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Profiling the Variability of *Eucalyptus* Essential Oils with Activity against the Phylum Nematoda †

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Abstract: The genus *Eucalyptus* (Myrtaceae) comprises more than 800 species, mostly native to Australia. *Eucalyptus* shoots essential oils (EOs) are well-known for their extremely high qualitative and quantitative variation in terpenes (mainly mono- and sesquiterpenes). These EOs have a wide range of uses, from the taxonomic characterization of populations based on the chemical profiling of EO chemotypes, to industrial applications, including pharmaceutical, agrochemical, and in food and fragrances. In this study, we reviewed the available information concerning the chemical variability of EOs from *Eucalyptus* spp. assayed against nematodes. Among the most active EOs, those from *E. globulus*, *E. staigeriana*, and *E. citriodora* were more frequently used. EO chemical composition was mainly dominated by 1,8-cineole, limonene, *p*-cymene, citronellal and piperitone in varying proportions. Nematicidal activity of *Eucalyptus* EOs was reported against animal-parasitic nematodes, including gastrointestinal nematodes (e.g., *Haemonchus contortus*), plant-parasitic nematodes, such as root-knot nematodes (e.g., *Meloidogyne incognita* and *M. chitwoodi*) or the pinewood nematode *Bursapelenchus xylophilus*, and the free-living nematode *Caenorhabditis elegans*. Correlation between EO qualitative and quantitative composition with its respective activity may provide valuable information on the nematicidal specificity of EOs. This knowledge can be useful for devising environmentally safer pest management strategies in the conservation of ecosystems biodiversity.

Keywords: Bursapelenchus xylophilus; Caenorhabditis elegans; chemical profiling; essential oil; Eucalyptus; Haemonchus contortus; Meloidogyne chitwoodi; Meloidogyne incognita; nematicides; sustainable pest management

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1. Introduction

Essential oils (EOs) are volatile mixtures, exclusively obtained from plant material by hydro-, steam- or dry- distillation, or in the case of *Citrus* fruits, mechanically without heating [1]. These volatile mixtures are mainly composed of terpenes (mono-, sesqui-, and di-terpenes) and phenylpropanoids, and are usually dominated by one to three major components at relatively high amounts [2]. The genus *Eucalyptus* (Myrtaceae family) comprises more than 800 species, mostly native to Australia. *Eucalyptus* spp. have been extensively explored in the pharmaceutical and cosmetic industries due to their terpene-rich essential oils (EOs), namely high quantities of 1,8-cineole (also referred as Eucalyptol), an oxygenated monoterpenoid used extensively in flavorings, fragrances and cosmetics [3]. In traditional medicine, eucalypt leaves and 1,8-cineole are generally used as cough suppressants.

Eucalyptus spp. are well-known for the high foliar terpene qualitative and quantitative variations, at the *taxa*, population, and individual levels, and a large number of chemotypes have been identified [4–9]. Terpenes are a large class of secondary metabolites, with an important ecological role in mediating plant-plant and plant-animal interactions. In the Myrtaceae family, terpenes have been mostly implicated in defensive roles against herbivores and pathogens [7]. EO chemotypes are defined by qualitative and quantitative differences in the EO chemical composition among populations of the same species, due to genetic variations in the regulation of terpene biosynthesis [4,10,11]. The occurrence of EO chemotypes has been strongly associated with geographical variation and can reflect the different environmental conditions to which plants are exposed (e.g., altitude, solar exposition, or soil type) [1]. In addition, the frequent occurrence of EO chemotypes within *Eucalyptus* spp. could implicate differences in biological activities and should be carefully evaluated. *Eucalyptus* EOs have a wide range of biological activities including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal [12]. The phylum Nematoda (or Nemathelminthes) comprise roundworms and eelworms (parasites of plants). Nematodes are present in every ecosystem being found in freshwater, marine and terrestrial environments, and can be parasitic (parasites of plants, insects, humans and other animals) or non-parasitic (free-living). They are vermiform, unsegmented, bilaterally symmetrical pseudocelomates, having a pseudocoelom lined with mesoderm, in one side, and with endoderm on the other side. The nematode body is protected by the cuticle, a very complex and evolutionarily plastic structure that functions as protection and is involved in body movement and maintaining shape. Nematodes have digestive, reproductive, nervous and excretory systems, but lack circulatory or respiratory systems [13].

