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Chemical composition and phytotoxic potential of *Eucalyptus globulus* essential oil against *Lactuca sativa* and two herbicide-resistant weeds: *Avena fatua* and *Amaranthus hybridus*

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Abstract

Weed control in sustainable agriculture requires new bioherbicidal molecules to replace synthetic herbicides that have damaged the environment and generated resistance in weeds. This study was conducted to investigate the chemical composition of *Eucalyptus globulus* essential oil and to explore its bioherbicidal potential on the germination and radicle growth inhibition. The phytotoxic effects of *E. globulus* essential oil (1, 10 and 20 µL mL^{-1}) were tested in comparison to those of the synthetic herbicide Glyphosate (1, 10 and 20 µL mL^{-1}) in bioassays of germination and radicle growth of *Lactuca sativa* and the resistant weeds *Avena fatua* and *Amaranthus hybridus*. Gas Chromatography with Flame Ionization Detector and Gas Chromatography-Mass Spectroscopy analysis showed that major monoterpenes comprised 1,8-Cineole (86.94%), α-pinene (7.71%), d-limonene (2.65%), and p-cymene (1.48%). The seed germination and radicle length exhibited different degrees of inhibition in response to the concentration of *E. globulus* essential oil. At some concentrations, both the Glyphosate herbicide and the *E. globulus* essential oil demonstrated the same phytotoxicity against the resistant weeds *A. fatua* and *A. hybridus.* Essential oil bioactivity Lethal Concentration (LC_{50}) in the majority of cases was lowest for *A. hybridus*, followed by *A. fatua* and *L. sativa.* Based on the results, it can be concluded that *E. globulus* essential oil possesses phytotoxic potential and could be explored as a bioherbicide for resistant weeds management programs.
Keywords: bioherbicide, allelochemicals; phytotoxicity; herbicide-resistant weeds.

Composición química y potencial fitotóxico de *Eucalyptus globulus* sobre *Lactuca sativa* y dos malezas resistentes a herbicidas: *Avena fatua* y *Amaranthus hybridus*

Resumen

El control de las malezas en una agricultura sustentable requiere de nuevas moléculas bioherbicidas que sustituyan a los herbicidas sintéticos que han dañado al medio y generado resistencia en las malezas. El presente estudio, se realizó para determinar la composición química del aceite esencial de *Eucalyptus globulus* y explorar su potencial bioherbicida sobre la germinación y la inhibición del crecimiento radicular. Los efectos fitotóxicos del aceite esencial de *E. globulus* (1, 10 y 20 µL mL^{-1}) se compararon con los ocasionados por el herbicida sintético Glifosato (1, 10 y 20 µL mL^{-1}) mediante bioensayos de germinación y crecimiento de las raíces de *Lactuca sativa* y de las malezas resistentes a los herbicidas *Avena fatua* y *Amaranthus hybridus.* El análisis por Cromatografía de Gases con Detector de Ionización de Flama y Cromatografía de Gases-Espectrometría de Masas mostró que los monoterpenos principales fueron 1,8-cineol (86,94%), α-pineno (7,71%), d-limoneno (2,65%) y p-cimeno (1,48%). La germinación de la semilla y la longitud de la radícula de ambas malezas exhibieron diferentes grados de inhibición en respuesta a la concentración del aceite esencial de *E. globulus*. En algunas concentraciones, tanto el herbicida Glifosato como el aceite esencial de *E. globulus* mostraron la misma fitotoxicidad contra las malezas estudiadas *A. fatua* y *A. hybridus*. La bioactividad de la Concentración Letal ($LC_{50,}$ por sus siglas en inglés) del aceite esencial en la mayoría de los casos fue menor para *A. hybridus,* seguido de *A. fatua* y *L. sativa.* Con base en los resultados, se puede concluir que el aceite esencial de *E. globulus* posee potencial fitotóxico y podría ser explorado como un bioherbicida para programas de manejo de malezas resistentes.
Palabras clave: bioherbicida, aleloquímico, fitotóxico, malezas resistentes a herbicidas.

