IECAG 2021

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Proceedings

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

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† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://iecag2021.sciforum.net/.

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) can be a viable environment friendly alternative that 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 EC_{50} values than the nematodicide fosthiazate. The study of EOs against cyst nematodes is still preliminary in comparison to other PPNs. Future works must expand this research line 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

Citation: Faria, J.M.S.; Vicente, C. Essential oils and volatiles as nematodicides against the cyst nematodes *Globodera* and *Heterodera* †. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

The cyst nematodes (CNs) from genus *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 restrict quarantine regulations are imposed [2,3]. For example, in Europe, PCNs are responsible for potato yield losses estimated at €220 million/year, while in East Africa (Kenya) potato losses are approximately $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) 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 by 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 nematodicides. 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 to 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 nematodicidal 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 species and family of the plant source.

2. Nematodicidal Essential Oils

Research on nematodicidal 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 respective EO half maximal effective concentration (EC_{50}), 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 3 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 basilicium*, *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.*

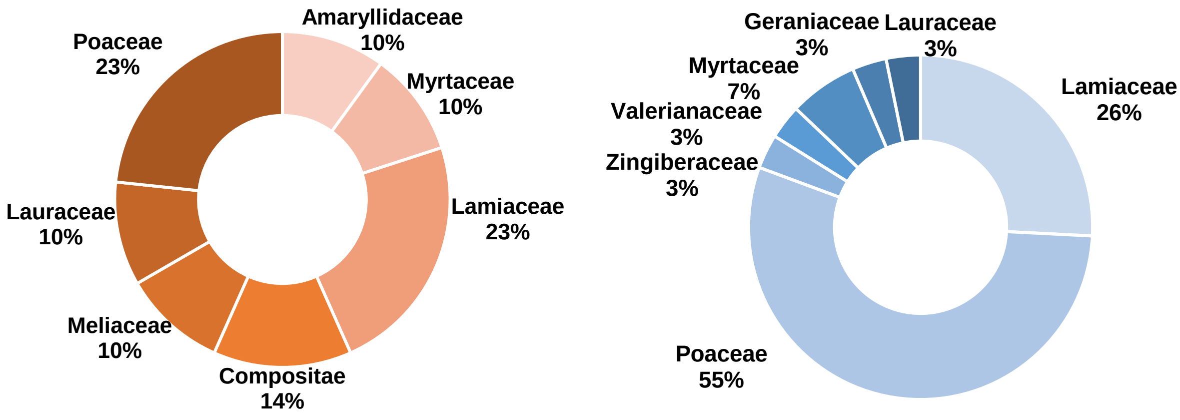

Figure 1. Families of plant sources of the EOs used in the bioassays against the cyst nematodes *Globodera* (**a**) and *Heterodera* (**b**). Percentages (%) correspond to EOs of a family over all EOs.

2.2. Activity against Heterodera

The activity of EOs against the genus *Heterodera* is reported by 8 publications [16–23]. EOs were reported against *H. avenae*, *H. cajani*, *H. schachtii* and on undefined HET species. Twenty plant species were used as sources for EO extraction, mainly from Poaceae and Lamieaceae families, over 32 bioassays (Figure 1b). The highest activities were obtained for EOs extracted from *Hyssopus cuspidatus*, *Kaempferia galanga*, *Mentha canadensis*, *Ocimum basilicum* and *Valeriana amurensis*. These EOs showed EC_{50} values that ranged from 0.09 to 0.48 mg/mL against J2 juveniles (Figure 2a).

