BRILL

Nematology 0 (2019) 1-9

Nematology

brill.com/nemy

Activities of essential oils from three Brazilian plants and benzaldehyde analogues against *Meloidogyne incognita*

Aline Ferreira BARROS [1,*], Vicente Paulo CAMPOS [1], Denilson Ferreira DE OLIVEIRA [2], Fabiola de Jesus SILVA [1], Iselino Nogueira JARDIM [2], Viviane Aparecida COSTA [3], Carlos Augusto Rodrigues MATRANGOLO [3], Regina Cássia Ferreira RIBEIRO [3] and Geraldo Humberto SILVA [4]

[1] *Departamento de Fitopatologia, Universidade Federal de Lavras, CP3037, 37200-000 Lavras, Minas Gerais, Brazil*
[2] *Departamento de Química, Universidade Federal de Lavras, CP3037, 37200-000 Lavras, Minas Gerais, Brazil*
[3] *Departamento de Ciências Agrárias, Universidade Estadual de Montes Claros, CP 91, 39440-000, Janaúba, Minas Gerais, Brazil*
[4] *Instituto de Ciências Exatas, Universidade Federal de Viçosa, CP 22, 38810-000, Rio Paranaíba, Minas Gerais, Brazil*

Received: 19 March 2019; revised: 27 May 2019
Accepted for publication: 27 May 2019

Summary – There is a demand for novel products for the control of plant-parasitic nematodes, so we characterised the effectiveness of some plant essential oils against *Meloidogyne incognita* and verified the efficiency of the major component from the most toxic oils and their analogues using *in vitro* and *in vivo* assays. In this study, the essential oils from *Piptadenia viridiflora*, *Hyptis suaveolens* and *Astronium graveolens* against *M. incognita* were evaluated, but only *P. viridiflora* oil showed toxicity toward *M. incognita*. Benzaldehyde was its main component according to GC-MS analysis. In *in vitro* assays, benzaldehyde (100 and 200 μg ml^{-1}) and its oxime (400 μg ml^{-1}) caused a higher rate of *M. incognita* second-stage juvenile (J2) mortality than the nematicide carbofuran (170 μg ml^{-1}). Reductions of more than 90% in the number of galls and eggs, even greater than that observed with carbofuran, were observed in the assay where the J2 were placed in solutions of benzaldehyde and its oxime 48 h prior to tomato plant inoculation. Application of benzaldehyde together with *M. incognita* J2 to the substrate resulted only in a reduction in the number of eggs (42-65%); however, its oxime reduced both the number of galls (43-84%) and eggs (23-89%). Therefore, the *P. viridiflora* oil, its major component benzaldehyde, and the analogue benzaldehyde oxime are toxic to *M. incognita*. In two different *in vivo* assays, benzaldehyde oxime was confirmed as toxic to *M. incognita* with a greater efficacy than benzaldehyde.

Keywords – *Astronium graveolens*, benzaldehyde oxime, *Hyptis suaveolens*, nematicide, nematode control, pesticide, *Piptadenia viridiflora*, root-knot nematodes.

Plant-parasitic nematodes are responsible for approximately 10% reduction in agricultural production worldwide (McCarter, 2008). In the genus *Meloidogyne*, *M. incognita* (Kofoid & White) Chitwood, which has a wide host range, is possibly the world's most damaging crop pathogen (Trudgill & Blok, 2001). Consequently, its control is of great importance for agribusiness, and the use of commercial nematicides has been indispensable. However, despite the benefits for agricultural production, use of chemical nematicides is becoming limited due to potential environmental problems and human and animal health concerns (Abdel-Rahman *et al.*, 2013). These problems have justified the withdrawal of several commercial nematicides from the agrochemical market (Spurgeon, 1997; Council of the European Union, 2003) and, thus, there is a demand for novel products for the control of plant-parasitic nematodes.

To address the market demand, many studies have been carried out with extracts and essential oils from plants, microorganism cultures, and fungal and bacterial filtrates, demonstrating the potential of several naturally occurring substances against phytopathogenic agents (Chitwood, 2002; Campos *et al.*, 2010; D'Addabbo *et al.*, 2014). However, there is still a need to focus effort on research studying the toxicity of various natural products toward phytonematodes. We have become interested

* Corresponding author, e-mail: afbarros2004@yahoo.com.br

DOI 10.1163/15685411-00003276

in three plant species with known antimicrobial activity, namely: *Astronium graveolens* Jacq. (Anacardiaceae), *Hyptis suaveolens* (L.) Poit. (Lamiaceae), and *Piptadenia viridiflora* (Kunth) Benth. (Fabaceae-Mimosoideae) (Asekun *et al.*, 1999; Trentin *et al.*, 2011; Hernández *et al.*, 2013). *Hyptis suaveolens* extract has nematicidal activity against *M. incognita*, *Heterodera sacchari*, *Helicotylenchus* spp., *Scutellonema bradys* and egg masses of *Meloidogyne* spp., whilst the extract of *Piptadenia viridiflora* has nematicidal activity against *Haemonchus contortus* (a nematode parasite of ruminants) (Olabiyi, 2008; Agbenin, 2014; Fabiyi *et al.*, 2015; Izuogu *et al.*, 2016; Morais-Costa *et al.*, 2016). However, the activities of essential oils of these three plant species against nematodes have not yet been studied.