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Introduction

The overuse and misuse of huge amounts of synthetic herbicides for weed management has resulted in the emergence of herbicide-resistant weed biotypes and environmental contamination. The current worldwide demand for cheaper, more environmentally friendly weed management technologies has motivated a number of studies on allelochemicals or allelophatins as phytotoxic compounds to be used as bioherbicides (Dorota, Urszula, Renata & Agnieszka, 2013; Rassaeifar, Hosseini, Haji, Zandi & Moradi, 2013; Radhakrishnan, Alqarawi & Abd_Allah, 2018). Allelochemicals are plant secondary metabolites and in the case of essential oils are synthesized during the isoprenoid pathway (Dorota *et al.,* 2013). On the other hand, they are considered a potential source of novel molecules with herbicidal action for the chemical industry, the necessity of which is due to the emergence of weeds that are herbicide-resistant to synthetic molecules (Bhowmik & Inderjit, 2003; Jabran, Mahajan, Sardana & Chauhan, 2015). There are currently 467 unique cases of herbicide-resistant weeds globally, with 249 species and among them, the most resistant of these are *Avena fatua*, *Amaranthus hybridus*, *Chenopodium album*, *Setaria viridis*, *Echinochloa crus-galli*, *Elusine indica*, *Kochia scoparia*, and *Conyza canadensis* (Heap, 2018). Glyphosate has become the world's most widely used herbicide (Duke & Powles, 2008), and new studies have been done on its effect on seed germination and radicle growth (Ismail, Chuah, Salmijah, Teng & Schumacher, 2002; Yanniccari, Istilart, Giménez & Castro, 2012; Gomes *et al*., 2017).

Mode of action of some allelochemicals is similar to synthetic herbicides. These features have allowed them to be considered for possible use in weed management as bioherbicides (Dorota *et al.,* 2013). Phenolic acids and volatile oils released from the leaves, bark, and roots of certain *Eucalyptus* spp. have harmful effects on other plant species (Florentine & Fox, 2003; Tang, Chen, Li & Huang, 2014; Puig, Reigosa, Valentão, Andrade & Pedrol, 2018). The phytotoxic effect of *E. globulus* essential oil against seed germination and seedling growth of some weeds has been demonstrated (Batish *et al.,* 2007; Rassaeifar *et al.,* 2013; Morsi & Abdelmigid, 2016). *E. globulus* ssp. (blue gum) is one of the most widely planted eucalypts in temperate parts of the world (Barbour, Otahal, Vaillancourt & Potts, 2008). Considering that allelochemicals of *E. globulus* could have potential use as bioherbicides, the main scope of this study was to characterize the chemical composition of eucalypt oil and to assess the phytotoxicity (on germination and radicle length) against one vegetable crop (*L. sativa*) and two herbicide-resistant weeds *(A. fatua* and *A. hybridus*) and compare it against herbicide Glyphosate, to provide insights into the suitability of this oil as a bioherbicide for weed management.

Materials and methods

Seeds of *L. sativa* Great Lakes variety, *A. fatua* and *A. hybridus* were used in this experiment. *E. globulus* essential oil obtained by steam distillation was provided by a German trading company (PRIMAVERA®).

Quantification and Identification of essential oil main components

Gas Chromatography with Flame Ionization Detector (GC-FID)

Quantitative GC analyses were carried out using a Hewlett-Packard 5890 Series II GC apparatus equipped with FID and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) (J & W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1} for integration purposes. Injector and detector temperatures were 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 L min^{-1} and 30 p.s.i. inlet pressure in split mode (1:30). The injection volume was 0.5 µL of diluted solution (0.01) of oil in n-hexane. The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. Analyses were carried out in triplicate.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The GC-MS analysis was carried out using a Varian 220-MS IT GC system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. fitted with the same column and temperature program as that for the GC experiments, with the following parameters: carrier gas = helium; flow rate = 1 mL min^{-1}; split mode (1:30); injected volume = 1 µL of diluted solution (0.01) of oil in n-hexane. Identification of the components was based on GC retention indices with reference to a homologous series of C8-C40 n-alkanes calculated using the Van den Dool and Kratz equation and by computer matching against the mass spectral library of the GC/MS data system (NIST 98 and WILEY) and co-injection with authentic standards as well as other published mass spectra. Area percentages were obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

