Essential Oils and Volatiles as Nematodicides against the Cyst Nematodes *Globodera* and *Heterodera* †

Jorge M. S. Faria 1,2,*, and Cláudia Vicente 1,2,

1 MED, Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Évora University, Pólo da Mitra, Ap. 94, 7066-554 Évora, Portugal; claudia.vicente@iniav.pt
2 INIAV, I.P., National Institute for Agricultural and Veterinary Research, Quinta do Marquês, 2780-159 Oeiras, Portugal
* Correspondence: fariajms@gmail.com
† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021.

Abstract: The cyst nematodes of the genera *Heterodera* (HET) and *Globodera* (GLO) are among the most damaging obligate plant parasitic nematodes (PPNs) that parasitize cereals, rice, potatoes and soybean. In the absence of resistant crops, soil fumigation of pesticides provides a good strategy for population control. However, synthetic nematicides can cause negative environmental and public health impacts and are feared to lead to the development of resistance and immunity. The use of essential oils (EOs) could be a viable environmentally friendly alternative, which has been poorly explored on cyst nematodes but has shown very good results on other PPNs. The present work reviews the existing bibliography on the biological activity of EOs against GLO and HET. EOs from *Allium sativum*, *Eucalyptus globulus*, and *Salvia officinalis* were the most active against GLO egg hatching. The EOs extracted from *Hyssopus cuspidatus*, *Kaempferia galanga*, *Mentha canadensis*, *Ocimum basilicum*, and *Valeriana amurensis* had the highest activity against HET J2 juveniles. Ethyl *p*-methoxycinnamate, a phenylpropanoid ester, was the EO volatile with the highest toxicity against HET, showing lower EC50 values than the nematocide fosthiazate. The study of EOs against cyst nematodes is still preliminary in comparison to other PPNs. Future works must expand this line of research and explore greener practices in cyst nematode pest management.

Keywords: biopesticides; cyst nematodes; essential oils; ethyl *p*-methoxycinnamate; *Globodera*; *Heterodera*; nematodicides; phytochemicals; sustainable pest management; volatiles

1. Introduction

The cyst nematodes (CNs) from the genera *Heterodera* (HET) and *Globodera* (GLO) are classified as the second most economically and scientifically important plant-parasitic nematode (PPN) group worldwide. These obligatory endoparasitic nematodes are highly distributed in global temperate regions and affect the productivity of essential food crops (e.g., potatoes, cereals, brassicas, tomatoes and sugar beet) [1]. Within this group, the soybean cyst nematode (SCN), *Heterodera glycines*; the potato cyst nematode (PCN), *Globodera pallida* and *G. rostochiensis*; and the cereal cyst nematode (CCN), *Heterodera avenae* and *H. filipjevi*, are considered the most damaging pests to food security with difficult to assess economical losses, and for which restricted quarantine regulations are imposed [2,3]. For example, in Europe, PCNs are responsible for potato yield losses estimated at EUR 220 million/year, while in East Africa (Kenya) potato losses are approximately USD 127 million/year, with only 9.9 t/ha of the potential yield of 40 t/ha [4].

The biology of cyst nematodes is quite exquisite. Similarly to the root-knot nematodes (RKNs), CNs are able to induce host-cell differentiation to establish a unique feeding structure for their development and reproduction (i.e., syncytium for CNs and giant cells...
for RKNs) [5,6]. Briefly, CN eggs are retained in the swollen females (cyst-like) and protected by a hardened cuticle. Upon stimulation by plant root exudates, eggs hatch and the infective J2 juveniles migrate towards the host root system, entering and moving intracellularly into the inner cortex, where a suitable cell to form the syncytium is selected [3]. An array of parasitism proteins (so called effectors) are secreted by the CNs to trigger root cell reprogramming as neighboring cells are incorporated into the syncytium, forming a multinucleate and highly metabolically active feeding structure [7]. The juveniles become sedentary, feeding on plant nutrients until reaching the adult stage. After mating, males leave the roots. On the contrary, females eventually die, and their cuticle undergoes tanning by polyphenol oxidases forming the cyst, which contains hundreds of embryonated eggs [3]. After the host plant dies, cysts are released from the roots into the soil, remaining dormant until the next susceptible host grows in its vicinity.

