Journal of Allelochemical Interactions

July 2018. JAI 4 (2): **17-26**

Phytotoxic potential of *Lantana camara, Eucalyptus camaldulensis, Eriocephalus africanus, Cistus ladanifer* and *Artemisia gallica* aqueous extracts to control weeds

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ABSTRACT

Weed management is necessary in natural and agricultural ecosystems. The most used control method in developed countries has been the application of chemical herbicides, which has caused many problems in human health and the environment as well as the development of resistant weeds due to the repeated use of herbicides with the same mode of action. Natural products could be an alternative to synthetic herbicides for weed management. The society is demanding new solutions and research of bioherbicides has increased in the last years. Aqueous extracts from some plant species contain allelopathic compounds that can inhibit the germination and the development and growth of other plants or organisms. In this work the phytotoxic potential of aqueous extracts from Mediterranean plants are studied in order to find new solutions for integrated weed management.

Keywords: weed control, natural herbicides, aqueous extracts, seed germination.

Introduction

In agricultural and natural ecosystems weeds are always present and is necessary to stablish adequate management and control measures in order to avoid their proliferation and spreading, causing interferences and losses in crop production and quality of harvests and decreases in biodiversity in natural areas. In the last century, the use of synthetic herbicides has become the main method for weed control in developed countries (Gaba *et al*., 2016). The use of herbicides and other agrochemical products allow to increase crop yields and food production, facing the necessities of a world growing population (Alexandratos and Bruinsma, 2012). However, during the last decades, many studies evidenced that agrochemical residues did spread in the environment, causing significant contamination of terrestrial and aquatic ecosystems, toxic effects on human health and poisoning human foods (Carvalho, 2017). In the European Union, Directive 2009/128/EC aims to achieve a sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of Integrated Pest Management (IPM) and of alternative approaches or techniques, such as non-chemical alternatives to pesticides. Also the development of resistant weeds to many synthetic herbicides is promoting the research of new tools for weed management. The use of biopesticides and related alternative management products is increasing, including semiochemicals and plant-incorporated protectants (PIPs), as well as botanical and microbially derived chemicals (Seiber *et al*., 2014).

Aqueous extracts from different plant species have been reported to contain allelochemical compounds that can inhibit germination and growth of weeds (Heisey, 1990; Chon *et al*., 2003; Puig *et al*., 2018). In this work, the allelopathic potential of the aqueous extracts from five species: *Lantana camara* L., *Eucalyptus camaldulensis* Dehnh., *Eriocephalus africanus* L., *Cistus ladanifer* L. and *Artemisia gallica* Willd was investigated against five important weeds in Mediterranean crops in order to find new alternatives for weed management. *L. camara*

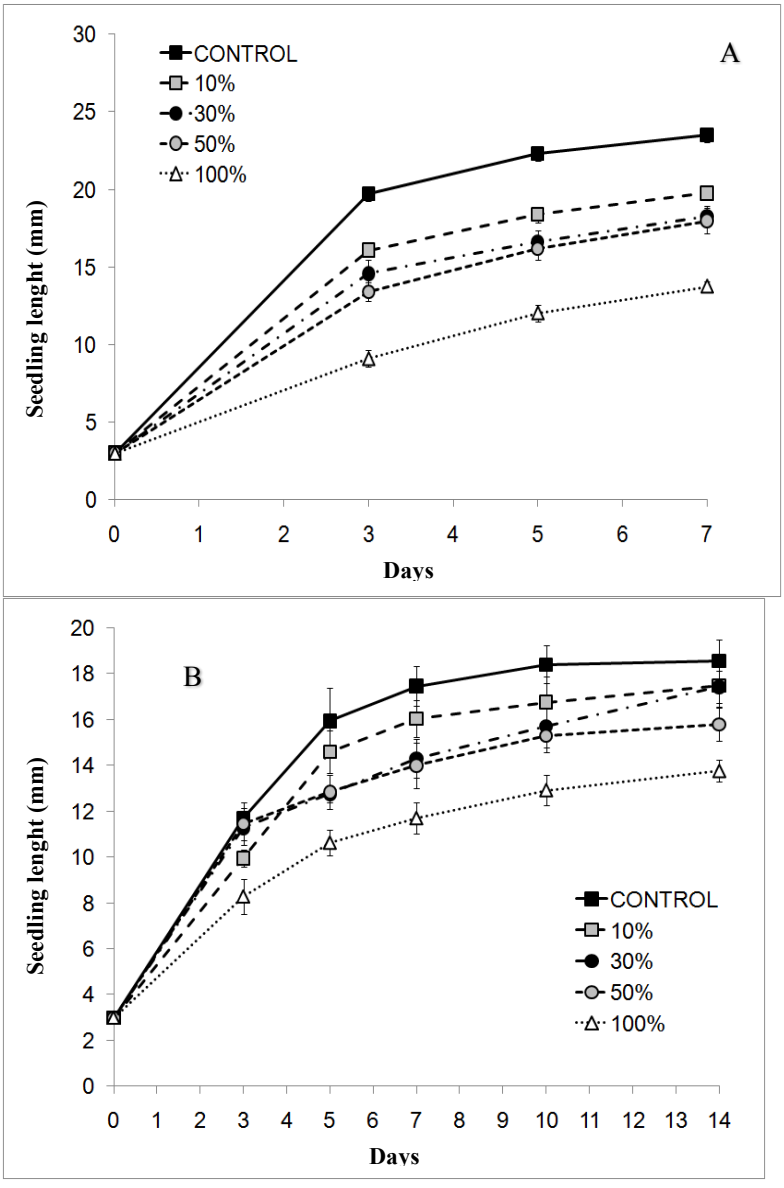

Figure 1.
Effect of *L. camara* aqueous extract on *A. hybridus* (A) and *C. album* (B) seedling length.

