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ORIGINAL PAPER

(E)-cinnamaldehyde from the essential oil of *Cinnamomum cassia* controls *Meloidogyne incognita* in soybean plants

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Abstract Among the main problems faced by soybean producers is the nematode *Meloidogyne incognita*. Because nematicides that are more efficient and less toxic to humans and the environment than those available are desirable to control this pathogen, this work aimed at studying the essential oil of *Cinnamomum cassia*, which has been described as active in vitro against the nematode *Bursaphelenchus xylophilus*. At the concentration of 62 μg mL^{-1}, it performed better than the nematicide carbofuran at 173 μg mL^{-1} in an in vitro assay with *M. incognita* eggs and second-stage juveniles. The main components of this oil were identified by gas chromatography–mass spectrometry analysis and submitted to in vitro assays with the nematode, which showed (E)-cinnamaldehyde (83.3% of the oil) as responsible for the nematicidal activity. Emulsions of the oil (500 μg mL^{-1}) and this aldehyde (416 μg mL^{-1}) reduced the numbers of *M. incognita* galls and eggs in soybean plants to values statistically equal to those obtained with carbofuran (415 μg mL^{-1}). Vapors of the essential oil and (E)-cinnamaldehyde were also as active as the fumigant nematicide Basamid against *M. incognita* according to an in vitro assay. Cultivation of soybean plants in substrate inoculated with *M. incognita* eggs and treated with Basamid at 0.25 g (L of substrate)$^{-1}$ or (E)-cinnamaldehyde at 1.0 mL (L of substrate)$^{-1}$ caused a reduction in the nematode population to values statistically equal to each other. These results make (E)-cinnamaldehyde very promising for the development of new products to control *M. incognita* in soybean fields.

Keywords Biopesticide · *Trans*-cinnamaldehyde · *Cinnamomum* · Root-knot nematode · *Glycine max* · *Meloidogyne* spp.

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Key message

- New products to control the nematode *Meloidogyne incognita* in soybean fields are desired.
- To meet this demand, the essential oil of *Cinnamomum cassia* was studied.
- Emulsions of the essential oil and its main component, (E)-cinnamaldehyde, reduced the population of *M. incognita* in soybean plants to values statistically equal to those observed for the nematicide carbofuran.
- Vapor of (E)-cinnamaldehyde reduced the population of the nematode to values statistically equal to those obtained with the fumigant nematicide Basamid.

Introduction

Native to Southeast Asia, soybean [*Glycine max* (L.) Merr.] is one of the most cultivated oleaginous plants in the world (Subramanyam et al. 2012). The USA is the major

producer of soybeans, followed by Brazil (USDA 2015), which produced about 96 million tons in the 2015/2016 harvest (CONAB 2016). Among the main problems faced by Brazilian soybean producers are diseases like those caused by root-knot nematodes (*Meloidogyne* spp.), which restrict the productivity of soybean (Soares et al. 2004). For example, *Meloidogyne incognita* (Kofoid and White) Chitwood causes losses of over 55% in soybean production in areas infested by this pathogen (Machado 2015). *M. incognita* is usually controlled in soybean plantations by commercial nematicidal substances, the use of which is generally more efficient than other methods to control plant-parasitic nematodes. However, these substances may persist in the environment and have side effects on humans and other non-target organisms (Sousa et al. 2015). Therefore, new nematicides that are more efficient and less toxic to humans and to the environment than the commercial products available are greatly welcome.

Plant metabolites are potentially useful to the development of new products able to circumvent the above-mentioned problems because some of them are known to be active against plant-parasitic nematodes (Douda et al. 2010). Some components of essential oils extracted from plants are among these metabolites (Ntalli and Caboni 2012; Regnault-Roger et al. 2012; Andrés et al. 2012), which can act alone or synergistically against nematodes. In addition to killing these organisms, these metabolites can attract or repel them, and stimulate or inhibit the eclosion of second-stage juveniles (J2) of these phytoparasites (Chitwood 2002; Faria et al. 2016).

