

Proceeding Paper

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

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Abstract: The 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 antine-matodal activity of the monoterpene alcohols geraniol, linalool, menthol or α -terpineol, which are generally found in high amounts in EOs of some aromatic and medicinal plants, was analyzed on the root knot nematode *Meloidogyne ethiopica*. Geraniol showed intense and lasting antine-matodal activity, suggesting that EOs rich in this compound can be used in the development of nematocidal biopesticides to integrate sustainable pest management strategies against this pest.

Keywords: biopesticides; essential oils; geraniol; linalool; menthol; monoterpenes; plant parasitic nematodes; sustainable pest management; α -terpineol



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1. Introduction

Plant parasitic nematodes (PPNs) are highly damaging pests that cause severe losses in the quality and quantity of many economically important crops, e.g., potato and tomato. Infestation with root knot nematodes (RKNs), 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 USD 150 billion. RKNs 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 [3]; in Brazil, Chile, and Peru [4]; in Turkey [5]; in Slovenia [6]; in Italy [7]; and in Greece [8]. However, in 2017, populations from Turkey, Greece, Italy and Slovenia were reclassified as *M. luci* [9], leaving some uncertainty about its distribution in Europe. *Meloidogyne ethiopica* has been 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, which 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, in addition to having negative environmental and human health impacts [10]. 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 [11]. 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 [12]. 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, antiallergic and anticancer [13].

In the present work, the nematocidal activity of four monoterpene alcohols (geraniol, linalool, menthol and α -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 α -terpineol (purity \geq 95%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was acquired from Fisher Chemicals (New Hampshire, USA).

2.2. *Meloidogyne 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 sterilized 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 μ L aliquots of the 5 mL solution where the eggs hatched.

2.3. Direct Contact Bioassays

Bioassays were performed as previously described [15], 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 μ L of aqueous solution, were added to 5 μ L 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 [14,16]. The contents were thoroughly mixed, and 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 μ L of methanol. After 24, 48, 72 and 96 h, nematodes were observed using an inverted microscope (Diaphot, Nikon, Japan (40 \times)). RKNs' mobility was tested by mechanical probing. Complete mortality was only considered if every RKN 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 [15]:

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. The results were presented as averages and standard errors 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, very strong mortality ($98.1 \pm 0.5\%$) 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 $23.1 \pm 2.8\%$ of mortality, but decreased thereafter (Figure 1b).

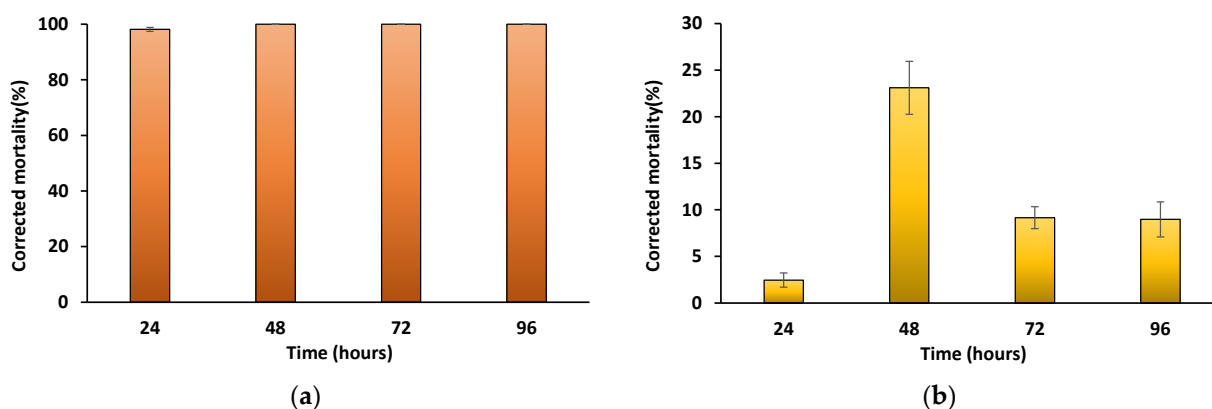


Figure 1. Corrected mortality (%) of *Meloidogyne ethiopica* juveniles (J2) after 24, 48, 72 or 96 h of direct contact with the monoterpene alcohols geraniol (a) or linalool (b) at 1 mg/mL. Values presented are the average and standard error of six replicates of 50 nematodes.