(Verbenaceae) is used as ornamental plant but it is also considered an invasive weed in many countries and one of the worst weeds in the world (Mersie and Singh, 1987; Guisalberti, 2000). *L. camara* may produce and release several types of secondary metabolites, including phenolic acids, flavonoids, terpenes and terpenoids. Some of them are allelochemicals that inhibit the growth of other organisms, like plants (weeds and crops), ferns and algae (Mersie and Singh, 1987; Sharma *et al*., 2005; Kong *et al*., 2006; Labruzzo *et al*., 2017). *E. camaldulensis* (Myrtaceae) is also used as ornamental plant. In California, the annual vegetation adjacent to natural stands of *E. camaldulensis* often is inhibited severely. Volatile and water-soluble toxins were found in *E. camaldulensis* tissues: cineole, α-pinene, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid and gallic acid (Del Moral and Muller, 1970). Aqueous extracts, essential oils and leaf litters from *E. camaldulensis* have shown phytotoxic effects (Ahmed *et al*., 2008; Verdeguer *et al*., 2009, Fikreyesus *et al*., 2011). *C. ladanifer* (Cistaceae) is a Mediterranean shrub that grows on siliceous soils (Juhren, 1966). Studies of *C. ladanifer* shrublands demonstrated the allelopathic effects of exudates secreted by leaves of this species on the adjacent vegetation (Malato-Beliz *et al*., 1992;

López-González, 2001). The allelopathic effects of *C. ladanifer* on germination of many Mediterranean species was studied concluding that this species may influence the composition and structure of Mediterranean communities where it is present and may reduce the area occupied by numerous herbaceous species (Herranz *et al*., 2006). *E. africanus* (Asteraceae) is an aromatic shrub native of South Africa, naturalized in most Mediterranean areas as ornamental plant. The essential oil from this species, with Artemisia ketone as main compound controlled *A. hybridus* germination (Verdeguer *et al*., 2009). *A. gallica* (Asteraceae) is a Mediterranean halophyte species native from France (Viehoever and Capen, 1923) and endemic from the South of Europe (Ladero *et al*., 1984). The phytotoxic potential of this species has not been previously studied.

Materials and Methods

Weed seeds

Seeds of *Amaranthus hybridus* L., *Portulaca oleracea* L., *Chenopodium album* L., *Conyza canadensis* (L.) Cronq. and *Parietaria judaica* L. were extracted from fully ripe weeds collected in agricultural fields and ruderal areas in Valencia and Castellón province (Spain) between October 2005 and August 2008. The plants were dried for 15 days at room temperature. Afterwards, the seeds were extracted. Uniform healthy seeds were selected and stored at room temperature until herbicidal testing.

Aqueous extracts

Aerial parts from *Lantana camara* L., *Eucalyptus camaldulensis* Dehnh. and *Eriocephalus africanus* L. were collected from gardens of Valencia and Burjasot (Valencia province, Spain) between May 2005 and June 2009. *Cistus ladanifer* L. aerial parts were collected from Guadarrama mountain range (San Lorenzo del Escorial, Spain) between June 2006 and June 2009, and aerial parts from *Artemisia gallica* Willd were collected from the marshes of Puzol (province of Valencia, Spain) and Torreblanca (province of Castellón, Spain) between January 2007 and June 2009.

Aqueous extracts were obtained from leaves of fresh plants. For each 20 g of leaves, 200 mL of distilled water were added and the mix was introduced into a bath at 80 °C for 15 min. The obtained aqueous solution was then filtered, and the leaves were extracted again with 100 mL of distilled water for each 20 g in a water bath at 80 °C for another 15 min. The solution was filtered and the two obtained filtrates were combined. This extract was considered the basic concentration (100%) and was stored at -40 °C until it was used for phytotoxicity tests. The other concentrations tested (50, 30 and 10%) were prepared diluting the original extract with distilled water.

In order to determine their composition, aqueous extracts from all the studied species were lyophilized and two samples of each were prepared at a concentration of 3 mg mL^{-1}, one in water and one in methanol. The composition of the aqueous extracts was analysed by HPLC-MS (High Performance Liquid Chromatography) using a Waters Acquity HPLC-PDA system (Waters Corp., Milford, USA) coupled to a Q-TOF micromass spectrometer. The system was equipped with a binary solvent pump, an automatic sample injector, a column compartment and a PDA 2996 detector, connected to the Waters Masslynx 4.1 software. An Acquity BEH C18 column (2.1 x 100 mm, 1.7 μm) was used at 30 °C. The mobile phase consisted of 0.1% formic acid in ultrapure

Table 1.
Germination of *A. hybridus*, *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seeds treated with *L. camara* aqueous extracts.

