

Antifeedant effects of common terpenes from Mediterranean aromatic plants on *Leptinotarsa decemlineata*

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Abstract

Essential oil terpenes are secondary metabolites produced in different biological pathways as pollinator attraction signals, but also as defense mechanisms against insect pests, herbivores, microorganisms, competing plants, etc. In this context, we have evaluated a total of 24 terpenes commonly found in Mediterranean aromatic plants, including 8 monoterpene hydrocarbons, 9 oxygenated monoterpenes, 2 esterified monoterpenes, 3 sesquiterpene hydrocarbons and 2 oxygenated sesquiterpenes in order to determine their antifeedant effects on the Colorado potato beetle (*Leptinotarsa decemlineata* Say), a major pest of potato crops. Terpene hydrocarbons showed low antifeedant activity, whereas the oxygenated sesquiterpene (-)- α -bisabolol with an inhibition activity of 96.3% was the most active, followed by carvacrol (90.9%), (+)-Terpinen-4-ol (87.1%) and thymol (81.5%). Other terpenes like (1S)-(-)-verbenone (72.9%), (+/-)-camphor (63.4%) and linalyl acetate (60.7%) showed moderate activity. Subsequently, terpenes showing antifeedant activity against this insect were also tested in allelopathic assays to determine potential damage to the crops. Results showed phytotoxic effects for (1S)-(-)-verbenone on *Lactuca sativa* germination, and for carvacrol on both the seed germination and the leaf and root growth of *Lolium perenne*. In conclusion, (-)- α -bisabolol is the best option to develop natural antifeedant formulations against *L. decemlineata* on the basis of its high antifeedant and low phytotoxic activities.

Keywords: Antifeedant activity, *Leptinotarsa decemlineata*, (-)- α -bisabolol, (+)-Terpinen-4-ol, carvacrol

1. Introduction

Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae) or the Colorado potato beetle is a common phytophagous insect that generates severe yield losses of potato crops along America, Europe and Asia. This pest is widely known for its high fertility rates and for its capability of developing fast resistances to traditional synthetic agrochemicals (Alyokhin *et al.*, 2006; 2007). Thus, there is an increasing interest in new and safe agrochemicals to avoid resistance and minimize environmental issues. Essential oils (EOs) obtained from the distillation of aromatic and medicinal plants, have been considered as potential exploitable insecticides to control and repel different insects because of their low health and ecological risks (Tampe *et al.*, 2016; Isman, 2000). These EOs are complex mixtures of terpenes, which are secondary metabolites produced by aromatic plants involved, among others, in defense mechanisms against phytophagous pests, microorganism diseases, herbivore organisms, etc. The major terpenes found in EOs are the monoterpenes (C₁₀), but they also contain sesquiterpenes (C₁₅) or even traces of some diterpenes (C₂₀). These compounds are common in essential oils of different plants from diverse families including Asteraceae (Leonardi *et al.*, 2013), Lamiaceae (Herraiz-Peñalver *et al.*, 2015), Pinaceae (Sadeghi *et al.*, 2013), Solanaceae (Murungi *et al.*, 2013), etc. Additionally, recent studies have suggested that the addition of micronutrients and the inoculation of aromatic plants with mycorrhiza can enhance the production of different terpenes as well as other secondary metabolites, increasing the yield of biocidal compounds from natural sources (Yadegari, 2015; Jugran *et al.*, 2015).

The chemical composition of the EO of each species depends on the population, variety, cultivar, ecotype, the organs from where they are produced, or even on the phenologic cycle (Herraiz-Peñalver *et al.*, 2015).

Such variability may well reduce the possible resistance mechanisms of *L. decemlineata* but make rather difficult or even impossible to predict the bioactivity of EOs unless they are directly tested on the insect. In this sense, the individual assays of terpenes might indicate the potential insecticidal activity of EOs with a known chemical composition and facilitate the selection of chemotypes with a higher content of bioactive terpenes. Nonetheless, synergisms or antagonisms among plant compounds may respectively increase or reduce bioactivities, as previously observed for the flavonoid taxifolin on a multiple insecticide-resistant Colorado potato beetle strain (Wang *et al.*, 2016). Besides, the synergistic effect of diverse terpenes on *Culex quinquefasciatus* Say has also been observed (Pavela, 2015). Consequently, these aspects should be considered by agrochemical industry when developing commercial biopesticides.