According to the literature, benzaldehyde has been detected in different natural products (Kudalkar *et al.*, 2012; Barros *et al.*, 2014; Ullah *et al.*, 2015; Jardim *et al.*, 2017) and its *in vitro* nematicidal activity has been proven in studies with *M. incognita* (Ntalli *et al.*, 2011; Jardim *et al.*, 2017); however, there remains a need to investigate the activity of this substance against *M. incognita* in *in vivo* studies. Previous studies have involved the application of benzaldehyde together with an organic amendment for the control of *M. incognita* in soybean and tomato (Chavarría-Carvajal *et al.*, 2001; Kokalis-Burelle *et al.*, 2002). Other studies have demonstrated that preplant incorporation of benzaldehyde into glasshouse soil reduces *M. incognita* population in cotton (Bauske *et al.*, 1994).

Although benzaldehyde has high efficacy against phytopathogens, instability in soil causes a reduction in efficiency of field application. To overcome this problem, it is necessary to search for similar benzaldehyde molecules (analogues) with greater stability under environmental conditions. The effects of benzaldehyde oxime, the product of the reaction of benzaldehyde with hydroxylamine, and benzoic acid, an oxidation product of benzaldehyde (Rappoport & Liebman, 2008; O'Neil, 2013), on nematodes remain to be studied in detail.

In the present study, the *in vitro* activities of essential oils of *A. graveolens*, *H. suaveolens* and *P. viridiflora* against *M. incognita* were verified or established. The most active oil against *M. incognita* was subjected to analysis using gas chromatography coupled to mass spectrometry (GC-MS) to identify its major component. Subsequently, an *in vitro* assay was performed with the major component against *M. incognita* in comparison with its analogues. Finally, an *in vivo* assay in tomato was performed to compare the effectiveness of the major component and the most efficacious analogue.

Materials and methods

Plant collection and extraction of essential oils

The leaves of *P. viridiflora*, *H. suaveolens* and *A. graveolens* were collected during the morning in Minas Gerais state, Brazil (March and April). Part of each sample was taken to the Institute of Biological Sciences at the UFMG (Herbário BHCB) for botanical identification at the species level, and voucher specimens were deposited under the codes 191976, 191977 and 191968, respectively.

A leaf sample (approximately 150 g fresh weight) of each plant species was ground in a blender with distilled water, and the resulting suspension was transferred to a round-bottomed flask (1000 ml) fitted to a Clevenger distillation apparatus. The steam distillation process lasted approximately 4 h. The essential oils were separated from the water by decantation and subsequently treated with anhydrous sodium sulphate. The oils were stored at −10°C until further use.

Yields of the extracted essential oils were calculated as a percentage, using the following formula: Yield (%) = (MO/MFP) × 100, where: MO = mass of essential oil obtained after extraction, without water (g) and MFP = fresh mass of the plant used for the extraction (g).

Collection of *M. incognita* eggs and second-stage juveniles

Pure *M. incognita* populations were multiplied in tomato plants (*Solanum lycopersicum* 'Santa Clara') and maintained in a glasshouse for approximately 3 months. Galled roots were separated from the soil, washed in water, cut into 0.5 cm segments, and eggs extracted (Hussey & Barker, 1973). Some of the extracted eggs were used for a hatching assay; the remainder were placed in a hatching chamber and incubated at 28°C for 2 days using the Baermann funnel technique (Baermann, 1917), and hatched second-stage juveniles (J2) were used in further assays.

In vitro assay with essential oils against *M. incognita* second-stage juveniles

Initially, stock emulsions of the essential oils were prepared in an aqueous solution of Tween-80® (0.01 g

ml^{-1}) at a concentration of 1200 μg ml^{-1}. The essential oil emulsions (each 100 μl) and an aqueous suspension (20 μl) containing approximately 20 J2 of *M. incognita* were placed in microtubes (1.5 ml). The final concentration of each essential oil was 1000 μg ml^{-1}. The negative control was the solution used for dissolving the compound, 0.01 g ml^{-1} Tween-80®, and carbofuran (2,3-dihydro-2,2-dimethyl-1-benzofuran-7-yl N-methylcarbamate, 98%; Aldrich) at a final concentration of 180 μg ml^{-1} was used as a positive control. The samples were incubated at 25°C for 48 h, after which they were transferred to polypropylene microplates, and mobile and immobile nematodes were counted under a microscope. Subsequently, one drop of a freshly prepared 1.0 mol l^{-1} NaOH solution was added to each well and the nematodes were counted again. J2 that changed their body shape within 3 min were considered alive, whereas those remaining still were considered dead (Chen & Dickson, 2000; Oliveira *et al.*, 2003). The data were transformed into percentages prior to being subjected to statistical analysis.

Composition of the essential oil of *P. viridiflora*

Analysis of the *P. viridiflora* essential oil was performed using a gas chromatograph coupled to a mass spectrometer (model QP2010, Shimadzu). An RTX®-5MS-Restek capillary column of 30 m (length) × 0.25 mm (internal diam.) × 0.25 μm (phase thickness) was employed, using helium at 1.0 ml min^{-1} as the carrier gas. The following conditions were adopted: *i*) split/splitless injector temperature, 220°C; *ii*) split ratio, 1:20; *iii*) initial temperature of the column, 60°C; *iv*) elevation rate of the column temperature, 2°C min^{-1} up to 200°C and then 5°C min^{-1}; *v*) final temperature of the column, 250°C; *vi*) temperature of the interface between the gas chromatograph and the mass spectrometer, 220°C; *vii*) ionisation of each molecule in the spectrometer, electron impact at 70 eV; *viii*) range of mass/charge (*m*/*z*) analyses in the mass spectrometer, 45-400; and *ix*) mass spectrum acquisition time, 0.5 s (Adams *et al.*, 2007).