Concentration (%)	Germination (% ± s.e.)				
	Amaranthus hybridus	***Portulaca oleracea***	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	45.0 ± 2.7 a	74.0 ± 10.2 a	90.0 ± 3.2 a	36.0 ± 10.9 a	56.6 ± 5.0 a
10	34.0 ± 1.9 ab	68.0 ± 4.4 a	50.0 ± 3.9 b	0.0 ± 0.0 b	41.3 ± 6.2 a
30	32.0 ± 4.6 ab	78.0 ± 3.4 a	35.0 ± 5.5 c	0.0 ± 0.0 b	59.0 ± 3.4 a
50	21.0 ± 4.6 bc	67.0 ± 4.6 a	3.0 ± 1.2 d	0.0 ± 0.0 b	60.8 ± 7.2 a
100	16.0 ± 4.8 c	71.0 ± 6.0 a	3.0 ± 2.0 d	8.0 ± 4.6 b	42.5 ± 8.0 a

Table 2.
Effect of *L. camara* aqueous extract on seedling length of *P. oleracea*, *C. canadensis* and *P. judaica.*

Concentration (%)	Seedling length (mm ± s.e.)		
	Portulaca oleracea	***Conyza canadensis***	***Parietaria judaica***
0 (control)	8.19 ± 0.49 a	1.79 ± 0.18 a	19.20 ± 1.79 a
10	7.80 ± 0.57 a	-	17.41 ± 0.69 a
30	8.20 ± 0.48 a	-	17.30 ± 1.64 a
50	9.13 ± 0.58 a	-	16.97 ± 1.20 a
100	7.49 ± 0.46 a	0.94 ± 0.43 a	22.04 ± 2.40 a

Table 3.
Germination of *A. hybridus*, *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seeds treated with *E. camaldulensis* aqueous extract.

Concentration (%)	Germination (% ± s.e.)				
	Amaranthus hybridus	***Portulaca oleracea***	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	45.0 ± 2.7 a	74.0 ± 10.2 a	90.0 ± 3.2 a	36.0 ± 10.9 a	56.6 ± 5.0 a
10	22.0 ± 3.4 b	60.0 ± 8.9 ab	86.0 ± 3.7 a	0.0 ± 0.0 b	49.0 ± 4.0 a
30	2.0 ± 1.2 c	64.0 ± 4.3 ab	69.0 ± 6.8 b	0.0 ± 0.0 b	42.0 ± 2.5 a
50	0.0 ± 0.0 c	41.0 ± 9.8 b	63.0 ± 2.5 b	0.0 ± 0.0 b	45.0 ± 6.1 a
100	1.0 ± 1.0 c	39.0 ± 7.0 b	74.0 ± 4.0 b	0.0 ± 0.0 b	52.0 ± 5.1 a

water (1: 1,000, v/v, phase A) and 0.1% acetonitrile in formic acid (1: 1,000, v/v, phase B). The gradient used was 100% at 80% A in 3 min, 80% at 50% A in 7 min, 50% at 0% A in 3 min, maintaining 100% B 1 min and returning to 100% A in 2 min. The flow was 0.2 mL min^{-1}. The temperature of the sample injector was maintained at 20 °C and the injection volume was 5 µL. The ultraviolet spectra were acquired between 210 and 500 nm with a resolution of 1.2 nm and 20 points/second of sampling speed. The analysis of the mass spectra was carried out by electrospray ionization in positive mode. The mass spectrometer was calibrated with sodium formate (10 ng μL^{-1} in 90:10 propan-2-ol: water). The conditions of analysis were the following: capillary voltage 3.0 kV, cone voltage 30 eV, desolvation temperature 300 °C, source temperature 120 °C, cone gas flow 50 L h^{-1}, desolvation gas flow 650 L h^{-1}, collision energy 10 eV. The mass spectra were acquired in centroid mode, in a range of exploration of mass-load ratio 100 to 1400, with an exploration time of 0.3 s and a time between explorations of 0.1 s.

Seed germination and seedling growth tests

Sets of 20 seeds each with five replicates per treatment, were germinated in Petri dishes (9 cm diameter) between four layers of filter paper (50 g/m^2) wetted with 4 mL of distilled water (control) or aqueous extracts from the studied species at concentrations of 10, 30, 50 and 100%. In accordance with previous assays, weed seeds were incubated in a germination chamber APG–GROW at 30.0 ± 0.1 ºC 16 h in light and 20.0 ± 0.1 ºC 8 h in dark. To evaluate the phytotoxic potential of the aqueous extracts, images of the seeds and seedlings in the Petri dishes were recorded after 3, 5, 7, 10 and 14 d of incubation in *A. hybridus* and *P. oleracea*, after 5, 7, 10 and 14 d in *C. canadensis* and *P. judaica* and after 7, 10 and 14 d in *C. album*, because of differences in the starting of seed germination. The images were processed with the software Image Tool, counting the number of seed germinated in order to obtain the germination percentage and seedling length (hypocotyl plus radicle) was measured.