The aim of this work was to assay in choice test the antifeedant capacity of 24 common terpenes widely identified in essential oils of aromatic and medicinal plants from Mediterranean areas against the Colorado potato beetle. This information could be helpful in the selection of essential oils with higher ratios of active compounds and presumably, with a higher bioactivity against this pest. Subsequently, terpenes that showed significant antifeedant activity against *L. decemlineata* were also assayed for their phytotoxicity in order to identify potential damage to the plants.

2. Materials and Methods

2.1. Terpene standards

Acetone and terpenes including monoterpenes (hydrocarbons, oxygenated and esterified) and sesquiterpenes (hydrocarbons, oxygenated) were acquired

from Sigma-Aldrich (St. Louis, MO, USA) (Table 1). AS 220/C/2, Radom, Poland) and dissolved in acetone. Terpenes were weighed in a precision balance (Radwag (5 µg/µL) previous to the assays on the insects.

Table 1. Terpenes assayed for their antifeedant activities against *Leptinotarsa decemlineata*.

Compounds tested	MF	MW (g/mol)	PubChem ID	Source	Purity
<i>Monoterpene hydrocarbons</i>					
<i>p</i> -cymene	C ₁₀ H ₁₄	134.22	7463	synthetic	≥97.0%
(+)-camphene	C ₁₀ H ₁₆	136.23	92221	synthetic	≥90.0%
(<i>S</i>)-(-)-limonene	C ₁₀ H ₁₆	136.23	439250	n.d.	≥99.0% (sum of enantiomers)
(<i>R</i>)-(+)-limonene	C ₁₀ H ₁₆	136.23	440917	n.d.	≥99.0% (sum of enantiomers)
Myrcene	C ₁₀ H ₁₆	136.23	31253	synthetic	≥95.0%
(+/-)- α -pinene	C ₁₀ H ₁₆	136.23	6654	n.d.	98.0%
(-)- β -pinene	C ₁₀ H ₁₆	136.23	440967	n.d.	99.0%
γ -terpinene	C ₁₀ H ₁₆	136.23	7461	n.d.	≥97.0%
<i>Oxygenated monoterpenes</i>					
Carvacrol	C ₁₀ H ₁₄ O	150.22	10364	synthetic	≥98.0%
Thymol	C ₁₀ H ₁₄ O	150.22	6989	n.d.	≥99.0%
(<i>IS</i>)-(-)-verbenone	C ₁₀ H ₁₄ O	150.22	92874	synthetic	≥93.0%
(+/-)-camphor	C ₁₀ H ₁₆ O	152.23	2537	synthetic	≥95.5%
(-)-borneol	C ₁₀ H ₁₈ O	154.25	439569	n.d.	97% (predominantly endo)
Eucalyptol	C ₁₀ H ₁₈ O	154.25	2758	natural	≥99.0%
Linalool	C ₁₀ H ₁₈ O	154.25	6549	n.d.	97.0%
(+)-Terpinen-4-ol	C ₁₀ H ₁₈ O	154.25	11230	n.d.	pharmaceutical standard
α -terpineol	C ₁₀ H ₁₈ O	154.25	17100	n.d.	90.0%
<i>Esterified monoterpenes</i>					
(-)-bornyl acetate	C ₁₂ H ₂₀ O ₂	196.29	93009	n.d.	95.0%
Linalyl acetate	C ₁₂ H ₂₀ O ₂	196.29	8294	synthetic	≥97.0%
<i>Sesquiterpene hydrocarbons</i>					
β -caryophyllene	C ₁₅ H ₂₄	204.35	5281515	synthetic	≥80.0%
Farnesene	C ₁₅ H ₂₄	204.35	5281516	synthetic	mixture of isomers
(-)- α -gurjunene	C ₁₅ H ₂₄	204.35	15560276	n.d.	≥97.0% (sum of enantiomers)
<i>Oxygenated sesquiterpenes</i>					
Caryophyllene oxide	C ₁₅ H ₂₄ O	220.35	14350	synthetic	95.0%
(-)- α -bisabolol	C ₁₅ H ₂₆ O	222.37	442343	n.d.	≥93.0%

