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ORIGINAL ARTICLE

Insecticidal activity of some plant essential oils against the Opuntia cochineal scale insect, *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae)

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Abstract *Opuntia ficus-indica* (L.) is largely cultivated in Morocco because of its remarkable adaptation to arid and semi-arid climates and significant economic importance. However, in recent years, a severe invasion of these species plantations by *Dactylopius opuntiae* has been detected in many regions in Morocco. The present study evaluates under laboratory conditions the toxicity of some Moroccan plant essential oils (EOs) against the mobile stages (adult males and crawlers) of *D. opuntiae* in direct contact and fumigation bioassays. All EOs tested were found to be toxic to adult males and crawlers of *D. opuntiae*, but with variable degrees. *Thymus vulgaris* EO exhibited the highest activity against adult males in both contact and fumigation assays. EOs obtained from *Mentha suaveolens* subsp. *timija*, *Chenopodium ambrosioides*, *Mentha piperita*, *Myrtus communis* and *Laurus nobilis* expressed a moderate activity. *Rosmarinus officinalis* displayed relatively the weakest insecticidal effect. The results suggest that these EOs, particularly those obtained from *T. vulgaris* and *C. ambrosioides*, could present potential plant-based insecticidal agents against this prickly pear cactus pest.

Keywords *Dactylopius opuntiae* · *Opuntia ficus-indica* · Essential oils · Insecticidal activity

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Introduction

The prickly pear (*O. ficus-indica* L. Miller) cactus is native to Mexico, but is now cultivated on a large scale in many parts of the world (Griffith, 2004). Its wide large cultivation is due to its high economical and ecological importance. In fact, the prickly pear is widely exploited for its fruits and fresh stems (cladodes) as a vegetable source for humans and fodder for livestock, especially in arid and semi arid regions. In Morocco, *O. ficus-indica* is not considered as weed, but rather a productive crop, due to its usefulness in various fields (Bouharroud et al., 2016). Unfortunately, during

the last years (at the end of 2014), many prickly pear plantations were attacked by the destructive cochineal *D. opuntiae* (Hemiptera; Dactylopiidae), which has proved to be the most aggressive species in the genus *Dactylopius* (Mazzeo et al., 2019). In some newly cultivated areas in the Mediterranean Basin (e.g., Israel, Spain, and Morocco), it has become a davestating agnet (Bouharroud et al., 2016, 2018). In Morocco, this scale insect was first observed in 2014 in the Sidi Bennour region, located 120 km northwest of Marrakech (Bouharroud et al., 2016), and has since spread rapidly to other regions of the country (Abda, Doukkala, Chaouia, Rhamna and Youssoufia). Tens of thousands of hectares of cactus have been destroyed, causing severe socio-economic and environmental losses (El Aalaoui et al., 2019).

D. opuntiae was introduced to some countries to produce essentially the carmine dye in cosmetics, and food industries. The cochineal feeds on the cladode tissue, which can lead to chlorosis and premature dropping of cladodes and fruits (Vanegas-Rico et al., 2010), and results in a decrease in the production of the cactus crop (Da Silva Santos et al., 2016). In other countries, *D. opuntiae* was intoduced as a biological agent to cope with the cactus invasions. For this reason, the cochineal was introduced into several areas (e.g. Australia, India and South Africa) where cactus was considered as noxious weeds. Thus, *D. opuntiae* causes a serious damage leading eventually to reduced nopal yield and plant productivity (Kavirindi et al., 2010). Due to the economical and ecological impact of *D. opuntiae,* the life cycle of the cochineal has been described in many previous studies (Palafox-Luna et al., 2018; El Aalaoui et al., 2019). In fact, the females have three biological stages including egg and nymph with two instars and adults, whereas males develop through egg, nymph, pre-pupa, pupa and adult stages. The females are sessile and typically disperse during the crawler stage. Adult females remain attached to the cladode and are protected by white waxy filamentous secretions, while adult males become winged. The crawlers move upwards on the cladodes of their host to facilitate dissemination by wind.

D. opuntiae is spreading rapidly in the Mediterranean Basin, where it has become a serious pest of prickly-pear crops (Mazzeo et al., 2019). Therefore, the control of this invasive and destructive pest is urgent to protect the areas cultivated with *O. ficus-indica*. One of the available solutions is using conventional synthetic insecticides (Kavirindi et al., 2010). Generally, these products have an acceptable efficacy, yet, since these compounds are associated with environmental concerns as they are found to cause contamination problems and undesirable effects on non-target organisms (Arias-Estévez et al., 2008; Dawar et al., 2016). Alternatives, new sustainable and eco-friendly compounds, are required.