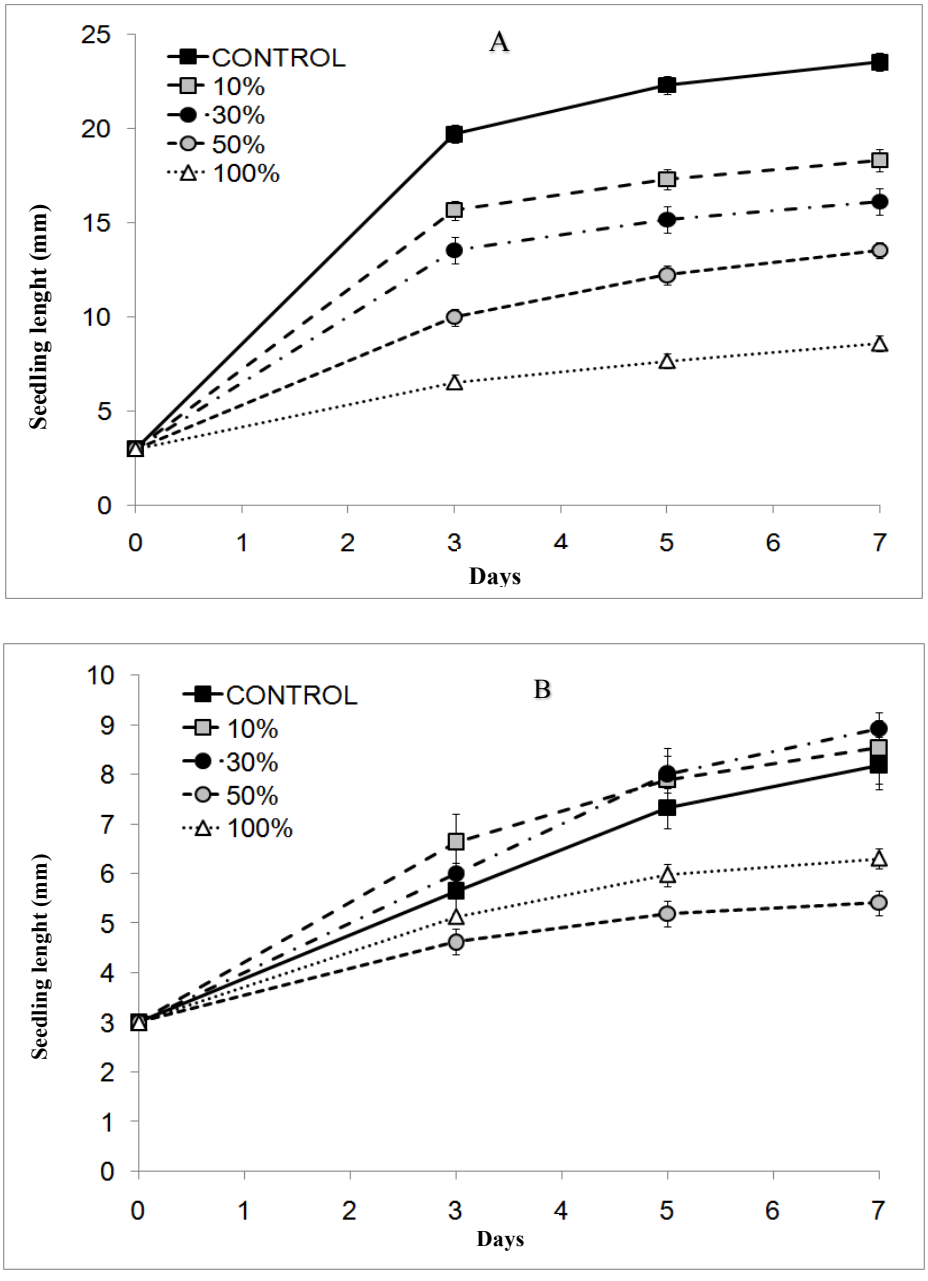

Figure 2.
Effect of *E. camaldulensis* aqueous extract on *A. hybridus* (A) and *P. oleracea* (B) seedling length.

Statistical analysis

Tests were conducted in a randomised complete design with five replications. Data were submitted to analysis of variance. Previously, homoscedasticity was checked with Cochran's, Bartlett's and Levene's tests. Percentage values were arcsin transformed. Length values were transformed to y=log(x+1). Means were compared using Fisher's least significant difference (LSD) test ($p \leq 0.05$).

Results and Discussion

L. camara aqueous extract showed the strongest activity against *C. canadensis* (Table 1), since the concentrations of 10, 30 and 50% completely inhibited its germination, while the 100% reduced it by 77.8%, without significant differences between concentrations. In contrast, it showed no effect on the germination and growth of *P. oleracea* and *P. judaica* (Tables 1 and 2), neither on *C. canadensis* seedlings length (Table 2).

Table 4.
Effect of *E. camaldulensis* aqueous extracts on *C. album*, *C. canadensis* and *P. judaica* seedling length.