n.d.: not defined

2.2. Insect rearing

Male and female adults of *Leptinotarsa decemlineata* Say (Coleoptera:Chrysomelidae) were collected in May 2016 from a natural untreated potato crop in Cuenca (Spain) and reared on potato foliage (*Solanum tuberosum* L.) in growth chambers at 22 ± 1 °C and >70% relative humidity, with a constant photoperiod of 16:8h (L:D). Potato plants were 6-7 weeks old (prior to flowering), about 25-30 cm high and cultivated with no plant treatments both at the crop fields of *Centro de Investigación Agroforestal de Albaladejito* (Cuenca, Spain) and in growth chambers as described above.

2.3. In vitro antifeedant activity

Feeding assays were tested in triplicate in choice tests with four male and female *L. decemlineata* individuals (Figure 1) as described in Rodilla *et al.* (2008). In six Petri dishes, containing 4 potato leaf disks (1.0 cm²): 2 control (acetone) and 2 treated with the terpene at an initial dose of 50 µg/cm². Experiments were performed in growth chambers (22 °C, 70% RH, 16:8 L:D), and finished when insects fed at least 50% of control or treatment disks. Subsequently, non-consumed foliar areas were calculated by means of Image J Version 1.37 r, 2010 (<http://rsb.info.nih.gov/ij/>). The feeding inhibition capacity of each compound was calculated as follows: % FI = $[1 - (T/C)] \times 100$, where T and C were the non-consumed area of treatment or control disks, respectively. The effective concentration to obtain 50% (EC₅₀) or 90% (EC₉₀) feeding inhibition was calculated by statistical logarithmic regression.

2.4. Allelopathic effects

Terpenes were assayed with seeds of lettuce (*Lactuca sativa* L. var. *Carrascoy*, dicotyledonous) and English ryegrass (*Lolium perenne* L., monocotyledonous)

(Fitó; Barcelona, Spain, <http://www.semillasfito.com/>) as described in Moiteiro *et al.* (2006). Four 2.5 cm diameter paper filters were treated either with 20 µL of acetone (control) or with 20 µL of the treatment (5 µg terpene/µL acetone, 100 µg of terpene per filter), and placed in 12-well plates (Falcon, San Jose, CA, USA). Then, 10 dry seeds of *L. sativa* or 10 one-day-hydrated seeds of *L. perenne* were respectively introduced per well, followed by 700 µL of distilled water. Finally, the plate was inserted inside plastic zipper bags, to avoid desiccation, and incubated in growth chambers (22 °C, 70% RH, 16:8 L:D). Germination was monitored each 24 hours up to a total of 7/10 days (*L. sativa*/ *L. perenne*). Twenty-five newly emerged seedlings roots of *L. sativa* or roots and leaves of *L. perenne* were measured by the Image J Version 1.37 r, 2010 (<http://rsb.info.nih.gov/ij/>). Phytotoxic effects (%) were calculated as follows: $100 - [(T/C) \times 100]$, where T and C were the number of germinated seeds or the length of roots/leaves of the treated and control seeds, respectively.

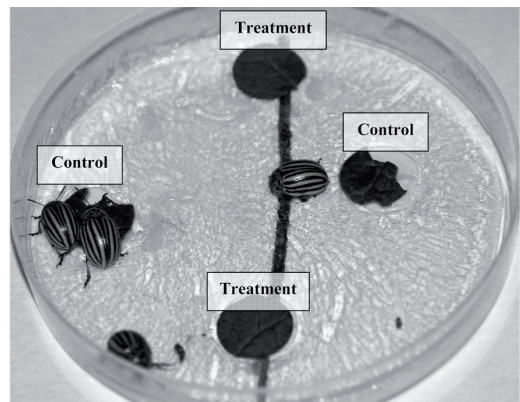


Figure 1. Choice feeding assay example.