Botanical pesticides such as essential oils (EOs) display very important physical and chemical activities. Over the years, they have shown good potential in the control of insect pests (Lee et al., 2003, 2004). Also, botanical pesticides have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because they reputedly pose little threat to the environment and to human health (El-Wakeil, 2013). Compared to the other natural and safer materials (e.g. mineral oils), EOs showed to be sometimes more efficacious against some pest insects with less host plant toxicity (Zheng et al., 2002; Sertkaya et al., 2010). Hence, the objective of the present study was to evaluate, under laboratory conditions, the insecticidal activity of EOs extracted from seven Moroccan native plants namely, *Mentha suaveolens* L. subsp. *timija* (Briq.) Harley, *Chenopodium ambrosioides* L., *Mentha piperita* L., *Myrtus communis* L., *Thymus vulgaris* L., *Laurus nobilis* L., and *Rosmarinus officinalis* L. against the mobile stages (adult males and crawlers) of *D. opuntiae*.

Materials and methods

Biological material

Tests were conducted using insects of the same age collected directly from cladodes naturally infested with *D. opuntiae*. These cladodes were not previously treated with insecticide to avoid any negative interference. The individuals were collected from cladodes upon the time of the bioassays in a growth chamber at 26 ± 1 °C, with a relative humidity (RH) of 70–85% and 12: 12 h light: dark photoperiod. To obtain insects of the same age, pieces (4 cm diameter) of infested cladodes have been placed in Petri dishes for 24 hours under the above mentioned conditions. The pieces of cladodes used were hand-cleaned before under the binocular magnifying glass (Leica Microsystems GmbH, Wetzlar, Germany) to examine the active adult males and crawlers collected after 24 hours from the Petri dishes, and were used for the tests.

Plant materials and extraction of EOs

The aerial parts of *M. suaveolens* subsp. *timija*, *C. ambrosioides, M. piperita, M. communis, T. vulgaris, L. nobilis* and *R. officinalis* were harvested from different locations in Morocco (Table 1). The plants were collected by hand, stored in plastic bags, and transported to the laboratory. One of the authors did the identification, and voucher specimens were deposited at the Laboratory of Water, Biodiversity and Climate change, Faculty of Sciences, Semlalia, Cadi Ayyad University, Marrakech, Morocco. Plant materials were dried in the shade at room temperature (≈ 25 °C) and subjected to steam-distillation, using a Clevenger-type apparatus (Borosil 3,451,029 Clevenger Apparatus) for 3 h until total recovery of oil. The extraction of the EOs was performed three times (3 × 100 g) and EOs obtained were dried over anhydrous sodium sulphate and stored at 4 °C in the dark until gas chromatography analysis and biological tests. The yield was calculated in % (v/w, mL/100 g) based on dry plant materials.

Gas chromatography/mass spectrometry (GC/MS) analyses

The GC/MS analysis of EOs was carried out on an Agilent GC-MSD system (Agilent Technologies 6890/5973) with helium (high purity) as the carrier gas at a constant linear velocity of 37 cm/s. The transfer, source and quadrupole temperatures were 280 °C, 230 °C, and 150 °C, respectively, operating at 70 eV ionization energy and scanning the m/z range 41–450. The column used was an Agilent DB5 ms capillary column (30.0 m × 0.25 mm ID × 0.25 µm film thickness; Model Number: 122-5532) programmed from 60 °C to 246 °C at 3 °C/min. EO samples (60 µL) were diluted with acetone (2 mL). The injection volume was 1.0 µL, the split ratio was 1:50, and the injector temperature was 260 °C. Identification of the individual components was based on (i) comparison with the mass spectra of authentic reference compounds where possible and by reference to WILEY275, NBS75K, and Adams terpene library: (Adams, 2007); (ii) comparison of their retention indices (RI) on a DB5 (apolar, 5% phenyl polysilphenylene-siloxane), calculated relative to the retention times of a series of C-9 to C-24 n-alkanes, with linear interpolation, with those of authentic compounds or published data. For semi-quantitative purposes, the normalized peak area of each compound was used without any correction factors to establish abundance.