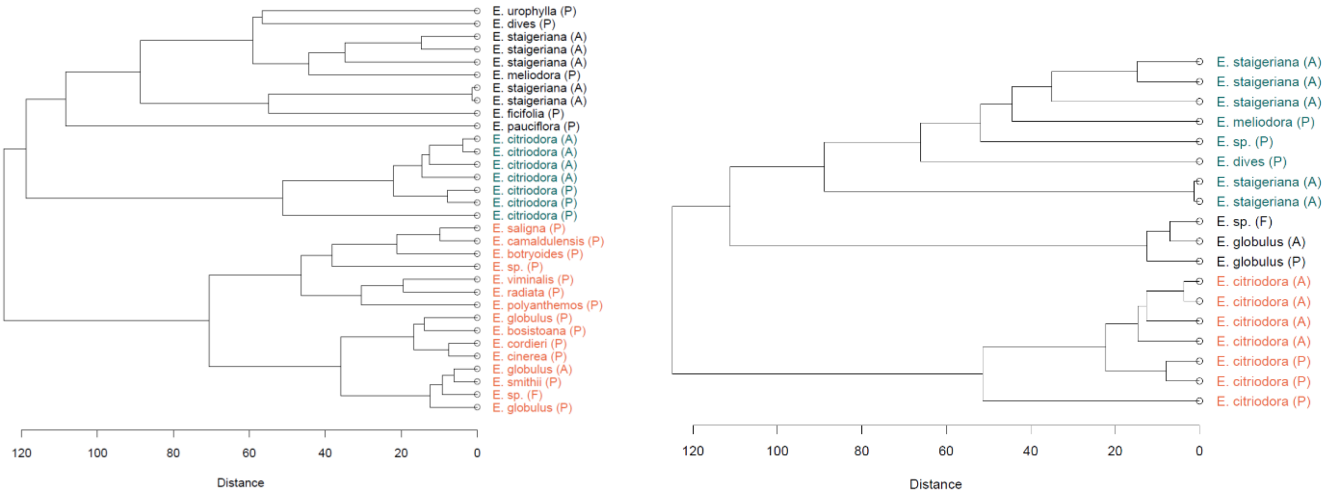
In this study, we reviewed the available information concerning the chemical variability of EOs from *Eucalyptus* spp. assayed against nematodes (phylum Nematoda). Research was performed with Web of Science® search engine, in all available databases, on published works reporting the composition of EOs used against nematodes, using the topics "*Eucalyptus*", "nematode" and "essential oil". Information on the *Eucalyptus* species and EO composition (≥1%) was collected when available. A total of 17 publications was retrieved reporting on the nematicidal activity of eucalypt EOs [14–30]. These reports were published in journals dedicated to Parasitology (60%), Veterinary Sciences (47%) and Agronomy (20%). Publications dated from 2006 to 2021, with 2011, 2013 and 2015 being the years with the most publications. The listed publications were cited 350 times, with an average of 23.3 citations per work. The citing reports were published in journals specialized in Parasitology (22%), Veterinary Sciences (16%), Food Science Technology (11%) and Plant Sciences (11%).

2. Chemical Variability of *Eucalyptus* Essential Oils

Chemical composition was reported for a total of 32 EOs tested against plant-parasitic (21), animal-parasitic (10) and free-living nematodes (1). *E. citriodora*, *E. globulus* and *E. staigeriana* were the most studied *Eucalyptus* species, with seven, three and five EOs tested, respectively. Cluster analysis was performed to determine the similarity between the composition of the tested EOs. Three main clusters were obtained (Figure 1a). EOs from *E. citriodora* were grouped in one single cluster, revealing a high similarity, and low variability, between EO composition. *E. citriodora* EO main components were citronellal and isopulegol. EOs from *E. globulus* were grouped together with EOs from *E. saligna*, *E. camaldulensis*, *E. botryoides*, *E. viminalis*, *E. polyanthemos*, *E. bosistoana*, *E. cordieri*, *E. cinerea*, *E. smithii* and two unidentified *Eucalyptus* species. EOs from *E. globulus* revealed an extremely high degree of similarity with *E. smithii*, *E. bosistoana*, *E. cordieri* and *E. cinerea*. All *Eucalyptus* spp. included in this cluster were rich in 1,8-cineole and α-pinene. The remaining cluster comprised the EOs from *E. staigeriana* and *E. urophylla*, *E. dives*, *E. meliodora*, *E. ficifolia* and *E. pauciflora*. The composition of the EO from these species showed high variability. *E. ficiflora* and *E. pauciflora* EOs were rich in α-pinene and limonene (only for *E.*

