# How monoterpene hydrocarbons could be an alternative insecticide for effective control of Lepidoptera defoliators?

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¿Cómo los hidrocarburos monoterpénicos podrían ser un insecticida alternativo para el control efectivo de los Lepidópteros defoliadores?

Com els hidrocarburs monoterpès podrien ser un insecticida alternatiu per al control efectiu dels defoliadors de lepidòpters?

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## ABSTRACT

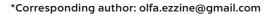
In Tunisia, Orgyia trigotephras (Lepidoptera, Erebidae) is considered among the most harmful pests for forests that cause significant damage to kermes oak and its maquis. Thus, the aim of this study was to evaluate the insecticidal effect of essential oils of Eucalyptus camaldulensis, E. kirtoniana, Thymus algerienis and T. *capitatus* toward the 3<sup>rd</sup> instar larvae of *O. trigotephras*. The chemical composition of essential oils was studied using GC-MS. Different concentrations of essential oils were used for contact action and compared to synthetic insecticide deltamethrin as a reference product. The insecticidal effects of the essential oils were evaluated by measuring the lethal time of 50% of larvae ( $LT_{50}$ ) and the lethal time of 100% of larvae ( $LT_{100}$ ). Eucalyptus essential oils were found effective when compared with the essential oil of *Thymus* spp. and deltamethrin. Their high toxicity may be attributed to their richness in monoterpenes hydrocarbons. Compounds belonging to monoterpenes hydrocarbons may constitute an alternative insecticide for controlling Lepidoptera defoliators outbreaks.

**Keywords:** Essential oil, *Eucalyptus, Thymus,* Larvae, Contact action, Toxicity

## RESUMEN

En Túnez, Orgyia trigotephras (Lepidoptera, Erebidae) está considerada entre las plagas más dañinas para los bosques y causa importantes daños a la coscoja y su maquis. Así, el objetivo de este estudio fue evaluar el efecto insecticida de los aceites esenciales de Eucalyptus camaldulensis, E. kirtoniana, Thymus algerienis y T. capitatus hacia las larvas de tercer estadio de O. trigotephras. La composición química de los aceites esenciales se estudió mediante GC-MS. Se utilizaron diferentes concentraciones de aceites esenciales para la acción de contacto y se compararon con el insecticida sintético deltametrina como producto de referencia. Los efectos insecticidas de los aceites esenciales se evaluaron midiendo el tiempo letal del 50% de las larvas (LT50) y el tiempo letal del 100% de las larvas (LT100). Los aceites esenciales de eucalipto resultaron eficaces en comparación con el aceite esencial de Thymus spp. y deltametrina. Su alta toxicidad puede atribuirse a su riqueza en hidrocarburos monoterpénicos. Los compuestos pertenecientes a hidrocarburos monoterpénicos pueden constituir un insecticida alternativo para controlar los brotes de Lepidópteros defoliadores.

**Palabras clave:** Aceite esencial, Eucalipto, Timo, Larvas, Acción de contacto, Toxicidad :





### RESUM

A Tunísia, Orgyia trigotephras (Lepidoptera, Erebidae) es considera una de les plagues més nocives per als boscos que causen danys importants a l'alzina i la seva màquia. Així, l'objectiu d'aquest estudi va ser avaluar l'efecte insecticida dels olis essencials d'Eucalyptus camaldulensis, E. kirtoniana, Thymus algerienis i T. capitatus cap a les larves de 3r estadi d'O. trigotephras. La composició química dels olis essencials es va estudiar mitjancant GC-MS. Es van utilitzar diferents concentracions d'olis essencials per a l'acció de contacte i es van comparar amb l'insecticida sintètic deltametrina com a producte de referència. Els efectes insecticides dels olis essencials es van avaluar mesurant el temps letal del 50% de les larves (LT50) i el temps letal del 100% de les larves (LT100). Els olis essencials d'eucaliptus es van trobar eficaços en comparació amb l'oli essencial de Thymus spp. i deltametrina. La seva alta toxicitat es pot atribuir a la seva riquesa en hidrocarburs monoterpens. Els compostos pertanyents als hidrocarburs monoterpès poden constituir un insecticida alternatiu per controlar els brots de defoliadors de lepidòpters.