Bioassays with adult males

Bioassays with adult males were carried out using direct contact method on glass Petri dishes (9 × 2 cm), which were covered with filter paper (9 cm) previously treated with various concentrations of EOs of *M. suaveolens* subsp. *timija, C. ambrosioides M. piperita*,

Table 1 Locality, harvesting time and essential oil yield of the plants studied

Plants Species	Species code	Local name	Harvesting place	Harvesting time	Voucher specimen	Latitude/Longitude	Oil Yield[a] mL/100 g
Mentha suaveolens subsp. *timija*	MS	Timija	Ourika	March 2019	MST-67	31°23'N-07°42'W	0.57 ± 0.07
Chenopodium ambrosioides	CA	Lmkhinza	Ouahat Sidi brahim	Juin 2019	CHA-13	31°43′ N-07°58′ W	1.62 ± 0.08
Mentha piperita	MP	Likama	Oulad Dlim	March 2018	MPIP-12	32°01 N-08°13 W	1.97 ± 0.09
Myrtus communis	MC	Rihane	Ouezzane	May 2018	MRC-07	34°47' N-05° 34' W	0.29 ± 0.01
Thymus vulgaris	TV	Zaater	Oulad Dlim	March 2018	THV-05	32°01 N-08°13 W	1.03 ± 0.02
Laurus nobilis	LN	Rand	Azilal	May 2018	LAN-07	31°52'N-06°28 W	2.32 ± 0.11
Rosmarinus officinalis	RO	Azir	Rich	September 2018	ROF-11	32° 16' N/04°36' W	1.85 ± 0.03

[a]yield of EOs determined based on their volume/weight of the sample used for distillation

M. communis, *T. vulgaris*, *L. nobilis* and *R. officinalis* (Table 2). The entire concentration range was chosen after the completion of the preliminary tests. Each EO concentration was diluted in 1 mL of acetone. After the evaporation of the solvent (1.30 ± 0.04 min), 10 adult males were placed inside each Petri dish and the experiment was repeated five times for each treatment. The Petri dishes were kept in an incubator under controlled conditions as follow: 26 ± 1 °C, 70 ± 10% RH, and a photoperiod of 12:12. Petri dishes treated with acetone alone were included as negative control and incubated at the same conditions. After 24 h of incubation, numbers of dead and live adult males were recorded.

The second part of this study consists on evaluating the fumigant toxicity of the tested EOs. The assessment was conducted using filter paper squares (2 cm diameter) attached to the inner side of screw caps of 125 mL Plexiglas vials (length (without cap) 98 mm, external diameter 54 mm), and impregnated with different concentrations of plant EOs tested (Table 2). Caps were screwed tightly on the glass vials, each of which contained 10 adult males. Each concentration along with the negative control were replicated five times, and the mortality was recorded 24 h post treatment.

Bioassay with mobile crawlers

The contact toxicity bioassay of EOs with the crawlers was carried out by placing 10 individuals in each

Table 2 Plants species, insect stages, method used and the different concentrations of plants essential oil

Plants Species	Insect stage	Method	Concentration
Mentha suaveolens subsp. *timija*	Adult males	Contact	0-0.003-0.006-0.012 and 0.025
		Fumigation	0-0.8-1.6-3.2 and 6.4
	Crawlers	Contact	0- 0.0008- 0.0016- 0.0031 and 0.0063
		Fumigation	0-0.4-0.8-1.6 and 3.2
Chenopodium ambrosioides	Adult males	Contact	0-0.003-0.006-0.012 and 0.025
		Fumigation	0-0.8-1.6-3.2 and 6.4
	Crawlers	Contact	0-0.0008-0.0016-0.0031 and 0.0063
		Fumigation	0-0.2-0.4-0.8 and 1.6
Mentha piperita	Adult males	Contact	0-0.005-0.011-0.022 and 0.044
		Fumigation	0-0.8-1.6-3.2 and 6.4
	Crawlers	Contact	0-0.002-0.005-0.011 and 0.022
		Fumigation	0-0.4-0.8-1.6 and 3.2
Myrtus communis	Adult males	Contact	0-0.0062-0.0125-0.0251 and 0.0503
		Fumigation	0-0.8-1.6-3.2 and 6.4
	Crawlers	Contact	0-0.0008-0.0016-0.0031 and 0.0063
		Fumigation	0-0.4-0.8-1.6 and 3.2
Thymus vulgaris	Adult males	Contact	0-0.0028-0.0056-0.0113 and 0.0226
		Fumigation	0-0.1-0.2-0.4 and 0.8
	Crawlers	Contact	0-0.0008-0.0016-0.0031 and 0.0063
		Fumigation	0-0.4-0.8-1.6 and 3.2
Laurus nobilis	Adult males	Contact	0-0.011-0.022-0.044 and 0.088
		Fumigation	0-0.4-0.8-1.6 and 3.2
	Crawlers	Contact	0-0.011-0.022-0.044 and 0.088
		Fumigation	0-0.4-0.8-1.6 and 3.2
Rosmarinus officinalis	Adult males	Contact	0-0.039-0.078-0.157 and 0.314
		Fumigation	0-1.6-3.2-6.4 and 12.8
	Crawlers	Contact	0-0.019-0.039-0.078 and 0.157
		Fumigation	0-3.2-6.4-12.8 and 25.6