Bioassay

The bioassay consisted of the treatments: distilled water (negative control); Roundup® (Glyphosate isopropylamine salt) as positive control (at 1, 10 and 20 µL mL^{-1} distilled water), essential oil (1, 10 and 20 µL mL^{-1}) and distilled water + Tween® 20 (polyoxyethylene [20] sorbitan monolaurate), at 5% (v/v). Treatments were distributed in a completely randomized design with 30 replicates each. The synthetic herbicide Roundup® is recommended to apply at 2 - 4 L ha^{-1} (10 - 20 µL mL^{-1}).

E. globulus essential oil effects were evaluated on germination and radicle length in the previously mentioned species. The experiment was repeated during two periods.

One seed of each species was placed onto non-toxic paper (Whatman No. 1 filter paper) about 4 cm from the top edge, leaving a 3 - 4 cm gap on the sides; each seed was then covered with a second sheet of moist paper. The sheet of paper was rolled and placed upright in a deep-bottomed test tube, and 5 mL of each treatment to be evaluated was added (Gold, 2009). The test tubes were sealed with a paraffin film and placed in a growth chamber set at 22 °C ± 1 °C, 50% ± 1% RH, for 16:8 h light/dark cycles. After 7 days, the number of germinated seeds and radicle lengths were measured by unrolling the paper carefully to avoid tearing it or damaging the radicles of the young seedlings. Mortality was calculated based on seeds not germinated. The percentage of Radicle Growth Inhibition (RGI) was calculated by the difference between growth of Treatments (T) and the negative Control (C) using the following equation:

$$RGI = (C - T)/C) \times 100$$

Statistical data analysis

All measured variables were tested for normality (Shapiro–Wilk W test) and homoscedasticity (Bartlett test). Kruskal–Wallis non-parametric analysis of variance was utilized when data violated the normality assumption and could not be corrected employing a transformation. One-way analysis of variance (ANOVA) and Tukey test ($p < 0.05$) were also performed to detect possible differences among the treatments. Linear regression analysis was used for quantifying the relationship between the essential oil concentration and seed germination and radicle length of the three species studied. The 50% Lethal Concentration (LC_{50}) (concentration causing 50% mortality compared to the control) was calculated for each treatment by probit analysis, based on mortality (seeds not germinated) obtained at each concentration of the samples (SAS, 2012).

Results and discussion

Essential oil main components

GC-FID and GC-MS analysis demonstrated that the oil is mainly a mixture of monoterpenes, sesquiterpenes and alcohols. Dominant among the constituents were the monoterpenes, constituting approximately 97.41% of the oil (Table I). Major monoterpenes included the oxygenated monoterpene 1,8-cineole (77.91%), p-cymene (10.19%) and the monoterpene hydrocarbon α-pinene (4.33%).

Previous studies have found that major constituents of *Eucalyptus* spp. essential oils are 1,8-cineole (49.07 to 83.59%) and α-pinene (1.27–26.35%) (Zhang, An, Wu, Liu & Stanton, 2012; Sebei, Sakouhi, Herchi, Khouja & Boukhchina, 2015; Morsi & Abdelmigid, 2016). 1,8-cineole is the most important compound of the genus *Eucalyptus* and is largely responsible for a variety of its pesticidal properties (Batish, Singh, Kohli & Kaur, 2008) and its antibacterial activity against Gram-positive and Gram- bacteria, related with the presence of this oxygenated compound (Ben *et al.,* 2011). Different composition may be due to genetic variability between species of *E. globulus*, environmental factors and extraction procedures.

Table I. Essential oil main components of *Eucalyptus globulus* and optical rotation.

