Pakistan Journal of Nematology (2017) 35 (1): 93-104

ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk

http://dx.doi.org/10.18681/pjn.v35.i01.p93-104

Toxicity of essential oils extracted from *Corymbia citriodora* and *Eucalyptus camaldulensis* leaves against *Meloidogyne incognita* under laboratory conditions

A.M. El-Baha[1], A. A. El-Sherbiny[2†], M. Z. M. Salem[1], N. Shaarawy[3] and N. H. Mohamed[3]

[1] *Forestry and Wood Technology Department, Faculty of Agriculture El-Shatby, Alexandria University, Alexandria, Egypt*

[2] *Nematology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Plant Protection Research Station, Sabaheya, Alexandria, Egypt*

[3] *Forestry & Wood Technology Department,Horticulture Research Institute, Agricultural Research Center, Antoniades Gardens, Alexandria, Egypt*

† Corresponding author: amr_elsherbiny_68@yahoo.com

Abstract

Essential oils (EOs) were extracted from *Corymbia citriodora* and *Eucalyptus camaldulensis* fresh leaves and tested for their nematicidal activity at four different concentrations viz., 125, 250, 500 and 1000 mg/l against root-knot nematode, *Meloidogyne incognita* under laboratory conditions. All concentrations significantly inhibited egg hatching (31.24–66.35%) and mortality of second stage juvenile. Inhibition of egg hatching (%) and mortality (%) of J_2 were increased linearly with increasing concentration of EOs. *Corymbia citriodora* EO was more effective than *E. camaldulensis* in inhibiting egg hatchability and suppressing J_2 viability. Probit analysis results showed median inhibitory concentration (IC_{50}) values of 412.7 and 615.9 mg/l for *C. citriodora* and *E. camaldulensis* leaves EOs, respectively. In addition, the lethal concentrations (LC) causing 50% J_2 mortality (LC_{50}) of *C. citriodora* and *E. camaldulensis* oils were 235.9 and 327.7 mg/l, for *C. citriodora* and *E. camaldulensis* leaves EOs, respectively. Essetial oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Isopulegol (53.68%), citronellol (15.26%) and isopulegol acetate (15.25%) were found to be major constituents of *C. citriodora*, while eucalyptol (55.36%) and α-pinene (14.87%) were the major components of *E. camaldulensis* leaves EO. Because of the high nematicidal performance of studied EOs, further trials are required to investigate their efficacy in controlling nematode infection and to use them as alternatives to synthetic nematicides in integrated nematode management.

Keywords: Essential oils, nematicidal activity, *Corymbia citriodora*, *Eucalyptus camaldulensis*, *Meloidogyne incognita*, gas chromatography- mass spectrometry (GC-MS).

The root-knot nematodes, *Meloidogyne* spp., are predominant and economically important soil pests attacking more than 150 host plant species in Egypt (Ibrahim *et al.*, 2010). Plants infested with root- knot nematodes have distinctive symptoms viz., chlorosis of the leaves, poor and reduced growth, and low response to fertilizers and irrigation (Rahman, 2003).

Nematode management often depends on the application of potent chemical nematicides, but due to their high cost, negative environmental and public health impacts botanical nematicides are considered as cheap, safe and environment-friendly alternatives. Plant essential oils are among the major types of botanical products used as nematicides; for the protection of plants from insects, nematodes and microorganisms. Generally, they degrade quickly and there is less possibility to kill beneficial soil microflora and microfauna than synthetic nematicides (Singh & Prassad, 2014).

Essential oils (EOs) are formed by aromatic plants as secondary metabolites; they are volatile, natural, complex compounds characterized by a strong odour. Steam or hydro-distillation method has been used for the extraction of essential oils from plants (Azmir *et al*., 2013). EOs are used in the cosmetic, preservation of foods and as therapeutics in the herbal medicine because of their antiseptic, analgesic, sedative, anti-inflammatory, spasmolytic, bactericidal, fungicidal, virucidal and anthelmintic activities (Garg, 2005; Bakkali *et al*., 2008).

During the last three decades, many plant EOs and their components have been reported to possess nematicidal properties against plant nematodes (Oka *et al*., 2000; Pérez *et al*., 2003; Bai *et al*., 2011; Faria, 2015). Therefore, the present research study was carried out to assess the nematicidal activity of the EOs extracted from fresh leaves of *Corymbia citriodora* and *Eucalyptus camaldulensis* at different concentrations on *M. incognita* under laboratory conditions.

Corymbia citriodora Hook. (syn. *Eucalyptus citriodora* Hook.) is a woody tree belonging to family Myrtaceae. It is inhabitant of Queensland, Australia and is widespread in the tropics and several other regions of the world. It is a tall, evergreen, fast-growing and graceful tree, generally known as lemon-scented gum; it has been grown for the production of essential oil, fuel wood and timber and is a source of nectar in honey production (Hill & Johnson, 1995; Hussain & Ali, 2013).