Concentration (%)	Seedling length (mm ± s.e.)		
	Chenopodium album	***Conyza canadensis***	***Parietaria judaica***
0 (control)	18.44 ± 0.29 a	1.79 ± 0.18	19.20 ± 1.79 a
10	15.43 ± 0.73 a	-	16.36 ± 0.90 a
30	17.08 ± 1.25 a	-	22.00 ± 1.64 a
50	14.69 ± 0.75 a	-	17.55 ± 1.47 a
100	9.96 ± 0.47 b	-	16.90 ± 0.79 a

This extract was very active against the germination of *C. album*, reducing it at all the doses tested, being the maximum inhibition of 96.7% at the two highest concentrations, without significant differences between them (Table 1). However, only these two concentrations reduced the length of the seedlings, by 14.9 and 25.9% respectively (Fig. 1B).

Finally, only the two higher concentrations of the extract controlled the germination of *A. hybridus*, decreasing it by 53.3 and 64.4% respectively, without significant differences between them (Table 1), while all the concentrations inhibited the growth of the seedlings, up to 41.6%, at 100% (Fig. 1A).

The aqueous extract of *E. camaldulensis* showed also the greatest effect on *C. canadensis*, since it completely inhibited its germination at all concentrations applied (Table 3). In *A. hybridus* all concentrations inhibited the germination (Table 3), without significant differences between the 3 higher doses, which showed great activity, reducing the germination of *A. hybridus* from 95.6% until totally controlled. Likewise, all the concentrations of this extract inhibited *A. hybridus* seedling growth, with significant differences between all of them, and being the highest observed growth reduction of 63.6% (Figure 2A).

Only the two stronger concentrations of the extract (50 and 100%) had inhibitory effect on *P. oleracea* germination, without significant differences between them, reducing it by 44.6 and 47.3% respectively (Table 3). These concentrations reduced the growth of *P. oleracea* seedlings, without differences among them, being the maximum reduction of 33.9% (Figure 2B). The 3 highest concentrations of the extract showed an inhibitory effect on the germination of *C. album*, with a maximum reduction of 30% (Table 3), whereas only 100% concentration reduced seedling length, by 46% (Table 4). In *P. judaica* this extract showed no effect, neither on its germination (Table 3) nor on its growth (Table 4).

The aqueous extract of *E. africanus* showed the strongest activity against *C. canadensis* germination, with practically complete inhibition at concentrations of 10-50%, without significant differences between concentrations (Table 5). There was an abnormal result at the 100% concentration, as this extract showed no significant differences compared to the control (Table 5). The same results were obtained on the growth of *C. canadensis* seedlings (Table 6), being the maximum inhibitory effect achieved by this extract of 92.7%.

Table 5.
Germination of *A. hybridus*, *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seeds treated with *E. africanus* aqueous extract.

Concentración (%)	Germination (% ± s.e.)				
	Amaranthus hybridus	***Portulaca oleracea***	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	45.0 ± 2.7 a	74.0 ± 10.2 a	90.0 ± 3.2 a	36.0 ± 10.9 a	56.6 ± 5.0 a
10	30.0 ± 5.7 a	38.0 ± 9.8 b	72.0 ± 3.7 b	2.0 ± 1.2 b	29.1 ± 4.0 b
30	14.0 ±7.0 b	36.0 ± 2.9 b	63.0 ± 3.4 bc	0.0 ± 0.0 b	26.9 ± 6.8 bc
50	8.0 ± 6.8 bc	29.0 ± 1.9 b	54.0 ± 5.3 cd	1.0 ± 1.0 b	8.2 ± 6.5 d
100	0.0 ± 0.0 c	24.0 ± 5.8 b	42.0 ± 9.2 d	39.0 ± 11.7 a	9.5 ± 2.8 cd

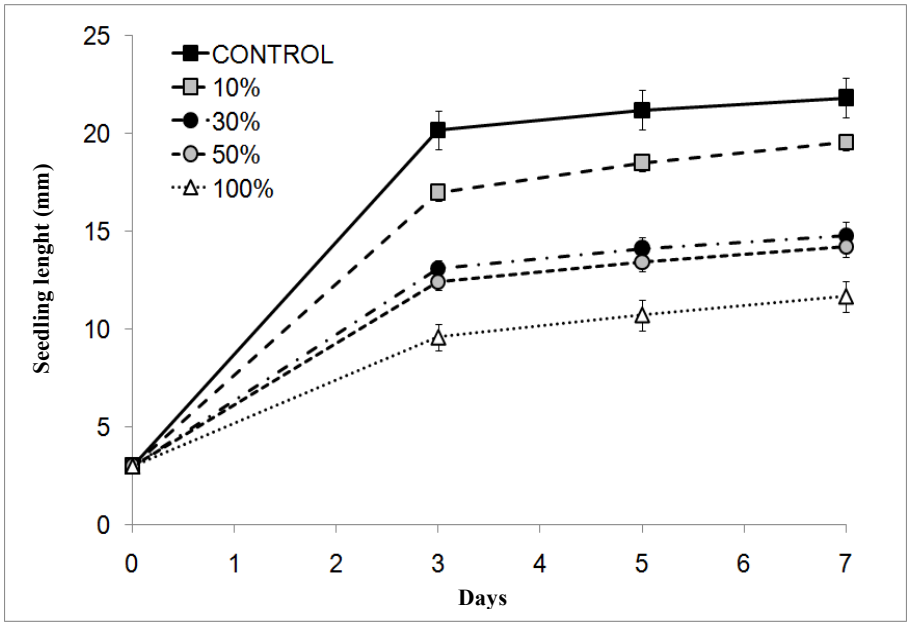

Figure 3.
Effect of *E. africanus* aqueous extract on *A. hybridus* seedling length.