**Paraules clau:** Oli essencial, Eucaliptus, Tim, Larves, Acció de contacte, Toxicitat

## INTRODUCTION

Forests play an essential role in the provision of a wide range of ecosystem services that are very crucial to human well-being<sup>1</sup>. Nevertheless, they are threatened by various environmental stress factors, mainly drought and biotic constraints such as pests<sup>2</sup> and diseases<sup>3</sup>. Among which, Lepidoptera is considered the most harmful defoliators causing significant damage to forests especially the larval stage which is the most destructive. In Tunisia, larvae of the moth Orgyia trigotephras (during the outbreak peak in 2005) caused a defoliation of more than 500 ha of evergreen shrubs in the northeast. It was recorded that 3rd Instar larvae feed on the surface resulting in skeletonization and a burnt appearance<sup>4</sup>. The high mobility of larvae allows them to feed on several host plants mostly Fagaceae Quercus coccifera L. and Anacardiaceae Pistacia lentiscus L.<sup>4</sup>. This situation led to serious concern for the conservation of Mediterranean vegetation specific to this region<sup>5</sup>. Likewise, larvae of O. trigotephras caused considerable damage to P. lentiscus in 2009/2010 in Italy6.

In order to deal with insect pests, synthetic chemical pesticides are widely used throughout the world. Although, the use of these products may cause adverse effects on human health<sup>78,9</sup> and the environment<sup>10,11</sup> (Singh et al., 2018; Yatoo et al., 2022), including non-target organisms<sup>11,12</sup>causing the disturbance of the biological balance. Hence, biopesticides based on essential oils and plant extracts are an eco-friendly alternative to chemical pesticides<sup>13,14,15</sup>. Therefore, the use of natural plant products can be a potential method as part of an integrated management strategy in the forests. In this context, essential oils extracted from aromatic and medicinal plant species were tested for their insecticidal activity against larvae of Lepidoptera as Lymantria dispar<sup>16,17</sup>, Thaumetopoea pityocampa<sup>18,19</sup> and O. trigotephras<sup>20,21,22,23</sup>. Plant species of Myrtaceae and Lamiaceae families present sources of medicinal virtues since ancient times and were used in traditional medicine<sup>24,25</sup>. Within Myrtaceae family, *Eucalyptus* species are the most popular plants possessing a wide range of health-promoting activities<sup>26</sup>. Furthermore, their essential oils present potential interest in controlling insects and fungal pathogens in agriculture, market garden greenhouse and forestry owing to their antifungal and insecticidal properties<sup>27,28</sup>. Likewise, essential oils from species of the Lamiaceae family are reported to be effective against several pests and are worthy of exploitation in pesticide formulation<sup>29</sup>. In fact, Essential oils of E. camaldulensis were used for insecticidal activity against mosquitoes<sup>30</sup>, and the Braconid Habrobracon hebetor<sup>31</sup>. These substances were also used against *Ectomyelois ceratoniae*<sup>32</sup>. Likewise, Eucalyptus kirtoniana was not used against herbivores. As far as Thymus spp. were tested for its insecticidal activity against aphid<sup>32</sup>, coleopteran<sup>33</sup>, dipteran<sup>34</sup> and stored product insects<sup>35</sup>. In this context, the present study aims to test the insecticidal effect of essential oils from two Myrataceae species (Eucalyptus camaldulensis and *Eucalyptus kirtoniana*) and two Lamiaceae species (Thymus algeriensis and Thymus capitatus) against the 3<sup>rd</sup> Instar larvae of *Orgyia trigotephras*.

## MATERIALS AND METHODS

#### Insects

Larvae of *Orgyia trigotephras* were collected from Jebel Ben Oulid, located in Northeastern Tunisia. At the laboratory larvae were sorted and conserved in groups of 50/box ( $21 \times 10 \times 10$  cm) at a temperature of  $25\pm2^{\circ}$ C and fed every two days on fresh leaves of *Q. coccifera* according to the protocol of Akkari et al.<sup>22</sup>.