ficiflora); *E. meliodora* EO contained mainly *p*-cymene, 1,8-cineole, and cryptone; *E. urophylla* EO contained mainly α-phellandrene and 1,8-cineole; *E. dives*

EO contained, piperitone, α-phellandrene, and *p*-cymene; and *E. staigeriana* showed a high variability in EO composition, with three EOs rich in limonene and the remaining two EO were rich in geranial, geraniol and methyl geranate.



(**a**) (**b**)

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition (≥1%) of all *Eucalyptus* essential oils reported (**a**) and of those with activity against nematodes (**b**), based on distance and using unweighted pair-group method with arithmetic average (UPGMA) method. A—animal-parasitic nematodes, P—plant-parasitic nematodes and F—free-living nematodes.

3. Chemical Composition of Active EOs

A total of 18 EOs were reported with a high activity against nematodes. Cluster analysis revealed three major clusters, with *E. citriodora* EOs included in one single cluster, *E. globulus* and one unidentified *Eucalyptus* sp. grouped together in another cluster, and *E. staigeriana* clustered together with *E. dives* and one unidentified *Eucalyptus* sp. (Figure 1b). No correlation could be established between EO clustering and activity against specific nematode groups. Despite the small number of reported nematicidal chemically characterized EOs some preliminary considerations could be made regarding activity against the groups of nematodes analyzed. Only one EO was tested against free-living nematodes, thus no substantial comparison can be made with the remaining nematode types. The oxygenated monoterpene 1,8-cineole could be found in EOs active against all nematode types, in amounts ≥20% (Table 1, Figure 2). The hydrocarbon monoterpene *p*-cymene was found in amounts ≥20% in EOs active against plant-parasitic nematodes while the hydrocarbon monoterpene limonene was only found in amounts ≥20% in EOs active against animal-parasitic nematodes.

Table 1. Minimum and maximum percentages of main components (≥20%) of *Eucalyptus* EOs with activity against plant-parasitic nematodes, animal-parasitic nematodes and free-living nematodes.

EO Components (%)	Plant Parasitic Nematodes	Animal Parasitic Nematodes	Free Living Nematodes
p-Cymene	3.1–25.0	2.6	3.8
1,8-Cineole	1.3–91.5	1.7–83.9	82.6
Limonene	-	7.0-72.9	7.7
Citronellal	35.8–83.8	5.5–71.8	-
Piperitone	40.2	-	-

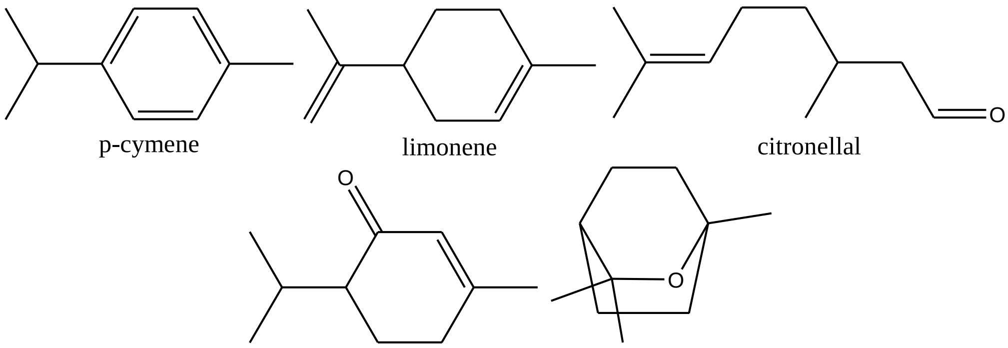


Figure 2. Chemical structures of the main compounds found on nematicidal *Eucalyptus* essential oils.