The essential oil was dissolved in acetone to a concentration of 10 mg ml^{-1}, and 1 μl was injected into the gas chromatograph. A solution of homologous linear alkanes, containing C9-C20 carbon atoms, was used as an external standard. All mass spectra were compared with those in the NIST 05 Mass Spectral Library, 2005, and all peaks in the chromatogram with a similarity index <85% were considered unidentified. For each of the remaining peaks, the arithmetic index (AI) was calculated according to the following formula: $AI = (100P_z + 100((RT - RTP_z)/(RTP_{z+1} - RTP_z)))$, where P_z is the number of carbon atoms in the linear alkane with a retention time immediately below that of the substance to be identified in the chromatogram; RT is the retention time (min) of the substance to be identified in the chromatogram; RTP_z is the retention time (min) of the linear alkane with a number of carbon atoms equal to P_z; and RTP_{z+1} is the retention time (min) of the linear alkane with a number of carbon atoms equal to $P_z + 1$. Substances with calculated AI values corresponding to an error $\geqslant$3% in relation to the AI described by Adams (2007) were considered unidentified.

In vitro activity of benzaldehyde and its analogues against *M. incognita* J2

Benzaldehyde (99%), benzaldehyde oxime (97%) and benzoic acid (99.5%) (Fig. 1) were supplied by Sigma-Aldrich. Sodium benzoate was prepared from benzoic acid. Initially, stock solutions of the substances in aqueous Tween-80® (0.01 g ml^{-1}) were prepared at a concentration of 2000 μg ml^{-1}. Sodium benzoate was obtained *in situ* from the neutralization of benzoic acid with sodium hydroxide, both diluted in Tween-80® (0.01 g ml^{-1}) at a stock concentration of 2000 μg ml^{-1}. The substances diluted in Tween-80® (500 μl) were placed in microtubes (1.5 ml) together with the aqueous suspension (500 μl) containing 200 J2 of *M. incognita*. In the case of benzaldehyde, the final concentrations of the solutions were 50, 100 and 200 μg ml^{-1}, whereas for benzaldehyde oxime and benzoic acid the final concentrations were 100, 200 and 400 μg ml^{-1}. Sodium benzoate was used at a single concentration of 400 μg ml^{-1}. Negative controls were an aqueous solution of Tween-80® (0.01 g ml^{-1}) and distilled water. The commercial nematicide, carbofuran (final concentration, 170 μg ml^{-1}), was used as a positive control. The samples were incubated at 25°C for 48 h, after which time they were homogenised, and 100 μl was transferred to the wells of a 96-well polypropylene plate. Mobile, im-

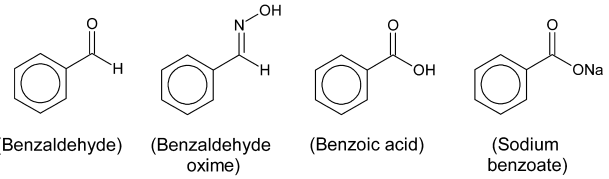

Fig. 1. Chemical structure of benzaldehyde and analogues that were studied in the present work.

mobile and dead nematodes were subsequently counted under a microscope.

Hatching of J2 of *M. incognita* in the presence of benzaldehyde and its oxime

An aqueous suspension (500 μl) containing 1000 eggs of *M. incognita* was placed in a microtube (1.5 ml) together with the benzaldehyde solution (500 μl) (final concentrations, 600 and 900 μg ml^{-1}) or its oxime (final concentrations, 600 and 900 μg ml^{-1}). As negative and positive controls, solutions of Tween-80® (0.01 g ml^{-1}) and carbofuran at a final concentration of 415 μg ml^{-1} were used, respectively. Following incubation at 25°C for 7 days, the samples were transferred to a counting box and the hatched J2 were evaluated.

Infectivity and reproduction of *M. incognita* in tomato plants following J2 exposure to benzaldehyde and its oxime

An aqueous suspension (500 μl) containing 700 *M. incognita* J2 was placed in microtube (1.5 ml) together with the benzaldehyde solution (500 μl) (final concentrations, 100 μg ml^{-1}) or its oxime (final concentration, 200 and 400 μg ml^{-1}) in Tween-80® (0.01 g ml^{-1}). Negative controls were an aqueous solution of Tween-80® (0.01 g ml^{-1}) and distilled water. Carbofuran (final concentration, 170 μg ml^{-1}), was used as a positive control. The samples were incubated at 25°C for 48 h, after which they were homogenised, and approximately 20 J2 were transferred to the wells of a 96-well polypropylene plate. Mobile, immobile and dead nematodes were subsequently counted under a microscope. The remaining suspension, containing approximately 680 J2, was dispersed in 4 ml distilled water, and distributed into four holes around the stem of a tomato seedling 30 days after sowing, which was kept in a plastic cup (300 ml) containing Tropstrato® substrate (pine bark, vermiculite, PG Mix 14-16-18, potassium nitrate, simple superphosphate and turf; Vida Verde). Following inoculation, the seedlings were kept in a glasshouse for 35 days, after which each root system was removed from the cup and washed thoroughly with water. Roots were subsequently air-dried with paper and weighed, the galls were counted and the eggs were extracted (Hussey & Barker, 1973). The eggs were counted in a Peters chamber under an optical microscope. The number of galls (infectivity) and eggs (reproduction) were calculated per g root.