#### Plant material and essential oil extraction

Branches from the two Myrtaceae species (Eucalyptus camaldulensis and Eucalyptus kirtoniana) and the two Lamiaceae species (Thymus algeriensis and Thymus capitatus) originating from Cap Bon, region of northeastern Tunisia were harvested and separately placed in plastic bags. Plant species identification was done courtesy of Pr. Mohamed BOUSSAID and voucher specimens (EC01, EK01, TA01, and TC01) were deposited with the Herbarium of the National Institute for Research in Rural Engineering Water and Forest (INRGREF) for future reference. Leaves were removed manually, air-dried at room temperature (20-25°C) for two weeks and grounded into fine powders. For the extraction, the method of Riahi et al.<sup>36</sup> was used. Briefly for each species, 100 g of dry matter were used for essential oil extraction by hydro-distillation using a Clevenger-type apparatus. Sodium sulfate anhydrous was added in order to remove water after essential oil extraction. The extracted essential oils were stored in Eppendorf safe-lock tubes and stored at 4° C.

#### **Chemical composition**

The chemical composition of essential oils was analysed by GC-MS using an Agilent 7890A gas chromatograph equipped with an HP-5MS fused silica column (30 m × 0.25 mm), and coupled with a mass spectrometer (HP 5972) following the method of Messaoud et al.<sup>37</sup>. Each essential oil (1  $\mu$ L) was injected in triplicate in the split mode (1:20). Helium was used as gas carrier at 1.2 mL/min. The used program was isothermal at 70 °C, followed by 50–240 °C at a rate of 5 °C/min, then held at 240 °C for 10 min. The total electronic impact mode at 70 eV was used. Terpenic compounds were identified by comparison of their retention indices determined with reference to a homologous series of C9–C24 and by comparison of their mass spectra with those recorded in NIST08 and W8N08 libraries.

#### Preparation of test solutions

Four different concentrations of essential oils (0.05; 0.10; 0.50 and 1%) were prepared to test their insecticidal activity by contact action against larvae of *O. trigotephras.* The larvicidal effect of essential oils was appreciated by comparison to a standard chemical insecticide deltamethrin (reference product, provided by Atlas Agro-Tunisia) at a concentration of 0.015% used as positive control. Ethanol (96%) used for essential oil dilutions was used as negative control<sup>22,23</sup>.

#### Larvicidal activity

A total of 200 larvae of the  $3^{rd}$  instar were used for the evaluation of the larvicidal activity. According to Kanat and Alma<sup>18</sup>, an aliquot of 10µl of essential oils at different concentrations were poured using a micropipette (10µl) onto 10 larvae placed in Petri dishes (R= 9cm). For each essential oil concentration, six replications were performed. Ethanol and deltamethrin were deposited on the back of larvae. The effects of the essential oils, deltamethrin and ethanol (96%) were evaluated using Akkari et al.<sup>22</sup> method by measuring the Lethal Time for killing 50% of larvae, (LT<sub>50</sub>) the Lethal Time for killing 100% of larvae (LT<sub>100</sub>).

#### **Statistical analysis**

Statistical analyses were performed using the SPSS-10.0 software package for Windows. Percentages of terpene compounds and measurements of insecticidal action were assessed as Means  $\pm$  Standard deviations (SD). Variations of terpene composition, terpenic classes, and insecticidal activity between the *Eucalyptus* and *Thymus* species were determined using the student test. The correlations between terpenic classes and larvicidal activity expressed as LT<sub>50</sub> and LT<sub>100</sub> were determined by the Pearson test.

## RESULTS

#### Essential oils' chemical composition and characterization

A total of 53 terpenic compounds were identified for the four studied species, with 20 for *Eucalyptus cama*-

## *ldulensis*, 29 for *Eucalyptus kirtoniana*, 24 for *Thymus algeriensis*, and 12 for *Thymus capitatus* (Table 1).

For Myrtaceae species, *p*-cymene and cryptone were the major compounds of *E. camaldulensis* essential oil while  $\alpha$ -pinene, *p*-cymene, and 1.8-cineole were the main compounds of *E. kirtoniana* essential oil (Table 1). The two species were characterized by their richness in monoterpene hydrocarbons (48.14 % for *E. camaldulensis* and 41.52 % for *E. kirtoniana*). The student test showed highly significant differences (P < 0.001) in all terpenic compound contents, in monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, and ketones between the two species.

