biology and life sciences forum

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*Proceeding Paper*

# Bioactivity of Monoterpene Alcohols as an Indicator of Biopesticidal Essential Oils against the Root Knot Nematode *Meloidogyne ethiopica* †

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† Presented at the 1st International Electronic Conference on Horticulturae, 16–30 April 2022; Available online: https://iecho2022.sciforum.net/.

**Abstract:** Application of pesticides remains one of the most efficient control methods for phytophagous parasites in crops. Essential oils (EOs) are complex mixtures of highly active compounds that can be used as biopesticides against plant parasitic nematodes. In the present work, the antinematodal activity of the monoterpene alcohols geraniol, linalool, menthol or $\alpha$-terpineol, which are generally found in high amounts on EOs of some aromatic and medicinal plants, was analyzed on the root knot nematode *Meloidogyne ethiopica*. Geraniol showed an intense and lasting antinematodal activity, suggesting that EOs rich in this compound can be used in the development of nematicidal biopesticides to integrate sustainable pest management strategies against this pest.

**Keywords:** biopesticides; essential oils; geraniol; linalool; menthol; monoterpenes; plant parasitic nematodes; sustainable pest management; $\alpha$-terpineol

**Citation:** Faria, J.M.S.; Rusinque, L.; Vicente, C.S.L.; Inácio, M.L. Bioactivity of Monoterpene Alcohols as an Indicator of Biopesticidal Essential Oils against the Root Knot Nematode *Meloidogyne ethiopica*. *Biol. Life Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editors:

Published: 16 April 2022



## 1. Introduction

Plant parasitic nematodes (PPN) are highly damaging pests that cause severe losses in the quality and quantity in many economically important crops, e.g., potato and tomato. Infestation with root-knot nematodes (RKN), one of the oldest known PPN groups, is generally devastating for agricultural fields, and is one of the main concerns for farmers. Global agricultural *losses* due to *RKN* infestation are believed to be over 150 billion USD. RKN are sedentary obligate endoparasites that attack plant roots by inducing characteristic galls (root-knots) where reproduction occurs [1]. *Meloidogyne ethiopica* was first described by Whitehead in 1968 from a single egg mass culture on tomato (type host) from the Mlalo region of Tanzania [2]. It has been currently reported in Ethiopia, Kenya, Mozambique, South Africa, Tanzania and Zimbabwe (Whitehead 1968, 1969); in Brazil, Chile, Peru [3]; in Turkey [4] and in Europe in Slovenia [5], Italy [6] and Greece [7]. However, in 2017, populations from Turkey, Greece, Italy and Slovenia were reclassified as *M. luci* [8], leaving some uncertainty about its distribution in Europe. *Meloidogyne ethiopica* is included in the EPPO alert list since 2011, due to its polyphagia and wide host range. In agricultural soils, RKN populations are commonly controlled by resorting to several management practices, either using naturally resistant crop varieties, cultural control through non-host cover crops and crop rotation, or the application of soil fumigants. These are pesticides of synthetic and hemisynthetic origin, that kill or disrupt the feeding or reproductive behavior of nematodes, generally acting as nervous system toxins. Although highly effective, these compounds are broad spectrum pesticides and, unfortunately, damage many natural and beneficial soil organisms, besides having negative environmental and human health impacts [9]. This has led to many of the commercial formulations containing these chemicals being banned from agricultural use in recent years. In the search for more ecofriendly alternatives, essential oils (EOs) have been extensively screened for bioactivity against many RKNs [10]. EOs are obtained from medicinal and aromatic plants by distillation, and expression in the case of *Citrus*, and are complex mixtures of very active chemicals, being mainly composed of terpenes (mono-, sesqui- and a few diterpenes) and phenylpropanoids [11]. Monoterpenes are the predominant components of EOs (90%) and are composed of two isoprene units (C10), which can be arranged into acyclic or cyclic structures. They are commonly involved in plant-animal and plant-plant interactions, and have been attributed several biological activities, e.g., antibacterial, antioxidant, anti-allergic and anticancer [12].

In the present work, the nematicidal activity of four monoterpene alcohols (geraniol, linalool, menthol and $\alpha$-terpineol), commonly found as major constituents in the EOs of medicinal and aromatic plants, was screened against juveniles (J2) of the RKN *Meloidogyne ethiopica*, in direct contact bioassays.

## 2. Materials and Methods

### 2.1. Chemicals

Pure analytical grade standard compounds were acquired from commercial sources: geraniol (purity $\geq$ 98.5%), linalool (purity $\geq$ 99%), menthol (purity $\geq$ 98%) and $\alpha$-terpineol (purity $\geq$ 95%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was acquired from Fisher Chemicals (NH, USA).

