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Fumigant Toxicity and Feeding Deterrent Activity of Essential Oils from *Lavandula dentata*, *Juniperus procera*, and *Mentha longifolia* against the Land Snail *Monacha obstructa*

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Abstract: Land mollusks are one of the most destructive agricultural pests worldwide, the management of which depends on synthetic molluscicides. However, many of these molluscicides are harmful to nontarget organisms. Hence, there is a need to develop alternative ecofriendly molluscicides that are less impactful toward nontarget organisms. So, an investigation into the fumigant toxicity and feeding deterrent effect of essential oils (EOs) from *Lavandula dentata* L. (Lamiaceae), *Juniperus procera* Hochst. (Cupressaceae), and *Mentha longifolia* (L.) Huds. (Lamiaceae) against the land snail *Monacha obstructa* (Pfeiffer, 1842) (Hygromiidae) was performed. *L. dentata* EO exhibited the highest fumigant toxicity with LC_{50} values of 8.68 µL/L air and 7.24 µL/L air after 24 h and 48 h exposure periods, respectively. Its main components were camphor, 1,8-cineole, fenchone, and *β*-myrecene. The fumigant toxicity of *J. procera* EO was lower than that of *L. dentata*, with LC_{50} values of 25.63 µL/L air and 20.11 µL/L air after 24 h and 48 h exposure periods, respectively. The major constituents of *J. procera* EO were *α*-pinene, *p*-cymene, and *β*-ocimene. The analysis of *M. longifolia* EO showed that pulegone, and menthol were the major constituents. However, it displayed no fumigant toxicity up to 50 µL/L air. The three EOs exhibited a strong feeding deterrent effect at sublethal concentrations. The EOs extracted from *L. dentata*, *J. procera* and *M. longifolia* are promising ecofriendly botanical molluscicides against the land snail *M. obstructa*.

Keywords: essential oils; land snails; molluscicidal activity; feeding deterrent activity

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1. Introduction

Herbivorous land mollusks are considerable agricultural pests in different regions of the world. They cause economic damage to vegetables, fruits, field crops, medicinal plants, and ornamentals [1]. Land snails attack fruits, flowers, leaves, buds, tree trunks, and even the roots causing great injury to the plants [1,2]. They also cause wounds in plants, allowing pathogens to infect the injured plants. Furthermore, their bodies, shells, excrement, and mucus contaminate mechanically collected crops, reducing their value [3–5]. Moreover, land mollusks can transmit a number of pathogens and parasites to humans and domestic animals [6,7]. Warm and humid habitats having an abundance of food in a closed system, e.g., greenhouses, are ideal conditions for the survival and growth of snail and slug pests [8]. *M. obstructa* is a voracious agricultural pest snail that causes considerable damage to many agricultural crops [9,10]. In addition, it is an intermediate host of the trematode parasite *Brachylaima* [11]. The management of snail pests all over the world depends on the use of molluscicides via only four active ingredients (iron phosphate,

sodium ferric EDTA, metaldehyde, and methiocarb). Nevertheless, the efficacy of these molluscicides is very inconstant and influenced by environmental conditions [12]. In addition, poisoning cases affecting pets, birds, and domestic animals via metaldehyde have been reported [13–15]. Therefore, there is a pressing need to develop novel ecofriendly molluscicidal products that are more reliable and have less impact on nontarget organisms. Terrestrial snails locate and detect food items at a distance principally via olfaction [16]. Consequently, plants that contain EOs can affect mollusk feeding behavior [17]. Therefore, the development of biorational products that contain EOs as their active constituents is one of the available options.

Plants produce diverse secondary metabolites as self-protective agents against pest attack [18]. Plant EOs display different biological activities against many pests and may act as contact, fumigant, repellent, and/or feeding deterrents [19]. The genus *Lavandula* is endemic to the Mediterranean region, Canary Islands, India, and the Arabian Peninsula [20]. Different *Lavandula* species are cultivated worldwide due to their medicinal and economic importance for pharmaceutical and cosmetic industries [21,22]. Bioactive EOs extracted from *L. dentata* exhibit antibacterial, antifungal, and antiprotozoal activities [23–25]. The genus *Juniperus* is distributed in many parts of the world as an indigenous plant with approximately 70 identified species [26]. The EOs from *Juniperus* species display antibacterial, antifungal, and larvicidal actions [27–29]. More than 30 described species of *Mentha* grow in Africa, Asia, Australia, Europe, and North America [30]. Several biological activities have been ascribed to *Mentha*, e.g., antimicrobial, antiviral, and bio-pesticidal [31].