The germination of *A. hybridus* was completely inhibited by the extract of *E. africanus* at the 100% concentration (Table 5), with no significant differences between the activity of this dose and 50% (82.2% inhibition). All concentrations significantly reduced the length of the seedlings (Fig. 3), with the greatest reduction in growth of 46.5%. All the concentrations of the extract significantly inhibited the germination of *P. oleracea* (48.6-67.6%) without differences between them (Table 5). However, the extract did not control the seedling growth of this weed (Table 6). The germination of *C. album* was reduced by all the concentrations of the extract (Table 5), with the maximum inhibition of 53.3%, but only the two major concentrations reduced the growth of the seedlings, in 25.6% and 50%, respectively (Table 6).

On *P. judaica* germination, all the concentrations of the extract exerted an inhibitory effect (Table 5), being the maximum reduction of 85.5% at the concentration of 50%, without differences with 100% (83.2% inhibition). All the concentrations of the extract reduced *P. judaica* seedling length (Table 6), but the length of the seedlings treated with 50% did not show significant differences with the control seedlings. The 100% concentration achieved the greatest reduction in growth, 66.8% (Table 6).

The aqueous extract of *C. ladanifer*, as the previous extracts, showest the strongest activity against *C. canadensis* germination, the concentration of 50% reduced it by 97.2%, without significant differences

Table 6.
Effect of *E. africanus* aqueous extract on *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seedling length.

Concentration (%)	Seedling length (mm ± s.e.)			
	Portulaca oleracea	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	8.38 ± 0.63 a	20.58 ± 0.37 a	1.79 ± 0.18 a	19.20 ± 1.79 a
10	8.56 ± 0.19 a	17.60 ± 0.65 ab	0.97 ± 0.59 b	10.11 ± 1.31 bc
30	8.67 ± 0.60 a	17.77 ± 0.64 a	-	12.64 ± 0.66 b
50	7.88 ± 0.60 a	15.32 ± 0.68 b	0.13 ± 0.13 b	14.74 ± 0.55 ab
100	7.00 ± 0.77 a	10.30 ± 0.42 c	2.37 ± 0.30 a	6.37 ± 0.51 c

Table 7.
Germination of *A. hybridus, P. oleracea, C. album, C. canadensis* and *P. judaica* seeds treated with *C. ladanifer* aqueous extract.

Concentration (%)	Germination (% ± s.e.)				
	Amaranthus hybridus	***Portulaca oleracea***	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	97.0 ± 2.0 a	90.0 ± 5.2 a	78.0 ± 6.8 a	36.0 ± 10.9 a	56.6 ± 5.0 a
10	83.8 ± 6.0 bc	81.2 ± 5.8 a	76.0 ± 7.3 a	24.0 ± 7.8 ab	51.0 ± 5.6 ab
30	90.0 ± 1.6 bc	92.0 ± 3.0 a	82.0 ± 3.0 a	2.0 ± 1.2 c	49.0 ± 5.8 abc
50	96.9 ± 2.4 ab	92.0 ± 2.5 a	73.0 ± 3.4 a	1.0 ± 1.0 c	38.0 ± 5.4 bc
100	76.5 ± 3.4 c	75.0 ± 5.2 a	78.0 ± 4.9 a	7.0 ± 3.0 bc	36.0 ± 1.9 c

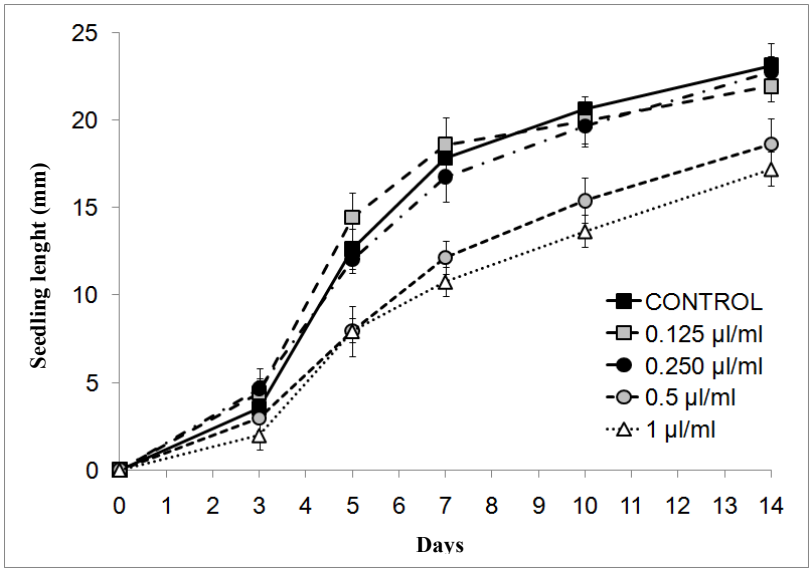

Figure 4.
Effect of *C. ladanifer* aqueous extract on *A. hybridus* seedling length.