Contact method in $\mu L/cm^2$, fumigation method in $\mu L/L_{air}$

glass Petri dish, similarly as described for the adult males. However, in this case, the filter paper (9 cm) was previously treated by different EO concentrations diluted in 1 mL of acetone (Table 2). After 5 h of incubation under controlled conditions in an incubator with temperature (26 ± 1 °C) and humidity (70 ± 10% RH), dead and live crawlers were counted using a binocular stereomicroscope (×40). Crawlers that did not move after stimulation with a needle were recorded as dead. Negative control without oils was incubated at the same conditions. The experiment was carried out in five replicates.

The crawlers were similarly treated using the fumigation method as described for the adult males. Nevertheless, in this case, filter paper discs (2 cm diameter) were impregnated with a different set of EOs concentrations (Table 2). After 5 h in an incubator with temperature and humidity controlled at 26 ± 1 °C, 70 ± 10% RH, dead and alive crawlers were counted using a binocular stereomicroscope (×40). Crawlers that did not move after stimulation with a needle were recorded as dead. Negative control with no oil was incubated at the same conditions. The experiment was carried out in five replicates.

Data analysis

Probit analysis was conducted to estimate lethal concentration leading to 50% and 90% mortality (LC_{50} and LC_{90}) with the 95% confidence interval using SPSS 12.0 Statistical Software. LC values were considered significantly different when their respective 95% confidence intervals did not overlap.

Results

Hydro distillation of the aerial parts of the studied plants gave pale yellowish to deep yellow oils with a yield ranging from 0.29 ± 0.01% to 2.32 ± 0.11% (v/w), based on dry weight (Table 1). The chemical composition of the EOs was analyzed by GC-MS and the results (percentage content of each compound, retention time (RT) and structural subclass) are summarized in supplementary Table S1. In total, 136 compounds were identified, accounting for 94.97–97.54% of the total oils. Oxygenated monoterpenes constituted the principal compound class in *M. suaveolens* subsp. *timija* (75.94%), *L. nobilis* (70.40%), *M. piperita* (63%), *R. officinalis* (61.22%), *T. vulgaris* (53.84%), and *M. communis* (38%) EOs. *C. ambrosioides* EO was composed mainly by monoterpene hydrocarbons (53.54%). The main chemical constituents of *M. suaveolens* subsp. *timija* were menthone (40.42%) and pulegone (19.22%). In the EO of *L. nobilis*, the most abundant compounds were found to be 1-8-cineole (37.50%), linalool (14.09%). Whereas, *M. piperita* was rich in 1-8-cineole (29.90%) and menthofuran (18.30%). Concerning *R. officinalis* EO, the major constituents were found to be 1-8-cineole (31.13%) and 2-bornanone (17.56%). The most abundant compounds in *T. vulgaris* EO was thymol (43.04%), followed by p-cymene (19.05%), while *M. communis* EO was found to be rich in α-pinene (20.80%) and 1-8-cineole (20.47%). The predominant constituents of *C. ambrosioides* EO were terpinolene (37.91%), p-cymene (13.43%) and isoascaridol (12.04%).

In the direct contact bioassay, the toxicity of tested EOs for adult males of *D. opuntiae* are presented in Table 3. From the data, it appears that all studied EOs showed toxicity against adult males of *D. opuntiae*, but with variable degrees. No mortality was recorded in the controls. Comparatively, EOs obtained from *C. ambrosioides* (LC_{50} = 0.004 µL/cm^2, LC_{90} = 0.009 µL/cm^2), *T. vulgaris* (LC_{50} = 0.006 µL/cm^2, LC_{90} = 0.025 µL/cm^2) and *M. suaveolens* subsp. *timija* (LC_{50} = 0.008 µL/cm^2, LC90 = 0.030 µL/cm^2) exhibited the highest potency. *M. communis* (LC_{50} = 0.013 µL/cm^2, $LC_{90=}$ 0.052 µL/cm^2) and *M. piperita* (LC_{50} = 0.014 µL/cm^2, LC_{90} = 0.059 µL/cm^2) EOs showed relatively intermediate activity, while the lowest activity was observed for EOs obtained from *L. nobilis* (LC_{50} = 0.024 µL/cm^2, LC_{90} = 0.093 µL/cm^2) and *R. officinalis* (LC_{50} = 0.055 µL/cm^2, LC_{90} = 0.131 µL/cm^2). However, significant difference was observed between *R. officinalis* EO and *M. suaveolens* subsp. *timija*, *T. vulgaris*, *C. ambrosioides* and *M. piperita* EOs, while no significance difference was noticed between *R. officinalis* EO and *L. nobilis* EO regarding the lethal concentration 50 (LC_{50}). Also, a significant difference was noticed between *C. ambrosioides* EO and M. piperita, *M. communis*, and *L. nobilis* EOs. Regarding the lethal concentration 90 (LC_{90}), a significant difference was observed between *C. ambrosioides* EO and *L. nobilis* and *R. officinalis* EOs.