Concerning Lamiaceae species, *T. algeriensis* essential oil revealed high amounts in  $\alpha$ -pinene, 1.8-cineole, camphor and elemol. Whereas, *T. capitatus* essential oil was characterized by the dominance of carvacrol (Table 1). The two species showed high contents in oxygenated monoterpenes (45.21% for *T. algeriensis* and 78.50% for *T. capitatus*). All identified compounds varied significantly (P < 0.001) between the two species. Likewise, significant variations were noted in monoterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes (P < 0.001) between the two species. However, no significant variation was revealed in sesquiterpene hydrocarbons (P = 0.440).

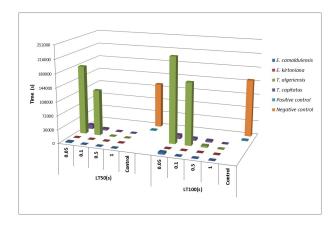


Fig.1. Larvicidal activity of essential oils in comparison with negative and positive controls

#### Larvicidal activity of essential oils

The essential oils of Myrtaceae species showed larvicidal activity against the larvae of *O. trigotephras* in comparison with negative control (Fig. 1). However, this activity expressed as lethal time for killing 50% and 100 % of larvae, varied significantly between the two Myrtaceae species (P < 0.001) for all concentrations. Indeed, *E. kirtoniana* essential oil was more efficient than that of *E. camaldulensis* by revealing shorter lethal times especially at the highest concentration (1%) (LT<sub>50</sub> = 22.43±0.7 sec and LT<sub>100</sub> = 25.31±0.43 sec). Thus, *E. kirtoniana* essential oil was even more active than deltamethrin (Table 2, Fig. 1).

The essential oils from Lamiaceae species revealed larvicidal activity against the larvae of *O. trigotephras* compared to the negative control. Furthermore, they

