences in environmental conditions could be responsible for this.

Essential oils from several plant species have been shown to have nematicidal activity on root-knot nematodes *in vitro* and in soil (Chatterjee *et al.*, 1982; Sangwan *et al.*, 1985; Soler-Serratos* et al.*, 1996). Results from the present *in vitro* and *in planta* experiments indicated that essential oil compounds from *C. coronarium* flowerheads directly affect nematode biology by interfering with nematode hatching and $J_2$ viability, as reported for other phytochemical compounds (Chandrawdana *et al.*, 1994; Chitwood, 2002). The influence of the essential oil on egg hatch and $J_2$ survival of *M. artiellia* is probably related to its nematicidal activity against other *Meloidogyne* spp. Thus the data suggest that the essential oil from *C. coronarium* has potential to be used in nematode control. Additionally, as essential oils from *C. coronarium* have also been reported to have insecticidal and fungicidal activities (Pérez *et al.*, 1999; Alvarez-Castellanos *et al.*, 2001), treatment of soil with essential oils or their components could serve as a means of soil disinfection. However, the practical use of essential oils or organic amendments to control *M. artiellia* on chickpea has constraints: the direct costs associated with the cultivation of such plant species or their incorporation into soil. Further investigations are needed to confirm the nematicidal activity of these compounds on other root-knot nematodes affecting high-value crops (those produced or marketed at relatively greater risk or cost of production, such as nursery crops, fruits and vegetables) before they can be incorporated into an integrated pest management system.

In conclusion, these results suggest that species of Asteraceae may be used in crop rotations as antagonistic plants or green manures to reduce *Meloidogyne* populations. Dry preparations of these plants or extracts of the active principles could be used as nematicides.

Acknowledgements

This research, part of a PhD study by M.P.P., was supported by grant SC98-022 and a fellowship from the Instituto Nacional de Tecnologías Agroalimentarias (INIA). The authors thank Drs N. Greco and M. Di Vito (Bari, Italy) for kindly providing the populations of *Meloidogyne artiellia*, and J. Martín Barbarroja IAS-CSIC for technical assistance.

References


\[ f(nematode reproduction rate) = \text{final nematode density per plant} / \text{initial nematode population density} \]

Table 3 Influence of essential oil from *Chrysanthemum coronarium* on the growth of chickpea cv. PV 61 and reproduction of *Meloidogyne artiellia*

<table>
<thead>
<tr>
<th>Essential oil concentration (µL per 500 cm³ soil)</th>
<th>Nematode inoculum (eggs + J₂ cm⁻³ soil)</th>
<th>Plant growth</th>
<th>R²f</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1 17 a</td>
<td>12 76 a</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>1 21 a</td>
<td>14 20 a</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>1 05 a</td>
<td>14 61 a</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>1 13 a</td>
<td>16 09 a</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>1 24 a</td>
<td>15 61 a</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>1 13 a</td>
<td>12 16 a</td>
</tr>
</tbody>
</table>

*Data are the averages of two experiments with five replicated plants per treatment combination in each experiment. Means followed by the same letter do not differ significantly (P > 0.05) according to Fisher’s protected LSD test.

$R²$ (nematode reproduction rate) = final nematode density per plant/initial nematode population density per plant. Data were transformed to log $(X + 1)$ for analysis.