The data presented in Table 4 showed that all EOs revealed an interesting insecticidal potency against the adult males using fumigation method, whereas

Table 3 Insecticidal activities (LC_{50}- LC_{90}) of the tested EOs against adult males of *D. opuntiae* using contact bioassay

Essential oils	LC_{50} (µL/cm^2) (95% LC)[a]	LC_{90} (µL/cm^2) (95% LC)	Slope ± SE	χ^2	Df[b]
Mentha suaveolens subsp. *timija*	0.008 (0.004-0.013)	0.030 (0.016-0.270)	3.17 ± 0.699	1.165	2
Chenopodium ambrosioides	0.004 (0.002-0.006)	0.009 (0.006-0.032)	3.025 ± 1.229	1.452	2
Mentha piperita	0.014 (0.007-0.025)	0.059 (0.030-0.780)	3.075 ± 0.661	0.651	2
Myrtus communis	0.013 (0.007-0.022)	0.052 (0.029-0.383)	3.198 ± 0.686	0.209	2
Thymus vulgaris	0.006 (0.003-0.010)	0.025 (0.013-0.215)	3.279 ± 0.626	3.261	2
Laurus nobilis	0.024 (0.011-0.040)	0.093 (0.053-1.017)	3.020 ± 0.714	3.121	2
Rosmarinus officinalis	0.055 (0.028-0.078)	0.131 (0.089-0.456)	3.111 ± 1.080	0.785	2

Ten adult males were used for each repetition (five repetitions for each concentration)

LC values are considered significantly different when 95% LC did not overlap

SE standard error

[a]95% lower and upper confidence limits are shown in parenthesis

[b]Degree of freedom

the controls did not showed any mortality. *T. vulgaris* (LC_{50}=0.330 µL/L_{air}, LC_{90}=1.150 µL/L_{air}) and *L. nobilis* (LC_{50}=0.811 µL/L_{air}, LC_{90}=2.584 µL/L_{air}) EOs exhibited relatively the best activity. Intermediate activities were recorded for *C. ambrosioides* (LC_{50} values of 1.017 µL/L_{air} and LC_{90} of 2.899 µL/L_{air}). *M. communis* (LC_{50}=2.049 µL/L_{air}, LC_{90}=3.683 µL/L_{air}), *M. piperita* (LC_{50}=2.372 µL/L_{air}, LC_{90}=6.019 µL/L_{air}), *M. suaveolens* subsp. *timija* (LC_{50}=2.448 µL/L_{air}, LC_{90}=9.288 µL/L_{air}) and *R. officinalis* (LC_{50}=3.571 µL/L_{air}, LC_{90}=8.645 µL/L_{air}) EOs exhibited relatively significant lower insecticidal potency compared to *T. vulgaris and L. nobilis* EOs. Regarding LC_{50}, there is a significant difference between *T. vulgaris M. suaveolens* subsp. *timija* and *R. officinalis* EOs activities. Nevertheless, it appears that there was no significant difference between activities of EOs regarding the LC_{90}.

Concerning the crawlers of *D. opuntiae*, all EOs tested were toxic (Table 5), while no mortality was observed in negative control. The relatively greatest efficacy was observed with EOs obtained from *M. suaveolens* subsp. *timija* (LC_{50}=0.003 µL/cm^2, LC_{90}=0.009 µL/cm^2), *T. vulgaris* (LC_{50}=0.003 µL/cm^2, LC_{90}=0.009 µL/cm^2) and *C. ambrosioides* (LC_{50}=0.003 µL/cm^2, LC_{90}=0.018 µL/cm^2). EOs extracted from *M. communis* (LC50=0.005 µL/cm^2, $LC_{90=}$ 0.017 µL/cm^2) and *M. piperita* (LC_{50}=0.006 µL/cm^2, LC_{90}=0.012 µL/cm^2) showed relatively intermediate efficacies. The lowest potency was observed for *L. nobilis* (LC_{50}=0.020 µL/cm^2, LC_{90}=0.050 µL/cm^2) and *R. officinalis*