Application of benzaldehyde and its oxime to substrate infested with *M. incognita* containing tomato seedlings

In each plastic cup (300 ml) containing Tropstrato® substrate a 25-day-old tomato plant 'Santa Clara' was transplanted. Benzaldehyde was dissolved in an aqueous solution of Tween-80® (0.01 g ml^{-1}) to concentrations of 600, 1000, 1400 and 1800 μg ml^{-1}, while benzaldehyde oxime solutions were prepared at concentrations of 1000, 1600, 2200 and 2800 μg ml^{-1}. Negative controls were solution of Tween-80® (0.01 g ml^{-1}) and distilled water. Carbofuran (final concentration, 830 μg ml^{-1}), was used as a positive control. At the time of inoculation, each solution (4.0 ml) was combined with an aqueous suspension (4.0 ml) containing 700 J2, which reduced the concentrations by half: benzaldehyde (final concentrations, 300, 500, 700 and 900 μg ml^{-1}), benzaldehyde oxime (final concentrations, 500, 800, 1100 and 1400 μg ml^{-1}) and carbofuran (final concentration: 415 μg ml^{-1}). The suspensions were placed around the stem in four holes approximately 2 cm deep in the substrate. Following inoculation, the plants were kept in a glasshouse for 45 days. Irrigation and fertilisation of the inoculated seedlings were carried out when necessary. Each root system was removed from the substrate and washed with water. The galls were counted and the number of eggs per g root was estimated according to methodology described above.

Statistics and data analysis

All the assays were performed twice using a completely randomised experimental design with five replicates per treatment. In the case of the *in vitro* assay with *M. incognita* J2, the values were converted to percentages prior to statistical analysis. The results were previously submitted to normality tests (Shapiro-Wilk) and homogeneity of error variance (Bartlett). Only the data for galls from the 'Infectivity and reproduction of *M. incognita* J2 in a tomato plant following J2 exposure to benzaldehyde and benzaldehyde oxime' experiment were transformed to $\sqrt{x}$. The results were analysed by analysis of variance. When the F-test was significant, the means were compared using the Scott-Knott test ($P < 0.05$) (Scott & Knott, 1974). The SigmaPlot® version 12.0 and Sisvar® version 5.6 software were used to prepare the graphs and to perform statistical analysis, respectively.

Table 1. *In vitro* mortality of *Meloidogyne incognita* second-stage juveniles (J2) when in contact with plant essential oils and controls.

Controls and plant species	Concentration (μg ml^{-1})[a]	J2 dead (%)[b]
Carbofuran (control)	180	58.3 b
Tween-80, 0.001 g ml^{-1} (control)	–	7.6 a
Astronium graveolens	1000	1.1 a
Hyptis suaveolens	1000	11.5 a
Piptadenia viridiflora	1000	85.9 b

[a] Final concentration, after combining the sample solution to be tested with the J2 suspension.
[b] Means followed by the same letter do not differ according to the Scott-Knott (1974) test ($P < 0.05$).

Results

Oil yields

The yields of essential oils from leaves of *P. viridiflora*, *H. suaveolens* and *A. graveolens* were 0.054, 0.039 and 0.043%, respectively.

In vitro activity of essential oils against *M. incognita* J2

There was a significant difference among the effects of the three essential oils on *M. incognita* J2 mortality ($P < 0.05$). Only *P. viridiflora* oil showed a high nematicidal activity (85.9%), similar to the positive control, carbofuran. The other oils failed to differ from the negative control (Table 1).

Composition of *P. viridiflora* essential oil

GC-MS analysis showed that benzaldehyde was the main component of the essential oil of *P. viridiflora*, corresponding to approximately 98% of the total essential oil produced. The similarity of the mass spectrum obtained for this substance relative to that in the spectral bank was 99%. With respect to the AI, the value obtained was 961, while in the literature (Adams, 2007) the value quoted is 952, which corresponds to a difference of less than 1.0%.

In vitro activity of benzaldehyde and its analogues against *M. incognita* J2

Benzaldehyde (final concentrations, 100 and 200 μg ml^{-1}), benzaldehyde oxime (final concentrations, 200 and 400 μg ml^{-1}), benzoic acid (final concentration, 400 μg ml^{-1}), and carbofuran (final concentration, 170 μg ml^{-1}) caused greater ($P < 0.0001$) *M. incognita* J2 immobility and mortality than the negative controls (distilled water and Tween-80®). Although at a concentration of 50 μg ml^{-1} the mortality caused by benzaldehyde failed to differ from the negative controls, the immobility at this concentration was higher than that observed with the negative controls. For benzaldehyde (final concentrations, 100 and 200 μg ml^{-1}) and benzaldehyde oxime (final concentration, 400 μg ml^{-1}), J2 mortality was higher (75.3-96.5%) than that observed with carbofuran (64.2%). Considering only immobility, the effect of benzoic acid (final concentration, 400 μg ml^{-1}) and benzaldehyde oxime (final concentration, 200 μg ml^{-1}) failed to differ from carbofuran (170 μg ml^{-1}). Sodium benzoate had no effect on the immobility or mortality of J2 (Fig. 2).