### 2.2. Meloidogne ethiopica Juveniles

The reference isolate of *Meloidogyne ethiopica* was provided by the European reference laboratory for nematology (EUR Nem ANSES-ILVO) and maintained in the laboratory of nematology at INIAV (NemaINIAV), in tomato plants *Solanum lycopersicum* L., cv. Ox heart by periodic subculturing in a growth chamber at 25 ± 2 °C [14]. Pots were filled with a sterilised mixture of soil, sand and substrate (1:1:1). Tomato seeds were previously germinated on hydrated filter paper in Petri dishes, at 23 °C, and transferred to the pots. Tomato plants were inoculated with a single initial egg mass and, after 60 days, newly developed egg masses were used to inoculate new tomato seedlings.

The second-stage *M. ethiopica* juveniles (J2) used for direct contact bioassays were obtained from egg masses handpicked from cultured infected tomato roots, at the end of the 60-day period. Egg masses were maintained in moist chambers at 25 °C for hatching. Nematodes were counted by sampling five replicates of 100 μL aliquots of the 5 mL solution where the eggs hatched.

### 2.3. Direct Contact Bioassays

Bioassays were performed as previously described [13], using flat bottom 96 well microtiter plates (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). In each well, 50 ± 5 mixed life-stage RKNs, in 95 μL aqueous solution, were added to 5 μL of a 20 mg compound/mL of methanol solution, to obtain a final concentration of 1 mg/mL. This concentration was selected for the present preliminary screening based on previous reports on the activity of terpenoids on PPNs, including RKNs [14,15]. Contents were thoroughly mixed, plates were covered with plastic film and aluminum foil and maintained at 25 ± 1 °C in an orbital shaker at 80 r.p.m. Control bioassays were performed with 5 μL of methanol. After 24, 48, 72 and 96 h, nematodes were observed using an inverted microscope [Diaphot, Nikon, Japan (40×)]. RKNs mobility was tested by mechanically probing. Complete mortality was considered only if every RKNs showed no movement, even after mechanical stimulation. Each compound was bioassayed 6 times.

*2.4. Data treatment*

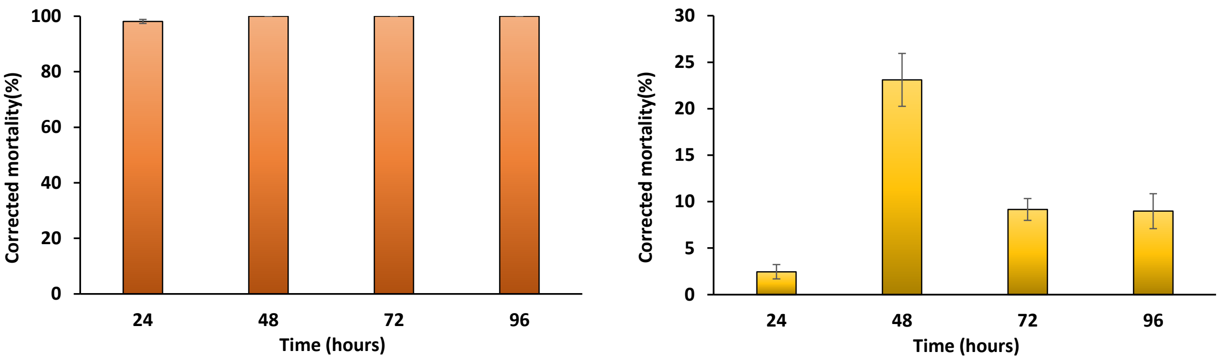
Average corrected mortality was determined by comparing the percentage mortality caused by each monoterpene alcohol with that of the methanol control, using the Schneider-Orelli formula according to [13]:

Corrected mortality percentage = [(mortality percentage in treatment − mortality percentage in control)/(100 − mortality percentage in control)] × 100

The activities of each monoterpene alcohol at each time point were classified as complete (100%), very strong (90–99%) strong (60–89%), moderate (37–59%), weak (11–36%) and low or inactive (>10%) [14]. Statistical analysis was performed with SPSS version 27 statistics software. Results were presented as average and standard error of replicates.

## 3. Results

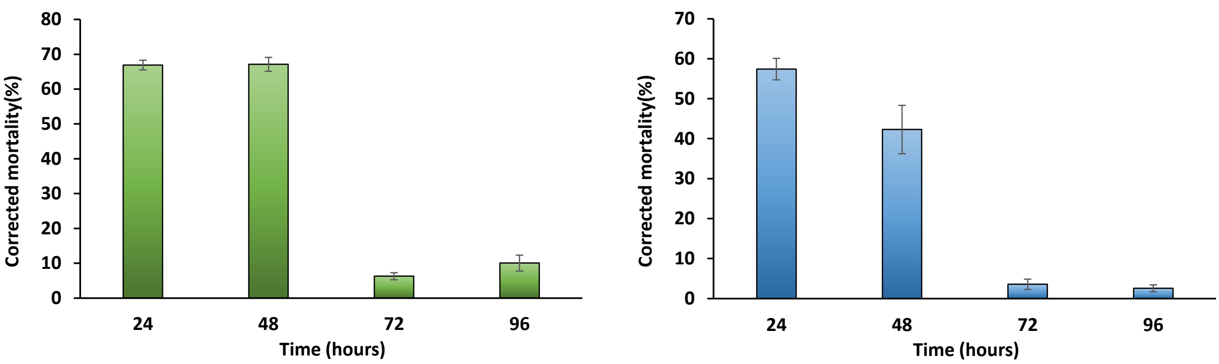
J2 juveniles of *M. ethiopica* displayed different patterns of mortality according to the monoterpene alcohol applied. For geraniol, after 24 h a very strong mortality (98.1 ± 0.5%) was reached and, in the following time-points, complete mortality was recorded (Figure 1a). The application of linalool at 1 mg/mL, was less effective in controlling *M. ethiopica*, a peak of activity was reached at 48 h, with 23.1 ± 2.8% of mortality, but decreased thereafter (Figure 1b).