2.5. Statistical analysis

Logarithmic regression tests were carried out by means of the statistical software STATGRAPHICS Centurion XVI Version 16.1.18 (32-bits) (Statpoint Technologies, Inc., Herndon, VA, USA, 2010). Data were expressed as means \pm SE (Standard Error). The adjusted coefficient of determination (R_2) was determined from mean values in logarithmic regression tests. Additionally, EC_{50} and EC_{90} values along with their interval bilateral limits (lower and upper) were also predicted at 95% confidence level.

3. Results

3.1. Antifeedant effects

None of the monoterpene hydrocarbons demonstrated potential activity ($FI \leq 60\%$) (Table 2). In contrast, five oxygenated monoterpenes presented antifeedant activity especially carvacrol and (+)-terpinen-4-ol, both showing inhibition percentages of around 90% at $50 \mu\text{g}/\text{cm}^2$. Thymol was also very effective ($>80\%$) whilst (-)-verbenone and camphor had a moderate activity, with percentages in the range of 60-75% at the same concentration. In the case of esterified monoterpenes, only linalyl acetate showed a moderate activity. Finally, the oxygenated sesquiterpene (-) α -bisabolol was the terpene with the strongest activity against *L. decemlineata*, showing EC_{50} and EC_{90} values considerably lower than that of any other terpene. The chemical structures of active terpenes are represented in Figure 2.

3.2. Allelopathic capacities

As observed in Table 3, none of the terpenes that presented antifeedant activity against *L. decemlineata* showed noticeable phytotoxicity against the seeds of

L. sativa. Germination was within normal ratios for all assayed terpenes, except for (-)-verbenone, that showed a total inhibition of the germination during the first 24 h although all the seeds germinated after 48 h. On the contrary, the (+)-terpinen-4-ol showed a certain stimulant effect on the root development of *L. sativa*. Allelopathic assays on *L. perenne* seeds showed moderate negative effects for the carvacrol, as deduced from the inhibition of the germination of seeds (26.8%) and a diminution on the development of root (50.9%) and leaf (60.8%). Finally, thymol (33.0%) and (-)- α -bisabolol (43.2%) also presented certain negative effects on the normal development of *L. perenne* leaves.

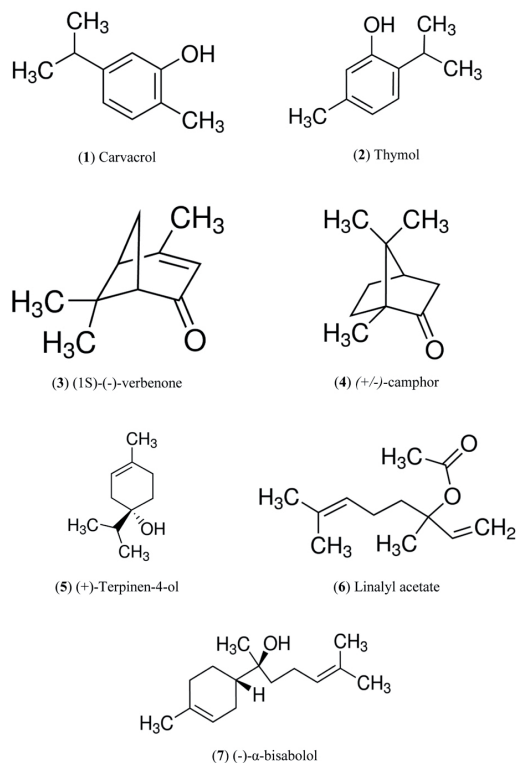


Figure 2. Molecular structures of antifeedant terpenes against adults of *Leptinotarsa decemlineata* (Source: www.sigmaaldrich.com)

Table 2. Feeding inhibition percentages of twenty four terpenes against adults of *Leptinotarsa decemlineata*.