Cinnamomum cassia (L.) J. Presl (Lauraceae Juss.), known as Chinese cassia or Chinese cinnamon, is a tree native to China and other countries of Southeast Asia, where it has been used as a spice or a medicine since ancient times (Geng et al. 2011). Nematicidal activity has been attributed to the essential oils obtained from barks of the trunk or brunches of this plant, since they were active in vitro against adults of the pine wood nematode [*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle] (Kong et al. 2007). However, no report about the efficiency of these oils or their components is described in the literature for the control of plant-parasitic nematodes in soybean fields. As essential oils and their components apparently can decompose into non-toxic compounds and usually have few harmful effects on non-target organisms, an investigation into the essential oil of *C. cassia* and its main components was carried out to contribute to the development of a new fumigant nematicide to control *M. incognita* in fields for planting soybeans. To achieve such a goal, the objectives of the present work were to: (1) investigate the in vitro activity of the essential oil of *C. cassia* against *M. incognita*; (2) identify the main components of this essential oil through analysis by gas chromatography–mass spectrometry (GC–MS); (3) evaluate the in vitro activity against *M. incognita* by the main components of the essential oil obtained from *C. cassia*; (4) evaluate, under greenhouse conditions, the efficiency of the main component as a fumigant to prevent the development of *M. incognita* in soybean plants.

Materials and methods

M. incognita and chemicals

M. incognita was sampled from artificially infested tomato plants (*Solanum lycopersicum* L. cv. Santa Clara) grown under greenhouse conditions. Eggs were extracted from 60-day nematode-infested roots according to the method described by Hussey and Barker (1973). Eggs retained in a 500-mesh sieve (American Society for Testing and Materials, ASTM) were used in the experiments or transferred to a Baermann funnel (Whitehead and Heming 1965), and those second-stage juveniles (J2) of *M. incognita* that hatched after 24–48 h were collected to be used in the experiments.

The essential oil from barks of *C. cassia* was supplied by Empresa Ferquima Ind. Com. Ltda. (Vargem Grande Paulista, São Paulo, Brazil). It was obtained by hydrodistillation in January 2015 (lot 218) and is valid until January 2018. (E)-cinnamaldehyde (≥99%), *o*-methoxycinnamaldehyde (≥96%), benzaldehyde (≥99%) (Fig. 1) and carbofuran (98%) were supplied by Sigma-Aldrich Co. (Milan, Italy); while Basamid (98%) was purchased from BASF (Ludwigshafe, Germany).

In vitro motility and mortality of *M. incognita* J2 exposed to emulsions of the essential oil of *C. cassia*

According to the method described by Chen and Dickson (2000) and adapted by Amaral et al. (2003), an aqueous suspension (20 µL) containing approximately 20 *M. incognita* J2 and 100 µL of aqueous 0.01 g/mL Tween 80® solutions containing the essential oil of *C. cassia* at six different concentrations (1200, 600, 300, 150, 74.4 and 37.2 µg mL^{-1}) were put into 350-µL wells of a 96-well polypropylene plate. The final concentrations of the essential oil in the wells were 1000, 500, 250, 125, 62 and

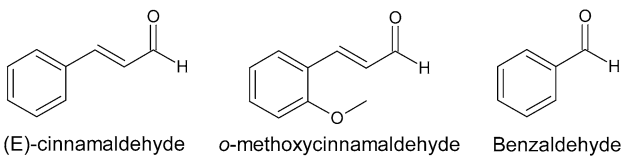

Fig. 1 Chemical structures of the main components of the essential oil obtained from *Cinnamomum cassia*

31 μg mL^{-1}. Water, Tween 80® at 0.01 g mL^{-1} and carbofuran (2,3-dihydro-2,2-dimethyl-1-benzofuran-7-yl *N*-methylcarbamate; final concentration 173 μg mL^{-1}) were used as controls. Six replicates were employed for each treatment. The plates were sealed with parafilm and kept at 26 °C for 48 h. Then, mobile and immobile nematodes were counted under a microscope, and one drop of a freshly prepared 1.0 mol L^{-1} NaOH solution was added to the content of each well and nematodes were counted again. J2 that changed their body shape within 3 min were considered to be alive, whereas the nematodes not responding to the addition of NaOH were considered dead.