Control of CN populations is a challenging task due to the fact that they can persist in soil for long periods (up to 20 years), without a proper host plant, and withstanding extreme conditions of temperature and desiccation [8]. Pest management is normally performed by (a) improving cultural practices in order to increase plant tolerance and/or decrease CN hosts in the field; (b) through cultural control, by introducing crop rotation or cover crops with non-host plants and (c) chemical control using (hemi)synthetic chemical nematocides. Pest management through cultural control can be an environmentally sustainable practice, but often provides no short-term farm income and may involve further expenses in additional equipment [9]. Chemical control is performed by the application of potent synthetic chemicals that kill or disrupt the feeding or reproductive behavior of nematodes and, although highly efficient, can show extremely negative environmental and public health impacts [10]. The use of essential oils (EOs), chemical mixtures of natural products, has begun to be regarded as a potential sustainable chemical control strategy against PPNs [10,11]. EOs are mostly composed of terpenoids (mainly mono- and sesquiterpenes) and phenolic compounds, such as phenylpropanoids, that can often display additive, synergistic and antagonistic component interactions associated with their biological activities. Additionally, these mixtures have the advantage of not accumulating in the environment and having a broad range of activities, which diminishes the risk of developing resistant pathogenic strains [12]. The nematocidal activities of EOs have been previously described for several PPNs [13] but biological assays against the cyst nematodes HET and GLO are still very few. In the present work, a bibliographic survey was performed on available publications reporting EOs tested against HET and GLO. Information was compiled on EO activity and chemical composition as well as the species and family of the plant source.

2. Nematocidal Essential Oils

Research on nematocidal EOs was performed with Web of Science® and Google Scholar® search engines, in all available databases, on published works reporting direct contact bioassays, using the topics “Heterodera” or “Globodera” and “essential oil”. Information on the family and species of the plant source used for EO extraction and the respective EO half maximal effective concentration (EC50) was collected when available. Only nine reports were found for the cyst nematodes.

2.1. Activity against Globodera

The activity of EOs extracted from plants against GLO was reported by three publications. Assays were performed on G. rostochiensis or on undefined GLO species [14–16]. EOs extracted from a total of 10 plant species were used in 24 bioassays. EOs used in the bioassays belonged mostly to plants from the Lamiaceae and Poaceae families (Figure 1a). The species used were Allium sativum, Azadirachta indica, Cinnamomum camphora, Cymbopogon martinii, Eucalyptus globulus, Linum usitatissimum, Ocimum basilicum, Salvia officinalis, Tagetes erecta, and Thymus vulgaris. The highest hatching inhibition percentages were obtained for the EOs of A. sativum, E. globulus, and S. officinalis.
3. Nematodicidal Volatiles from Essential Oils

In five publications, the main compounds of the most successful EOs against the genus HET were also tested, to pinpoint the compound(s) responsible for nematodicidal activity. The compounds with the highest activity were ethyl cinnamate, ethyl \( p \)-methoxy cinnamate, isovaleric acid, \textit{trans}-cinnamaldehyde and \( \alpha \)-terpineol. The EC\(_{50}\) values ranged from 0.08 to 0.21 mg/mL (Figure 2b). The most successful EO component was ethyl \( p \)-methoxy cinnamate, a phenylpropanoid ester that showed an EC\(_{50}\) value lower than that of the commercial nematocide fosthiazate. Highly active compounds contained oxygen in their structure, a very electronegative element, and, with the exception of isovaleric acid and \( \alpha \)-terpineol, were aromatic compounds (Figure 3).
To assess the potential environmental and human health dangers for the use of EOs or EO components against CNs, some toxicological parameters are summarized for the most active compounds (Table 1). These EO compounds are reported to possess lower toxicities to mammals (higher LD$_{50}$ values, in feeding tests with rats) and be lower potential environmental hazards than the commonly used nematodicide fosthiazate. Isovaleric acid and trans-cinnamaldehyde also appear to possess additional biological activities, which may be useful against multiple plant pests.