Based on the comprehensive review of Radwan and Gad [32], there is insufficient data available regarding the fumigant toxicity and feeding deterrent activity of EOs from aromatic plants against land snails. Therefore, this study aimed to investigate the chemical composition of EOs from *L. dentata*, *J. procera*, and *M. longifolia*. Furthermore, the fumigant toxicity and feeding deterrent activities of these isolated EOs were evaluated against the land snail pest *M. obstructa*.

2. Materials and Methods

2.1. Collection and Maintenance of Snails

Adult specimens of the land snail *M. obstructa* (Pfeiffer 1842) were selected from a clover field (24.834111° N, 46.487667° E) in Al-Wasil region, east of Riyadh, Saudi Arabia. The snails were collected early in the morning and maintained in polythene containers (40 cm × 30 cm × 20 cm) at the laboratory (25 ± 2 °C, 30 to 40% RH, 10/14 light/dark regime of lightening) for 10 days of acclimatization. They were fed fresh lettuce leaves *ad libitum*.

2.2. Collection of Plants

The aerial parts of *L. dentata* and *J. procera* were collected from the Asir area (18.103281° N, 42.871289° E), southwest of Saudi Arabia in the spring of 2017. The *M. longifolia* plants were obtained from a local market in Riyadh city. The plants were identified in the herbarium of the Botany Department, College of Science, King Saud University.

2.3. Isolation of Essential Oils

The aerial parts of the three identified plants were dried at laboratory temperature (25 ± 2 °C) for 3 days and then underwent hydro-distillation for 3 h. The obtained distilled EOs were dried over anhydrous Na_2SO_4. The oils were kept at −20 °C in dark glass vials, firmly closed with Teflon screw caps until experimental use.

2.4. Analysis of Essential Oils

Analyses of the oils were performed using GC/MS (Agilent Model 7890 MSD, Santa Clara, CA, USA). Aliquots of diluted oils in n-hexane (1 µL of 1 ppm concentration) were injected into the GC/MS apparatus by autosampler with split-less mode. The GC was equipped with a HP-5MS capillary column (30 m × 0.25 mm internal diameter and 0.25 µm

film thickness). The temperature programming was performed as column temperature at 40 °C for 10 min, and programmed at the rate of 5 °C/min to 200 °C, hold for 5 min., 10 °C/min to 290 °C and finally held isothermally for 5 min. The detector and injector temperatures were 290 and 280 °C, respectively. The carrier gas used was helium (99.999% purity) at a flow rate of 1.2 mL/min. In addition, there were the following significant quadrupole MS operating parameters: electron-ionization (EI) at 70 eV with scan mass range of 30 to 600 m/z. The components were identified by comparing their retention time values with the retention time values of authentic standards, and mass spectra with National Institute of Standards and Technology (NIST 2017). The analysis and processing of the results were controlled using MASSHUNTER software (Agilent, Santa Clara, CA, USA).

2.5. Fumigant Toxicity Assay

Glass bottles with a volume of 1000 mL were used as fumigation chambers to assay the fumigant toxicity of the EO vapors against adult *M. obstructa*. The EOs were placed in 1 mL narrow opening glass vials, which were hung inside the fumigation chambers. The bottles were quickly secured with their screw caps and sealed with Parafilm to prevent the escape of the snails and EO vapors. The snails were exposed to five different concentrations of the EOs of *L. dentata* ranged from 5 to 15 µL/L air as well as for *J. procera* and for *M. longifolia* ranged from 10 to 50 µL/L air. Three replicates with 10 snails each were used for each concentration. A parallel set-up lacking the EOs served as a control. The snails were fed on lettuce throughout the experiment. Mortality was assessed after 24 h and 48 h exposure periods and the snails were considered dead if they did not react to palpation with a thin needle.