In vitro hatching of *M. incognita* J2 from eggs exposed to emulstion of the essential oil of *C. cassia*

This experiment was set up similarly to that described above, but with 60 eggs of the nematode per well instead of 20 J2. The experiment time was also changed, as the evaluation was made seven days after the beginning of the experiment. This comprised counting intact eggs and hatched J2 (alive or dead).

GC–MS analysis of the essential oil of *C. cassia*

A gas chromatograph coupled to a mass spectrometer (model QP2010, Shimadzu, Japan), equipped with a RTX®-5MS capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness; Restek), was employed in this work, which used helium at 1.0 mL min^{-1} as carrier gas. According to Adams (2007), the following conditions were adopted: (1) split/splitless injector temperature: 220 °C; (2) split ratio: 1:20; (3) initial temperature of the column: 60 °C; (4) elevation rate of the column temperature: 2 °C min^{-1} up to 200 °C and then 5 °C min^{-1}; (5) final temperature of the column: 250 °C; (6) temperature of the interface between the gas chromatograph and the mass spectrometer: 220 °C; (7) ionization of each molecule in the spectrometer: electron impact at 70 eV; (8) range of mass/charge (*m/z*) analyses in the mass spectrometer: 45–400; and (9) mass spectrum acquisition time: 0.5 s. The essential oil of *C. cassia* was dissolved in acetone to a concentration of 10 mg mL^{-1}, and 1 μL of this solution was injected in 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 to those in the NIST 05 Mass Spectral Library, 2005, and all peaks in the chromatogram with similarity index below 90% were considered unidentified. For each of the remaining peaks, the arithmetic index (AI) was calculated according to the following formulae: $AI = \{100P_z + 100[(RT - RTP_z)/(RTP_{z+1} - RTP_z)]\}$, where P_z = number of carbon atoms of the linear alkane with retention time immediately below that of the substance to be identified in the chromatogram; RT = retention time (min) of the substance to be identified in the chromatogram; RTP_z = retention time (min) of the linear alkane with number of carbon atoms equal to P_z; and RTP_{z+1} = retention time (min) of the linear alkane with number of carbon atoms equal to $P_z + 1$. Substances with calculated values of AI corresponding to an error ≥3% in relation to the AI described by Adams (2007) were considered not identified.

In vitro mortality of *M. incognita* J2 exposed to emulsions of the main components from the essential oil of *C. cassia*

This experiment was carried out as described above for the essential oil of *C. cassia*, but instead of this oil the following substances were evaluated: (E)-cinnamaldehyde (final concentrations: 833, 416, 208, 104, 52 and 26 μg mL^{-1}), *o*-methoxycinnamaldehyde (final concentrations: 71, 36 and 18 μg mL^{-1}) and benzaldehyde (final concentrations: 19, 10 and 5 μg mL^{-1}) (Fig. 1).

In vitro hatching of *M. incognita* J2 from eggs exposed to emulsions of (E)-cinnamaldehyde

This experiment was carried out as described above for the essential oil of *C. cassia*, with (E)-cinnamaldehyde (Fig. 1) at final concentrations of 833, 416, 208, 104, 52 and 26 μg mL^{-1}.