Compound	RI[a]	LR[b]	%	Method of identification
α-thujene	924	923	0,15 ± 0,00	RI, MS
α-pinene	932	930	4,33 ± 0,09	RI, MS,CI
sabinene	969	970	0,52 ± 0,00	RI, MS
β-pinene	975	976	1,97 ± 0,03	RI, MS, CI
myrcene	988	989	0,62 ± 0,04	RI, MS
α-phellandrene	1002	1003	0,18 ± 0,00	RI, MS
p-cymene	1020	1021	10,19 ± 0,37	RI, MS, CI
1,8-cineole	1026	1025	77,91 ± 1,14	RI, MS, CI
Z-β-ocimene	1032	1034	0,72 ± 0,01	RI, MS
γ-terpinolene	1054	1055	0,17 ± 0,00	RI, MS
terpinolene	1086	1089	0,65 ± 0,00	RI, MS, CI
Total			97,41 ± 1,09	

[a]Retention indices calculated from retention times in relation to those of a series of n-alkanes on a 30m DB-5 capillary column. [b]Linear retention indices from the literature. RI = retention index, MS = mass spectrum, CI = co-injection with authentic standards.

Data analyzed by germination

ANOVA values indicated that differences between treatments and between species were significant ($p < 0.01$) and species × treatments ($p < 0.01$). There was not a significant difference for each species between the treatments comprising distilled water + Tween® and distilled water.

L. sativa exhibited the highest number of seeds germinated in all treatments. With regard to *A. fatua* and *A. hybridus*, Glyphosate treatment at 20 µL mL^{-1} completely inhibited germination (Table II).

Essential oil treatments showed the highest average of germination in *L. sativa*, which has a middle-sized seed compared with *A. fatua* and *A. hybridus*. Batish *et al.* (2008) concluded that eucalyptus oil exhibits species-specific toxicity and that the toxic effect was more noticeable in small-seeded crops such as *Amaranthus viridis* compared with large-seeded *Raphanus sativus*. Treatment with essential oil at concentrations of 10 and 20 µL mL^{-1} caused greatest inhibition ($p < 0.01$) of germination in *A. hybridus*, overcoming the inhibitory effect achieved in the species *L. sativa* and *A. fatua* (Table II). This indicates that *A. hybridus* is more sensitive to be inhibited in its germination by the essential oil in comparison with the other two species studied. This shows that *A. hybridus* is a species in which the phytotoxicity caused by the *E. globulus* essential oil occurs at low doses. Regarding the *E. globulus* essential oil LC_{50}, the lowest concentration was *A. hybridus* (< 1 µL mL^{-1}), followed by the dose for *A. fatua* (11.78 µL mL^{-1}) and *L. sativa* (> 20 µL mL^{-1}).

Inhibition of germination caused by *E. globulus* essential oils and Glyphosate treatments on three seeds were highest for *A. hybridus*, followed by *A. fatua* and then by *L. sativa*. However, the inhibition of germination caused by Glyphosate was higher than the effect of essential oil on all concentrations. Batish, Setia, Singh & Kohli (2004) found that *E. globulus* essential oils caused inhibition in the germination and radicle growth of *A. viridis, Cassia occidentalis, E. crus-galli, R. sativus, Triticum aestivum* and *Zea mays*. This result suggests that *E. globulus* essential oils could possess a greater capacity to severely affect the germination, although the different plants species involved prevent direct comparisons. This could be related with volatile monoterpenes, eucalyptol (1,8-cineole), and camphor, which can induce nuclear abnormalities and increasing vacuole numbers (Nishida, Tamotsu, Nagata, Saito & Sakai, 2005; Pawlowski, Kaltchuk-Santos, Zini, Caramao & Soares, 2012). Batish *et al.* (2007) speculated that eucalypt oil inhibits the mitotic activity of growing cells; however, the mechanism of the inhibitory effect remains unknown. On the other hand, α-pinene, the third major component of *E. globulus* essential oil acts under at least two mechanisms: uncoupling of oxidative phosphorylation, and inhibition of electron transfer (Nishida *et al.*, 2005) and allelochemicals might inhibit seed germination by suppressing the synthesis of gibberellins and indole acetic acid (Chu *et al.,* 2014).