The monoterpenic aldehyde citronellal was found in amounts ≥20% in EOs active against plant- and animal-parasitic nematodes while the ketone piperitone was only found in amounts ≥20% in EOs active against plant-parasitic nematodes (Table 1, Figure 2).

4. Conclusions

Chemical variability is an important trait of *Eucalyptus* EOs. In this study, EO composition from *Eucalyptus* spp. assayed against nematodes was reviewed. Cluster analysis grouped EOs from *E. citriodora* and *E. globulus* in two clusters based on the EO main components, namely citronellal and 1,8-cineole, respectively. *E. staigeriana* EO showed high variability in the EO main components, namely limonene, geranial, geraniol and methyl geranate. Concerning nematicidal EOs, *p*-cymene and 1,8-cineole were ubiquitously present, yet high proportions of limonene and piperitone were exclusive to animal- or plant-parasitic nematodes, respectively. Citronellal showed high relative amounts for both animal- and plant-parasitic nematodes. Although supported by very few data, some chemical trends seem to indicate that specificity may occur for nematicidal EOs. A greater number of studies is necessary to understand how *Eucalyptus* EO chemical variability can influence nematicidal activity. This knowledge can be very valuable for the establishment of precision biocides with neutral environmental impacts.

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References

1. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226, doi:10.1002/ffj.1875.
2. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475, doi:10.1016/j.fct.2007.09.106.
3. Pino, J.A.; Marbot, R.; Quert, R.; García, H. Study of essential oils of *Eucalyptus resinifera* Smith, *E. tereticornis* Smith and *Corymbia maculata* (Hook.) K. D. Hill & L. A. S. Johnson, grown in Cuba. *Flavour Fragr. J.* **2002**, *17*, 1–4, doi:10.1002/ffj.1026.