Table 8.
Effect of *C. ladanifer* aqueous extract on *P. oleracea, C. album, C. canadensis* and *P. judaica* seedling length.

Concentration (%)	Seedling length (mm ± s.e.)			
	Portulaca oleracea	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	8.98 ± 0.24 b	20.05 ± 1.65 a	1.79 ± 0.18 a	19.20 ± 1.79 a
10	14.63 ± 0.94 a	18.55 ± 0.29 a	1.36 ± 0.35 a	21.15 ± 2.34 a
30	14.85 ± 0.79 a	18.87 ± 1.62 a	0.70 ± 0.44 a	20.23 ± 1.44 a
50	10.36 ± 0.70 b	18.74 ± 1.19 a	0.49 ± 0.49 a	16.94 ± 1.16 a
100	10.55 ± 0.68 b	15.33 ± 1.26 a	1.39 ± 0.60 a	14.95 ± 1.71 a

Table 9.
Germination of *A. hybridus*, *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seeds treated with *A. gallica* aqueous extract.

Concentration (%)	Germination (% ± s.e.)				
	Amaranthus hybridus	***Portulaca oleracea***	***Chenopodium album***	***Conyza canadensis***	***Parietaria judaica***
0 (control)	97.0 ± 2.0 a	90.0 ± 5.2 a	78.0 ± 6.8 a	93.0 ± 3.4 a	67.1 ± 4.6 a
10	86.7 ± 6.3 ab	97.0 ± 1.2 a	88.0 ± 3.0 a	0.0 ± 0.0 b	53.7 ± 8.3 a
30	91.0 ± 4.6 ab	94.0 ± 2.4 a	82.0 ± 3.4 a	3.0 ± 3.0 b	36.0 ± 8.6 a
50	70.0 ± 8.9 b	91.0 ± 1.0 a	68.8 ± 3.4 a	3.0 ± 3.0 b	48.2 ± 7.1 a
100	35.0 ± 10.6 c	97.0 ± 2.0 a	73.8 ± 3.8 a	0.0 ± 0.0 b	36.2 ± 8.8 a

with concentrations 30 and 100% (Table 7). However, this extract had no effect on *C. canadensis*, *C. album* and *P. judaica* seedling growth (Table 8). On *C. album* the extract showed no effect (Tables 7 and 8), while in *P. judaica* the two highest concentrations reduced germination 32.9 and 36.4% (50 and 100%, respectively) without differences between them (Table 7). This extract did not control *P. oleracea* germination (Table 7) and concentrations 10 and 30% produced a stimulatory effect on the growth of its seedlings (Table 8). The 100% concentration was the most effective against the germination of *A. hybridus*, reducing it by 21.1% (Table 7). Both this concentration and 50% inhibited the growth of the seedlings, 25.6 and 19.5% respectively, without significant differences between them (Figure 4).

The aqueous extract of *A. gallica* also affected more the germination of *C. canadensis*, since all the concentrations reduced it drastically, without significant differences between them (Table 9). This extract had no effect on the germination of *P. oleracea*, *C. album* and *P. judaica* (Table 9). Only the two largest concentrations (50 and 100%) inhibited the germination of *A. hybridus*, 27.9 and 63.9% respectively, the difference between them being significant (Table 9). The concentrations of 30 and 50% inhibited the growth of *C. canadensis* seedlings by 71.4 and 78.7%, without differences between them (Table 10). There were no significant effects of the extract on the growth of seedlings of *A. hybridus*, *C. album* and *P. judaica*, while all concentrations stimulated the growth of *P. oleracea* seedlings (Table 10).

From the results obtained, we can affirm that the activity of the extracts depends on several factors: one of them is the species against which they act (Lee *et al*., 2002), since in some cases they are selective, inhibiting the germination of certain weeds, but without showing effects or even stimulating the germination of crops or other species of weeds, as in the case of the extracts of *Cistus ladanifer* L. and *Lavandula stoechas* L., which inhibited *in vitro* the germination of *Phalaris minor* L., the main weed in wheat cultivation in the south of Portugal, while stimulating the growth of the latter (Dias *et al*., 1995). The species *Alcea pallida* Waldst. & Kit. was the only one that showed resistance to the

Table 10.
Effect of *A. gallica* aqueous extract on *A. hybridus*, *P. oleracea*, *C. album*, *C. canadensis* and *P. judaica* seedling length.