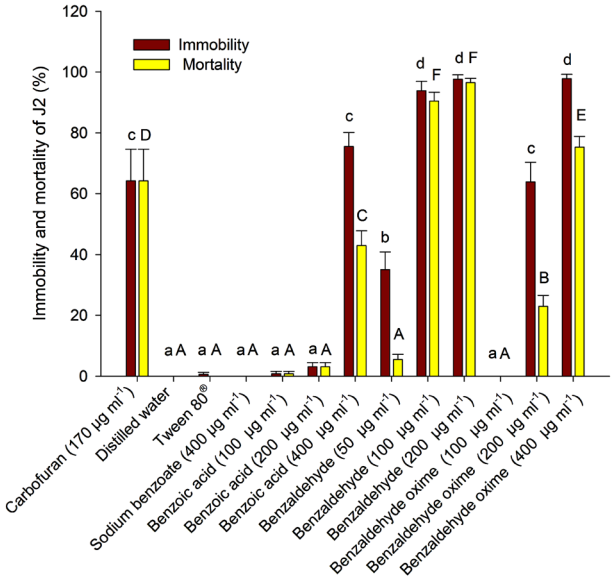

Fig. 2. Immobility and *in vitro* mortality of second-stage juveniles (J2) of *Meloidogyne incognita* in solutions of benzaldehyde and its analogues. Negative controls: distilled water and Tween-80®. Positive control: carbofuran at 170 μg ml^{-1}. Bars indicate the standard error of the mean. Mean values with the same lower-case (immobility) and upper-case (mortality) letter do not differ significantly according to the Scott-Knott test (Scott & Knott, 1974) ($P < 0.05$).

Hatching of *M. incognita* J2 in the presence of benzaldehyde and its oxime

There was no significant difference between the hatching of *M. incognita* J2 at different concentrations of benzaldehyde and its analogues ($P = 0.0534$); the number

of J2 hatched in these treatments ranged from 78 to 91, while the negative control was 88. Carbofuran (415 μg ml^{-1}) also had no effect on J2 hatching, with only 105 J2 hatched (data not shown).

Infectivity and reproduction of *M. incognita* in tomato plants following J2 exposure to benzaldehyde and its oxime

The high immobility and/or mortality of *M. incognita* J2 in the presence of benzaldehyde (100 μg ml^{-1}) and benzaldehyde oxime (200 and 400 μg ml^{-1}) as compared with the negative controls was confirmed. Following incubation of J2 with solutions of benzaldehyde and its oxime for 48 h prior to inoculation of tomato plants, significant reductions were observed in the number of galls ($P < 0.0001$) and eggs per g root ($P < 0.0001$) as compared with the negative (distilled water and Tween-80®) and positive (carbofuran) controls. The reductions ranged from 91 to 100% for the number of galls per g root, and from 97 to 100% for the number of eggs per g root; this was greater than that seen with the positive control carbofuran (Fig. 3).

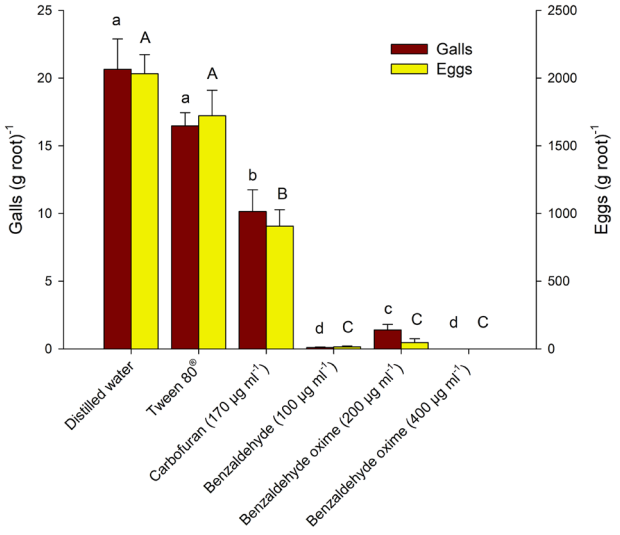

Fig. 3. Number of galls and eggs of *Meloidogyne incognita* in tomato roots following inoculation of second-stage juveniles previously exposed to benzaldehyde (100 μg ml^{-1}) and benzaldehyde oxime (200 and 400 μg ml^{-1}) for 48 h. Negative controls: distilled water and Tween-80®. Positive control: carbofuran at 170 μg ml^{-1}. Bars indicate the standard error of the mean. Data for galls were transformed to $\sqrt{x}$. Means with the same lower-case (galls) and upper-case (eggs) letter do not differ significantly according to the Scott-Knott test (Scott & Knott, 1974) ($P < 0.05$).

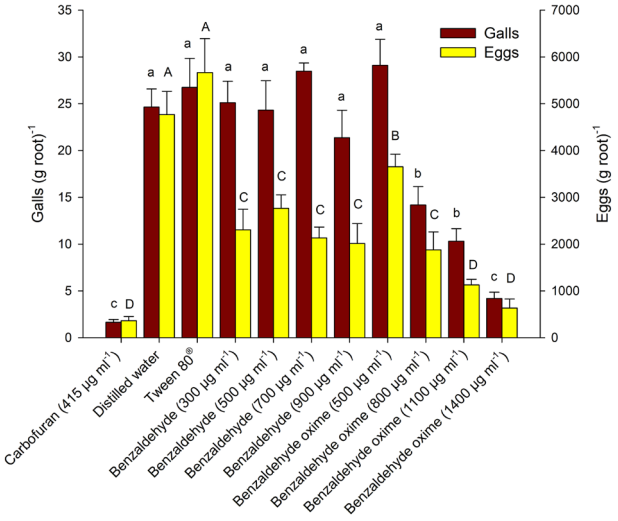

Fig. 4. Number of galls and eggs following inoculation of second-stage juveniles of *Meloidogyne incognita* together with the application of benzaldehyde (300, 500, 700 and 900 μg ml^{-1}) and benzaldehyde oxime (500, 800, 1100 and 1400 μg ml^{-1}) in tomato seedling-containing substrate under glasshouse conditions. Negative controls: distilled water and Tween-80®. Positive control: carbofuran at 415 μg ml^{-1}. Bars indicate the standard error of the mean. Means with the same lower-case (galls) and upper-case (eggs) letter do not differ significantly according to the Scott-Knott test (Scott & Knott, 1974) ($P < 0.05$).