4. Keszei, A.; Brubaker, C.L.; Foley, W.J. A molecular perspective on terpene variation in Australian Myrtaceae. *Aust. J. Bot.* **2008**, *56*, 197–213, doi:10.1071/BT07146.
5. Keszei, A.; Brubaker, C.L.; Carter, R.; Köllner, T.; Degenhardt, J.; Foley, W.J. Functional and evolutionary relationships between terpene synthases from Australian Myrtaceae. *Phytochemistry* **2010**, *71*, 844–852, doi:10.1016/j.phytochem.2010.03.013.
6. Andrew, R.L.; Keszei, A.; Foley, W.J. Intensive sampling identifies previously unknown chemotypes, population divergence and biosynthetic connections among terpenoids in *Eucalyptus tricarpa*. *Phytochemistry* **2013**, *94*, 148–158, doi:10.1016/j.phytochem.2013.05.002.
7. Padovan, A.; Keszei, A.; Külheim, C.; Foley, W.J. The evolution of foliar terpene diversity in Myrtaceae. *Phytochem. Rev.* **2014**, *13*, 695–716, doi:10.1007/s11101-013-9331-3.
8. Bustos-Segura, C.; Dillon, S.; Keszei, A.; Foley, W.J.; Külheim, C. Intraspecific diversity of terpenes of *Eucalyptus camaldulensis* (Myrtaceae) at a continental scale. *Aust. J. Bot.* **2017**, *65*, 257, doi:10.1071/BT16183.
9. Faria, J.M.S.; Lima, A.S.; Mendes, M.D.; Leiria, R.; Geraldes, D.A.; Figueiredo, A.C.; Trindade, H.; Pedro, L.G.; Barroso, J.G.; Sanches, J. *Eucalyptus* from Mata Experimental do Escaroupim (Portugal): Evaluation of the essential oil composition from sixteen species. *Acta Hortic.* **2011**, *925*, 61–66, doi:10.17660/ActaHortic.2011.925.7.
10. Figueiredo, A.C. Biological properties of essential oils and volatiles: Sources of variability. *Nat. Volatiles Essent. Oils* **2017**, *4*, 1–13.
11. Rodrigues, A.M.; Mendes, M.D.; Lima, A.S.; Barbosa, P.M.; Ascensão, L.; Barroso, J.G.; Pedro, L.G.; Mota, M.M.; Figueiredo, A.C. *Pinus halepensis*, *Pinus pinaster*, *Pinus pinea* and *Pinus sylvestris* essential oils chemotypes and monoterpene hydrocarbon enantiomers, before and after inoculation with the pinewood nematode *Bursaphelenchus xylophilus*. *Chem. Biodivers.* **2017**, *14*, e1600153, doi:10.1002/cbdv.201600153.
12. Batish, D.R.; Singh, H.P.; Kohli, R.K.; Kaur, S. Eucalyptus essential oil as a natural pesticide. *For. Ecol. Manag.* **2008**, *256*, 2166–2174, doi:10.1016/j.foreco.2008.08.008.
13. Basyoni, M.M.A.; Rizk, E.M.A. Nematodes ultrastructure: Complex systems and processes. *J. Parasit. Dis.* **2016**, *40*, 1130–1140, doi:10.1007/s12639-015-0707-8.
14. Ibrahim, S.K.; Traboulsi, A.F.; El-Haj, S. Effect of essential oils and plant extracts on hatching, migration and mortality of Meloidogyne incognita. *Phytopathol. Mediterr.* **2006**, *45*, 238–246, doi:10.14601/Phytopathol_Mediterr-1828.
15. Macedo, I.T.F.; Bevilaqua, C.M.L.; Oliveira, L.M.B. de; CamurÇa-Vasconcelos, A.L.F.; Vieira, L. da S.; Oliveira, F.R.; Queiroz-Junior, E.M.; Portela, B.G.; Barros, R.S.; Chagas, A.C.S. Atividade ovicida e larvicida in vitro do óleo essencial de *Eucalyptus globulus* sobre *Haemonchus contortus*. *Rev. Bras. Parasitol. Vet.* **2009**, *18*, 62–66, doi:10.4322/rbpv.01803011.
16. Macedo, I.T.F.; Bevilaqua, C.M.L.; de Oliveira, L.M.B.; Camurça-Vasconcelos, A.L.F.; Vieira, L. da S.; Oliveira, F.R.; Queiroz-Junior, E.M.; Tomé, A. da R.; Nascimento, N.R.F. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. *Vet. Parasitol.* **2010**, *173*, 93–98, doi:10.1016/j.vetpar.2010.06.004.
17. Macedo, I.T.F.; Bevilaqua, C.M.L.; Oliveira, L.M.B. de; Camurça-Vasconcelos, A.L.F.; Vieira, L. da S.; Amóra, S. dos S.A. Evaluation of *Eucalyptus citriodora* essential oil on goat gastrointestinal nematodes. *Rev. Bras. Parasitol. Vet.* **2011**, *20*, 223–227, doi:10.1590/S1984-29612011000300009.
18. Ntalli, N.G.; Ferrari, F.; Giannakou, I.; Menkissoglu-Spiroudi, U. Phytochemistry and nematicidal activity of the essential oils from 8 greek lamiaceae aromatic plants and 13 terpene components. *J. Agric. Food Chem.* **2010**, *58*, 7856–7863, doi:10.1021/jf100797m.
19. De Aquino Mesquita, M.; e Silva Júnior, J.B.; Panassol, A.M.; de Oliveira, E.F.; Vasconcelos, A.L.C.F.; de Paula, H.C.B.; Bevilaqua, C.M.L. Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes. *Parasitol. Res.* **2013**, *112*, 3161–3165, doi:10.1007/s00436-013-3492-2.
20. Ribeiro, W.L.C.; Macedo, I.T.F.; dos Santos, J.M.L.; de Oliveira, E.F.; Camurça-Vasconcelos, A.L.F.; de Paula, H.C.B.; Bevilaqua, C.M.L. Activity of chitosan-encapsulated *Eucalyptus staigeriana* essential oil on Haemonchus contortus. *Exp. Parasitol.* **2013**, *135*, 24–29, doi:10.1016/j.exppara.2013.05.014.
21. Ribeiro, J.C.; Ribeiro, W.L.C.; Camurça-Vasconcelos, A.L.F.; Macedo, I.T.F.; Santos, J.M.L.; Paula, H.C.B.; Araújo Filho, J.V.; Magalhães, R.D.; Bevilaqua, C.M.L. Efficacy of free and nanoencapsulated *Eucalyptus citriodora* essential oils on sheep gastrointestinal nematodes and toxicity for mice. *Vet. Parasitol.* **2014**, *204*, 243–248, doi:10.1016/j.vetpar.2014.05.026.
22. Laquale, S.; Candido, V.; Avato, P.; Argentieri, M.P.; D'Addabbo, T. Essential oils as soil biofumigants for the control of the root-knot nematode *Meloidogyne incognita* on tomato. *Ann. Appl. Biol.* **2015**, *167*, 217–224, doi:10.1111/aab.12221.
23. Ribeiro, W.L.C.; Camurça-Vasconcelos, A.L.F.; Macedo, I.T.F.; dos Santos, J.M.L.; de Araújo-Filho, J.V.; de Carvalho Ribeiro, J.; de Abreu Pereira, V.; de Araújo Viana, D.; de Paula, H.C.B.; Bevilaqua, C.M.L. In vitro effects of Eucalyptus staigeriana nanoemulsion on *Haemonchus contortus* and toxicity in rodents. *Vet. Parasitol.* **2015**, *212*, 444–447, doi:10.1016/j.vetpar.2015.07.019.