Table 4 Insecticidal activities (LC_{50}- LC_{90}) of the tested EOs against adult males of *D. opuntiae* using fumigation bioassay

Essential oils	$LC_{50\ (air)}$ (95% LC)[a]	$LC_{90\ (air)}$ (95% LC)	Slope ± SE	χ^2	Df[b]
Mentha suaveolens subsp. *timija*	2.448 (1.440-4.500)	9.288 (4.879-18.576)	3.171 ± 0.955	0.191	2
Chenopodium ambrosioides	1.017 (0.370-1.523)	2.899 (1.882-12.811)	2.947 ± 0.955	0.191	2
Mentha piperita	2.372 (1.628-3.618)	6.019 (3.865-19.247)	3.802 ± 0.834	2.523	2
Myrtus communis	2.049 (1.516-2.788)	3.683 (2.724-7.743)	3.767 ± 1.337	0.192	2
Thymus vulgaris	0.330 (0.205-0.598)	1.150 (0.624-9.293)	3.298 ± 0.718	0.458	2
Laurus nobilis	0.811 (0.446-1.256)	2.584 (1.562-7.752)	3.307 ± 0.770	1.757	2
Rosmarinus officinalis	3.571 (2.391-5.190)	8.645 (5.777-19.101)	3.771 ± 0.885	1.656	2

Ten adult males were used for each repetition (five repetitions for each concentration)

LC values are considered significantly different when 95% LC did not overlap

SE standard error

[a]95% lower and upper confidence limits are shown in parenthesis

[b]Degree of freedom

Table 5 Insecticidal activities (LC_{50}- LC_{90}) of the tested EOs against crawlers of *D. opuntiae* using contact bioassay

Essential oils	LC_{50} (µL/cm^2) (95% LC)[a]	LC_{90} (µL/cm^2) (95% LC)	Slope ± SE	χ^2	Df[b]
Mentha suaveolens subsp. *timija*	0.003 (0.002-0.006)	0.009 (0.006-0.048)	3.48 ± 0.858	0.784	2
Chenopodium ambrosioides	0.003 (0.002-0.013)	0.018 (0.007-5.205)	2.650 ± 0.664	0.292	2
Mentha piperita	0.006 (0.004-0.008)	0.012 (0.009-0.032)	3.605 ± 1.019	0.174	2
Myrtus communis	0.005 (0.003-0.016)	0.017 (0.008-0.958)	2.868 ± 0.762	0.120	2
Thymus vulgaris	0.003 (0.002-0.005)	0.009 (0.005-0.069)	3.357 ± 0.732	2.019	2
Laurus nobilis	0.020 (0.013-0.029)	0.050 (0.034-0.142)	3.565 ± 0.934	0.288	2
Rosmarinus officinalis	0.051 (0.034-0.078)	0.139 (0.088-0.480)	3.701 ± 0.800	1.983	2

Ten crawlers were used for each repetition (five repetitions for each concentration)

LC values are considered significantly different when 95% LC did not overlap

SE standard error

[a]95% lower and upper confidence limits are shown in parenthesis

[b]Degree of freedom

(LC_{50}=0.051 µL/cm^2, LC_{90}=0.139 µL/cm^2) EOs. From a statistical point of view, a significance difference was found between *R. officinalis* EO and all other EOs. Also, a significant difference has been observed between *L. nobilis* EO and *M. suaveolens* subsp. *timija, T. vulgaris* and *M. piperita* EOs.

The results of fumigation toxicity showed that all EOs tested were toxic against *D. opuntiae* crawlers, but with variable degrees (Table 6). The relatively best activity was exhibited by EOs obtained from *C. ambrosioides* (LC_{50}=0.755 µL/L_{air}, LC_{90}=2.201 µL/L_{air}) and *T. vulgaris* (LC_{50}=0.787 µL/L_{air}, LC_{90}=1.272 µL/L_{air}). *M. suaveolens* subsp. *timija* (LC_{50}=1.427 µL/L_{air}, LC_{90}=3.011 µL/L_{air}), *M. piperita* (LC_{50}=1.447 µL/L_{air}, LC_{90}=3.834 µL/L_{air}), *M. communis* (LC_{50}=1.997 µL/L_{air}, LC_{90}=8.639 µL/L_{air}) and *L. nobilis* (LC_{50}=2.357 µL/L_{air}, LC_{90}=8.367 µL/L_{air}) EOs exhibited relatively intermediate insecticidal efficacy. *R. officinalis* EO (LC_{50}=10.162 µL/L_{air}, LC_{90}=24.141 µL/L_{air}) exhibited relatively lowest activity. The latter is significantly different compared to all other EOs regarding the LC50 values. Also, a significant difference was observed between *T. vulgaris* EO and *L. nobilis, M. suaveolens* subsp. *timija*, and *M. communis* EOs. The same tendency has been showed between *C. ambrosioides* EO and *L. nobilis* EO. Regarding the lethal concentration 90 (LC_{90}), a significant difference has been observed between *R. officinalis* EO and *T. vulgaris*, *M. suaveolens* subsp. *timija*, *C. ambrosioides* and *M. piperita* EOs. A significant difference was observed also between *M. communis* and *L. nobilis* EOs and *T. vulgaris* EO.