Treatments	Dose ($\mu\text{g}/\text{cm}^2$)	% FI ^a	R ² (%) ^b	EC ₅₀ (Limits) ^c	EC ₉₀ (Limits) ^d
<i>Monoterpene hydrocarbons</i>					
<i>p</i> -cymene	50.0	44.60 \pm 10.6	n.d.	n.d.	n.d.
(+)-camphene	50.0	37.28 \pm 9.1	n.d.	n.d.	n.d.
(<i>S</i>)-(-)-limonene	50.0	50.87 \pm 12.2	n.d.	\approx 50	n.d.
(<i>R</i>)-(+)-limonene	50.0	38.45 \pm 10.0	n.d.	n.d.	n.d.
Myrcene	50.0	35.57 \pm 11.0	n.d.	n.d.	n.d.
(+/-)- α -pinene	50.0	51.66 \pm 10.4	n.d.	\approx 50	n.d.
(-)- β -pinene	50.0	33.70 \pm 12.3	n.d.	n.d.	n.d.
γ -terpinene	50.0	18.62 \pm 10.7	n.d.	n.d.	n.d.
<i>Oxygenated monoterpenes</i>					
Carvacrol	50.0	90.92 \pm 7.2	95.20	6.64 (1.4-11.3)	49.36 (28.8-255.5)
	25.0	79.62 \pm 10.1			
	12.5	67.35 \pm 9.4			
	6.3	44.73 \pm 10.5			
Thymol	50.0	81.54 \pm 7.6	92.75	10.76 (4.3-16.8)	n.d.
	25.0	73.58 \pm 9.5			
	12.5	57.52 \pm 12.2			
	6.3	33.66 \pm 11.0			
(1 <i>S</i>)-(-)-verbenone	50.0	72.90 \pm 9.7	99.77	25.41 (15.2-38.6)	n.d.
	25.0	50.41 \pm 11.9			
	12.5	25.10 \pm 10.5			
(±)-camphor	50.0	63.36 \pm 11.5	98.35	25.17 (12.2-42.6)	n.d.
	25.0	48.68 \pm 10.9			
	12.5	38.01 \pm 10.2			
(-)-borneol	50.0	21.15 \pm 6.6	n.d.	n.d.	n.d.
Eucalyptol	50.0	46.98 \pm 9.2	n.d.	n.d.	n.d.
Linalool	50.0	41.70 \pm 12.2	n.d.	n.d.	n.d.
(±)-Terpinen-4-ol	50.0	87.08 \pm 5.0	98.06	10.31 (4.4-15.9)	52.66 (30.9-221.9)
	25.0	74.44 \pm 8.8			
	12.5	57.00 \pm 13.0			
	6.3	35.79 \pm 12.1			
α -terpineol	50.0	39.97 \pm 10.9	n.d.	n.d.	n.d.
<i>Esterified monoterpenes</i>					
(-)-bornyl acetate	50.0	29.00 \pm 11.5	n.d.	n.d.	n.d.
Linalyl acetate	50.0	60.65 \pm 10.2	n.d.	40.14 (19.6-302.8)	n.d.
	25.0	27.05 \pm 11.5			
<i>Sesquiterpene hydrocarbons</i>					
β -caryophyllene	50.0	34.70 \pm 12.2	n.d.	n.d.	n.d.
Farnesene	50.0	46.56 \pm 13.0	n.d.	n.d.	n.d.
(-)- α -guriunene	50.0	36.57 \pm 12.2	n.d.	n.d.	n.d.
<i>Oxygenated sesquiterpenes</i>					
Caryophyllene oxide	50.0	30.71 \pm 7.3	n.d.	n.d.	n.d.
(-)- α -bisabolol	50.0	96.25 \pm 1.9	95.44	0.94 (0.5-1.5)	24.58 (10-70.5)
	25.0	91.11 \pm 7.8			
	12.5	87.02 \pm 8.2			
	6.3	69.30 \pm 13.5			
	3.1	67.48 \pm 11.3			
	1.6	51.84 \pm 11.3			
	0.8	49.71 \pm 11.1			

Table 3. Phytotoxic effects of the antifeedant terpenes at 100 µg/well.