2.6. Feeding Deterrence Assay

To determine if the food was avoided by snails exposed to sublethal concentrations of essential oil vapors, 5 g of fresh lettuce leaves was offered to the snails inside the fumigation chambers. The lettuce leaves and snails were exposed to different three sublethal concentrations of *L. dentata* (0.75, 1.25, and 2.5 µL/L air), *J. procera* and *M. longifolia* (5, 10, and 15 µL/L air) oils. A further control of no EO treatment was also included. There were three replicates per treatment with 10 snails each. After 24 h and 48 h exposure periods, percentages of the consumed lettuce leaves were recorded based on the wet residual weight.

2.7. Data Analysis

The statistical toxicity indices LC_{50} and LC_{95} were estimated as described by Finney [33] via the LdP line program (Ehab Soft, Cairo, Egypt). The feeding deterrence index (FDI) was calculated with the following equation:

$$FDI\ (\%) = [(C - T)/C] \times 100 \quad (1)$$

where C is the lettuce consumption (g) in the control and T is the lettuce consumption (g) in the treatment [34]. An analysis of variance (ANOVA) was used to analyze the results of the feeding deterrence treatment and the Tukey's test was applied using the SPSS 22 statistical program (IBM, Armonk, NY, USA).

3. Results

3.1. Chemical Composition of Essential Oils

The chemical composition analysis of *L. dentata* EO by GC/MS allowed for the identification of a total of 8 compounds, accounting for 99.16% of the total oil (Table 1). The identified compounds are divided into oxygenated monoterpenes (86.81%) and monoterpene hydrocarbons (12.35%) chemical classes. According to the analysis results, camphor was the most abundant compound in *L. dentata* EO (45.74%), followed by 1,8-cineole (eucalyptol) (18.63%), fenchone (18.06%), and β-myrecene (9.02%).

Table 1. Chemical constituents identified from the essential oil of *Lavandula dentata*.

No.	Compound	Chemical Class	Molecular Formula	RT (min)	Area (%)	RI
1	α-Pinene	Bicyclic monoterpene	$C_{10}H_{16}$	8.78	0.86	937
2	β-Myrecene	Acyclic monoterpene	$C_{10}H_{16}$	11.997	9.02	992
3	Limonene	Cyclohexane monoterpene	$C_{10}H_{16}$	13.26	1.56	1026
4	1,8-Cineole	Oxygenated cyclic monoterpene	$C_{10}H_{18}O$	14.00	18.63	1032
5	γ-Terpinene	Cyclohexane monoterpene	$C_{10}H_{16}$	16.22	0.91	1060
6	Fenchone	Oxygenated bicyclic monoterpene	$C_{10}H_{16}O$	16.70	18.06	1096
7	cis-Verbinol	Oxygenated bicyclic monoterpene	$C_{10}H_{16}O$	16.99	4.38	1141
8	Camphor	Oxygenated bicyclic monoterpene	$C_{10}H_{16}O$	17.17	45.74	1145
	Total identified				99.16	
	Grouped components (%)					
	Monoterpene hydrocarbons				12.35	
	Oxygenated monoterpenes				86.81	
	Others				0.84	

RT is retention time in minutes and RI is retention index.

In the *J. procera* EO, 5 compounds were specified, which represented 97.87% of the EO composition (Table 2). Monoterpene hydrocarbons represented the major components of the oil (89.31%) followed by sesquiterpene hydrocarbons (5.25%), and oxygenated monoterpenes (3.31%). The oil components were α-pinene (53.70%), *p*-cymene (24.83%), β-ocimene (10.78%), γ-elemene (5.25%), and thymol (3.31%).

Table 2. Chemical constituents identified from the essential oil of *Juniperus procera*.

No.	Compound	Chemical Class	Molecular Formula	RT (min)	Area (%)	RI
1	α-Pinene	Bicyclic monoterpene	$C_{10}H_{16}$	8.7809	53.70	937
2	*p*-Cymene	Cyclic monoterpene	$C_{10}H_{14}$	12.0992	24.83	1014
3	β-Ocimene	Acyclic monoterpene	$C_{10}H_{16}$	12.7914	10.78	1048
4	Thymol	Oxygenated cyclic monoterpene	$C_{10}H_{14}O$	24.9006	3.31	1290
5	γ-Elemene	Cyclic Sesquiterpene	$C_{15}H_{24}$	25.7675	5.25	1342
	Total identified				97.87	
	Grouped components (%)					
	Monoterpene hydrocarbons				89.31	
	Oxygenated monoterpenes				3.31	
	Sesquiterpene hydrocarbons				5.25	
	Others				2.13	

RT is retention time in minutes and RI is retention index.