Effect of emulsions of the essential oil of *C. cassia* and (E)-cinnamaldehyde on the infectivity and reproduction of *M. incognita* in soybean plants

Seeds of soybean (*Glycine max* L. cv. BRS-284) susceptible to *M. incognita* were sown on a commercial substrate (Tropstrato®, Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda., Mogi Mirim, São Paulo, Brazil) contained in 300-mL plastic pots. Twenty days later, plants were used in the experiment. An aqueous suspension (24 mL), containing about 3000 *M. incognita* J2 and emulsions (24 mL) of the essential oil of *C. cassia* (500, 250 and 125 μg mL^{-1}) or (E)-cinnamaldehyde (416, 208 and 104 μg mL^{-1}) (Fig. 1), in Tween 80 at 0.01 g mL^{-1}, was combined, resulting in eight different suspensions. Water, Tween 80® at 0.01 g mL^{-1} and carbofuran (415 μg mL^{-1}) were used as controls. A sample (8 mL, containing 500 J2) of each suspension was added to the substrate of each soybean plant through four equidistant holes (0.4 cm wide × 1.5 cm deep) around the stem. Plants were kept for 48 h in a room with no sun incidence and then moved to a greenhouse, where they were maintained for 30 days. After this period of time, roots

were removed, carefully washed, dried on paper towels and weighed. After counting galls, roots underwent eggs extraction according to the method described by Hussey and Barker (1973). Eggs retained in the 500-mesh (ASTM) sieve were suspended in 50 mL water and counted in a Peters chamber under a microscope. This experiment was carried out with six replicates for each treatment, under a random design.

In vitro mortality of *M. incognita* J2 exposed to vapors of the essential oil of *C. cassia* and (E)-cinnamaldehyde

Adapting the method described by Barros et al. (2014), sand (30 g) was sterilized by autoclaving at 120 °C for 30 min and poured into each Supelco™ SPME flask (28 mm wide × 80 cm deep, Sigma-Aldrich, Bellefonte, PA, USA). Two Eppendorf tubes (0.5 mL) were partially immersed in the sand of each flask, and 100 µL of the sample to be evaluated was poured into one of the tubes. The flask was immediately sealed with a screw cap internally coated with silicone and kept at 28 °C for 72 h. Employing a syringe with a needle to punch the silicon septa of the Supelco™ SPME flask, an aqueous suspension containing about 1000 *M. incognita* J2 was injected into each empty Eppendorf tube. After 48 h at 28 °C, the flasks were opened for the homogenization of the J2 suspension, from which 20 µL was withdrawn, diluted with 100 µL of water and submitted to mobile and immobile J2 count under a microscope. According to the method of Chen and Dickson (2000), adapted by Amaral et al. (2003), one drop of a freshly prepared 1.0 mol L^{-1} NaOH solution was added and immobile J2 under a microscope were considered dead. The products evaluated in this experiment were essential oils of *C. cassia* and (E)-cinnamaldehyde (Fig. 1). This experiment was carried out under a random design, with six replicates per treatment, employing the commercial fumigant nematicide Basamid® [(80 mg; BASF (Ludwigshafen, Germany)] and water as positive and negative controls, respectively. This experiment was carried out three times, resulting in similar values. Therefore, only one set of data is presented.

In vitro hatching of *M. incognita* J2 from eggs exposed to vapors of the essential oil of *C. cassia* and (E)-cinnamaldehyde

This experiment was set up similarly to that described above, but with a suspension containing *M. incognita* eggs (3000 eggs mL^{-1}) instead of J2. The experiment time was also changed, as the evaluation was made seven days after the beginning of the experiment. This comprised counting intact eggs and hatched J2 (alive or dead). This experiment was carried out three times, resulting in similar values. Therefore, only one set of data is presented.

Effect of vapor of (E)-cinnamaldehyde on the infectivity and reproduction of *M. incognita* in soybean plants