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

**Figure 1.** Corrected mortality (%) of *Meloidogyne ethiopica* juveniles (J2) after 24, 48, 72 or 96 h of direct contact with the monoterpene alcohols geraniol (**a**) or linalool (**b**) at 1 mg/mL. Values presented are the average and standard error of six replicates of 50 nematodes.

For both menthol and $\alpha$-terpineol, the highest activities were obtained at 24 and 48 h. Strong mortalities were observed for menthol, 66.9 ± 1.4 and 67.1 ± 2.0%, respectively, and moderate mortalities for $\alpha$-terpineol, 57.4 ± 2.7 and 42.3 ± 6.0%, respectively. After 72 or 96 h, these pure compounds showed very low mortalities (Figure 2a,b).




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

**Figure 2.** Corrected mortality (%) of *Meloidogyne ethiopica* juveniles (J2) after 24, 48, 72 or 96 h of direct contact with the monoterpene alcohols menthol (**a**) or α-terpineol (**b**) at 1 mg/mL. Values presented are the average and standard error of six replicates of 50 nematodes.

## 4. Discussion

Geraniol showed a stronger and more lasting activity against *M. ethiopica* juveniles. After 24 h almost all nematodes were motionless and remained unresponsive until the end of the experimental time. The activity of linalool was mostly observed after 48 h, but faded with time. Good antinematodal activities were obtained by menthol and α-terpineol but their effects were shorted lived, after 72 h activities were greatly reduced. Geraniol and linalool are acyclic monoterpenoids, however while the first is a primary alcohol, the latter is a tertiary alcohol, which may have influenced differentially the nematicidal activity (Figure 3). The position of the alcohol functional group has previously been reported to affect nematicidal strength in many compounds [16]. Geraniol has shown very good activities against RKN, e.g., *M. incognita* [17] and *M javanica* [18]. This monoterpenoid is of high commercial importance for the flavour and fragrance industries, and its high biologic activities along with its low toxicity makes it a promising natural pest control agent. The data presented here suggests that the use of geraniol-rich essential oils can be a sustainable alternative for the control of *M. ethiopica*, particularly in low-income countries, where farmers struggle to effectively manage plant parasitic nematode-caused diseases.

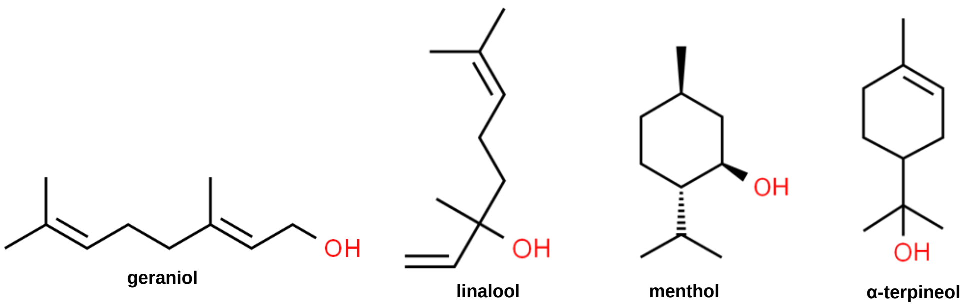



**Figure 3.** Chemical structure of geraniol, linalool, menthol and α-terpineol.

## 5. Conclusion

Geraniol and/or geraniol-rich essential oils can provide a basis for the development of stronger nematicides to be used in organic farming. This approach to pest management is advantageous for high-income country farmers, which are pressured to produce more using less pesticides, as well as for low-income country farmers, that many times lack the means to control soil nematode diseases.

**Author Contributions:** Conceptualization, J.M.S.F. and L.R.; methodology, J.M.S.F. and L.R.; software, J.M.S.F.; formal analysis, J.M.S.F. and L.R.; investigation, J.M.S.F. and L.R.; resources, J.M.S.F., L.R., C.S.L.V. and M.L.I.; data curation, J.M.S.F.; writing—original draft preparation, J.M.S.F. and L.R.; writing—review and editing, J.M.S.F., L.R., C.S.L.V. and M.L.I. 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.

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