The components identified in *M. longifolia* EO are listed in Table 3. In this EO, 10 compounds were detected, which represented 99.82% of the oil composition. Oxygenated monoterpenes represent the major components of the oil (93.45%). The results of GC/MS analysis showed that the primary components were pulegone (56.53%), menthol (18.257%), and menthofuran (7.748%).

Table 3. Chemical constituents identified from the essential oil of *Mentha longifolia*.

No.	Compound	Chemical Class	Molecular Formula	RT (min)	Area (%)	RI
1	α-Pinene	Bicyclic monoterpene	$C_{10}H_{16}$	8.7809	1.23	937
2	*p*-Cymene	Cyclic monoterpene	$C_{10}H_{14}$	12.0992	1.15	1014
3	*p*-Mentha-3,8-diene	Cyclic monoterpene	$C_{10}H_{16}$	14.9971	3.99	1074
4	1,8-Cineole	Oxygenated cyclic monoterpene	$C_{10}H_{18}O$	17.0024	0.889	1032
5	Menthofuran	Oxygenated bicyclic monoterpene	$C_{10}H_{14}O$	17.4746	7.748	1150

Table 3. *Cont.*

No.	Compound	Chemical Class	Molecular Formula	RT (min)	Area (%)	RI
6	Borneol	Oxygenated cyclic monoterpene	$C_{10}H_{18}O$	17.8174	4.303	1161
7	Menthol	Oxygenated cyclic monoterpene	$C_{10}H_{20}O$	19.4928	18.257	1169
8	Benzofuran 4,7-dimethyl	Benzofuran	$C_{10}H_{10}O$	19.8227	5.011	1222
9	Pulegone	Oxygenated cyclic monoterpene	$C_{10}H_{16}O$	20.0232	56.53	1235
10	Neryl acetate	Oxygenated acyclic monoterpene	$C_{12}H_{20}O_2$	22.8371	0.712	1367
	Total identified				99.82	
	Grouped components (%)					
	Monoterpene hydrocarbons				6.37	
	Oxygenated monoterpenes				93.45	
	Others				0.18	

RT is retention time in minutes and RI is retention index.

3.2. Fumigant Toxicity

The fumigant molluscicidal activity of the three evaluated EOs is presented in Table 4. The EO of *L. dentata* displayed the highest fumigant toxicity against *M. obstructa* with LC_{50} values of 8.68 µL/L air and 7.24 µL/L air after 24 h and 48 h exposure periods, respectively. The fumigant toxicity of *J. procera* was lower than that of *L. dentata*, with LC_{50} values of 25.63 µL/L air and 20.11 µL/L air after 24 h and 48 h exposure periods, respectively. The EO of *M. longifolia* did not exhibit fumigant molluscicidal activity up to 50 µL/L air.

Table 4. Fumigant toxicity indices of *Lavandula dentata*, *Juniperus procera*, and *Mentha longifolia* essential oils against the land snail *Monacha obstructa*.

Essential Oil	Exposure Time (h)	LC_{50} (µL/L Air)	95% Fiducial Limits		LC_{95} (µL/L Air)	95% Fiducial Limits		Slope ± SE	χ^2	R^2
			Lower	Upper		Lower	Upper			
Lavandula dentata	24	8.68	8.24	9.23	13.79	12.42	16.03	8.19 ± 0.83	0.59	0.997
	48	7.24	6.99	7.49	10.44	9.84	11.30	10.34 ± 0.86	2.58	0.995
Juniperus procera	24	25.63	24.34	26.88	42.53	39.49	46.86	7.48 ± 0.61	2.98	0.994
	48	20.11	19.06	21.09	30.24	28.19	33.32	9.29 ± 0.93	1.78	0.98
Mentha longifolia	24	>50	-	-	>50	-	-	-	-	-
	48	>50	-	-	>50	-	-	-	-	-

3.3. Feeding Deterrent Activity

The results of lettuce consumption showed a remarkable reduction in the snails' feeding activity after 24 h and 48 h of exposure to sublethal concentrations of *L. dentata*, *J. procera*, and *M. longifolia* EOs compared with that of the untreated control. The FDI indicated that the isolated EOs exerted a potent feeding deterrent effect (Table 5). The FDI ranged from 86.2% to 97.1% for *L. dentata*, from 87.13% to 95.63% for *J. procera*, and from 79.77% to 98.4% for *M. longifolia*.