M. incognita eggs (150,000) were added to 1 L of a commercial substrate (Tropstrato®, Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda., Mogi Mirim, São Paulo, Brazil) contained in 2-L polyethylene terephthalate bottles. (E)-cinnamaldehyde (Fig. 1) was then splashed on the substrate to the following concentrations: 1.0, 0.5 and 0.2 mL (L of substrate)$^{-1}$. The commercial fumigant nematicide Basamid at 0.25 g (L of substrate)$^{-1}$ and water (1.0 mL) was used as positive and negative controls, respectively. All bottles were cap-closed, and the resulting mixtures were homogenized and remained standing at 28 °C for three days. The bottles were then opened, and after five days, the substrate inside was poured into cells (121.2 cm^3) of a 72-cell Styrofoam tray. Twenty-day-old soybean plants (*Glycine max* L. cv. BRS-284), susceptible to *M. incognita*, were transferred to the tray, which was kept in a greenhouse for 30 days. After this period of time, roots were carefully washed, dried with a paper towel and weighed. After counting galls, *M. incognita* eggs were extracted by the method described by Hussey and Barker (1973). Eggs retained in the 500-mesh (ASTM) sieve were suspended in 50 mL water and counted in a Peters chamber under a microscope. This experiment was carried out with six replicates for each treatment, under a random design. This experiment was carried out twice, resulting in similar values. Therefore, only one set of data is presented.

Statistical analyses

For the in vitro assays with *M. incognita* eggs and J2, values were transformed into a percentage and submitted to analysis of variance (ANOVA). Means were compared according to the Scott and Knott (1974) test ($P \leq 0.05$). The statistical calculation was carried out with the software SISVAR (Ferreira 2011). For the experiments with soybean plants, the statistical calculation was done without transformations of values into a percentage.

Results

In vitro activity against *M. incognita* by emulsions of the essential oil from *C. cassia*

When dissolved in an aqueous Tween 80® solution, the essential oil of *C. cassia* was very active against *M.*

incognita J2, as it caused 100% immobility and mortality of the nematode at a concentration of 62 μg mL^{-1}, while only 63% of the nematode individuals were dead after exposure to the commercial nematicide carbofuran at 173 μg mL^{-1} (Table 1). A similar result was observed when eggs of the nematode were exposed to the same emulsions, since the percentage of J2 hatching from eggs in contact with the essential oil at 62 μg mL^{-1} was about the same as observed for carbofuran at a concentration almost three times higher.

GC–MS analysis of the essential oil of *C. cassia*

The main components of the essential oil of *C. cassia* were (E)-cinnamaldehyde (83.3%), *o*-methoxycinnamaldehyde (7.1%), (E)-cinnamyl acetate (2.0%) and benzaldehyde (1.9%) (Fig. 1). These four compounds corresponded to about 94.3% of this essential oil constituents and presented a similarity index to their database spectra above 90% (Table 2). Furthermore, the arithmetic indexes calculated for them were very close (errors ≤ 0.63%) to those described in the literature for the same substances (Adams 2007).

In vitro activity against *M. incognita* by emulsions of the main components in the essential oil from *C. cassia*

(E)-cinnamaldehyde (Fig. 1), the main component of the essential oil (Table 2), was very active against *M. incognita*, since it caused 100% immobility and 97% mortality of J2 at 52 μg mL^{-1}, while carbofuran at 173 μg mL^{-1} took J2 mortality to only 63% (Table 1). Furthermore, despite the differences between the concentrations of these two emulsions, the percentages of J2 hatched from eggs exposed to them were statistically equal. It is also worth mentioning that (E)-cinnamaldehyde at concentrations corresponding to 83.3% of those employed for the essential oil, which is the same percentage of this substance in the essential oil according to the GC–MS analysis (Table 2), caused J2 mortality for about 90% of those observed for the essential oil. Regarding benzaldehyde and *o*-methoxycinnamaldehyde (Fig. 1), their activities against the nematode were very low at the concentrations studied (Table 1), which reflected their percentages in the essential oil according to the GC–MS analysis (Table 2).

Effect of emulsions of the essential oil of *C. cassia* and (E)-cinnamaldehyde on the infectivity and reproduction of *M. incognita* in soybean plants

Both the essential oil and its main component, (E)-cinnamaldehyde (Fig. 1), performed similarly to the commercial nematicide carbofuran, which reduced the number of galls and eggs of the nematode to values corresponding to about 14% and 7%, respectively, of those observed for water and Tween 80®, which were employed as controls (Table 3). In fact, (E)-cinnamaldehyde was somewhat more active than carbofuran, as with half of the concentration employed for this commercial nematicide afforded values statistically equal to those obtained with carbofuran.