Table II. Effect of *Eucalyptus globulus* essential oil and Glyphosate herbicide on average germination of *Avena fatua, Amaranthus hybridus* and *Lactuca sativa.*

Treatment	Species	Average germination
Distilled water	*L. sativa*	1.000 ± 0.00 a
	A. fatua	0.633 ± 0.08 efgh
	A. hybridus	0.867 ± 0.06 abcd
E. globulus		
1 µL mL^{-1}	*L. sativa*	0.933 ± 0.04 ab
	A. fatua	0.733 ± 0.08 cdefg
	A. hybridus	0.433 ± 0.09 i
10 µL mL^{-1}	*L. sativa*	0.800 ± 0.07 bcde
	A. fatua	0.533 ± 0.0 9 hi
	A. hybridus	0.233 ± 0.07 j
20 µL mL^{-1}	*L. sativa*	0.633 ± 0.08 efgh
	A. fatua	0.433 ± 0.09 i
	A. hybridus	0.200 ± 0.07 jk
Glyphosate		
1 µL mL^{-1}	*L. sativa*	0.600 ± 0.09 fghi
	A. fatua	0.567 ± 0.07 ghi
	A. hybridus	0.033 ± 0.03 kl
10 µL mL^{-1}	*L. sativa*	0.767 ± 0.07 bcdef
	A. fatua	0.200 ± 0.07 jk
	A. hybridus	0.000 ± 0.07 l
20 µL mL^{-1}	*L. sativa*	0.167 ± 0.06 jkl
	A. fatua	0.000 ± 0.07 l
	A. hybridus	0.000 ± 0.00 l
Distilled water +Tween® 20	*L. sativa*	1.000 ± 0.00 a
	A. fatua	0.700 ± 0.08 defch
	A. hybridus	0.900 ± 0.05 abc

The values refer to means ± S.E. For a given measurement, mean values with the same letter in the same column are not significantly different ($p < 0.05$ Tukey test).

A partial correlation analysis between seed germination and oil concentration (positive correlation) suggested that the phytotoxicity effects of the essential oil of *E. globulus* might cause inhibition of seed germination.

Data analyzed by radicle length

The three species demonstrated highest radicle growth ($p < 0.05$) in treatments with distilled water and distilled water + Tween®, probably showing that division and/or the cell growth level radicle were normal (Table III).

When comparing the effect of radicle growth among the three species evaluated, the species *A. fatua* exhibited highest

Table III. Effect of *Eucalyptus globulus* oil and Glyphosate herbicide on average radicle length and percentage of Radicle Growth Inhibition (RGI) of *Avena fatua, Amaranthus hybridus* and *Lactuca sativa.*

Treatment	Species	Average radicle length (mm)	RGI (%)
Distilled water			
	L. sativa	3.000 ± 0.14 d	0
	A. fatua	8.671 ± 0.30 b	0
	A. hybridus	2.436 ± 0.19 ef	0
E. globulus			
1 µL mL^{-1}	*L. sativa*	0.500 ± 0.08 g	85.3
	A. fatua	0.425 ± 0.04 h	95.1
	A. hybridus	0.177 ± 0.04 h	92.7
10 µL mL^{-1}	*L. sativa*	0.284 ± 0.02 h	90.5
	A. fatua	0.189 ± 0.03 h	97.8
	A. hybridus	0.157 ± 0.02 h	93.6
20 µL mL^{-1}	*L. sativa*	0.177 ± 0.02 h	94.1
	A. fatua	0.047 ± 0.02 h	99.5
	A. hybridus	0.233 ± 0.03 h	90.4
Glyphosate			
1 µL mL^{-1}	*L. sativa*	1.313 ± 0.12 f	56.2
	A. fatua	3.920 ± 0.65 c	54.8
	A. hybridus	0.200 ± 0.00 h	91.8
10 µL mL^{-1}	*L. sativa*	2.304 ± 0.27 f	23.2
	A. fatua	0.267 ± 0.12 h	96.9
	A. hybridus	0.000 ± 0.00 i	100.0
20 µL mL^{-1}	*L. sativa*	0.290 ± 0.06 h	90.3
	A. fatua	0.000 ± 0.00 i	100.0
	A. hybridus	0.000 ± 0.00 i	100.0
Distilled water + Tween®			
	L. sativa	3.101 ± 1.4 d	-
	A. fatua	9.861 ± 0.41 a	-
	A. hybridus	2.501 ± 0.18 e	-