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

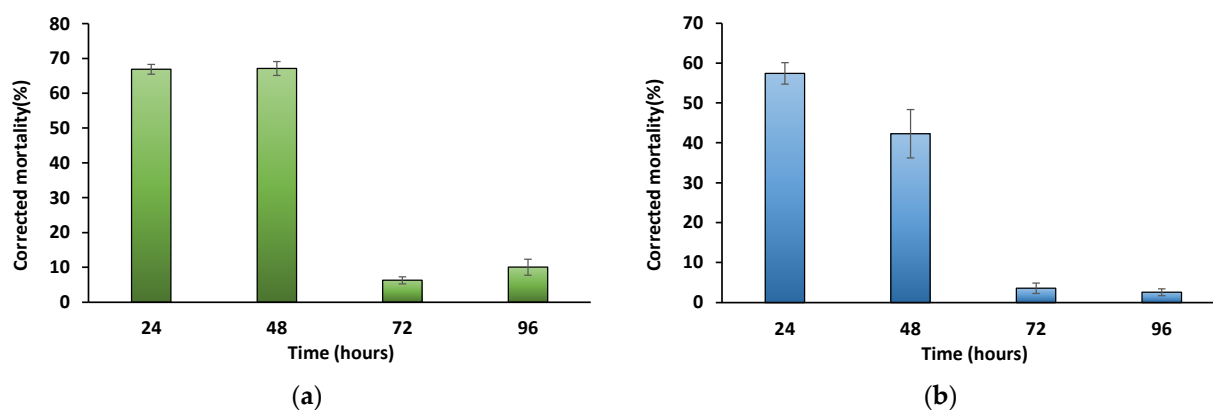


Figure 2. Corrected mortality (%) of *Meloidogyne ethiopica* juveniles (J2) after 24, 48, 72 or 96 h of direct contact with the monoterpene alcohols menthol (a) or α -terpineol (b) at 1 mg/mL. Values presented are the average and standard error of six replicates of 50 nematodes.

4. Discussion

Geraniol showed 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 with menthol and α -terpineol, but their effects were short 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 differentially influenced the nematicidal activity (Figure 3). The position of the alcohol functional group has previously been reported to affect nematicidal strength in many compounds [17]. Geraniol has shown very good activities against RKN, e.g., *M. incognita* [18] and *M. javanica* [19]. This monoterpene is of high commercial importance for the flavor 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 suggest 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.

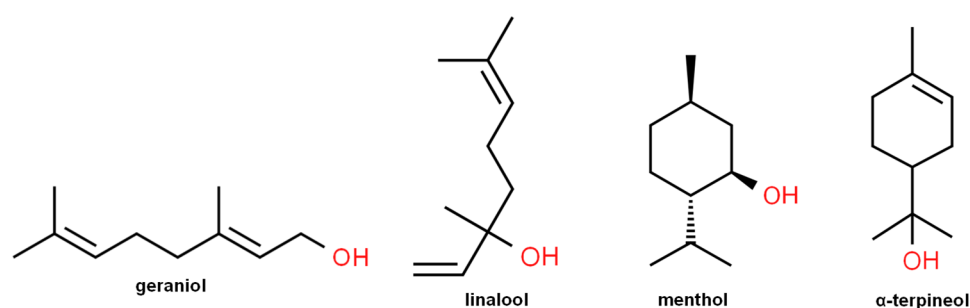


Figure 3. Chemical structure of geraniol, linalool, menthol and α -terpineol.

5. Conclusions

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, who oftentimes lack the means to control soil nematode diseases.

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