Just as in the in vitro assay with emulsions of the essential oil and (E)-cinnamaldehyde (Table 1), when the concentration of this substance corresponded to 83.3% of the essential oil, values were very close to each other, tending to be statistically equal for all parameters (Table 3).

Table 1 Mobility and mortality of *Meloidogyne incognita* second-stage juveniles (J2) exposed to emulsions of the essential oil of *Cinnamomum cassia* and its main components, and J2 hatching from *M. incognita* eggs exposed to these emulsions

Treatments	Concentration (μg mL^{-1})	Immobile J2 (%)[a]	Dead J2 (%)[a]	Hatched J2 (%)[a]
Essential oil	250	100 f	100 f	5 c
Essential oil	125	100 f	100 f	8 c
Essential oil	62	100 f	100 f	13 b
Essential oil	31	60 d	45 d	–
(E)-cinnamaldehyde	208	100 f	100 f	6 c
(E)-cinnamaldehyde	104	100 f	100 f	10 b
(E)-cinnamaldehyde	52	100 f	97 f	11 b
(E)-cinnamaldehyde	26	54 c	39 c	–
o-Methoxycinnamaldehyde	18	21 b	7 b	–
Benzaldehyde	5	21 b	7 b	–
Carbofuran (control)	173	71 e	63 e	12 b
Water (control)	–	7 a	3 a	44 a

[a] Means followed by the same letter in each column do not differ statistically according to the Scott and Knott (1974) test ($P \leq 0.05$)

Table 2 Main components of the essential oil of *Cinnamomum cassia* according to analysis by gas chromatography–mass spectrometry

Number	Component	RT[a] (min)	AI[b]	SI[c] (%)	Area (%)
1	Benzaldehyde	6.673	958	99	1.9
2	Benzene acetaldehyde	9.894	1041	95	0.9
3	*o*-Anisaldehyde	20.685	1241	91	0.8
4	(E)-cinnamaldehyde	22.680	1273	97	83.3
5	Coumarin	32.304	1431	94	0.9
6	(E)-cinnamyl acetate	33.062	1443	95	2.0
7	(E)-cinnamic acid	33.306	1447	91	1.3
8	*o*-Methoxycinnamaldehyde	38.113	1528	98	7.1
Total					98.2

[a] RT = retention time

[b] AI = calculated arithmetic index

[c] SI = similarity index

In vitro activity against *M. incognita* by vapors of the essential oil of *C. cassia* and (E)-cinnamaldehyde

The essential oil, (E)-cinnamaldehyde (Fig. 1) and the commercial fumigant nematicide Basamid immobilized 100% of the nematode, but they were a little different in regard to the values of dead and hatched J2 (Table 4). Basamid was a little better when the parameter of dead J2 was taken into account, while the essential oil was a little better when the number of hatched J2 was considered.

Effect of vapor of (E)-cinnamaldehyde on the infectivity and reproduction of *M. incognita* in soybean plants

In all concentrations, used (E)-cinnamaldehyde (Fig. 1) reduced the population of *M. incognita* on soybean plants. At 1.0 mL (L of the substrate)$^{-1}$, both numbers of galls and eggs of the nematode on roots of the plants were statistically equal to those observed for the treatment corresponding to the commercial fumigant nematicide Basamid (Table 5).

Discussion

There is no doubt about the high direct activity of the essential oil of *C. cassia* against *M. incognita*. At lower concentrations than that used for carbofuran emulsions, such oil increased the in vitro J2 mortality to values above that observed for this commercial nematicide, while reduced the number of J2 hatched to less than that obtained with carbofuran (Table 1). This result is in complete agreement with the activity of such oil against the pine wood nematode *B. xylophilus* (Kong et al. 2007).