	%GI ^a ± SE		%LI ^b ± SE		%GI ^a ± SE			%LI ^b ± SE	
	24h	48h	Root	72h	120h	168h	240h	Root	Leaf
<i>Oxygenated monoterpenes</i>									
Carvacrol	0.00 ± 0.0	0.00 ± 0.0	-17.03 ± 7.5 ^c	80.49 ± 8.1	64.29 ± 4.4	53.85 ± 8.5	26.76 ± 4.0	50.94 ± 9.9	60.78 ± 8.7
Thymol	10.00 ± 5.5	0.00 ± 0.0	-3.48 ± 9.5	39.02 ± 11.0	16.07 ± 11.9	7.69 ± 9.5	2.82 ± 9.8	20.95 ± 15.0	33.02 ± 11.7
(1S)-(-)-Verbenone	100.00 ± 0.0	0.00 ± 0.0	2.59 ± 5.4	21.95 ± 17.5	10.71 ± 18.1	4.62 ± 12.8	-1.41 ± 9.3	4.19 ± 17.6	21.19 ± 12.8
(+/-)-Camphor	0.00 ± 0.0	0.00 ± 0.0	-5.56 ± 5.2	19.51 ± 11.0	1.79 ± 13.9	6.15 ± 7.8	5.63 ± 5.8	-11.58 ± 15.6	-11.87 ± 15.2
(+)-Terpinen-4-ol	0.00 ± 0.0	0.00 ± 0.0	-28.78 ± 9.0	31.71 ± 15.3	7.14 ± 23.2	-4.62 ± 15.4	-4.23 ± 7.8	6.30 ± 15.4	12.48 ± 14.1
<i>Esterified monoterpenes</i>									
Linalyl acetate	0.00 ± 0.0	0.00 ± 0.0	-11.53 ± 7.0	12.20 ± 16.8	-7.14 ± 18.1	-7.69 ± 11.4	-1.41 ± 11.3	5.70 ± 17.7	-4.30 ± 19.6
<i>Oxygenated sesquiterpenes</i>									
α -bisabolol	7.50 ± 6.5	0.00 ± 0.0	-8.19 ± 7.8	19.51 ± 14.5	23.21 ± 13.2	13.85 ± 12.3	11.27 ± 10.4	17.38 ± 9.3	43.17 ± 6.5

^a %G: Germination Inhibition percentage; ^b %L: Length Inhibition percentage; ^c negative values indicate stimulant effects.

4. Discussion

4.1. Insecticidal activity

The antifeedant activity of the terpenes α -pinene, β -pinene, eucalyptol, β -caryophyllene and caryophyllene oxide against *L. decemlineata* have been previously investigated by Rodilla *et al.* (2008). Among them, β -caryophyllene and caryophyllene oxide showed high antifeedant activities in contrast to the low percentages that we have observed for these compounds. The activities reported in this study for β -pinene and α -pinene were also higher as compared with our data, whereas the activity of eucalyptol was similar in both works. Kostić *et al.* (2007) observed a moderate activity of camphor against *L. decemlineata* adults and larvae comparable to our results. These differences among studies might be related to the potential developing resistance of *L. decemlineata* against certain compounds, as previously reported (Alyokhin *et al.*, 2006; 2007). In agreement with our results, the antifeedant activity of some terpene standards has been also demonstrated against other beetles. Thus, Yildirim *et al.* (2013) observed a high activity for terpinen-4-ol against *Sitophilus zeamais* (Coleoptera: Curculionidae). Furthermore, Kim *et al.* (2010) assayed the antifeedant activities of different terpenes against the beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) and concluded that caryophyllene oxide, thymol, α -pinene,

carvacrol and myrcene were the most active, whereas γ -terpinene, *p*-cymene, camphene and linalool present low activities, which is in part consistent with our results on *L. decemlineata*. The strong insecticidal effects of hymol and carvacrol on *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) have also been reported by Szczepanik *et al.* (2012), and Erler (2005) demonstrated the high activity of carvacrol, γ -terpinene, thymol and terpinen-4-ol against the beetle *Tribolium confusum* (Coleoptera: Tenebrionidae) and the moth *Ephestia kuehniella* (Lepidoptera: Pyralidae). In addition, Gillette *et al.* (2014) proposed an effective mixture containing, among others, verbenone to avoid attacks from mountain pine beetles *Dendroctonus ponderosae* (Coleoptera: Curculionidae) on whitebark and limber pines. Summarizing, the above mentioned works are in agreement with the high antifeedant activities that we have observed for carvacrol, thymol and (+)-terpinen-4-ol against *L. decemlineata*.