<b>)</b> TO	<i>c</i> :			Myrtaceae		Lamiaceae	
N°	Compounds	RI	E. camaldulensis	E. kirtoniana	T. algeriensis	T. capitatus	
1	α-Pinene	938	2.99± 0.08 tr	22.04± 0.04 tr	13.6±2.1	tr	
2	Camphene	954	tr	tr	3.23±0.65	0.1±0.0	
3	Sabinene	978			0.8±0.2	0.2±0.2	
4	β-Pinene	980	tr	$0.68 \pm 0.03$	$2,9\pm0,4$	tr	
5	β-myrcene	994	tr	$0.1 \pm 0.01$	0.2±0.1 tr	0.7±0.0 tr	
6	α-Phellandrene	1002	$0.58 \pm 0.03$	$0.37 \pm 0.01$			
7	α-Terpinene	1018	$0.28 \pm 0.02$	$0.08 \pm 0.01$	$0.1 \pm 0.1$	$1.0 \pm 0.1$	
8	α-thujene	1024	tr	tr	tr	0.5±0.2	
9	<i>p</i> -cymene	1026	41.85±1.52	$14.74 \pm 0.02$	0.6±0.1	4.9±0.45	
10	β-Terpinene	1028	$2.16 \pm 0.35$	tr	tr	tr	
11	Limonene	1030	tr	$1.86 \pm 0.02$	tr	tr	
12	1.8-cineole	1033	2.87± 0.44	$12.01 \pm 1.21$	13.9±2.0	tr	
13	β -ocimene	1053	tr	tr	1.1±0.2	tr	
14	δ-Tepinene	1062	$0.21 \pm 0.07$	$10.55 \pm 0.02$	0.4±0.0	5.7±0.3	
15	Cymenene	1068	$0.64 \pm 0.1$	tr	tr	tr	
16	α -Thujone	1076	$0.43 \pm 0.09$	tr	tr	tr	
17	Linalool	1099	tr	tr	2.5±0.5	1.2±0.1	
18	Endo-fenchol	1112	tr	$1.03 \pm 0.02$	tr	tr	
19	α-campholenal	1125	tr	$0.32 \pm 0.02$	tr	tr	
20	Trans-pinocarveol	1138	tr	$9.46 \pm 0.06$	$1.5 \pm 0.1$	tr	
21	2-pinen-3-one	1142	tr	$2.2 \pm 0.02$	tr	tr	
22	Phellandral	1168	$5.07 \pm 0.18$	tr	tr	tr	
23	Borneol	1175	tr	$1.89 \pm 0.05$	$5.9 \pm 0.2$	0.5±0.3	
24	Terpinen-4-ol	1177	$3.67 \pm 0.13$	tr	$1.3 {\pm} 0.1$	0.3±0.2	
25	p-Menth-2-en-ol	1179	$2.38 \pm 0.20$	tr	tr	tr	
26	Cryptone	1180	$18.13 {\pm}~0.89$	tr	tr	tr	
27	$\alpha$ -carvomenthenol	1182	tr	$0.9 \pm 0.06$	tr	tr	
28	β-fenchol	1185	tr	$2.27 \pm 0.08$	tr	tr	
29	α-Terpineol	1191	$0.2 \pm 0.01$	tr	$1.6 \pm 0.2$	tr	
30	cis-piperitol	1194	$0.4 \pm 0.07$	tr	tr	tr	
31	Myrtenal	1197	tr	$0.27 \pm 0.03$	$1.2 \pm 0.1$	tr	
32	Carvone	1259	$0.25 \pm 0.01$	tr	tr	tr	
33	Bornyl acetate	1285	tr	$0.11 \pm 0.01$	tr	tr	
34	Thymol	1290	$2.02 \pm 0.12$	$0.53 \pm 0.02$	tr	tr	
35	Verbenone	1292	tr	tr	0.9±0.1	tr	
36	Carvacrol	1299	tr	tr	tr	76.5±4.0	
37	o-cymen-5-ol	1334	$1.71 \pm 0.06$	tr	0.1±0.0	tr	
38	2-carene	1387	tr	$0.1 \pm 0.01$	tr	tr	
39	α-gurjunene	1409	tr	$0.43 \pm 0.04$	tr	tr	
40	β-caryophyllene	1418	$0.31 \pm 0.02$	tr	2.1±1.4	2.8±0.2	
41	Aromadendrene	1442	tr	$3.36 \pm 0.03$	tr	tr	
42	Alloaromadendrene	1461	$0.66 \pm 0.07$	$1.11 \pm 0.05$	tr	tr	
43	δ-gurjunene	1476	tr	$3.31 \pm 0.03$	tr	tr	
44	δ-cadinene	1520	tr	$0.11 \pm 0.01$	tr	tr	
45	Camphor	1532	tr	tr	16.31±0.33	tr	
46	Elemol	1541	tr	tr	8.2±2.3	tr	
47	Caryophyllene oxide	1581	tr	tr	6.8±0.6	tr	
48	β-selinene	1586	tr	$4.41 \pm 0.04$	tr	tr	
49	Sapthulenol	1570	tr	4.41± 0.03	tr	tr	
50	Veridifloral	1590	tr	0.82± 0.01	3.3±0.9	tr	
51	β-eudesmol	1649	tr	tr	0.6±0.3	tr	
52	α-eudesmol	1656	tr	tr	0.6±0.2	tr	
53	Calarene	1674	tr	$4.41 \pm 0.03$	tr	tr	
55	Grouped compounds						
	Monoterpene hydrocarbons		48.14±1.46	41.52±0.09	22.93±2.31	13.1±0.98	
	Oxygenated monoterpenes		43.14±1.40 19.00±1.05	30.99±1.34	45.21±2.64	78.50±4.00	
			0.97±0.09	17.14±0.11	2.10±1.40	2.80±0.20	
	Sesquiterpenes hydrocarbons Oxygenated sesquiterpenes		0.97±0.09	5.23±0.04	$2.10 \pm 1.40$ 19.5±2.20	2.80±0.20 0	
	Oxygenated sesquiterp Ketones	001162		5.23±0.04 0		0	
	Total identified (%	<u> </u>	18.13±0.89		0		
	Total Identified (%	1	86.81±0.42	94.88±1.31	89.74±4.75	94.4±3.92	

 Table 1. Essential oils' chemical composition of the studied Myrtaceae and Lamiaceae species (Mean±SD).