Given the potential detected by in vitro tests for the utilization of essential oil of *C. cassia* in developing new nematicides, it was analyzed by GC–MS, which revealed (E)-cinnamaldehyde (Fig. 1) as the main constituent, accounting for approximately 83.3% of the oil (Table 2). This result is in total agreement with previous work carried out by other research groups, according to which the percentage of this aldehyde in such essential oil can vary in the 42–92% range (Kocevski et al. 2013; Kim et al. 2016; Khaled et al. 2015; Geng et al. 2011; Chou et al. 2013; Giordani et al. 2006). This wide range can be easily explained by factors such as the age of the plant, humidity

Table 3 Effect of emulsions of (E)-cinnamaldehyde and essential oil of *Cinnamomum cassia* on the numbers of *Meloidogyne incognita* galls and eggs on the roots of soybean plants, and on the mass of their roots

Treatments	Concentration (μg mL^{-1})	Galls[a]	Eggs[a]	Root mass (g)[a]
(E)-cinnamaldehyde	416	0 a	7 a	8.35 a
(E)-cinnamaldehyde	208	2 a	80 a	8.02 a
(E)-cinnamaldehyde	104	7 b	203 b	8.15 a
Essential oil	500	3 a	107 a	8.38 a
Essential oil	250	4 a	178 b	8.23 a
Essential oil	125	6 b	299 b	8.23 a
Carbofuran (control)	415	3 a	51 a	7.95 a
Tween 80® (control)		22 c	674 c	8.10 a
Water (control)		22 c	759 c	8.10 a

[a] Means followed by the same letter in each column do not differ statistically according to the Scott and Knott (1974) test ($P \leq 0.05$)

Table 4 Mobility and mortality of *Meloidogyne incognita* second-stage juveniles (J2) exposed to vapors of the essential oil of *Cinnamomum cassia* and (E)-cinnamaldehyde, and J2 hatched from the nematode eggs exposed to the same vapors

Treatment	Immobile J2 (%)[a]	Dead J2 (%)[a]	Hatched J2 (%)[a]
Essential oil	100 a	80 b	8 a
(E)-cinnamaldehyde	100 a	84 c	13 b
Basamid (control)	100 a	92 d	12 b
Water (control)	3 b	0 a	41 c

[a] Means followed by the same letter in each column do not differ statistically according to the Scott and Knott (1974) test ($P \leq 0.05$)

Table 5 Effect of (E)-cinnamaldehyde vapor on the numbers of *Meloidogyne incognita* galls and eggs on the roots of soybean plants, and on the mass of their roots

Treatments	Amount per liter of substrate	Galls[a]	Eggs[a]	Root mass (g)[a]
(E)-cinnamaldehyde	1.0 mL	19 a	603 a	2.18 b
(E)-cinnamaldehyde	0.5 mL	27 b	1335 b	2.26 b
(E)-cinnamaldehyde	0.2 mL	31 b	1746 b	2.26 b
Basamid (control)	0.25 g	18 a	322 a	1.94 a
Water (control)	–	53 c	2291 c	2.23 b

[a] Means followed by the same letter in each column do not differ statistically according to the Scott and Knott (1974) test ($P \leq 0.05$)

and method to prepare the essential oil (Lahlou 2004). The other constituents, in much lower concentrations according to the present work (Table 2), are also found in this essential oil according to these authors.

The assay with emulsions of benzaldehyde, *o*-methoxycinnamaldehyde and (E)-cinnamaldehyde (Fig. 1), at concentrations proportional to their amounts in the essential oil, revealed the latter substance as responsible for the nematicidal activity of such oil (Table 1). This result is consistent with the previously described in vitro activity of this substance against *B. xylophilus* (Kong et al. 2007) and *M. incognita* (Caboni et al. 2013). Apparently, it acts against the nematode inhibiting its V-ATPase enzyme, which is a vacuolar-type proton *trans*-locating ATPase that pumps protons across membranes, energized by ATP hydrolysis. This enzyme may be involved in nematode nutrition, osmoregulation, cuticle synthesis, neurobiology and reproduction (Caboni et al. 2013).

