



Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops

Mercedes Verdeguer^a, M. Amparo Blázquez^{b,*}, Herminio Boira^a

^a Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, C.P. 46022 Valencia, Spain

^b Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Avda Vicent Andrés Estellés s/n, C.P. 46100 Burjassot, Spain

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ABSTRACT

The essential oil composition of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* was analyzed by means of GC and GC–MS and bioassayed in order to determine their activity against *Amaranthus hybridus* and *Portulaca oleracea*. *E. camaldulensis* essential oil, with spathulenol as the main compound, was the most effective, completely inhibiting germination and seedling growth on both weeds. The essential oil of *E. africanus*, rich in artemisia ketone, showed activity similar to that of *E. camaldulensis* on *A. hybridus*, but it was not so effective against *P. oleracea*, and *L. camara* essential oil, with high percentages in sesquiterpene hydrocarbons, also showed higher phytotoxic activity against *A. hybridus*, inhibiting its germination and seedling length; however, it showed no effect against *P. oleracea* germination, whereas was effective in significantly reducing its seedling growth at all concentrations assayed. The results suggest the possible use of these essential oils as natural herbicides.

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1. Introduction

The continued use of synthetic herbicides has resulted in herbicide-resistant weeds as well as in negative impacts upon human health and the environment (Vyvyan, 2002). Nowadays, agricultural techniques are focused on sustainable agricultural production. In this sense, allelopathy offers an important tool for selective biological weed management through the production and release of allelochemicals from the leaves, flowers, seeds, stems and roots of plant materials (Weston, 1996).

Lantana camara L. (Verbenaceae), a neotropical aromatic shrub, is considered one of the 10 worst weeds of the world because it infests 14 crops in many tropical and subtropical countries (Oudhia, 2001). However, in Europe it grows as an ornamental plant. Allelopathic effects of *L. camara* on germination and seedling vigour of many agricultural crops, such as rice (Bansal, 1998; Oudhia and Tripathi, 1999), wheat (Oudhia and Tripathi, 2000) and soybean (Oudhia, 1999) have been reported. The leaves, roots and fruits of *L. camara* contain allelochemicals, mainly aromatic alkaloids and phenolic compounds (Ambika et al., 2003). The essential oil composition of *L. camara* leaves from different origins is characterized by a high content of sesquiterpene hydrocarbons (Ngassoum et al., 1999; Ouamba et al., 2006). However, the phytochemical group of the main compound is different depending on the part of the plant used to obtain the essential oil (Khan et al., 2003).

The allelopathic effects of some *Eucalyptus* species (Myrtaceae) have been also studied (May and Ash, 1990; Alves et al., 1999). Essential oils from *Eucalyptus* containing allelochemicals, like 1,8-cineol, limonene or α - and β -pinene are employed in medicine, cosmetic and pharmaceutical industries. In California, the annual vegetation adjacent to *Eucalyptus camaldulensis* Dehnh. is often severely inhibited by allelochemicals released by this species (Del Moral and Muller, 1970).

* Corresponding author. Tel.: +34 96 354 49 45; fax: +34 96 354 49 43.

E-mail addresses: merversa@doctor.upv.es (M. Verdeguer), amparo.blazquez@uv.es (M.A. Blázquez), hboira@eaf.upv.es (H. Boira).

On the other hand, no bioassays or field experiments had been performed to study the allelopathic potential of *Eriocophalus africanus* L. (Wild Rosemary, Asteraceae), an aromatic shrub native of South Africa, naturalized in most Mediterranean areas as an ornamental plant. Its essential oil composition (Merle et al., 2007), with a high content of artemisia ketone (>56%), a characteristic compound of the essential oils from plants with allelopathic properties of the *Artemisia* genus (Tan et al., 1998; Preston et al., 2002), guided us to select this species to compare its allelopathic potential with that known of *L. camara* and *E. camaldulensis*.