Eucalyptus camaldulensis Dehnh. (family Myrtaceae), is a big, long up to 125-160 meters and fast-growing evergreen woody tree indigenous to Australia. Eucalyptus trees do well in environments that sustain 60 ºC temperatures. They are familiar by their medicinal properties of the EO of their leaves (Sani *et al*., 2014). The *Eucalyptus* oil is a complex combination of a number of phytochemical constituents such as monoterpenes, sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. However, the exact composition and proportion varied with species (Brooker & Kleinig, 2006).

The objective of the present study was to determine the chemical composition of the essential oils of *Eucalyptus* species and to investigate their nematicidal efficacy against root-knot nematode, *M. incognita*.

Materials and Methods

Preparation of plant materials: Fresh leaves of the woody trees *Eucalyptus camaldulensis* were collected from Antoniades garden in Alexandria, and those of *Corymbia citriodora* were obtained from Shehab Mazhar botanical garden in Cairo. The leaves were gently washed by running tap water to remove dust and cut into small pieces by a stainless scissor as small particle size of the plant material help in the oil extraction efficiently (Silva *et al*., 1998).

Oils extraction: Essential oils of the studied tree species were extracted by the hydro-distillation method of herbage (approximately 1 kg fresh leaves, each) in a Clevenger apparatus (Kumar & Tripathi, 2011). Extracted EOs were gently collected into amber glass vials (ca.15ml) and stored in the refrigerator (4ºC) until proceeding of the bioassays.

Nematode inocula: The root knot-nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood was extracted from severely galled roots of the ornamental shrubs *Justicia adhatoda* (Family Acanthaceae) grown in Montaza garden, Alexandria. It was identified to the species level by the characteristics of its perineal pattern (Eisenback *et al*., 1981) and cultured on tomato plants (Gold Stone hybrid) grown in 17cm diam. plastic pots filled with a steam sterilized mixture of sand and clay (2:1) under greenhouse conditions (27±3ºC) for 60 days. Nematode eggs were extracted from tomato roots with 0.5% sodium hypochlorite (Hussey &

Barker, 1973) and the extracted eggs were either used immediately for egg hatchability test, or incubated in egg hatching plastic cups at the laboratory temperature (24±3°C) for 72 h to provide nematode J_2 for the test of juveniles viability (Al-Rajhi *et al.*, 1997).

Experimental techniques: Essential oils (EOs) of *C. citriodora* and *E. camaldulensis* were tested for their nematicidal activity towards *M. incognita* at four concentrations @125, 250, 500 and 1000 mg/l. Stock solutions of the extracted EOs were prepared by dissolving 0.5 ml of crude oil in 0.5 ml dimethylsulphoxide (DMSO) and the volume was made up to 4 ml with 0.5% Tween-80 and distilled water. These solutions were further diluted by adding the appropriate amounts of distilled water to obtain the double concentrations (250, 500, 1000 and 2000 mg/l). One milliliter of nematode juveniles suspension containing approximately 500 J_2, or 1000 nematode eggs were poured into loosely capped glass test vials (ca. 10 ml) over 1 ml of double concentrations in order to optimize the desired ones into test vials (Pandey *et al.*, 2000). An EOs-free treatment (distilled water) and a blank (DMSO+Tween-80) were prepared and inoculated with nematode J_2/eggs as previously mentioned to serve as controls. All treatments were replicated four times and kept under laboratory temperature (24±2°C).

Egg hatching was recorded after 7-days exposure to the treatments, and viability of nematode J_2 after 48 hours (Meyer *et al.*, 2006). Hatched eggs and activity of J_2 were examined under a stereo microscope using Peter's 1 ml eelworm counting slide. The inhibition (%) of egg hatching and J_2 mortality (%) were assessed as compared to the controls.

Chemical analysis of EOs: The studied EOs was chemically analyzed (GC and GC-MS) at the Institute of Graduate Studies and Research, Alexandria University.

A Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) instrument equipped with a FID and a DB-5 narrow bore column (length 10 m × 0.1 mm ID, 0.17 µm film thickness; Agilent, Palo Alto, CA, USA) was used. Helium was used as the carrier gas (flow rate of 1 ml min^{-1}), and the oven temperature program was: $^{-1}$ min^{-1}) with post run (off) at 280 °C. Oil samples (1 µl) were injected at 250 °C, with split/split-less injector (50:1 split ratio) in the split-less mode flow with 10 ml min^{-1} (Saleem *et al.*, 2014).

Gas chromatography mass spectrometry (GC-MS) was equipped with a ZB-5MS Zebron column (length 30 m × 0.25 mm ID, 0.25 µm film thickness; Agilent). Helium (average velocity 39cm s^{-1}) was used as the carrier gas and the oven temperature was held at 45°C for 2 min. then increased from 45 to 165°C (4°C $min.^{-1}$), and 165-280 °C (15 °C min^{-1}). All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer was scanned from m/z 50-500 at five scans per second. Peak area percent was used for obtaining quantitative data with the GC with HP-ChemStation software (Agilent Technologies) (Elansary & Ashmawy, 2013) without using correction factors. Identification of the EO constituents was performed on the basis of MS library search (NIST and Wiley) (Davies, 1990; Adams, 1995; Saleem *et al.*, 2016 a).