Table 5. Feeding deterrent activity of *Lavandula dentata*, *Juniperus procera*, and *Mentha Longifolia* essential oils against the land snail *Monacha obstructa*.

Essential Oil	Concentration (µL/L Air)	Feeding Deterrence Index (FDI) % ± SD	
		24 h	48 h
Lavandula dentata	0.75	86.20 ± 1.42 ^{a}	89.83 ± 1.33 ^{a}
	1.25	91.53 ± 1.36 ^{b}	92.33 ± 0.81 ^{a}
	2.5	93.37 ± 2.41 ^{b}	97.10 ± 2.90 ^{b}
Juniperus procera	5	87.13 ± 4.58 ^{a}	87.60 ± 3.38 ^{a}
	10	89.03 ± 6.11 ^{a}	92.97 ± 2.95 ^{a}
	15	93.97 ± 1.53 ^{a}	95.63 ± 0.70 ^{a}
Mentha longifolia	5	79.77 ± 2.38 ^{a}	84.33 ± 3.30 ^{a}
	10	92.47 ± 1.94 ^{b}	93.70 ± 1.85 ^{b}
	15	94.13 ± 0.59 ^{b}	98.40 ± 0.41 ^{b}

Differences between values with the same superscript in each row are not significant.

4. Discussion

In agreement with our results, *L. dentata* EO was found to be rich in camphor [35,36], 1,8-cineole [37–39], camphor and 1,8-cineole [25,40], and camphor and fenchone [41,42]. Nevertheless, other studies reported different major components in *L. dentata* EO, such as linalool, linalyl acetate, and α-terpinolene [43,44]. Consistent with our results, α-pinene was the major component in the EO of *J. procera* from East Africa and Saudi Arabia [45,46]. Among the components of the Saudi *J. procera* extract, α-pinene and α-humulene were reported by Abdelghany et al. [29]. However, eugenol and β-caryophyllene were reported as the major constituents in other samples of Saudi *J. procera* EO [47]. As a major compound, α-pinene was detected in the gum-resin and branch oils of *J. excelsa* in the Sultanate of Oman [48]. In line with our results, pulegone was the primary component in the EO of *M. longifolia* from Saudi Arabia with a total content of 61.66% and 40.7% [49,50]. Moreover, a high proportion of pulegone in the EO of *M. longifolia* from Iran has been documented [51]. The primary compounds in the wild *M. longifolia* subsp. *longifolia* grown in Turkey were menthone, pulegone, piperitone, and dihydrocarvon [52]. In Tunisia, *M. longifolia* EOs were grouped in two chemotypes, one rich in pulegone and the other rich in menthone [53]. However, 1,8-cineol, linalool, menthone, and trans-piperitone oxide were identified as the major components of the *M. longifolia* oil by other researchers [54].

Variation in the composition and abundance of EO components for different samples of the same plant species could be attributed to the time of harvesting, the nutritional condition of plants, genetic variations, extraction techniques, and environmental conditions (e.g., geographical, climatic, and seasonal) [55].