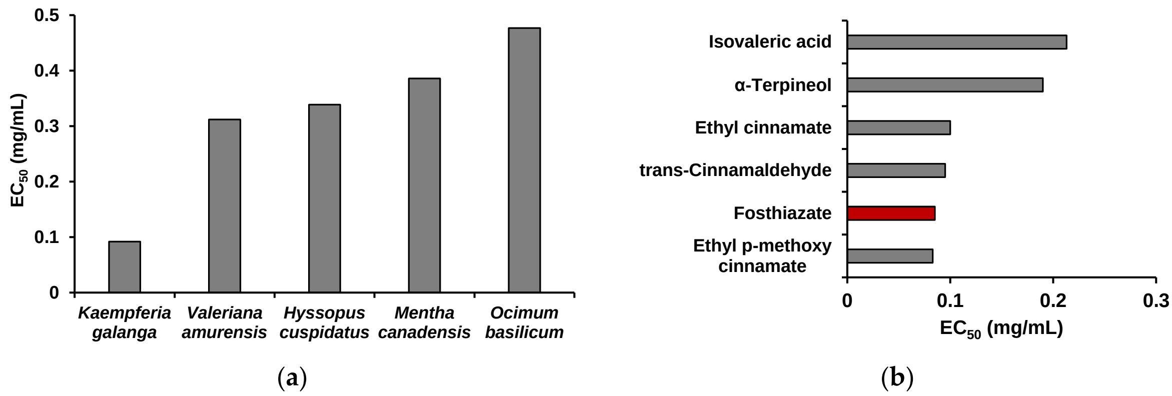

Figure 2. Half maximal effective concentrations (EC_{50}) reported for plant essential oils (EOs) (**a**) and EO main volatiles (**b**) in bioassays against species of the genus *Heterodera*. Fosthiazate is a commercial nematodicide.

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, trans-cinnamaldehyde and *α*-terpineol. The EC_{50} values reported 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 nematodicide fosthiazate. Highly active compounds contained oxygen in their structure, a very electronegative element, and, with the exception of isovaleric acid and α-terpineol, were aromatic compounds (Figure 3).

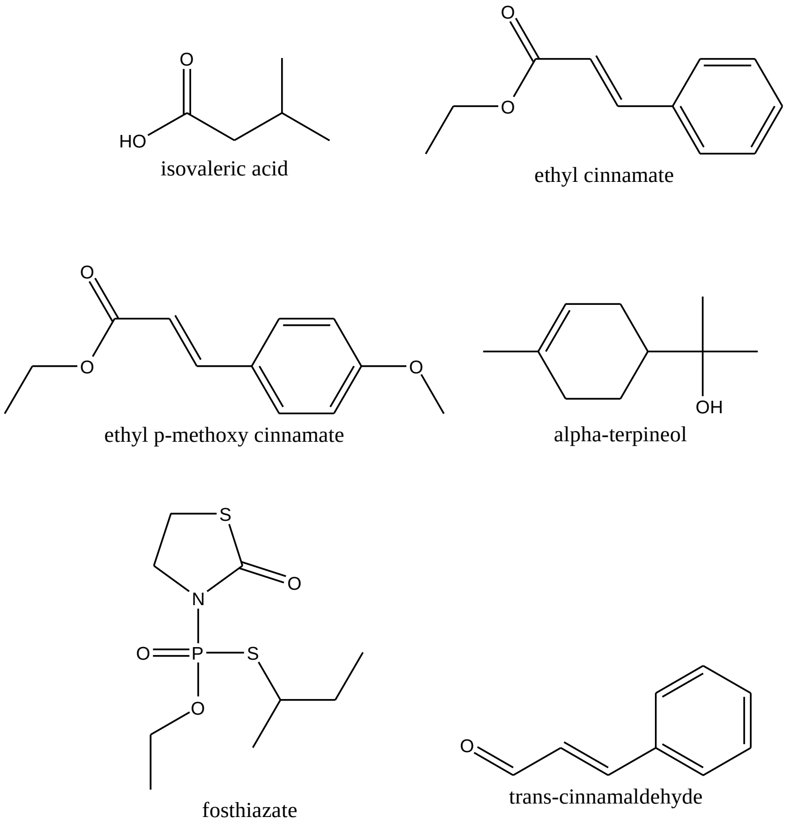

Figure 3. Chemical structure of the most active EO compounds against the genus *Heterodera* and the commercial nematicide fosthiazate.

To assess the potential environmental and human health dangers for the use of EOs or EO components against the CNs, some toxicological parameters were 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 common use 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) [1]	GHS Signal [4]	GHS Hazards [4]	Other Activities
Isovaleric acid	2000 [2]	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 [3]	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); [2] in μL/kg; [3] retrieved from [24]; [4] as defined by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of 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:

Informed Consent Statement:

Data Availability Statement:

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

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