Finally, *Amaranthus hybridus* L. (smooth pigweed) and *Portulaca oleracea* L. (purslane) are two serious weeds in summer crops in the Mediterranean area and are considered very aggressive in tropical and subtropical climates (Holm et al., 1991). So, the aim of the present work was to study the essential oil composition from three populations of *L. camara*, *E. camaldulensis* and *E. africanus* in order to know if these compositions have phytotoxic effects against *A. hybridus* and *P. oleracea* germination and seedling growth.

2. Materials and methods

2.1. Weeds

Mature seeds of annual weeds *A. hybridus* and *P. oleracea* were collected from parent plants growing in fields of Valencia area, in October 2005. The plants were dried for 15 days at room temperature, afterwards the seeds were extracted. Uniform healthy seeds were selected and stored at 4 °C until germination tests.

2.2. Essential oils

Fresh plant materials of *L. camara*, *E. camaldulensis* and *E. africanus* were collected from gardens of Valencia between May 2005 and October 2007 and were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus, yielding 0.04%, 0.71% and 0.43% v/w, respectively, of essential oil. All essential oils were stored at 4 °C until they were analyzed (diluted at 1% v/v in diethyl ether) or their allelopathic potential was tested.

2.3. Gas chromatography

Gas chromatography was performed using a Clarus 500GC Perkin–Elmer apparatus equipped with a flame ionization detector (FID), a Hewlett–Packard HP-1 (cross-linked methyl silicone) capillary column 30 m long and 0.2 mm i.d., with 0.33 µm film thickness. The column temperature program was 60 °C for 5 min, with 3 °C increases per min to 180 °C, then 20 °C increases per min to 280 °C which was maintained for 10 min. The carrier gas was helium at a flow-rate of 1 ml/min. Both the FID and injector port temperature were maintained at 250 °C and 220 °C respectively.

2.4. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry analysis was carried out with a Varian Saturn 2000 equipped with a Varian C.S VA-5MS capillary column 30 m long and 0.25 mm i.d. with 0.25 µm film thickness. The same working conditions used for GC and split mode injection (ratio 1:25) were employed. Mass spectra were taken over the *m/z* 28–400 range with an ionizing voltage of 70 eV. Kovats retention index was calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by MS (Adams, 2007) and their identity was confirmed by comparison of their RIs, relative to C₈–C₃₂ *n*-alkanes, and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST 98 library and in the literature.

2.5. Seed germination and growth seedling tests

Sets of 20 seeds each with five replicates per treatment were germinated in Petri dishes (9 cm diameter) between two layers of filter paper (Whatman No. 1) wetted with 4 ml of distilled water. Essential oils of *L. camara*, *E. camaldulensis* or *E. africanus*, were added at volumes of 0 (control, distilled water), 0.5, 1, 2, and 4 µl. According previous assays, *A. hybridus* and *P. oleracea* seeds were incubated alternating 30.0 ± 0.1 °C, 16 h in light and 20.0 ± 0.1 °C, 8 h in dark. To evaluate the phytotoxic activity of the essential oils, germination and seedling length, data were recorded after 3, 5, 7, 10 and 14 days.

2.6. Statistical analysis

Tests were conducted in a randomized complete design with five replications. Data were submitted to analysis of variance. Percentage values were previously arcsin transformed. The means were compared by Tukey test (*P* < 0.05).

3. Results

3.1. Chemical composition

A total of 123 compounds were identified by GC and GC/MS from the essential oils of *L. camara*, *E. camaldulensis* and *E. africanus*. The qualitative and quantitative composition of the three species, very different, is given in Table 1 where the compounds are classified by phytochemical groups and listed in order of their elution on a methyl silicone HP-1 column. Thirty-four compounds were identified in *L. camara* essential oil, accounting for 96% of the total oil composition. The monoterpene fraction commonly found in *E. camaldulensis* and *E. africanus* was devoid in the three populations of *L. camara* here analyzed but not in other *L. camara* species from different geographical origin (Rana et al., 2005). Substantial differences on these fractions were observed in the other two analyzed species. The monoterpene hydrocarbons ranged 7.09% in *E. africanus*, with α -pinene (3.09%), β -pinene (1.65%) and *p*-cymene (1.67%) being the major components. This fraction, with 26.38% of the total oil, represented the second most important group on *E. camaldulensis* essential oil, with *p*-cymene (21.92%) followed by α -pinene (1.29%) and *o*-cymene (1.21%) as the main compounds.