Table 6 Insecticidal activities (LC_{50}- LC_{90}) of the tested EOs against crawlers of *D. opuntiae* using fumigation bioassay

Essential oils	LC_{50} ($_{air}$) (95% LC)[a]	LC_{90} ($_{air}$) (95% LC)	Slope ± SE	χ^2	Df[b]
Mentha suaveolens subsp. *timija*	1.427 (1.034-2.089)	3.011 (2.064-7.919)	3.829 ± 1.032	2.555	2
Chenopodium ambrosioides	0.755 (0.506-1.308)	2.201 (1.281-6.240)	3.546 ± 0.778	1.778	2
Mentha piperita	1.447 (0.994-2.358)	3.834 (2.354-9.982)	3.697 ± 0.819	3.148	2
Myrtus communis	1.997 (1.209-6.945)	8.639 (3.659-19.006)	2.841 ± 0.709	0.941	2
Thymus vulgaris	0.787 (0.551-1.003)	1.272 (0.959-2.699)	3.515 ± 1.579	0.002	2
Laurus nobilis	2.357 (1.492-7.645)	8.367 (3.841-20.051)	2.972 ± 0.784	1.093	2
Rosmarinus officinalis	10.162 (7.140-15.288)	24.141 (15.862-70.436)	3.874 ± 0.880	2.591	2

Ten crawlers were used for each repetition (five repetitions for each concentration)

LC values are considered significantly different when 95% LC did not overlap

SE standard error

[a]95% lower and upper confidence limits are shown in parenthesis

[b]Degree of freedom

Discussion

This study has revealed the insecticidal activity of seven EOs obtained from Moroccan aromatic plants on adult males and crawlers of *D. opuntiae*, an aggressive pest of *O. ficus-indica* in Morocco. The major chemical constituents of all of the essential oils were monoterpenoids. Due to their high volatility, these compounds possess great fumigant activity that might be of useful for pest management (Saad et al., 2006). It has been shown that monoterpenoids kill insects by a nervous system dysfunction or neuromuscular action (Waliwitiya et al., 2009). In our study, the insecticidal activity varied with the examined development insect stage, the applied method and the plant EOs used. The present work corroborates the findings of several previous studies using EOs in different formulations and doses against *D. opuntiae*. It has been reported that involved mechanisms showed that EOs act on insects in several ways. These compounds can be toxic by direct contact and others act by fumigation (Basaid et al., 2020).