The values refer to means ± S.E. For a given measurement, mean values with the same letter in the same column are not significantly different ($p < 0.05$ Tukey test).

growth ($p < 0.05$) in distilled water + Tween®, followed by the treatment with water ($p < 0.01$). *L. sativa* had highest radicle lenght at the 1 µL mL^{-1} concentration of the essential oil compared with the other two species. No difference was observed in radicle growth and RGI among two other species at 10 and 20 µL mL^{-1} concentrations of the essential oil. The RGI caused by the essential oil concentrations of 10 and 20 µL mL^{-1} was statistically equal ($p < 0.05$) to that achieved by the Glyphosate concentration at 10 µL mL^{-1} on *A. fatua* and 1 µL mL^{-1} on *A. hybridus* and 20 µL mL^{-1} on *L. sativa*. The phytotoxic effect of the essential oil is equal to that of Glyphosate, although at higher concentrations, shows that it has the potential to cause effects similar to those of the synthetic herbicide, but most likely without its adverse effects on the environment. *A. fatua* showed significantly higher radicle lenght ($p < 0.05$) at 1 µL mL^{-1} concentration of Glyphosate compared with other seeds treated with different concentrations of Glyphosate and essential oil. This suggests that *A. fatua* resists the toxic effect of Glyphosate at its lowest concentration, exhibiting some type of resistance. This species has been reported as a herbicide-resistant weed (Heap, 2018). The increase in the concentration of *E. globulus* essential oil did not cause a significant increase in RGI. However, Rassaeifar *et al.* (2013), in working with *E. globulus* essential oil on seedling establishment of *Amaranthus blitoides* and *Cynodon dactylon*, found that when the concentration of the essential oil increases (0.00005 to 0.005 µL mL^{-1}), radicle length decreases.

Data analyzed by species

Avena fatua

In *A. fatua* germination, there was no significant difference between the essential oil in increasing concentrations (10 µL and 20 µL mL^{-1}), and Glyphosate at its lowest concentration (1 µL mL^{-1}). This indicated that the essential oil showed a phytotoxic effect equal to that of the Glyphosate. However, an increase in the concentration of the essential oil does not decrease the germination in *A. fatua*. Rassaeifar *et al.* (2013) showed that the germination percentage and germination rate of *A. blitoides* and *C. dactylon* decreased by increasing the concentration of *E. globulus* essential oil. On the other hand, El-Rokiek & Eid (2009) demonstrated that the germination percentage of wild oat seeds was negatively affected by the fresh and dry leaf extract of *Eucalyptus citriodora* at different concentrations.

Regression analysis of average radicle length exhibited a positive linear correlation with increasing concentrations of *E. globulus* essential oil ($R^2 = 0.97$; $p < 0.05$) where the RGI was 95.1% at 1 µL mL^{-1} and increased to 99.5% at the highest concentration (20 µL mL^{-1}). Similar results were found by Vishwakarma & Mittal (2014), who demonstrated that root development was 24% in high concentrations and reached 84% in low concentrations. The essential oil of *E. globulus* at three concentrations and Glyphosate (10 mL^{-1}) showed an equal capacity to inhibit root growth in *A. fatua.* These results indicated that the essential oil did not show strong inhibition of germination; however, its main effect was on the inhibition of radicle growth. This allows to suppose that the use of *E. globulus* essential oil can delay the growth and development of this weed and potentially reduce the negative effects resulting from the use of Glyphosate. The use of *E. globulus* essential oils instead of synthetic herbicides could have a lower ecological impact, lower selection pressure, and reduce health and environmental problems.