Differences in the oxygenated monoterpenes were also observed between the two species. With the exception of 1,8-cineole, terpinen-4-ol and mirtanal, which were identified in the two essential oils, the oxygenated monoterpenes found in *E. camaldulensis* were not found in *E. africanus* essential oil. This fraction, with 25 identified compounds in *E. camaldulensis*, accounted for 19.86% of the total oil, with the main compounds being cryptone (7.76%), terpinen-4-ol (2.56%), *p*-menth-1-en-7-al (2.09%), 1,8-cineole (1.92%) and cumin aldehyde (1.17%), whereas the 12 oxygenated monoterpenes identified in *E. africanus* accounted for 64.17% of the total oil composition. Artemisia ketone (56.46%) followed by yomogi alcohol (2.99%), artemisia alcohol (1.71%) and pinocarvone (1.13%), not found in *L. camara* or *E. camaldulensis*, were the main compounds of this fraction.

The greater differences comparing the three species appeared in the sesquiterpene hydrocarbons contents. In *L. camara*, it accounted for 88.96% of the total oil, whereas in *E. camaldulensis* only allo-aromadendrene with a percentage of 0.89% was identified and in *E. africanus* with 7 identified compounds, it was 3.64%. With 20 compounds identified, sesquiterpene hydrocarbons constitute the predominant group in *L. camara* essential oil. This fraction contained mainly β -caryophyllene (17.54%), α -humulene (7.43%), γ -muurolene (12.54%) and curcumene compounds α -curcumene (23.09%), γ -curcumene (14.64%) and β -curcumene (4.83%).

In the oxygenated sesquiterpene fraction qualitative and quantitative differences were also observed: zingiberenol (1.26%) and caryophyllene oxide (1.63%) were the main compounds in *L. camara*, whereas spathulenol derivatives (spathulenol 41.46%, isospathulenol 1.39% and spathulenol isomer 1.08%) and isobicyclogermacrene (1.92%) were the principal oxygenated sesquiterpenes of *E. camaldulensis* populations. Intermedeol (9.59%) and γ -eudesmol (5.64%), not detected in *L. camara* or *E. camaldulensis*, were the main compounds of these fractions in *E. africanus* essential oil.

Oxygenated diterpenes were found in *L. camara* (1.05%) and also in trace amount in *E. africanus*. Finally, a fraction of non-terpenic compounds was detected in *E. camaldulensis* and *E. africanus* (0.52%) essential oils. From this fraction only 5-methyl-3-hexen-2-one and 2-nonanone were identified in trace amounts in *E. camaldulensis*.

3.2. Germination tests

The essential oil of *E. camaldulensis* was the most effective, completely inhibiting both *A. hybridus* and *P. oleracea* seed germination (Table 2) whereas *L. camara* and *E. africanus* essential oils showed different activity depending on the weed assayed. The essential oil of *E. africanus* showed very phytotoxic effects against *A. hybridus* seeds, blocking their germination at all the concentrations applied, while it only reduced significantly *P. oleracea* germination at the highest concentrations (0.5 and 1 μ l/ml). Both concentrations decreased *P. oleracea* germination 23 and 24% respectively.

L. camara essential oil was not active against *P. oleracea* germination. No significant differences were found between the control and the concentrations assayed. On the other hand, this essential oil was effective in reducing *A. hybridus* germination at all the concentrations tested, not showing significant differences in the germination inhibition between concentrations: 0.125 μ l/ml reduced *P. oleracea* germination 93.10%, 0.25 μ l/ml 86.21% and 0.5 μ l/ml and 1 μ l/ml concentrations inhibited germination 94.25% and 96.55%, respectively.