To our knowledge, there are not previous works on the antifeedant activity of α -bisabolol against the Colorado potato beetle or other related beetles. However, some authors have searched for essential oils with a high content in this terpene due to its potential activity on different insects. Thus, Vila *et al.* (2010) propose the essential oil of *Morella parvifolia* (60% α -bisabolol) as a possible source of this compound, and De Andrade *et al.* (2004) reported a total α -bisabolol content of 90% in the heartwood essential

oil of a natural Brazilian population of *Vanillosmopsis pohlii*, which showed strong insecticidal effects against the silverleaf whitefly *Bemisia argentifolii* (Hemiptera: Aleyrodidae). Likewise, Kamatou and Viljoen (2010) have reviewed several aromatic plants with a high content in this compound including *Eremanthus erythropappus*, *Smyrniopsis aucheri* and *Salvia runcinata*. In addition, a possible larvicidal activity of the essential oil of *Plinia cerrocampaensis* against the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae) has been related to this compound since it is the major component of the oil (Vila et al., 2010). Salamon et al. (2016) have suggested different breeding programs to develop German chamomile (*Chamomila recutita*) with a high content in l-l- α -bisabolol as it is an important indicator of flower quality and value for cosmetic and pharmaceutical industry.

4.2. Phytotoxicity

The phytotoxic effects of essential oils and their individual components on different weeds, have been reviewed by Amri et al. (2013) who conclude that thymol and carvacrol present a high activity. For example, Azirak and Karaman (2008) observed that these terpenes present a high phytotoxicity against different weeds, whereas Vokou et al. (2003) have demonstrated moderate negative effects for these terpenes and no effect for linalyl acetate against *L. sativa*, as also observed in our study. Nevertheless, the above study also shows exceptional phytotoxicities for the (+)-terpinen-4-ol and camphor against *L. sativa*, which is in contradiction with our results. From the screening of twenty seven monoterpenes, including carvacrol, thymol, camphor and linalyl acetate, De Martino et al. (2010) have observed that high concentrations of camphor affected the germination and growth of *Raphanus sativus* and the growth of *Lepidium sativum*. They also observed an inhibition of both the

germination and growth of *L. sativum* seeds in the presence of high concentrations of thymol. As far as we know, no previous studies have been carried out on the phytotoxicity of verbenone and α -bisabolol. In general, carvacrol was the most detrimental terpene to seeds although its phytotoxicity could be reduced if it is used in synergism with other non-phytotoxic antifeedant compounds. Therefore, the use of these antifeedant terpenes is justified according to their moderate to low negative effects on germination and development of these model plants.

5. Conclusions

This work demonstrates that certain oxygenated terpenes present in essential oils are endowed of insecticidal activities against *L. decemlineata*, as previously observed for other insects. From the screening of a total of 24 terpenes, including monoterpenes (hydrocarbons, oxygenated and esterified) and sesquiterpenes (hydrocarbons, oxygenated), we can conclude that (-)- α -bisabolol, carvacrol, (+)-terpinen-4-ol and thymol were the most active against this pest, whereas (-)-verbenone, camphor and linalyl acetate presented certain antifeedant activities with inhibition percentages slightly above 60%. The allelopathic tests showed that only (-)-verbenone had negative effects on the germination of *Lactuca sativa* seeds after 24 hours of incubation, and carvacrol on the seed germination and on the leaf and root growth of *Lolium perenne*. Consequently, the use of carvacrol as antifeedant in potato crops should be considered with caution since it could affect the plant development. Nevertheless, carvacrol has been assayed on standard target seeds selected for the study of allelopathy capacities, so a study directly on potato plants is necessary to clarify this point. Thymol and α -bisabolol also presented moderate negative effects on the normal development of *L. perenne* leaves, whereas (+)-terpinen-4-ol was not

phytotoxic. However, the antifeedant activity (EC_{50}) of α -bisabolol was around 10 times stronger than that of (+)-terpinen-4-ol. In addition, the low toxicity of (-)- α -bisabolol to humans has been certified by the Food and Drug Administration (FDA, 2015) who has granted this compound with Generally Regarded As Safe (GRAS) and by the Cosmetic Ingredient Review (CIR) Expert Panel (Andersen, 1999). Consequently, α -bisabolol is the best option to develop natural antifeedant formulations against *L. decemlineata* on the basis of the high antifeedant and low phytotoxic activities of this terpene.

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