The experimental design: The bioassays were assigned as factorial experiments in complete randomized design (Steel & Torrie, 1980), where the oils are the first factor and their concentrations are the second one. Four replicates were used for each treatment. Analysis of variance (ANOVA) between treatments was statistically done by SAS software (SAS, 1997).

Prior to statistical analysis, J_2 mortality (%) was corrected using Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Mortality\%of treatment -Mortality\% of control}}{\text{100 \% Mortality of control}} \times 100$$

Later, data were subjected to the Probit analysis to estimate the inhibitory concentration (IC) of egg hatching by 50% (IC_{50}) and 95% (IC_{95}), and the lethal concentration (LC) causing juveniles mortality by 50% (LC_{50}) and 95% (LC_{95}) (Finney, 1971). Moreover, linear regression models were used to determine the relationship between tested concentrations of the oils and percentages of inhibition of egg hatching and juveniles' mortality (SAS, 1997).

Results and Discussion

Oils yield: The yield of EOs obtained by hydrodistillation of approximately 1 kg fresh leaves of *C. citriodora* and *E. camaldulensis* was 1.5% and 0.9% (w/w), respectively.

GC-MS chemical analysis of EOs: The chemical constituents of the EO of *C. citriodora* leaves by GC-MS were given in Table 1. The main components were isopulegol (53.68%), α-citronellol (15.26%), isopulegol acetate (15.25%) and eucalyptol (3.71%) as shown in Fig. 1A. These results are in conformity with the findings of earlier researchers who reported citronellal, isopulegol, citronellol, cetronellyl acetate and geraniol as the major components of the EO of *E. citriodora* (Rastogi & Mehrotra, 1998; Batish *et al*., 2006).

The chemical components of EO from leaves of *E. camaldulensis* by GC-MS were given in Table 2. The main constituents were eucalyptol (55.36%), α-pinene (14.87%), γ-terpinene (8.77%) and (−)-terpinen-4-ol (5.23%) as observed in Fig. 1B. Lima *et al*., (2013) found eucalyptol (46.74%), α-pinene (6.35%) and terpinen-4-ol (7.6%) as major components of *E. camaldulensis*. Eucalyptol exhibited antimicrobial properties against many pathogens as reported earlier (Rosato *et al*., 2007; Bakkali *et al*., 2008; Salem *et al*., 2015, 2016 b). Several authors confirmed that α-pinene,isopulegol, citronellol and eucalyptol were among the most effective terpenoids possessing high nematicidal activity (Choi *et al*., 2007; Echeverrigaray *et al*., 2010; Andrés *et al* ., 2012; Abdel-Rahman *et al*., 2013; Faria, 2015).

Egg hatchability percent and mortality of second stage juveniles: Results of the bioassays revealed that EO of *C. citriodora* was more effective than *E. camaldulensis* oil for inhibiting egg hatching and suppressed juveniles activity of *M. incognita* under laboratory conditions (Table 3). The concentration @ 1000 mg/l of *C. citriodora* oil exhibited the maximum inhibition of egg hatching (66.32%) and 100% juveniles' mortality; same trend was found with *E. camaldulensis* oil (60.05% and 96.33%, respectively). Considerable inhibition of egg hatching (49.13%) and juveniles' mortality (76.19%) were obtained by the concentration 500 mg/l of *C. citriodora* oil, while the minimum ones (33.34% and 26.7%, respectively) were given by the concentration 125 mg/l.

Inhibition of egg hatching after 7 days (Fig. 2A) and juveniles mortality after 48 days (Fig. 2B) were increased (P=0.0001) linearly with increasing concentration of the *C. citriodora* oil recording R^2 = 0.94 and 0.91, respectively. Inhibition of egg hatching after 7 days (Fig. 3A) and juveniles mortality after 48 days (Fig. 3B) were significant increased (P=0.0001) linearly with increasing concentration of the *E. camaldulensis* oil recording R^2 = 0.96 and 0.99, respectively. This result was in harmony with results obtained by Pandey *et al*., (2000) who indicated that EOs of *E. citriodora* and *E. hybrida* possess strong nematicidal properties against *M. incognita* under laboratory conditions. Batish *et al*., (2008) also reported that EO of *Eucalyptus* spp. had a strong nematicidal activity. EOs of *E. citriodora* and *E. hybrida* were toxic to *M. incognita* J_2 (Saxena *et al*., 1987). EOs of *E. camadulensis, E. saligna* and *E. urophylla* caused the mortality of *M. exigua* J_2 (Salgado *et al*., 2003). Based on results of the Probit analysis (Table 4), it was observed that the

Table 1. Chemical composition of the essential oil extracted from *Corymbia citriodora* leaves using GC-MS.