Tr: trace ; RI: Retention indice

were more potent than the deltamethrin. Regarding the two Lamiaceae species, highly significant differences were noted in  $LT_{50}$  and  $LT_{100}$  between them at concentrations of 0.05% and 0.05% (P < 0.005). Nevertheless, no significant difference was revealed at concentrations of 0.5% and 1% (P = 0.975 and P = 0.607, respectively) (Table 2).

Table 2. Larvicidal activity of essential oils on larvae of							
Orgyia trigotephras							

Essential oil	Essential oil C (%)		LT <sub>100</sub> (s)	
	0.05	2481.5±339.18	5329.23±1057.54	
E. camaldulensis	0.1	156.10±6.20	226.95±23.00	
E. camatautensis	0.5	125.46±9.09	90.23±8.22	
	1	73.90±2.96	89.21±3.29	
	0.05	78±2.54	$101.25 \pm 4.38$	
E. kirtoniana	0.1	42.13±0.43	46.56±0.71	
	0.5	32.23±0.23	34.51±0.34	
	1	22.43±0.37	25.31±0.43	
	0.05	181003±27917.50	224166.9±23918.50	
T. algeriensis	0.1	120261.2±24867.99	162535.07±18061.46	
1. augeriensis	0.5	423.84±117.55	4486.15±959.81	
	1	154.65±6.44	189.47±12.89	
	0.05	11134.4±1161,68	10173.94±873.23	
T. capitatus	0.1	4311.9±1846.1	6113.7±1593.13	
1. cupitatus	0.5	734.25±510.58	4434.75±1373.00	
	1	139.6±10.75	176.61±21.98	
Positive control (deltamethrin at 0.015%)		1587.33±11.79	1724±28.71	
Negative control (Ethanol 96%)		118616±41225.90	149783.66±39235.19	

C: essential oil concentration; LT<sub>50</sub>: Lethal time of 50% of larvae; LT<sub>100</sub>: Lethal time of 100% of larvae

Pearson test showed negative and highly significant correlations between larvicidal activity and monoterpene hydrocarbons or sesquiterpene hydrocarbons (Table 3), contrary to oxygenated monoterpenes which revealed positive and significant correlation with larvicidal activity.

 Table 3.
 Correlation coefficients (R) between terpenic

 classes and LT<sub>50</sub> or LT<sub>100</sub> (at 1%)

	LT 50	LT100	
Terpenic classes			
Monoterpene hydrocarbons	-0.801"	-0.815**	
Oxygenated monoterpenes	0.651°	0.674°	
Sesquiterpene hydrocarbons	-0.764"	-0.761**	
Oxygenated sesquiterpenes	0.422 <sup>ns</sup>	0.398 <sup>ns</sup>	
Ketones	-0.258**	-0.266**	

ns: not significant, \*: significant at p < 0.05, \*\*: highly significant at p < 0.01

## DISCUSSION

Among essential oils, *Eucalyptus* spp. and *Thymus* spp. possess a wide range of desirable properties worth exploiting for pest management<sup>33,38</sup>, because of their efficacy against fungi, bacteria, insects, mites, and weeds <sup>27,28,29,30,34,35,39</sup>. They provide a simple, inexpensive, and environment friendly (non-polluting and lesser or no toxicological concerns) alternative pest control<sup>34,40</sup>. *Eucalyptus* oil can directly act as a natural insect repellent to provide protection against mosquitoes and other harmful arthropods or serves antifeedant activity against herbivores<sup>35,40</sup>. No investigations in the literature on insecticidal activity of *Eucalyptus camaldulensis*, *Eucalyptus kirtoniana*, *Thymus algeriensis*, and *Thymus capitatus* against *Orgyia trigotephras*.