3.3. Seedling growth

As there was no germination of *A. hybridus* and *P. oleracea* seeds treated with *E. camaldulensis* essential oil, there was no growth of these seedlings. The phytotoxic effect of *E. africanus* essential oil on seedlings growth depended on the species tested: in *A. hybridus* it caused a significant decrease in seedlings length, which was nearly 0, with no significant differences within concentrations, while in *P. oleracea* it reduced seedlings length at all the concentrations tested (Fig. 1), showing significant differences between control and the two lower concentrations, and between the lower concentrations and the two highest concentrations: the lower concentrations, 0.125 μ l/ml and 0.25 μ l/ml decreased germination 69.51% and 67.22%, respectively, and concentrations of 0.5 μ l/ml and 1 μ l/ml inhibited germination 80.25% and 82.74%, respectively.

L. camara essential oil was the least active of the oils tested. It showed phytotoxic effects on the seedlings growth of the two species assayed, although its effect was stronger on *A. hybridus*. In this species the seedling length was significantly

Table 1Constituents of essential oils from *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* by GC and GC–MS analysis.

Compound	KI	<i>Lantana camara</i>	<i>Eucalyptus camaldulensis</i>	<i>Eriocephalus africanus</i>
Monoterpene hydrocarbons			26.38	7.09
Santolina triene	903	–	–	0.24 ± 0.00
Artemisia triene	924	–	–	0.28 ± 0.02
α -Thujene	931	–	0.27 ± 0.05	–
α -Pinene	939	–	1.29 ± 0.07	3.09 ± 0.35
Camphene	954	–	–	t
Thuja-2,4(10)-diene	960	–	0.09 ± 0.01	–
Verbenene	967	–	t	–
Sabinene	977	–	t	0.03 ± 0.01
β -Pinene	979	–	0.07 ± 0.00	1.65 ± 0.05
Myrcene	991	–	t	0.13 ± 0.01
α -Phellandrene	1006	–	0.10 ± 0.07	–
α -Terpinene	1017	–	0.08 ± 0.05	–
<i>p</i> -Cymene	1026	–	21.92 ± 1.61	1.67 ± 0.18
<i>o</i> -Cymene	1028	–	1.21 ± 0.10	–
Limonene	1030	–	1.11 ± 0.17	t
γ -Terpinene	1060	–	0.19 ± 0.08	t