Retention Time (min.)	Compound	Area (%)
5.88	3-Thujene	0.54
6.08	1S-α-Pinene	0.24
7.15	6,6-Dimethyl-3-methylenebicyclo[3.1.1]heptane	0.72
7.30	α-Pinene	0.46
8.97	cis-Terpin hydrate	3.71
9.82	(E)-2,5-Dimethyl-1,6-octadiene	0.37
9.91	3-Carene	0.81
10.32	γ-Pyronene	0.11
10.94	Pseudolimonene	0.12
11.81	(+)-Rose oxide	0.21
12.38	Pseudoionone	0.11
13.58	Isopulegol acetate	15.25
14.00	Isopulegol	53.68
14.20	(−)-Isopulegol	1.21
14.38	4-Isopropyl-5-methylhexa-2,4-dien-1-ol	0.57
14.76	α-Terpineol	0.50
16.4	α-Citronellol	15.26
17.21	(S)-(-)-Citronellic acid, methyl esterr	0.10
17.96	Nona-2,3-dienoic acid, ethyl ester	0.12
19.75	Neomenthoglycol	1.24
19.93	Exo-2-hydroxycineole acetate	0.51
19.99	Citronellic acid	0.24
20.38	2,6-dimethyl-2,6-Octadiene,	1.67
20.51	trans-p-Menthane-3,8-diol	0.28
21.36	Geranyl acetate	0.31
21.82	cis-Jasmone	0.18
22.44	Caryophyllene	0.96
27.32	Spatulenol	0.09
27.47	Caryophyllene oxide	0.45

Table 2. Chemical composition of the essential oil extracted from *Eucalyptus camaldulensis* leaves using GC-MS.

Retention Time (min.)	Compound	Area(%)
6.90	α-Pinene	14.87
9.21	α-Phellandrene	0.54
10.05	Cosmene	0.51
10.41	Eucalyptol	55.36
11.26	γ-Terpinene	8.77
12.24	Terpinolene	1.01
12.94	Isovaleric acid, isopentyl ester	0.80
15.51	(−)-Terpinen-4-ol	5.23
15.77	p,α,α-Trimethylbenzyl alcohol	0.44
16.00	α-Terpineol	2.88
17.38	α-Citronellol	0.30
19.50	Carvacrol	0.35
24.48	Aromadendrene	0.52
25.17	(-)-Alloaromadendrene	0.16
27.89	Elemol	0.16
28.19	Ledol	0.26
28.94	Globulol	1.81
29.15	Ledol	0.48
30.03	β-Eudesmol	0.47
30.90	a-Eudesmol	0.15

nematicidal activity of *C. citriodora* oil was more effective on viability of juveniles (LC_{50} = 233.8 mg/l and LC_{95} = 920.7 mg/l) than on eggs of *M. incognita* (IC_{50} and IC_{95} = 412.6 and 26588 mg/l). Consequently, our bioassay results were very similar to those obtained by Zia-Ul-Haq *et al*., (2010) and El-Sherbiny & Zein El-Din (2012). Moreover, the same result with *E. camaldulensis* oil was also found more effective on juveniles (LC_{50} = 324.2 mg/l and LC_{95} = 1664.3 mg/l) than on eggs of *M. incognita* (IC_{50} and IC_{95} = 615.3 and 68695.1 mg/l, respectively).

Conclusion

Essential oils of *C. citriodora* and *E. camaldulensis* offer a promising and strong nematicidal activity towards *M. incognita* under laboratory (*in vitro*) conditions. They may use as safe alternatives to the chemical nematicides in nematode management. It was well documented that the mode of action of synthetic nematicides against plant nematodes is due to disruption of the transport signal in the neurosystem by inhibition of acetylcholinesterase (AChE): an enzyme essential in the breakdown of acetylcholin, which is the transmitter substance for the signals to the muscular system. Consequently, the acetylcholin accumulates, which results in convulsion, paralysis and finally death of nematode (Opperman & Chang, 1990). Sadraei *et al*., (2003) found that essential oil of the medicinal plant *Ferula assafoetida* significantly inhibited AChE activity of experimental rats subjected to a study on its spasmolytic activity. Therefore, it is proposed, that mode of action of EOs of

Table 3. Nematicidal effect of two essential oils of *Corymbia citriodora* and *Eucalyptus camaldulensis* on egg hatchability (%) and the mortality (%) of second stage juveniles of *Meloidogyne incognita* under *in vitro* conditions.