Essential oils tested in the present study showed a contact insecticidal activity against the 3<sup>rd</sup> instar larvae of *Orgyia trigotephras*. Results showed that the two *Eucalyptus* essential oils were by far the most active in comparison with *Thymus* essential oils. These findings concur well with previous results of Oulebsir-Mohandkaci et al.<sup>39</sup> which revealed that *Eucalyptus globulus* essential oil is more effective than *Thymus vulgaris* essential oil towards the green peach aphid, *Myzus persicae*.

In the same context, Ben Slimane et al.<sup>20</sup> tested the toxicity of two *Eucalyptus* species against the 3<sup>rd</sup> instar larvae of *Orgyia trigotephras*,  $LT_{50}$  and  $LT_{100}$ , obtained after treatment with 0.5% were 1 min 52 sec and 2 min 38 sec for *E. lehmannii* and 1 min 7 sec and 1 min 39 sec for *E. globulus*, respectively. In our study, at the same concentration, *E. camaldulensis* showed a  $LT_{50}$  of 90.23 sec and  $LT_{100}$  of 125.46 sec. However, *E. kirtoniana* showed a  $LT_{50}$  of 32.23 sec and  $LT_{100}$  of 34.51 sec. For all used solutions, the insecticidal activity of *E. kirtoniana* was more important than that of *E. camaldulensis*.

Among the various components of Eucalyptus essential oil, 1, 8-cineole is considered the characteristic compound of the genus Eucalyptus, which defines the quality of *Eucalyptus* and is responsible for a variety of its pesticidal properties<sup>42</sup>. The highest 1,8-cineole content was recorded for E. kirtoniana (12.01%) which may give this species its larvicidal activity. However, it is not allowed to attribute the insecticidal activity of essential oil to a single compound as terpenic compounds are known to interact additively, synergistically, and/ or antagonistically<sup>27,42,43</sup>. *Eucalyptus kirtoniana* and *E*. camaldulensis essential oils were revealed to be rich in monoterpene hydrocarbons such as  $\alpha$ -pinene and *p*-cymene. Correlation analysis between this terpene class and insecticidal activity expressed as  $LT_{50}$  and  $LT_{100}$ showed highly negative significant correlations. Accordingly, the obtained results proved that as monoterpene hydrocarbons are abundant, the lethal time of larvae is short. This outcome supports previous studies reporting that monoterpene hydrocarbons showed higher toxicity in comparison with oxygenated monoterpenes against larvae of Colorado potato beetles<sup>44</sup>. Furthermore, Abdel-Sattar et al.<sup>45</sup> attributed the high insecticidal effects of Schinus molle essential oils towards Trogoderma *granarium* and *Tribolium castaneum* to their richness in monoterpenes hydrocarbons.

On the other hand, sesquiterpene hydrocarbons such as  $\alpha$ -gurjunene and  $\beta$ -caryophyllene revealed negative and significant correlations with  $LT_{50}$  and  $LT_{100}$  which may suggest the important insecticidal effect of this terpenic class against larvae of O. trigotephras. In this context, Russo et al.<sup>46</sup> reported that essential oils containing high amounts of sesquiterpene hydrocarbons, can be an excellent choice as natural insecticides because of their great effects at low concentrations and short times of exposure. The toxic activity of monoterpenes and sesquiterpenes against larvae is related to their capacity to penetrate the lipid bilayer of cells causing then lesions on larval cuticle and digestive tract<sup>47</sup>. Our results showed that the application of natural plant products such as *Eucalyptus kirtoniana* and *E*. camaldulensis essential oils which have a toxic effect against larvae of Orgyia trigotephras can be a potential method in environmental-friendly control management.

## CONCLUSION

In summary, natural products based on essential oils are developed in order to minimize the use of synthetic compounds which can be harmful to human health and the environment. The obtained results indicate notable toxicity of essentials oils against O. trigotephras. The use of natural products may be considered an important alternative insecticide for the control of this pest. Therefore, it will be interesting to study the insecticidal activity of essential oils by considering other modes of action and routes of penetration (inhalation and ingestion) and to identify the terpenic compounds responsible for the larvicidal activity against the young larvae. The present study is based on laboratory data, so it remains important to direct an applied approach to carry out it in the nursery and so in the field. Thus, it is necessary to develop formulations on the basis of these oils' essentials and the detailed action mechanism.

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