**Table 1.** Toxicological parameters for the most active EO compounds and the nematodicide fosthiazate.

| EO Compound                  | LD$_{50}$ Oral/Rat (mg/kg) | GHS Signal | GHS Hazards | Other Activities                        |
|------------------------------|----------------------------|------------|-------------|-----------------------------------------|
| Isovaleric acid              | 2000                       | Danger     | Causes severe skin burns and eye damage; causes serious eye damage | Used in fungicides, rodenticides, sedatives, narcotics, and other drugs |
| α-Terpineol                  | 4300                       | Warning    | Causes skin irritation; causes serious eye irritation |                               |
| Ethyl cinnamate              | 4000                       | Warning    | May be harmful if swallowed |                               |
| *trans*-Cinnamaldehyde       | 2220                       | Warning    | Causes skin irritation; may cause an allergic skin reaction; causes serious eye irritation; may cause respiratory irritation | Antifungal agent; corn rootworm attractant; dog and cat repellent |
| Ethyl p-methoxy cinnamate    | >2000                      | None       | Not classifiable according to GHS |                               |
| Fosthiazate                  | 57                         | Danger     | Toxic if swallowed; harmful in contact with skin; may cause an allergic skin reaction; toxic if inhaled; very toxic to aquatic life; very toxic to aquatic life with long lasting effects |                               |

1 retrieved from PubChem chemistry database of the National Institutes of Health (NIH). (www.pubchem.ncbi.nlm.nih.gov, accessed on 5 January 2021); 2 in µL/kg; 3 retrieved from [24]; 4 as defined by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations Economic Commission for Europe (UNECE).
4. Conclusions

In the present work, existing published information on the activity of EOs against the cyst nematodes HET and GLO was compiled and analyzed. Lamiaceae, Poaceae and Compositae plant families show potential as sources for EO-bearing plants with activity against cyst nematodes. Future projects with the aim of screening active EOs may benefit from exploring these families. Aromatic compounds with highly electronegative elements appear to be highly active and EOs rich in these components should be favored. Research on the mechanism of action of EOs on cyst nematodes is needed to ascertain the biological targets of the respective components, in order to devise sustainable and more ecofriendly pest management practices.

Author Contributions: Conceptualization, J.M.S.F.; methodology, J.M.S.F.; software, J.M.S.F.; investigation, J.M.S.F.; resources, J.M.S.F.; writing—original draft preparation, J.M.S.F. and C.V.; writing—review and editing, J.M.S.F. and C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the findings of this study are available from the corresponding author (Jorge M. S. Faria) upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jones, J.T.; Haegeman, A.; Danchin, E.G.J.; Gaur, H.S.; Helder, J.; Jones, M.G.K.; Kikuchi, T.; Manzanilla-López, R.; Palomares-Rius, J.E.; Wesemael, W.M.L.; et al. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol. 2013, 14, 946–961. [CrossRef] [PubMed]

2. Nicol, J.M.; Turner, S.J.; Coyne, D.L.; den Nijs, L.; Hockland, S.; Maafi, Z.T. Current Nematode Threats to World Agriculture. In Genomics and Molecular Genetics of Plant-Nematode Interactions; Jones, J.T., Gheysen, G., Fenoll, C., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 21–43.

3. Moens, M.; Perry, R.; Jones, J. Cyst Nematodes–Life Cycle and Economic Importance. In Cyst Nematodes; Perry, R., Moens, M., Jones, J., Eds.; CAB International: Heidelberg, Germany, 2018; pp. 1–26.

4. Mburu, H.; Cortada, L.; Haukeland, S.; Ronno, W.; Nyongesa, M.; Kinyua, Z.; Bargul, J.L.; Coyne, D. Potato Cyst Nematodes: A New Threat to Potato Production in East Africa. Front. Plant Sci. 2020, 11, 670. [CrossRef] [PubMed]

5. Gheysen, G.; Mitchum, M.G. How nematodes manipulate plant development pathways for infection. Curr. Opin. Plant Biol. 2011, 14, 415–421. [CrossRef]

6. Lilley, C.J.; Atkinson, H.J.; Urwin, P.E. Molecular aspects of cyst nematodes. Mol. Plant Pathol. 2005, 6, 577–588. [CrossRef] [PubMed]

7. Folkertsma, R.T.; Helder, J.; Gommers, E.F.; Bakker, J. Storage of potato cyst nematodes at −80 °C. Fundam. Appl. Nematol. 1997, 20, 299–302.