Oil (O)	Concentration at mg/l (C)						
	Control (Distilled water)	Blank (DMSO+Tween-80)	125	250	500	1000	Overall mean (O)
Egg hatchability (%) 7 days after treatment							
C. citriodora	219.0 ^{a} ± 4.69	215.25 ^{a} ± 6.24	143.5 ^{b} ± 2.08 (33.34%)*	125.25 ^{c} ± 9.32 (41.82%)	109.5 ^{d} ± 7.33 (49.13%)	72.5 ^{f} ± 6.35 (66.32%)	147.50 ^{B}
E. camaldulensis			148.0 ^{b} ± 2.94 (31.24%)	137.50 ^{b} ± 2.08 (36.13%)	123.0 ^{c} ± 9.06 (42.86%)	86.0 ^{c} ± 3.37 (60.05%)	154.79 ^{A}
Overall mean (C)	219.0 ^{A}	215.25 ^{A}	145.75 ^{B}	131.38 ^{C}	116.25 ^{D}	79.25 ^{E}	
Mortality(%) of second stage juveniles (J_2) 48 h after treatment							
C. citriodora	0.74 ^{e} ± 0.94	2.38 ^{e} ± 0.51	26.7 ^{e} ± 3.50	48.6 ^{c} ± 6.21	76.19 ^{b} ± 1.70	100.00 ^{a} ± 0.0	42.42 ^{A}
E. camaldulensis			22.3 ^{e} ± 3.94	36.71 ^{d} ± 2.23	52.00 ^{c} ± 3.56	96.33 ^{a} ± 2.23	35.10 ^{B}
Overall mean (C)	0.74 ^{E}	2.38 ^{E}	24.5 ^{D}	43.04 ^{C}	64.1 ^{B}	98.16 ^{A}	

Data are average of four replicates. Values (No. J_2/ml ± SD & Percentages of J_2 mortality ± SD) within a column/row superscripted by the same letter(s) are not significantly different at *P*= 0.05;* Inhibition (%) of egg hatching after using Abbott's formula (Abbott, 1925), where distilled water and blank used as controls; LSD of (O) = 3.44, LSD of (C) = 5.95 and LSD of (O*C) = 10.67. (Percentages of J_2 mortality ± SD) within a column/row superscripted by the same letter(s) are not significantly different at *P*= 0.05; LSD of (O) = 1.64, LSD of (C) = 2.84 and LSD of (O*C) = 24.01.

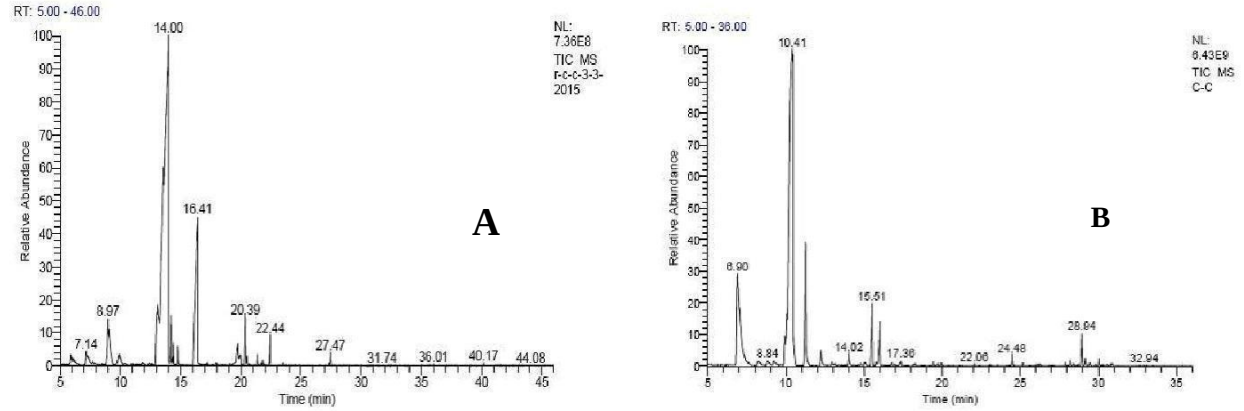

Fig. 1.(A-B). GC-MS chromatogram of chemical components presents in essential oil extracted from (A) *Corymbia citriodora* and (B) *Eucalyptus camaldulensis* leaves