<i>m</i> -Cymenene	1085	–	0.05 ± 0.02	–
Oxygenated monoterpenes		–	19.86	64.17
Dehydro-1,8-Cineole	993	–	t	–
Yomogi alcohol	996	–	–	2.99 ± 0.17
1,8-Cineole	1033	–	1.92 ± 0.32	0.06 ± 0.00
Artemisia ketone	1062	–	–	56.46 ± 1.99
<i>cis</i> -Linalool oxide	1076	–	0.07 ± 0.01	–
Artemisia alcohol	1082	–	–	1.71 ± 0.13
<i>trans</i> -Linalool oxide	1088	–	0.09 ± 0.06	–
Linalool	1100	–	0.46 ± 0.19	–
<i>cis</i> -Thujone	1106	–	0.07 ± 0.01	–
<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	1126	–	0.09 ± 0.02	–
α -Campholenal	1130	–	0.39 ± 0.03	–
<i>cis</i> - <i>p</i> -Mentha-2,8,dien-1-ol	1139	–	0.19 ± 0.03	–
<i>trans</i> -Pinocarveol	1141	–	–	0.55 ± 0.07
<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	1143	–	0.36 ± 0.01	–
Nerol oxide	1161	–	–	t
Pinocarvone	1166	–	–	1.13 ± 0.15
Terpinen-4-ol	1180	–	2.56 ± 0.43	0.03 ± 0.01
Cryptone	1185	–	7.76 ± 0.62	–
Artemisia ketone isomer	1186	–	–	0.68 ± 0.06
α -Terpineol	1192	–	0.93 ± 0.15	–
Myrtenal	1197	–	t	0.45 ± 0.02
Myrtenol	1199	–	–	t
Verbenone	1208	–	0.08 ± 0.01	–
<i>m</i> -Cumenol	1230	–	0.10 ± 0.00	–
Cumin aldehyde	1242	–	1.17 ± 0.10	–
Carvone	1245	–	t	–
Carvotanacetone	1249	–	0.07 ± 0.01	–
Piperitone	1255	–	0.10 ± 0.00	–
<i>p</i> -Menth-1-en-7-al	1279	–	2.09 ± 0.47	–
α -Terpinen-7-al	1285	–	t	–
Thymol	1293	–	0.38 ± 0.06	–
Carvacrol	1302	–	0.98 ± 0.11	–
3-oxo- <i>p</i> -Menth-1-en-7-al	1335	–	t	–
Geranyl acetate	1380	–	–	0.11 ± 0.01
Sesquiterpene hydrocarbons		88.96	0.89	3.64
α -Copaene	1378	0.25 ± 0.17	–	0.17 ± 0.10
β -Bourbonene	1388	0.24 ± 0.16	–	–
β -Elemene	1393	3.45 ± 0.65	–	–
Italicene	1404	0.78 ± 0.48	–	–
Sesquithujene	1405	0.84 ± 0.17	–	–
α -Cedrene	1413	0.64 ± 0.07	–	–
β -Caryophyllene	1419	17.54 ± 0.56	–	1.27 ± 0.50
β -Copaene	1432	0.05 ± 0.00	–	–
α - <i>trans</i> -Bergamotene	1436	t	–	–
<i>cis</i> - β -Farnesene	1445	0.73 ± 0.01	–	–
α -Humulene	1457	7.43 ± 0.64	–	0.09 ± 0.05
<i>allo</i> -Aromadendrene	1464	–	0.89 ± 0.21	0.03 ± 0.03
α -Acoradiene	1467	0.22 ± 0.02	–	–
β -Acoradiene	1470	t	–	–