24. Ribeiro, W.L.C.; Camurça-Vasconcelos, A.L.F.; dos Santos, J.M.L.; Macedo, I.T.F.; de Carvalho Ribeiro, J.; de Oliveira, E.F.; de Paula, H.C.B.; Bevilaqua, C.M.L. The use of *Eucalyptus staigeriana* nanoemulsion for control of sheep haemonchosis. *Pesqui. Vet. Bras.* **2017**, *37*, 221–226, doi:10.1590/s0100-736x2017000300004.
25. De Araújo-Filho, J.V.; Ribeiro, W.L.C.; André, W.P.P.; Cavalcante, G.S.; de C.M. Guerra, M.; Muniz, C.R.; Macedo, I.T.F.; Rondon, F.C.M.; Bevilaqua, C.M.L.; de Oliveira, L.M.B. Effects of *Eucalyptus citriodora* essential oil and its major component, citronellal, on *Haemonchus contortus* isolates susceptible and resistant to synthetic anthelmintics. *Ind. Crops Prod.* **2018**, *124*, 294–299, doi:10.1016/j.indcrop.2018.07.059.
26. De Araújo-Filho, J.V.; Ribeiro, W.L.C.; André, W.P.P.; Cavalcante, G.S.; Rios, T.T.; Schwinden, G.M.; da Rocha, L.O.; Macedo, I.T.F.; de Morais, S.M.; Bevilaqua, C.M.L.; et al. Anthelmintic activity of *Eucalyptus citriodora* essential oil and its major component, citronellal, on sheep gastrointestinal nematodes. *Rev. Bras. Parasitol. Vet.* **2019**, *28*, 644–651, doi:10.1590/s1984-29612019090.
27. Kundu, A.; Dutta, A.; Mandal, A.; Negi, L.; Malik, M.; Puramchatwad, R.; Antil, J.; Singh, A.; Rao, U.; Saha, S.; et al. A Comprehensive in vitro and in silico Analysis of Nematicidal Action of Essential Oils. *Front. Plant Sci.* **2021**, *11*, 1–15, doi:10.3389/fpls.2020.614143.
28. Lanzerstorfer, P.; Sandner, G.; Pitsch, J.; Mascher, B.; Aumiller, T.; Weghuber, J. Acute, reproductive, and developmental toxicity of essential oils assessed with alternative in vitro and in vivo systems. *Arch. Toxicol.* **2021**, *95*, 673–691, doi:10.1007/s00204-020-02945-6.
29. Faria, J.M.S.; Barbosa, P.; Bennett, R.N.; Mota, M.; Figueiredo, A.C. Bioactivity against *Bursaphelenchus xylophilus*: Nematotoxics from essential oils, essential oils fractions and decoction waters. *Phytochemistry* **2013**, *94*, 220–228, doi:10.1016/j.phytochem.2013.06.005.
30. Faria, J.M.S.; Sena, I.; Ribeiro, B.; Rodrigues, A.M.; Maleita, C.M.N.; Abrantes, I.; Bennett, R.; Mota, M.; da Silva Figueiredo, A.C. First report on *Meloidogyne chitwoodi* hatching inhibition activity of essential oils and essential oils fractions. *J. Pest Sci.* **2016**, *89*, 207–217, doi:10.1007/s10340-015-0664-0.