Effect of benzaldehyde and its oxime applied to substrate infested with *M. incognita* containing tomato seedlings

Benzaldehyde oxime (800, 1100 and 1400 μg ml^{-1}) reduced the number of galls by 43-84% ($P < 0.0001$) as compared with the negative controls. At 1400 μg ml^{-1}, there was no difference in relation to the positive control (carbofuran at 415 μg ml^{-1}). Benzaldehyde, at all concentrations used, failed to reduce the number of galls as compared with the negative controls; however, it did reduce the number of eggs by 42-65%. Benzaldehyde oxime (500, 800, 1100 and 1400 μg ml^{-1}) reduced the number of eggs by 23-89%, and for the two highest concentrations, the values did not differ statistically from that of carbofuran (Fig. 4).

Discussion

Benzaldehyde was the major component of *P. viridiflora* oil, which has also been found in smaller proportions in other plants or essential oil from plants (Kim *et*

al., 2008; Barros *et al.*, 2014; Jardim *et al.*, 2017). In the present study, it was demonstrated that the nematicidal activity of *P. viridiflora* essential oil was due to benzaldehyde, which represented 98% of the total oil extracted. This substance caused immobility and mortality of *M. incognita* J2, which is in agreement with the *in vitro* nematicidal activity of benzaldehyde according to reports available in the literature (Kokalis-Burelle *et al.*, 2002; Kim *et al.*, 2008; Ntalli *et al.*, 2011; Jardim *et al.*, 2017). In addition, in the present study, benzaldehyde caused a significant reduction in the number of galls and eggs in tomato plants following exposure of J2 to a concentration of 100 μg ml^{-1} for 48 h prior to inoculation. However, a reduction in only the number of eggs was observed when solutions of this substance were applied to the substrate at various concentrations together with the nematode. Although there exist only a few studies on the *in vivo* nematicidal effect of benzaldehyde, it has been observed that this substance solely or in combination with other molecules reduces the number of galls caused by *M. javanica*, *M. arenaria* and *M. incognita* (Bauske *et al.*, 1994; Soler-Serratosa *et al.*, 1996; Calvet *et al.*, 2001; Oka, 2001). However, in the experiments described in the literature, the number of eggs was not evaluated. Benzaldehyde alone or with organic amendments has been shown to be efficient in reducing the plant-parasitic nematode population in planted soybean in naturally infested soil (Chavarría-Carvajal *et al.*, 2001). Similarly, a combination of chitin and benzaldehyde has also been demonstrated to reduce the number of galls caused by *M. incognita* in tomato plants, although benzaldehyde alone did not demonstrate a nematicidal effect in the same experiment (Kokalis-Burelle *et al.*, 2002).

These conflicting results for benzaldehyde may be due to its volatility and instability in soil. It is therefore necessary to search for alternatives that avoid losses in nematicidal activity, but at the same time solve the problem of rapid oxidation of benzaldehyde to benzoic acid (O'Neil, 2013). It is desirable to obtain analogues of benzaldehyde with nematicidal activities similar or superior to nematicides commonly available on the market, and with greater stability at the site of action. As a result, the efficacies of benzoic acid and benzaldehyde oxime were evaluated in the present study. Benzaldehyde oxime is a molecule of low volatility with much greater stability than benzaldehyde, from which it can be easily synthesised (Rappoport & Liebman, 2008). In the present study, when both substances were incubated with the nematode for 48 h prior to tomato plant inoculation, this trend was maintained, since at lower concentrations benzaldehyde caused a reduction in the number of eggs and galls per g root that was statistically equal to that observed for benzaldehyde oxime at higher concentrations. However, when benzaldehyde and its oxime came into contact with the nematode only at the moment of plant inoculation, the oxime was more active; this may be due to the higher oxime stability.

In the *in vivo* assay, when benzaldehyde and its oxime came into contact with the nematode at the time of plant inoculation, it was also observed that the reduction in the number of eggs produced by the nematode was proportionally greater than the reduction in gall number, which is in accordance with several authors working with other sources of natural products (Barros *et al.*, 2014; López *et al.*, 2017; Pimenta *et al.*, 2017). Exposing J2 to the molecules seems to change the physiological aspects that lead to the nutrition sink formation in the giant cells, furnishing fewer nutrients to the female for egg production, as explained by Barros *et al.* (2014).

With respect to benzoic acid dissolved in an aqueous solution of Tween-80®, *in vitro* nematicidal activity that was intermediate between that of benzaldehyde and its oxime was observed. However, when benzoic acid was dissolved in an aqueous solution of NaOH, forming sodium benzoate, such activity disappeared completely, suggesting that, because the pKa was in the order of 4.2 (Haynes, 2010), benzoic acid killed J2 by lowering the pH of the medium. This is in agreement with the fact that soil pH is one of the attributes that influences nematode populations, the best adaptation of which occurs at pH values greater than 5.0 (Babatola, 1981; Chen *et al.*, 2013).

It appears that the lack of efficacy of benzaldehyde and its oxime in reducing *M. incognita* J2 hatching may be explained by the protection that the lipid layer below the chitin of the egg exerts against the entry of molecules into the forming embryo (Gaugler & Bilgrami, 2004). However, this behaviour may be similar to other nematicidal substances, for instance carbofuran, which also failed to affect hatching in the present study.