(continued on next page)

Table 1 (continued)

Compound	KI	<i>Lantana camara</i>	<i>Eucalyptus camaldulensis</i>	<i>Eriocephalus africanus</i>
γ -Muurolene	1479	12.54 $\pm$ 1.43	–	–
α -Curcumene	1480	23.09 $\pm$ 2.10	–	–
γ -Curcumene	1482	14.64 $\pm$ 1.06	–	–
Bicyclogermacrene	1500	–	–	1.48 $\pm$ 0.29
α -Muurolene	1500	0.35 $\pm$ 0.03	–	–
β -Bisabolene	1507	0.67 $\pm$ 0.06	–	–
β -Curcumene	1518	4.83 $\pm$ 0.38	–	–
7- <i>epi</i> - α -Selinene	1522	–	–	0.30 $\pm$ 0.06
β -Sesquiphellandrene	1525	0.68 $\pm$ 0.04	–	–
δ -Cadinene	1526	–	–	0.30 $\pm$ 0.06
Oxygenated sesquiterpenes		6.21	48.27	21.26
10,11-epoxy-Calamenene	1492	–	0.59 $\pm$ 0.03	–
Kessane	1530	–	–	0.65 $\pm$ 0.29
Liguloxide	1536	–	–	1.26 $\pm$ 0.03
Elemol	1549	–	–	0.82 $\pm$ 0.37
<i>cis</i> -Sesquisabinene hydrate	1556	t	–	–
Liguloxide isomer	1558	–	–	0.09 $\pm$ 0.04
<i>E</i> -Nerolidol	1565	t	–	–
Germacrene-D-4-ol	1577	t	–	t
Spathulenol	1581	0.62 $\pm$ 0.02	41.46 $\pm$ 3.04	0.53 $\pm$ 0.23
Caryophyllene oxide	1586	1.63 $\pm$ 0.56	–	0.29 $\pm$ 0.04
β -Copaen-4- α -ol	1590	–	–	0.06 $\pm$ 0.03
Viridiflorol	1595	–	0.13 $\pm$ 0.02	t
Cedrol	1600	0.27 $\pm$ 0.03	–	–
Guaiol	1601	–	–	0.22 $\pm$ 0.10
Ledol	1604	–	0.76 $\pm$ 0.06	–
Spathulenol isomer	1610	–	1.08 $\pm$ 0.19	–
Humulene epoxide	1611	0.49 $\pm$ 0.06	–	–
Zingiberenol	1611	1.26 $\pm$ 0.26	–	–
10- <i>epi</i> - γ -Eudesmol	1625	–	–	0.02 $\pm$ 0.01
1- <i>epi</i> -Cubenol	1630	–	–	0.02 $\pm$ 0.01
Isospathulenol	1634	–	1.39 $\pm$ 0.30	–
γ -Eudesmol	1635	–	–	5.64 $\pm$ 1.38
Caryophylla-4,8-dien-5-ol	1640	–	–	0.95 $\pm$ 0.15
<i>tau</i> -Cadinol	1643	1.27 $\pm$ 0.10	–	t
<i>allo</i> -Aromadendrene epoxide	1644	–	–	–
<i>tau</i> -Muurolol	1645	–	0.48 $\pm$ 0.13	0.89 $\pm$ 0.42
β -Eudesmol	1650	–	–	0.03 $\pm$ 0.01
α -Eudesmol	1655	–	–	0.05 $\pm$ 0.02
α -Cadinol	1658	0.29 $\pm$ 0.04	–	–
Selin-11-en-4- α -ol	1661	–	–	0.03 $\pm$ 0.03
Intermedeol	1667	–	–	9.59 $\pm$ 0.89
Caryophyllenol	1672	–	–	0.12 $\pm$ 0.02
<i>epi</i> - β -Bisabolol	1674	0.39 $\pm$ 0.17	–	–
<i>epi</i> - α -Bisabolol	1687	t	–	–
Isobicyclogermacrenal	1734	–	1.92 $\pm$ 0.29	–
Oxygenated diterpenes		1.05	–	t
Phytol	1945	0.96 $\pm$ 0.32	–	t
Phytol isomer	1974	0.09 $\pm$ 0.02	–	–
Others		–	t	0.52
1-Octene	793	–	–	0.07 $\pm$ 0.02
1-Octen-3-ol	979	–	–	t
Hexanal	803	–	–	t
Butyl acetate	813	–	–	t
7-Methyl-1-Octene	852	–	–	0.24 $\pm$ 0.11
1-Nonene	889	–	–	0.04 $\pm$ 0.02
5-Methyl-3-Hexen-2-one	896	–	t	–
Benzaldehyde	966	–	–	0.03 $\pm$ 0.01
3-Octanone	984	–	–	0.10 $\pm$ 0.02
<i>cis</i> -3-Hexenyl acetate	1006	–	–	0.04 $\pm$ 0.02
2-Nonanone	1092	–	t	–
Total identified		96.22	95.40	96.84

Compounds listed in order of elution in the HP-1 column. T, traces (<0.04% *L. camara* and *E. camaldulensis* and <0.01% in *E. africanus*). RI, retention index relative to C₈–C₃₂ *n*-alkanes on the HP-1 column. Peak area percentages are calculated in GC on apolar HP-1 column. Values are means $\pm$ standard error.

Table 2

Effects of essential oils from *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* on *Amaranthus hybridus* and *Portulaca oleracea* seeds germination.