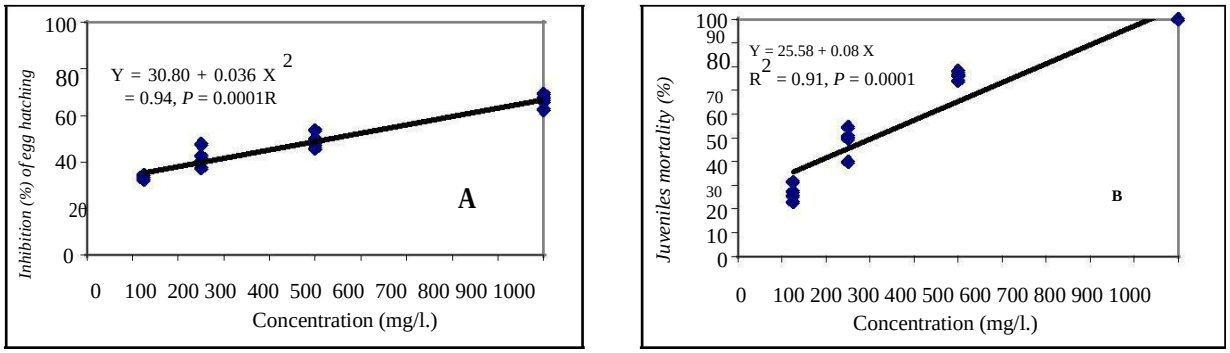

Fig. 2 (A, B). A. Inhibition (%) of egg hatching after 7 days; B. Corrected mortality % of *Meloidogyne incognita* after 48 days exposed to different concentrations of *Corymbia citriodora* essential oil under *in vitro* conditions.

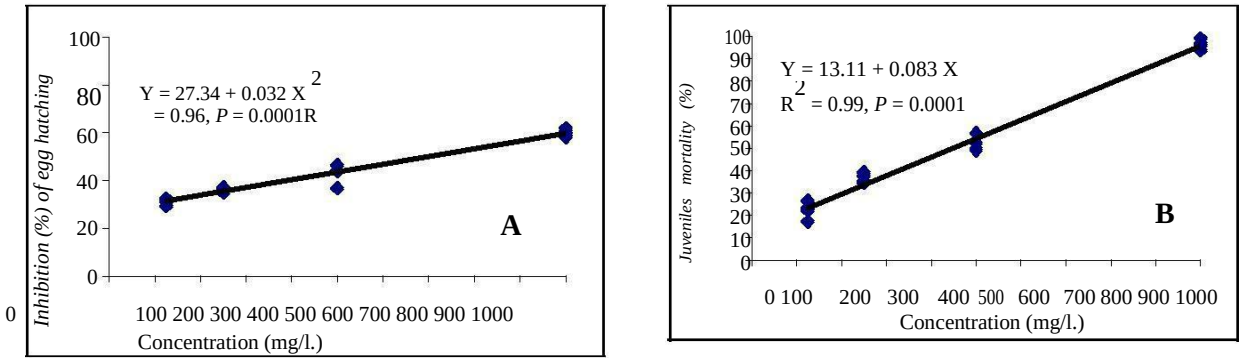

Fig. 3 (A, B). A. Inhibition (%) of egg hatching after 7 days; B. Corrected mortality % of *Meloidogyne incognita* after 48 days exposed to different concentrations of *Eucalyptus camaldulensis* essential oil under *in vitro* conditions.

Table 4. Probit analysis of the nematicidal activity of essential oils extracted from leaves of *Corymbia citriodora* and *Eucalyptus camaldulensis* on *Meloidogyne incognita* under laboratory conditions.

Oil	Analyzed conc.	Value (mg/l)	Fiducial limits* Lower	Fiducial limits* upper	Slope	Chi-sq
Inhibition (%) of egg hatching of *Meloidogyne incognita*						
C. citriodora	IC_{50}	412.7	299.0	570.1	0.908 ± 3.671E-02	1.018
	IC_{95}	26625.2	4392.9	165406.5		
E. camaldulensis	IC_{50}	615.9	399.0	954.3	0.804 ± 3.673E-02	1.543
	IC_{95}	68587.3	5833.1	837954.3		
Mortality (%) of J_2 of *Meloidogyne incognita*						
C. citriodora	LC_{50}	235.9	208.1	267.3	2.789 ± 6.533E-02	8.275
	LC_{95}	916.9	720.3	1168.4		
E. camaldulensis	LC_{50}	327.7	286.4	375.2	2.33 ± 0.050	19.98
	LC_{95}	1659.2	1198.2	2301.3		

Inhibitory concentration (IC), Lethal concentration (LC).
* Probit analysis values.

C. citriodora and *E. camaldulensis* against *M. incognita* in the current study may be due to the inhibition of AChE of nematode. Similarly, it was suggested that the components of EOs might involve in interrupting the nematode nervous system (Oka *et al.*, 2000). Andrés *et al*., (2012) reviewed other modes of action of EOs towards nematodes. It is concluded that further trials have to be conducted for the study of the toxicity of these EOs and their major constituents against nematodes that could be used as alternatives to chemical nematicides in integrated nematode management.

Acknowledgments

Authors would like to thank and appreciate efforts of Prof. Dr. A.M. Ebieda (Sugar Crops Research Institute, Agricultural Research Center, Egypt) in the Probit analysis of the bioassay tests.

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267.

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.

Adams, R. P. (1995). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Allured Publishing Corporation, Carol Stream, IL, USA.

Al-Rajhi, D. H., Al-Hazmi, A. S., Hussein, H. I., Ibrahim, A. A. M., Al-Yahya, F. A. & Mostafa, S. (1997). Nematicidal properties of *Rhazya stricta* and *Juniperus polycarpos* on *Meloidogyne javanica* in Saudi Arabia. *Alexandria Science Exchange*, 18, 135-142.