All of the EOs studied have already been tested as natural bio-insecticides against a variety of pest insects including *Ephestia kuehniella*, *Pochazia shantungensis*, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Tribolium castaneum* and proved their high effectiveness (Aouadi et al., 2020; Ben Jemâa et al., 2012; Chu et al., 2011; Harraz et al., 2015; Park et al., 2017; Pavela, 2008). Based on the results obtained in the present work, it appears that EOs obtained from *T. vulgaris* and *C. ambrosioides* exhibited the highest insecticidal activity in both direct contact and fumigation bioassays against the two mobile stages (adult male and crawlers) of *D. opuntiae*. The impact may be explained by the monoterpene richness. These compounds have several possible mode of action. Miyazawa and Yamafuji (2005) reported the potent inhibitor of acetylcholinesterase (AChE) activity of monoterpenes. These substances can act on the nervous system of invertebrates including the blockage of octopamine receptors sites (Enan, 2001). They are also considered as inhibitors of AChE (Park et al., 2001, 2003a, b) as well as tyramine receptors (Enan, 2005). The toxic effect of *T. vulgaris* EO may be explained by its richness in thymol, a phenolic monoterpene well known for its potent insecticide to many pest insects (Park et al., 2017). In addition, p-cymene, one of the major compounds of EO of this species has been reported to have effect to produce a high mortality in some insects (Martinez-Velazquez et al., 2011). Park et al. (2017) have indicated that the analogue insecticidal mode of action of thymol could be largely attributed to the methyl functional group with promising potential as first choice insecticide. The insecticidal effectiveness of *C. ambrosioides* EO towards the mobile stages of *D. opuntiae* may be explained by its richness in some monoterpenes recognized for their insecticidal potency such as terpinolene, p. cymene, ascaridol and thymol. A previous study have already shown that the use of extracts of *C. ambrosioides* have a potent effect on *D. opuntiae* using direct contact application (Vigueras et al., 2009). Furthermore, the EO extracted from this plant species has also proved a significant larvicidal activity against *Culex pipiens* (Harraz et al., 2015). Our results indicated that *L. nobilis* was more active in fumigation bioassay compared to the contact method. On the other hand, *R. officinalis* has presented the lower toxicity against the two mobile stages of the insect whatever the application methods. Indeed, 1.8-cineole and (+)-2-bornanone present in *Seriphidium brevifolium* which was tested against fire ants *Solenopsis invicta* suggested that (+)-2-bornanone did not show an insecticidal activity compared to 1.8-cineole (Feng et al., 2019). In this context, Liska et al. (2010) showed that 1.8 cineole has a better insecticidal activity against *T. castaneum* by fumigation toxicity compared to the contact toxicity. Pérez-Ramirez et al. (2014) demonstrated the ability of 1.8-cineole (99%) to prevent crawlers from establishing on non-infested cladodes. In our study, *M. piperita* and *M. communis* showed an intermediate efficacy against both stages and for de two methods used. In fact, several studies have reported the insecticidal effectiveness of *M. piperita* and *M. communis* on a range of insect pests, including stored-product insects *Callosobruchus maculates*, *Sitophilus oryzae* and *T. castaneum* (Rajkumar et al., 2019; Khani & Basavand, 2012), *Musca domestica* (Kumar et al., 2012), *Aedes aegypti* (Kumar et al., 2011), *Aedes albopictus* (Conti et al., 2010) and *Ephestia kuehniella* (Aouadi et al., 2020). As reported previously, the insecticidal activity of the EOs obtained from these aromatic species has been attributed to their richness in 1.8 cineole and pulegone. Concerning *M. suaveolens* subsp. *timija*, its EO was shown to be more active in contact method. The toxicity of *M. suaveolens* subsp. *timija* EO may be attributed to

the major components menthone (40.42%) and pulegone (19.22%). This result is in agreement with previous work (Laghzaoui et al., 2019), where it has been demonstrated the high contact efficacy of EOs of this aromatic plant on eggs hatching and larvae of the tick *Hyalomma aegyptium.* Also, the highest contact toxicity of *M. suaveolens* subsp. *timija* EO towards the red flour beetle, *T. castaneum* has been demonstrated in other previous work (Kasrati et al., 2015). Ramdani et al. (2021) showed that the use of *Mentha pulegium* EO, rich in pulegone, in combination with traditional fatty acid soap (soft soap) or in double sprays could be integrated in the management package for the control of the wild cochineal *D. opuntiae*, as a safe and natural alternative to chemical insecticides. Indeed, it has been reported that these two monoterpene compounds affect embryo development and molting stages (Pohlit et al., 2011).

Conclusion

This research work suggests that EOs tested are potentially effective, and therefore seem to offer an alternative measure for the control of the mobile stages of *D. opuntiae*. However, this activity varied, depending on plant EO, and the targeted mobile stages of the insect. The study showed that these EOs act on the two stages of insect in several ways. *M. suaveolens* subsp. *timija* EO appeared more efficacious by direct contact, while *L. nobilis* EO was more effective by fumigation. Interestingly, *T. vulgaris* and *C. ambrosioides* EOs were observed to have high toxicity towards the two mobile stages tested whatever the method used. However, the toxicity of the other EOs remains intermediate or weakest with regard to the treated stages or the method applied. Of the EOs tested, *R. officinalis* EO exhibited the weakest activity towards the mobile stage of the insect. The finding of the present study encourages the potential use of these EOs, particularly those extracted from *T. vulgaris* and *C. ambrosioides*, in different aqueous formulations (e.g. encapsulation) to be applied in integrated control strategies of this destructive pest insect. Additional studies focusing on the mixture between active EOs and on the main bioactive compounds are necessary. Also, an in vivo study to control the insect outdoor spread in the fields of *O. ficus indica* is the next phase of our research.

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Declarations

Competing interests The authors declare that they have no known competing interests.

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