8. Oka, Y.; Koltai, H.; Bar-Eyal, M.; Mor, M.; Sharon, E.; Chet, I.; Spiegel, Y. New strategies for the control of plant-parasitic nematodes. Pest Manag. Sci. 2000, 56, 983–988. [CrossRef]

9. Chitwood, D.J. Nematicides. In Encyclopedia of Agrochemicals; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2003; Volume 17, pp. 473–474, ISBN 9780123864543.

10. Chitwood, D.J. Phytochemical based strategies for nematode control. Annu. Rev. Phytopathol. 2002, 40, 221–249. [CrossRef] [PubMed]

11. 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. [CrossRef]

12. Andreis, M.F.; Gonzalez-Coloma, A.; Sanz, J.; Burillo, J.; Sainz, P. Nematicidal activity of essential oils: A review. Phytochem. Rev. 2012, 11, 371–390. [CrossRef]

13. Ibrahim, S.K.; Mama, M.; Israel, A.; Ibrahim, L. The Occurrence, Distribution and Control of Potato Cyst Nematodes in Lebanon. Am. J. Agric. Sci. 2017, 4, 51–57. [CrossRef]

14. Renço, M.; Andrea, C.; Sasanelli, N.; Toderas, I. Nematicidal activity of essential oils against the potato cyst nematode Globodera rostochiensis. Nat. Volatiles Essent. Oils 2015, 2, 122.
16. Marks, D. Nematicidal Composition. U.S. Patent 2009/0263520 A1, 7 October 2009.
17. Sangwan, N.K.; Verma, K.K.; Verma, B.S.; Malik, M.S. Nematicidal Activity of Essential Oils of Cymbopogon Grasses. *Nematologica* 1985, 31, 93–99. [CrossRef]
18. Sangwan, N.K.; Verma, B.S.; Verma, K.K.; Dhindsa, K.S. Nematicidal activity of some essential plant oils. *Pestic. Sci.* 1990, 28, 331–335. [CrossRef]
19. Li, H.T.; Zhao, N.N.; Yang, K.; Liu, Z.L.; Wang, Q. Chemical composition and toxicities of the essential oil derived from *Hyssopus cuspis*idatus flowering aerial parts against *Sitophilus zeamais* and *Heterodera avenae*. *J. Med. Plants Res.* 2013, 7, 343–348. [CrossRef]
20. Li, Y.C.; Ji, H.; Li, H.T. Gas chromatography-mass spectrometric analysis of nematicidal essential oil of *Valeriana amurensis* P Smirn ex Kom (Valerianaceae) roots and its activity against *Heterodera avenae*. *Trop. J. Pharm. Res.* 2015, 14, 1673–1678. [CrossRef]
21. Ji, H.; Li, Y.C.; Wen, Z.Y.; Li, X.H.; Zhang, H.X.; Li, H.T. GC-MS Analysis of Nematicidal Essential Oil of *Mentha canadensis* Aerial Parts against *Heterodera avenae* and *Meloidogyne incognita*. *J. Essent. Oil-Bear. Plants* 2016, 19, 2056–2064. [CrossRef]
22. Li, Y.C.; Ji, H.; Li, X.H.; Zhang, H.X.; Li, H.T. Isolation of nematicidal constituents from essential oil of *Kaempferia galanga* L. rhizome and their activity against *Heterodera avenae* Wollenweber. *Trop. J. Pharm. Res.* 2017, 16, 59–65. [CrossRef]
23. Maňašová, M.; Wenzlová, J.; Douda, O.; Zouhar, M.; Novotný, D.; Ryšánek, P.; Mazáková, J.; Chochola, J.; Pavlú, K.; Šarovská, L.; et al. Research on alternative methods of sugar beet protection against sugar beet cyst nematode *Heterodera schachtii* (Schmidt, 1871). *List. Cukrov. Repar.* 2017, 133, 276–284.
24. Umar, M.I.; Asmawi, M.Z.; Sadikun, A.; Atangwho, I.J.; Yam, M.F.; Altaf, R.; Ahmed, A. Bioactivity-Guided Isolation of Ethyl-p-methoxycinnamate, an Anti-inflammatory Constituent, from *Kaempferia galanga* L. Extracts. *Molecules* 2012, 17, 8720–8734. [CrossRef] [PubMed]