Treatment ($\mu\text{l/ml}$)	<i>Lantana camara</i>	<i>Eucalyptus camaldulensis</i>	<i>Eriocephalus africanus</i>
<i>Amaranthus hybridus</i> germination (%)			
0 (Control)	87.0 $\pm$ 6.4 a	87.0 $\pm$ 6.4 a	87.0 $\pm$ 6.4 a
0.125	6.0 $\pm$ 2.9 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b
0.250	12.0 $\pm$ 6.0 b	0.0 $\pm$ 0.0 b	1.0 $\pm$ 1.0 b
0.5	5.0 $\pm$ 2.7 b	0.0 $\pm$ 0.0 b	1.0 $\pm$ 1.0 b
1	3.0 $\pm$ 2.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b
<i>Portulaca oleracea</i> germination (%)			
0 (Control)	100.0 $\pm$ 0.0 a	100.0 $\pm$ 0.0 a	100.0 $\pm$ 0.0 a
0.125	95.0 $\pm$ 2.7 a	0.0 $\pm$ 0.0 b	94.0 $\pm$ 3.7 a
0.250	92.0 $\pm$ 3.0 a	0.0 $\pm$ 0.0 b	96.0 $\pm$ 1.9 a
0.5	94.0 $\pm$ 1.9 a	0.0 $\pm$ 0.0 b	77.0 $\pm$ 7.7 b
1	97.0 $\pm$ 1.2 a	0.0 $\pm$ 0.0 b	76.0 $\pm$ 10.0 b

Values are means $\pm$ standard error of five replicates of 20 seeds each after 14 days of incubation. Within each species, different letters in the same column indicate that means are different at the 95% level of probability (Tukey's multiple-range test, HSD).

reduced, being practically 0 at all the concentrations assayed, without differences between concentrations. In *P. oleracea*, however, the seedling length was reduced at all the concentrations applied (Fig. 2), and there were significant differences between the control and the lower concentrations, which reduced germination 34.23%, 39.78% and 41.51%, respectively and also there were significant differences between the highest concentration and the others. The highest concentration caused a decrease of 50.45% in *P. oleracea* seedling length.

A continuous decrease of the seedling growth rate was observed (Figs. 1 and 2).

4. Discussion

Essential oils from different aromatic plants belonging to Lamiaceae, Compositae, Myrtaceae and Verbenaceae families have been reported to have allelopathic properties (Dudai et al., 1999; Angelini et al., 2003). The allelopathic effect of the essential oils is related to the essential oil composition and the species on which they are applied. In this sense, our results corroborate that the great differences in the essential oils composition of *L. camara*, *E. camaldulensis* and *E. africanus* produced a strong or more selective phytotoxic effect against *A. hybridus* or *P. oleracea* germination or seedling growth.

According to other authors (Scrivanti et al., 2003; López et al., 2009), the essential oils, rich in oxygenated compounds, were more active than essential oils with high percentages of hydrocarbon compounds.

The essential oil composition of *L. camara* changes depending on the geographical origin. *L. camara* growing in Spain is an ornamental plant with an essential oil rich in sesquiterpene compounds, mainly hydrocarbonated and devoid of monoterpene compounds. In India *L. camara* has become an obnoxious weed. Its essential oil contains, besides sesquiterpene hydrocarbons, oxygenated monoterpenes, 1,8-cineole and linalool with percentages near 1% and monoterpene hydrocarbons

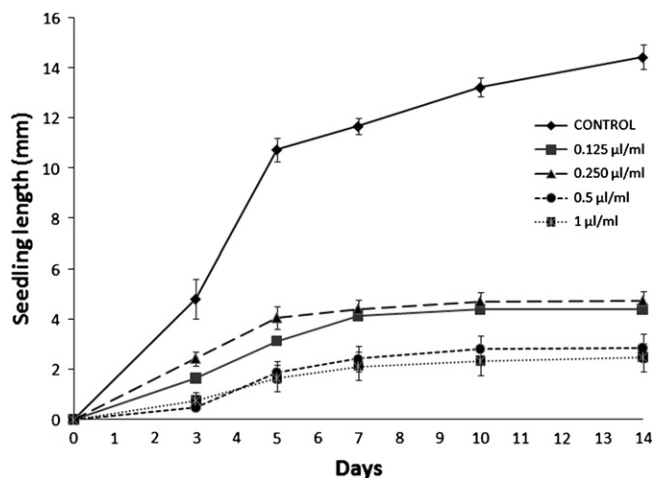


Fig. 1. Seedling length (mm) (mean $\pm$ SE) of *P. oleracea* control or treated with essential oil from *E. africanus* at concentrations of 0.125 $\mu\text{l/ml}$, 0.25 $\mu\text{l/ml}$, 0.5 $\mu\text{l/ml}$ and 1 $\mu\text{l/ml}$ measured for 14 days.