The present study is of great relevance due to the scarcity in the market of molecules that are efficient in the management of plant-parasitic nematodes as well as of low toxicity to the environment and humans. Benzaldehyde oxime has high toxicity to *M. incognita* and greater soil stability over benzaldehyde, being a good option in the management of this nematode. The toxicity of benzaldehyde oxime was not found in the literature. However, benzaldehyde has LD_{50} value (rates *via* oral

intake) of 1300 mg kg^{-1}. Interest from the industrial sector to work with this molecule in order to make it available to farmers will depend on future studies to verify the stability of benzaldehyde oxime in the field.

Acknowledgements

The authors gratefully acknowledge financial support and fellowships from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Abdel-Rahman, F.H., Alaniz, N.M. & Saleh, M.A. (2013). Nematicidal activity of terpenoids. *Journal of Environmental Science and Health, Part B* 48, 16-22. DOI: 10.1080/03601234.2012.716686

Adams, R.P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL, USA, Allured Publishing Corporation.

Agbenin, N.O. (2014). Effect of aqueous extract of some plants on soil micro-fauna and micro-flora *in vitro*. *Archives of Phytopathology and Plant Protection* 47, 205-211. DOI: 10.1080/03235408.2013.807034

Asekun, O.T., Ekundayo, O. & Adeniyi, B.A. (1999). Antimicrobial activity of the essential oil of *Hyptis suaveolens* leaves. *Fitoterapia* 70, 440-442. DOI: 10.1016/S0367-326X(99)00067-2

Babatola, J.O. (1981). Effect of pH, oxygen and temperature on the activity and survival of *Hirschmanniella* spp. *Nematologica* 27, 285-291. DOI: 10.1163/187529281X00511

Baermann, G. (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) larven in Erdproben. *Geneeskundig Tijdschrift voor Nederlandsch Indië* 57, 131-137.

Barros, A.F., Campos, V.P., Silva, J.C.P., Pedroso, M.P., Medeiros, F.H.V., Pozza, E.A. & Reale, A.L. (2014). Nematicidal activity of volatile organic compounds emitted by *Brassica juncea*, *Azadirachta indica*, *Canavalia ensiformis*, *Mucuna pruriens* and *Cajanus cajan* against *Meloidogyne incognita*. *Applied Soil Ecology* 80, 34-43. DOI: 10.1016/j.apsoil.2014.02.011

Bauske, E.M., Rodríguez-Kábana, R., Estaun, V., Kloepper, J.W., Robertson, D.G., Weaver, C.F. & King, P.S. (1994). Management of *Meloidogyne incognita* on cotton by use of botanical aromatic compounds. *Nematropica* 24, 143-150.

Calvet, C., Pinochet, J., Camprubí, A., Estaún, V. & Rodríguez-Kábana, R. (2001). Evaluation of natural chemical compounds against root-lesion and root-knot nematodes and side-effects on the infectivity of arbuscular mycorrhizal fungi. *European Journal of Plant Pathology* 107, 601-605. DOI: 10.1023/a:1017954315942

Campos, V.P., Pinho, R.S.C. & Freire, E.S. (2010). Volatiles produced by interacting microorganisms potentially useful for the control of plant pathogens. *Ciência e Agrotecnologia* 34, 525-535. DOI: 10.1590/S1413-70542010000300001

Chavarría-Carvajal, J.A., Rodríguez-Kábana, R., Kloepper, J.W. & Morgan-Jones, G. (2001). Changes in populations of microorganisms associated with organic amendments and benzaldehyde to control plant-parasitic nematodes. *Nematropica* 31, 165-180.

Chen, D., Lan, Z., Bai, X., Grace, J.B. & Bai, Y. (2013). Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe. *Journal of Ecology* 101, 1322-1334. DOI: 10.1111/1365-2745.12119

Chen, S.Y. & Dickson, D.W. (2000). A technique for determining live second-stage juveniles of *Heterodera glycines*. *Journal of Nematology* 32, 117-121.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40, 221-249. DOI: 10.1146/annurev.phyto.40.032602.130045

Council of the European Union (2003). Council Decision of 18 March 2003 concerning the non-inclusion of aldicarb in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. *Official Journal European Union* 76, 21-24.

D'Addabbo, T., Laquale, S., Lovelli, S., Candido, V. & Avato, P. (2014). Biocide plants as a sustainable tool for the control of pests and pathogens in vegetable cropping systems. *Italian Journal of Agronomy* 9, 137-145. DOI: 10.4081/ija.2014.616

Fabiyi, O.A., Osunlola, O.S. & Olatunji, G.A. (2015). *In vitro* toxicity of extracts from *Hyptis suaveolens* (L.) Poit. on eggs and second-stage juveniles of *Heterodera sacchari*. *Agrosearch* 15, 89-99. DOI: 10.4314/agrosh.v15i1.6

Gaugler, R. & Bilgrami, A.L. (2004). *Nematode behaviour*. Wallingford, UK, CAB International.

Haynes, W.M. (2010). *CRC handbook of chemistry and physics*, 91st edition. Boca Raton, FL, USA, CRC Press Inc.

Hernández, V., Mora, F., Araque, M., De Montijo, S., Rojas, L., Meléndez, P. & De Tommasi, N. (2013). Chemical composition and antibacterial activity of *Astronium graveolens* Jacq essential oil. *Revista latinoamericana de química* 41, 89-94.

Hussey, R.S. & Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter* 57, 1025-1028.