Andrés, M. F., Coloma, A. G., Sanz, J., Burillo, J. & Sainz, P. (2012). Nematicidal activity of essential oils: a review. *Phytochemistry Reviews*, 11, 371-390.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Shahina, F., Jahurul M. H. A., Ghafoor, K., Norulaini, N. A. N. & Omar, A. K. M. (2013) Techniques for extraction of bioactive compounds from plant materials. A review. *Journal of Food Engineering*, 117, 426-436. doi.org/10.1016/j.jfoodeng.2013.01.014

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils: a review. *Food and Chemical Toxicology*, 46, 446-475.

Bai, C. Q., Liu, Z. L. & Liu, Q. Z. (2011). Nematicidal constituents from the essential oil of *Chenopodium ambrosioides* aerial parts. *E-Journal of Chemistry*, 8 (S1), 143-148.

Batish, D. R., Singh H. P., Kohli, R. K. & Kaur, S. (2008). *Eucalyptus* essential oil as a natural pesticide. *Forest Ecology and Management*, 256, 2166-2174.

Batish, D. R., Singh, H. P., Setia, N., Kaur, S. & Kohli, R. K. (2006). Chemical composition and phytotoxicity of volatile essential oils from intact and fallen leaves of *Eucalyptus citriodora*. *Zeitschrift für Naturforschung* C, 61, 465-471.

Brooker, M. I. H. & Kleinig, D. A. (2006). *Field guide to Eucalyptus. vol.1*. South-eastern Australia, Third edition. Bloomings, Melbourne, 356 pp.

Choi, I. H., Kim, J., Shin, S. C. & Park, I. K. (2007). Nematicidal activity of monoterpenoids against the pinewood nematode (*Bursaphelenchus xylophilus*). *Russian Journal of Nematology*, 15, 35-40

Davies, N. W. (1990). Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20M phases. *Journal of Chromatography*, 503, 1-24.

Elansary, H. O. & Ashmawy, N. A. (2013). Essential oils of mint between benefits and hazards. *Journal of Essential Oil Bearing Plants*, 16, 429-438.

El-Sherbiny, A. A. & Zein El-Din, M. M. (2012). Nematicidal properties of *Ferula assafoetida* resin and its possible use in management of the root-knot nematode, *Meloidogyne incognita* on eggplants. *Egyptian Journal of Agronematology*, 11, 231-245.

Echeverrigaray, S., Zacaria, J. & Beltrão, R. (2010). Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathology*, 100, 199-203. doi: 10.1094/PHYTO-*100*-2-0199.

Eisenback, J. D., Hirschmann, H., Sasser, J. N. & Triantaphyllou, A. C. (1981). *A guide to the four most common species of root-knot nematodes (Meloidogyne spp.), with a pictorial key*. A Cooperative Publication of the Department of Plant Pathology and Genetics, North Carolina State University and USAID, Raleigh, North Carolina, USA.

Faria, J. M. S. (2015). *Essential oils as anti-nematode agents and their influence on in vitro nematode/plant co-cultures*. A Ph. D. thesis, University of Lisbon, Faculty of Sciences, Department of Plant Biology, Portugal, 290 pp.

Finney, D. J. (1971). *Probit analysis*. 3rd edition, Cambridge University Press, England, 318 pp.

Garg, S. C. (2005). Essential oils as therapeutics. *Natural Product Radiance*, 4, 18-26.

Hill, K. D. & Johnson, L. A. S. (1995). Systematic studies in eucalyptus. 7. A revision of the bloodwoods genus *Corymbia*. *Telopea*, 6, 185-504.

Hussain, S. S. & Ali, M. (2013). Volatile oil constituents of the leaves of *Eucalyptus citriodora* and influence on clinically isolated pathogenic microorganisms. *Journal of Scientific and Innovative Research*, 2, 852-858.

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, 1925-1928.

Ibrahim, I. K. A., Mokbel, A. A. & Handoo, Z. A. (2010). Current status of phytoparasitic nematodes and their host plants in Egypt. *Nematropica*, 40, 239-262.

Kumar, R. & Tripathi, Y. C. (2011). Getting fragrance from plants. In: *Training Manual on Extraction Technology of Natural Dyes & Aroma Therapy and Cultivation Value Addition of Medicinal Plants*, 1st edition, Forest Research Institute, Dehradun (India), 77-102 pp.

Lima, L. M., Babakhani, B., Boldaji, S. A. H., Asadi, M. & Boldaji, R. M. (2013). Essential oils composition and antibacterial activities of *Eucalyptus camaldulensis* Dehn. *International Journal of Medicinal and Aromatic Plants*, 3, 214-219.