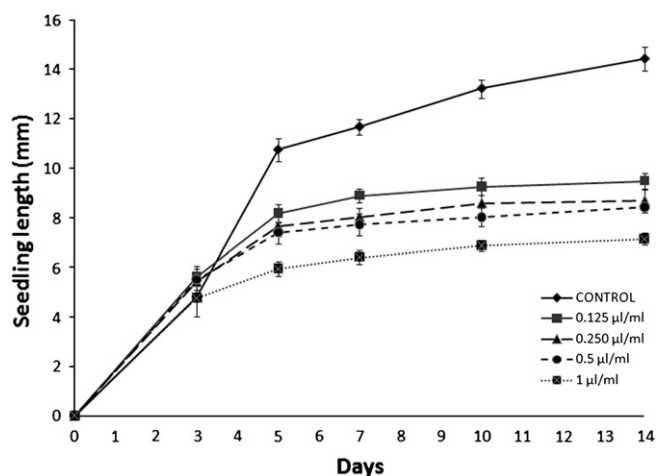


Fig. 2. Seedling length (mm) (mean $\pm$ SE) of *P. oleracea* control or treated with essential oil from *L. camara* at concentrations of 0.125 μ l/ml, 0.25 μ l/ml, 0.5 μ l/ml and 1 μ l/ml measured for 14 days.

such as sabinene or myrcene that reach 2.2% and 1.2%, respectively. It also contains davanone, an oxygenated sesquiterpene in large amounts (7.3%) (Rana et al., 2005). On the other hand aqueous extracts of *L. camara* had been widely described in India as allelopathic against many crops and weeds (Bansal, 1998). In previous assays (Verdeguez et al., 2007) we found that the aqueous extract of *L. camara* growing in Spain was not as effective against germination or seedling growth of *A. hybridus* and *P. oleracea* as its essential oil. Our results suggest that its essential oil could be used as a potential allelopathic substance.

Several authors suggested that monoterpene compounds are responsible for germination inhibition (Dudai et al., 2004; Armirante et al., 2006.). The minor effectiveness of *L. camara* compared with *E. camaldulensis* and *E. africanus* essential oils could be explained by the absence of a monoterpene fraction.

Essential oils from *Eucalyptus* species had shown strong inhibitory effects on germination of seeds of many crops and weeds (Sing et al., 2002; Azizi and Fuji, 2006). The essential oil composition of *E. camaldulensis* from different origins had been reported. Two groups of *E. camaldulensis* essential oils can be distinguished: those that contain 1,8-cineole as the main compound, which includes *E. camaldulensis* from Mali, Mozambique, Nigeria, Egypt and Iran (Chalchat et al., 2000; Pagula et al., 2000; Oyediji et al., 2000; Maximous, 2004; Sefidkon et al., 2006) and those that contain spathulenol, *p*-cymene and cryptone as main compounds and small quantities of 1,8-cineol, like the results we obtained, which are similar to *E. camaldulensis* from south Florida, Jerusalem and Greece (Pappas and Sheppard-Hanger, 2000; Chalchat et al., 2001; Tsiri et al., 2003). Both essential oil compositions were reported from *E. camaldulensis* clones growing in Australia (Dunlop et al., 2000). Our results suggest according to other authors (Angelini et al., 2003) that 1,8-cineol is not the principal responsible for the allelopathic effect from *Eucalyptus* sp. essential oil. The essential oil from *E. camaldulensis* assayed contained spathulenol as the main compound with percentage around 40%, whereas 1,8-cineole only reached 1.92 ± 0.32 of the total oil.

To conclude, it is interesting to note the results obtained with *E. africanus* essential oil: with the oxygenated monoterpene artemisia ketone as the main compound (56.46 ± 1.99); it was more active than *L. camara*, showing great selectivity against *A. hybridus*.

Further studies under field conditions are necessary to evaluate the possible use of these essential oils for their allelopathic activity.

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