Izuogu, N.B., Abolusoro, S.A. & Yakub, L.B. (2016). Nematicidal potential of aqueous extract of *Hyptis suaveolens* in the management of root-knot nematode, *Meloidogyne incognita* of some cowpea cultivars. *Croatian Journal of Food Science and Technology* 8, 15-19. DOI: 10.17508/CJFST.2016.8.1.05

Jardim, I.N., Oliveira, D.F., Silva, G.H., Campos, V.P. & de Souza, P.E. (2017). (E)-cinnamaldehyde from the essential oil of *Cinnamomum cassia* controls *Meloidogyne incognita* in soybean plants. *Journal of Pest Science* 91, 479-487. DOI: 10.1007/s10340-017-0850-3

Kim, J., Seo, S.M., Lee, S.G., Shin, S.C. & Park, I.K. (2008). Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valeriana wallichii*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *Journal of Agricultural and Food Chemistry* 56, 7316-7320. DOI: 10.1021/jf800780f

Kokalis-Burelle, N., Martinez-Ochoa, N., Rodríguez-Kábana, R. & Kloepper, J.W. (2002). Development of multi-component transplant mixes for suppression of *Meloidogyne incognita* on tomato (*Lycopersicon esculentum*). *Journal of Nematology* 34, 362-369.

Kudalkar, P., Strobel, G., Riyaz-Ul-Hassan, S., Geary, B. & Sears, J. (2012). *Muscodor sutura*, a novel endophytic fungus with volatile antibiotic activities. *Mycoscience* 53, 319-325. DOI: 10.1007/s10267-011-0165-9

López, L.E., Campos, V.P., Silva, A.P., Barros, A.F., Pedroso, M.P., Silva, J.C.P. & Terra, W.C. (2017). Volatile organic compounds from cottonseed meal are toxic to *Meloidogyne incognita*. *Tropical Plant Pathology* 42, 443-450. DOI: 10.1007/s40858-017-0154-4

McCarter, J.P. (2008). Molecular approaches toward resistance to plant-parasitic nematodes. In: Berg, R.H. & Taylor, C.G. (Eds). *Cell biology of plant nematode parasitism*. Berlin, Germany, Springer-Verlag, pp. 239-267.

Morais-Costa, F., Bastos, G.A., Soares, A.C., Costa, E.G., Vasconcelos, V.O., Oliveira, N.J., Braga, F.C., Duarte, E.R. & Lima, W.S. (2016). *In vitro* and *in vivo* action of *Piptadenia viridiflora* (Kunth) Benth against *Haemonchus contortus* in sheep. *Veterinary Parasitology* 223, 43-49. DOI: 10.1016/j.vetpar.2016.04.002

Ntalli, N.G., Ferrari, F., Giannakou, I. & Menkissoglu-Spiroudi, U. (2011). Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Management Science* 67, 341-351. DOI: 10.1002/ps.2070

Oka, Y. (2001). Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. *Nematology* 3, 159-164. DOI: 10.1163/156854101750236286

Olabiyi, T.I. (2008). Pathogenicity study and nematoxic properties of some plant extracts on the root-knot nematode pest of tomato, *Lycopersicon esculentum* (L.) Mill. *Plant Pathology Journal* 7, 45-49. DOI: 10.3923/ppj.2008.45.49

Oliveira, F.E.R., Oliveira, D.F., Campos, V.P. & Amaral, D.R. (2003). Purification of two substances from bulbs of onion (*Allium cepa* L.) with nematicidal activity against *Meloidogyne exigua* Goeldi. *Nematology* 5, 859-864. DOI: 10.1163/156854103773040763

O'Neil, M.J. (2013). *The Merck Index – an encyclopedia of chemicals, drugs, and biologicals*, 15th edition. Cambridge, UK, Royal Society of Chemistry.

Pimenta, L., Ferreira, M.A., Pedroso, M.P. & Campos, V.P. (2017). Wood-associated fungi produce volatile organic compounds toxic to root-knot nematode. *Scientia Agricola* 74, 303-310. DOI: 10.1590/1678-992x-2015-0472

Rappoport, Z. & Liebman, J.F. (2008). *The chemistry of hydroxylamines, oximes and hydroxamic acids*. Hoboken, NJ, USA, Wiley. DOI: 10.1002/9780470741962

Scott, A.J. & Knott, M. (1974). A cluster analysis method for grouping means in the analysis of variance. *Biometrics* 30, 507-512.

Soler-Serratosa, A., Kokalis-Burelle, N., Rodríguez-Kábana, R., Weaver, C.F. & King, P.S. (1996). Allelochemicals for control of plant-parasitic nematodes. 1. *In vivo* nematicidal efficacy of thymol and thymol/benzaldehyde combinations. *Nematropica* 26, 57-71.

Spurgeon, D. (1997). End agreed for ozone-destroying pesticide. *Nature* 389, 319. DOI: 10.1038/38564

Trentin, D.S., Giordani, R.B., Zimmer, K.R., Silva, A.G., Silva, M.V., Correia, M.T.S., Baumvol, I.J.R. & Macedo, A.J. (2011). Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles. *Journal of Ethnopharmacology* 137, 327-335. DOI: 10.1016/j.jep.2011.05.030

Trudgill, D.L. & Blok, V.C. (2001). Apomictic, polyphagous root-knoot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology* 39, 53-77. DOI: 10.1146/annurev.phyto.39.1.53

Ullah, I., Khan, A.L., Ali, L., Khan, A.R., Waqas, M., Hussain, J., Lee, I.-J. & Shin, J.-H. (2015). Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021. *Journal of Microbiology* 53, 127-133. DOI: 10.1007/s12275-015-4632-4