Studies concerning the use of EOs and/or their constituents as novel natural products in managing terrestrial mollusks are still in their early stages [12]. To the best of our knowledge, this is the first study on the fumigant toxicity and feeding deterrent activity of *L. dentata*, *J. procera*, and *M. longifolia* EOs against the land snail *M. obstructa*. In the present study, the fumigant toxicity of *L. dentata* EO (LC_{50} = 8.68 µL/L air) against *M. obstructa* was approximately two-fold of that previously reported (LC_{50} =16.3 µL/L air) against the land snail *Theba pisana* [41]. This difference in the fumigant toxicity could be attributed to the difference of snail species and/or the variation in the components of the two oils. The EO of *L. dentata,* used by Eshra et al. [41], contained only camphor and fenchone as the major components [56], while the purified oil in the present study contained camphor, 1,8-cineole, and fenchone. Camphor was reported as a toxic monoterpenoid by contact against the land snails *Cornu aspersum* and *T. pisana* [57,58]. The major components, camphor and 1,8-cineole, were reported as effective fumigants to *T. pisana* snails [58]. The monoterpene fenchone was a potent fumigant against *T. pisana* [58] and by contact against the helicid land snails *Massylaea vermiculata* and *T. pisana* [59]. Furthermore, *L. dentata* EO displayed contact toxicity against *T. pisana* snails [60].

The antibacterial, insecticidal, antifungal, and antitoxoplasmic activities of different *Juniperus* species were documented [28,29,61,62]. Teixeira et al. reported the molluscicidal activity of *J. brevifolia* EO against the freshwater snail *Radix peregra* [63]. However, there are no reports in the literature on the molluscicidal activity of *J. procera* against terrestrial mollusks. In comparison with *L. dentata* EO efficacy, *J. procera* EO exhibited lower fumigant toxicity against *M. obstructa*. The major monoterpenes, α-pinene and *p*-Cymene, displayed pronounced contact toxicity against *T. pisana* [64].

The fumigant toxicity of *Mentha* species EOs against agricultural and stored product arthropod pests, as well as vectors, is well documented [56,65–69]. Nevertheless, *M. longifolia* did not exhibit any molluscicidal activity via fumigation up to 50 µL/L air against *M. obstructa* in the present study.

The role of volatile organic compounds (VOCs) as olfactory signals for natural feeding deterrence to herbivores is well-known for several plants [70,71]. Different categories of plant chemicals have repellent or feeding deterrent activities against gastropod mollusks, including monoterpenes [72,73] tannins [74] and cinnamic acid [75].

The EOs of all tested plants in this study exhibited strong feeding deterrent effects against *M. obstructa*. The antifeedant effect of EOs against terrestrial snails was reported earlier by El-Zemity and Radwan [76], who found strong reduction in *T. pisana* snail feeding when exposed to fennel, rosemary, dill, and cinnamon oils. Tomas et al. concluded that the oxygenated monoterpenes (primarily pulegone) in *Clinopodium rouyanum* EO were active deterrents to herbivore mollusks and represented a good defense against terrestrial mollusks [77]. Menthol has been reported as a good repellent to the land gastropods *C. aspersum* and *Deroceras reticulatum* [57,78]. Also, fenchone was repellent to *D. reticulatum* [79]. Camphor was reported as a feeding deterrent to the herbivore snowshoe hares *Lepus americanus* and *Spodoptera littoralis* [80,81]. In addition, 1,8-cineole and *α*-pinene were potent feeding deterrents against *Tribolium castaneum* [82,83]. A multiple correlation analysis between the composition of the EOs of four Piperaceae species and their antifeedant activities showed strong positive relations for *α*-pinene, *β*-pinene, *δ*-3-carene, limonene, and linalool on *S. littoralis* [84]. According to the results of this study and literature, it can be concluded that pulegone, camphor, 1,8-cineole, menthol and *α*-pinene in the isolated EOs could be the responsible components, individually or synergistically, for the feeding deterrent effects of these oils.

For the practical application of the potential EOs as molluscicides, or feeding deterrents, against herbivore land mollusks, further studies regarding the development of novel EO formulations are required to increase the efficacy and durability. Progress in the pesticide formulation industry may offer better molluscicidal EO products that could achieve ecofriendly land mollusk control, particularly in greenhouses and agriculturally closed systems.

5. Conclusions

In conclusion, the major compounds identified by GC/MS analysis in *L. dentata* EO were camphor, 1,8-cineole, and fencholne; in *J. procera* EO were *α*-pinene, *p*-cymene, and *β*-ocimene; and in *M. longifolia* EO were pulegone, menthol, and menthofuran. All EOs extracted from the tested plants had powerful feeding deterrent effects against *M. obstructa*. Moreover, the EOs isolated from *L. dentata* and *J. procera* have promising potential as ecofriendly botanical molluscicides against the land snail *M. obstructa*.

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