Amaranthus hybridus

Regression analysis showed that germination of *A. hybridus* had a positive linear correlation with increasing concentrations of essential oil (R^2 = 0.83; $p < 0.05$). Likewise, previous investigation has shown that the essential oil of eucalyptus species increases the inhibition of germination by increasing the concentration of the essential oil (Rassaeifar *et al*., 2013; Vishwakarma & Mittal, 2014).

Treatments with essential oil demonstrated significantly greater inhibition ($p \leq 0.05$) on germination and radicle growth when compared with control (Table II and III). At the highest concentration of the essential oil (20 µL mL^{-1}), the inhibition on germination was statistically the same as that caused by Glyphosate at (1 and 10 µL mL^{-1}). Once again, we can suppose that the use of the essential oil of *E. globulus* instead of the synthetic herbicide Glyphosate could have similar phytotoxic effects but less negative consequences on the environment. Increasing concentrations of the essential oil inhibited germination in a different manner, but not radicle growth. Batish *et al.* (2007), studying the effect of the essential oil of *E. citriodora* on *Phalaris minor*, found that this caused a greater effect on seedling growth than on germination. Based on this, studying the effect of foliar application of essential oil of *E. globulus* on herbicide resistant species is a topic that could be analyzed in future research. In the present study, the magnitude of the phytotoxic effect was observed both in radicle growth and germination. The essential oil at its three concentrations possesses the same radicle growth inhibitory effect as Glyphosate at its lowest concentration (RGI, 90.4 and 91.8, respectively).

Lactuca sativa

In the species *L. sativa*, treatments showed significantly higher germination ($p \leq 0.01$) in distilled water and distilled water + Tween® and essential oil at its lowest concentration (1 µL mL^{-1}) compared with the other treatments.

Glyphosate demonstrated highest inhibition of germination at 20 µL mL^{-1}. Treatments with essential oil at the three concentrations (1, 10, and 20 µL mL^{-1}) exhibited inhibition on germination that was statistically equal to that of Glyphosate at 10 µL mL^{-1}; the essential oil at such concentrations is as effective in inhibiting germination as Glyphosate (Table II). Yamagushi, Gusman & Vestena (2011) and de Souza & Cardoso (2013) found that aqueous extracts of *E. globulus* at increasing concentrations (100,000–1,000,000 µL mL^{-1}) occasioned the increase of inhibition of germination and of RGI of *L. sativa*. However, in the present study the concentrations were lower, and RGI was statistically equal at increasing concentrations.

Treatments with the essential oil (10 and 20 µL mL^{-1}) showed RGI that was statistically equal to that of Glyphosate at 20 µL mL^{-1} and significantly higher than the controls (distilled water and distilled water + Tween®). On increasing the concentration of essential oil, inhibition of germination is increased. However, the RGI is statistically equal and is not affected by the increase in oil concentration from 10 to 20 µL mL^{-1}. Apparently, the emerged radicle of *L. sativa* exhibited Glyphosate resistance at its concentrations 1 and 10 µL mL^{-1}.

Conclusions

The major compounds of the *E. globulus* essential oil were monoterpenes 1,8-cineole (77.91%) and p-cymene (10,19%). Correlation analysis suggested that inhibition of seed germination and radicle growth might be caused by a phytotoxic potential of *E. globulus* essential oil. The latter showed differential bioactivity between the different plant species; being LC_{50} lowest for *A. hybridus*, followed by *A. fatua* and *L. sativa*.

The higher inhibition of root growth caused by essential oil compared to Glyphosate (at 1 µL mL^{-1}) can be used as a strategy to gradually affect the development of *A. fatua*, with a lower ecological cost. The higher the concentration of essential oil of *E. globulus*, the greater the inhibition of germination and the percentage of inhibition of radicle growth. At some of the evaluated concentrations, both the Glyphosate herbicide and the *E. globulus* essential oil demonstrated the same phytotoxicity against the resistant weeds *A. fatua* and *A. hybridus.*

E. globulus essential oil may be considered a potential source of new molecules with herbicidal action for the chemical industry and could be incorporated as a bioherbicide into agriculture. However, there is a need for the study of long-term crop–weed phytotoxic interactions under field conditions.

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