As the results so far discussed made clear the in vitro activity against *M. incongita* by the essential oil of *C. cassia* and its main component, (E)-cinnamaldehyde (Fig. 1), emulsions of both materials underwent an assay with soybean plants inoculated with *M. incognita* J2, which corroborated the potential of (E)-cinnamaldehyde to the development of new nematicides (Table 1). In almost all concentrations of this aldehyde, the numbers of galls and eggs in soybean plants were statistically equal to those observed in plants treated with the essential oil of *C. cassia* at concentrations equal to concentrations of (E)-cinnamaldehyde × 1.2. Thus, this substance really is responsible for the activity of the oil against the nematode, which is in accordance with its in vitro and in vivo activity against *Meloidogyne javanica* (Treub) Chitwood described in the literature (Oka 2001). Furthermore, in the present work this aldehyde was as efficient as carbofuran, which is a commercial nematicide used to reduce the populations of several plant-pathogenic nematodes, including *Meloidogyne* spp., in different cultures (Adegbite and Agbaje 2007; Khan et al. 2012; Jada et al. 2011). This result also seems in line with the work by Ntalli et al. (2016), who observed the nematicidal activities against *M. incognita*, *M. javanica* and *Meloidogyne arenaria* Chitwood, by (E,E)-deca-2,4-dienal and (E)-dec-2-enal, which are also aldehydes. It is worth mentioning that although some plant metabolites that are potentially useful for the control of plant pathogens can present phytotoxic effects (Roh et al. 2011), both essential oil and (E)-cinnamaldehyde have not affected root development (Table 3), suggesting that these materials are not phytotoxic. Actually, according to Oka (2001), this aldehyde can increase the shoot weight of tomato plants.

Despite the excellent results obtained with emulsions of (E)-cinnamaldehyde (Fig. 1) and the essential oil in the assay with soybean plants inoculated with *M. incognita* J2, it seemed very important to take into account the volatility and high sensitivity of (E)-cinnamaldehyde to oxidizing agents (López-Serna et al. 2016), which can cause its persistence in soil to be very low when compared to the commercial non-fumigant nematicides. Although these features sound undesirable for this class of nematicide, they are excellent for the development of a product characteristic of the fumigant class, which can be used to reduce the nematode population in the field before planting.

Therefore, the in vitro effect of vapors from (E)-cinnamaldehyde and essential oil of *C. cassia* on motility, mortality and hatching of *M. incognita* J2 was also studied. Values obtained for these vapors were very close to each other (Table 4), corroborating once again this aldehyde as the component accounting for the nematicidal activity of the essential oil. Furthermore, they were also close to values obtained with the commercial nematicide Basamid, which suggests that (E)-cinnamaldehyde has great potential for the development of new fumigant nematicides.

To assess the efficiency of (E)-cinnamaldehyde (Fig. 1) vapor against the nematode under conditions closer to those observed in the field, an experiment was carried out using substrate inoculated with *M. incognita* eggs, which correspond to most of the nematode population in fields (Evans and Perry 2009). The in vitro results were confirmed, as this aldehyde reduced both numbers of galls and eggs of the nematode to values close to those observed for the commercial nematicide Basamid (Table 5). Furthermore, these results are in accordance with the activity of this aldehyde against *M. incongita* in tomato plants, which was detected under conditions different from those employed in the present work (Ishibashi and Kubo 1987).

In view of the above-mentioned results, there is no doubt about the potential of (E)-cinnamaldehyde (Fig. 1) for the development of new products to control *M. incognita* in soybean fields. Furthermore, both this aldehyde and the product of its oxidation, (E)-cinnamic acid, present low toxicity to non-target organisms (Bickers et al. 2005), which is very important for the development of environmentally friendly nematicides.

Author contribution statement

INJ conceived, designed the research, conducted the experiments and wrote the manuscript. GHS carried out the GC–MS analysis and interpreted the results. VPC supervised the experiments with the nematode. PES conceived and designed the research. DFO supervised all the work and reviewed the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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