Concentration (%)	Seedling length (mm ± s.e.)		
	Chenopodium album	***Conyza canadensis***	***Parietaria judaica***
0 (control)	18.44 ± 0.29 a	1.79 ± 0.18	19.20 ± 1.79 a
10	15.43 ± 0.73 a	-	16.36 ± 0.90 a
30	17.08 ± 1.25 a	-	22.00 ± 1.64 a
50	14.69 ± 0.75 a	-	17.55 ± 1.47 a
100	9.96 ± 0.47 b	-	16.90 ± 0.79 a

oils tested against it and other weeds (Azirak and Karaman, 2008). Another factor is their composition, and the concentration applied. The aqueous extracts were analysed by HPLC-Mass Spectrometry using methanol-water or acetonitrile-water as the mobile phase. The complexity of the aqueous extracts obliged to postpone their in-depth analysis for further studies through the use of standard compounds. However, from the observation of the ultraviolet spectrum and the corresponding positive-mode electrospray mass spectra, could be deduced that *E. camaldulensis* and *E. africanus* extracts contained phenolic acids, and flavonoids both to the genin state and to the glycosides derived mainly from kaempferol. It is interesting to note the presence of phenylpropanoids as chlorogenic acid in *E. africanus* extract.

Aqueous extracts from *E. africanus* and *E. camaldulensis* were the most active. They showed good potential for weed control. In further studies their activity should be studied in greenhouse and field conditions and their composition should be more investigated.

References

Ahmed R, Hoque ATMR, Hossain MK (2008) Allelopathic effects of leaf litters of *Eucalyptus camaldulensis* on some forest and agricultural crops. J Forest Res 19: 19-24.

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. FAO, Rome.

Azirak S, Karaman S (2008) Allelopathic effect of some essential oils and components on germination of weed species. Acta Agr Scan Sect B 58: 88-92.

Carvalho FP (2017) Pesticides, environment, and food safety. Food Energy Sec 6(2): 48-60.

Chon S, Kim Y, Lee J (2003) Herbicidal potential and quantification of causative allelochemicals from several Compositae weeds. Weed Res 43: 444-450.

Dias LS, Pereira IP, Dias AS (1995) Evaluation of Mediterranean-type vegetation for weedicide activity. Allelopathy J 2: 197-204.

Fikreyesus S, Kebebew Z, Nebiyu A, Zeleke N, Bogale S (2011) Allelopathic effects of *Eucalyptus camaldulensis* Dehnh. on germination and growth of tomato. Am-Eur J Agric Environ Sci 11: 600-608.

Gaba S, Gabriel E, Chadœuf J, Bonneu F, Bretagnolle V (2016) Herbicides do not ensure for higher wheat yield, but eliminate rare plant species. Sci Rep 6: 30112.

Guisalberti EL (2000) *Lantana camara* L. (Verbenaceae). Fitoterapia 71: 467-486.

Heisey R.M. (1990). Allelopathic and herbicidal effects of extracts from Tree of Heaven (*Ailanthus altissima*). Am J Bot 77(5): 662-670.

Herranz JM, Ferrandis P, Copete MÁ, Duro EM, Zalacain A (2006) Effect of allelopathic compounds produced by *Cistus ladanifer* on germination of 20 Mediterranean taxa. Plant Ecol 184: 259-272.

Juhren MC (1966) Ecological observations on *Cistus* in the Mediterranean vegetation. Forest Sci 12: 415–426.

Kong CH, Wang P, Zhang CX, Zhang M.X, Hu F (2006) Herbicidal potential of allelochemicals from *Lantana camara* against *Eichhornia crassipes* and the alga *Microcystis aeruginosa*. Weed Res 46: 290-295.

Ladero M, Navarro F, Valle CJ (1984) Contribución al conocimiento de la flora halófila de la depresión del Duero. Stud Bot 3: 263-266.

Lee SY, Shim KC, Kil JH (2002) Phytotoxic effect of aqueous extracts and essential oils from southern marigold (*Tagetes minuta*). New Zeal J Crop Hort Sci 30: 161-169.

López-González G (2001).Los árboles y arbustos de la Península Ibérica e Islas Baleares. Mundi-Prensa, Madrid.

Labruzzo A, Carrubba A, Di Marco G, Ebadi MT (2017) Herbicidal potential of aqueous extracts from *Melia azedarach* L., *Artemisia arborescens* L., *Rhus coriaria* L. and *Lantana camara* L. Allelopathy J 41 (1): 81-92.

Malato-Beliz J, Escudero JC, Buyolo, T (1992) Application of traditional indices and of diversity to an ecotonal area of different biocoenes. The state of the art in vegetation science. Int Assoc Veget Sci, Toledo, Spain

Mersie W, Singh M (1987) Allelopathic effect of lantana on some agronomic crops and weeds. Plant Soil 98: 25-30.

Puig CG, Reigosa MJ, Valentão P, Andrade PB, Pedrol N (2018) Unravelling the bioherbicide potential of *Eucalyptus globulus* Labill: Biochemistry and effects of its aqueous extract. PLoS ONE 13(2): e0192872. https://doi.org/10.1371/journal.pone.0192872

Seiber JN, Coats J, Duke SO, Gross AD (2014) Biopesticides: state of the art and future opportunities. J Agr Food Chem 62: 11613–11619.

Sharma GR, Raghubanshi AS, Singh JS (2005) *Lantana* invasion: an overview. Weed Biol Manag 5: 157-165.

Verdeguer M, Blázquez MA, Boira H (2009) Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochem Syst Ecol 37: 362-369.

Viehoever A, Capen RG (1923) New sources of Santonin. J Amer Chem Soc 45: 1941-1944.

RECEIVED May, 15, 2018
ACCEPTED June, 30, 2018