Meyer, S. L. F., Zasada, I. A., Roberts, D. P., Vinyard, B. T., Lakshman, D. K., Lee, J. K., Chitwood, D. J. & Carta, L. K. (2006). *Plantago lanceolata* and *P. rugelii* extracts are toxic to *Meloidogyne incognita* but not to certain microbes. *Journal of Nematology*, 38, 333-338.

Oka, Y., Nacar, S., Putievsky, E., Ravid, U., Yaniv, Z. & Spiegel, Y. (2000). Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology*, 90, 710-715. doi: 10.1094/PHYTO.2000.90.7.710.

Opperman, C. H. & Chang, S. (1990). Plant-parasitic nematodes acetylcholinesterase inhibition by carbamate and organophosphate nematicides. *Journal of Nematology*, 22, 481-488.

Pandey, R., Kalra, A., Tandon, S., Mehrotra, N., Singh, H. N. & Kumar, S. (2000). Essential oils as potent sources of nematicidal compounds. *Journal of Phytopathology*, 148, 501-502. DOI: 10.1046/j.1439-0434.2000.00493

Pérez, M. P., Navas-Cortés, J. A., Pascual-Villalobos, M. J. & Castillo, P. (2003). Nematicidal activity of essential oils and organic amendments from Asteraceae against root-knot nematodes. *Plant Pathology*, 52, 395-401. DOI: 10.1046/j.1365-3059.2003.00859

Rahman, L. (2003). *Root knot disease and its control*. Agfact AB.1, third edition.

Rastogi, R. P. & Mehrotra, B. N. (1998). *Compendium of Indian medicinal plants*, Vol. 5, CDRI, Lucknow and NISCAIR, New Delhi, India.

Rosato, A., Vitali, C., De Laurentis, N., Armenise, D. & Milillo, M. (2007). Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine*, 14, 727-732.

Sadraei, H., Ghannadi, A. & Malekshahi, K. (2003). Compostion of the essential oil of asa-foetida and its spasmolytic action. *Saudi Pharmaceutical Journal*, 11, 136-140.

Salgado, S. L. M., Campos, V. P., Cardos, M. D. G. & Salgado, A. P. S (2003). Hatching and mortality of second-stage juveniles of *Meloidogyne exigua* in essential plant oils. *Nematol. Brasil*., 27, 17-22.

Salem, M. Z. M., Abdel-Majeed, A. & Hayssam, M. A. (2014). Stem wood and bark extracts of *Delonix regia* (Boj. Ex. Hook): Chemical analysis and antibacterial, antifungal and antioxidant properties. *BioResources*, 9, 2382-2395.

Salem, M. Z. M., Ashmawy, N. A., Elansary, H. O. & El-Settawy, A. A. (2015). Chemotyping of diverse *Eucalyptus* species grown in Egypt and antioxidant and antibacterial activities of its respective essential oils. *Natural Product Research*, 29, 681-685.

Salem, M. Z. M., Zayed, M. Z., Ali, H. M. & Abd El-Kareem, M. S. M. (2016a). Chemical composition, antioxidant and antibacterial activities of extracts from *Schinus molle* wood branch growing in Egypt. *Journal* of *Wood Science*, 62, 548-561. DOI 10.1007/s10086-016-1583-2

Salem, M. Z. M., Zidan, E. Y., Mansour, M. A. M., El-Hadidi, M. N. N. & Abo Elgat, A. A. W. (2016b). Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi. *International*

Biodeterioration and Biodegradation, 106, 88-96.

Sani, I., Abdulhamid, A. & Bello, F. (2014). *Eucalyptus camaldulensis*: Phytochemical composition of ethanolic and aqueous extracts of the leaves, stem, bark, root fruits and seeds. *Journal of Scientific and Innovative Research*, 3, 523-526.

SAS, (1997). *SAS/STAT User's Guide*: *Statistics*, *Version 6.12*. SAS Institute Inc. Cary, NC., USA.

Saxena, D. B., Goswami, B. K. & Tomar, S. S. (1987). Nematicidal activity of some EOs against *Meloidogyne incognita*. *Indian Perfume*, 31, 150-154.

Silva, G. L., Lee, I. & Kinghorn, A. D. (1998). Special problems with the extraction of plants. In: Cannell, R. J. P. (Eds). *Natural Products Isolation*, Humana Press Inc., Totowa, New Jersey, USA, 343-363 pp.

Singh, A. U. & Prassad, D. (2014). Management of plant-parasitic nematodes by the use of botanicals. *Journal of Plant Physiology and Pathology*, 2, 1. Doi.org/10.4172/2329-955X.1000116.

Steel, R. G. D. & Torrie, T. H. (1980). *Principles and procedures of statistics*. 2nd edition. Mc Graw Hill, New York, USA.

Zia-ul-Haq, M., Ahmad, M. & Akhter, M. (2010). Nematicidal activity of selected flora of Pakistan. *Pakistan Journal of Botany*, 42, 2119-2123.

(Accepted: Feb 2, 2016)