UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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Dept. of Agroforest Ecosystems

Herbicidal activity of Eucalyptus camaldulensis essential oil against problematic summer weeds in Mediterranean crops.

Master's Thesis

European Master Degree in Plant Health in Sustainable Cropping Systems

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Herbicidal activity of *Eucalyptus camaldulensis* essential oil against problematic summer weeds in Mediterranean crops

Master Thesis

M.Sc. Plant Health

Faculty of Agricultural Sciences

Georg-August-University Göttingen, Germany

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Abstract

Weeds and herbicides are important stress factors for crops. Weeds are responsible for great losses in crop yields, more than 50% in some crops if left uncontrolled. Herbicides have been used as the main method for weed control since their development. However, their widespread use has resulted in various toxicological effects on the environment and human health, besides resulting in the emergence of herbicide-resistant weed biotypes. To overcome these problems, there is an urgent need to search for novel compounds, particularly natural plant products, with potential herbicidal activity. The essential oil composition (EO) of was analyzed by GC. The main components were Spathulenol (31.29%), ρ-Cymene (20.38%) and Cryptone (17.00%). The herbicidal potential of the essential oil of *Eucalyptus camaldulensis* e.g., efficacy and damage level in the greenhouse against two weeds: *Echinochloa crus-galli* and *Amaranthus retroflexus* in pre- and post-emergence trials. For this purpose, the EO with spathulenol as the main component was applied in different concentrations by spraying and irrigation with pelargonic acid and glyphosate as reference. The results showed that the essential oil was effective in every dose for inhibition of germination in pre- emergence assays. In post emergence assays the dose 32 was effective in killing the Amaranthus plants with 60% efficacy by spraying. We conclude that the oil was more effective when applied by spraying and that Amaranthus species was more sensitive than *Echinochloa*. The results suggest the possible use of the oil as natural herbicide. Further research is needed to determine the effective doses for different species.

Key words: Natural Herbicides, Weeds, Herbicidal Action, *Eucalyptus camaldulensis*, Essential Oil, *Amaranthus retroflexus* L., *Echinocloa crus-galli*

1. Introduction

1.1 Weed resistance and necessity of natural herbicides

The rapid growth in world population is a serious problem, which needs to be addressed thoroughly. Such increase requires a similar increment in agricultural and food production in addition to resources such as water, energy, labor, etc. (Verdeguer *et al*., 2020a). To achieve such a goal, it seems a bit hard to increase arable land without affecting the natural habitat (Verdeguer *et al*., 2020a), so an alternative solution is to increase plant protection (Rassaeifar *et al*., 2013; Verdeguer *et al*., 2020a).

Weeds are a major problem affecting plant crop production with direct crop productivity losses up to 50% (Muñoz *et al.*, 2020; Verdeguer *et al*., 2020a). Synthetic chemical herbicides were used earlier for weed management (Muñoz *et al.*, 2020). However, the over- and misuse of such herbicides, despite increasing productivity, had negative impact in increasing weed resistance to herbicides (Verdeguer *et al*., 2009; Ataollahi *et al*., 2014; Grichi *et al*., 2016; Andert and Gerowitt, 2020), environmental contamination (Ataollahi *et al*., 2014; Andert and Gerowitt, 2020), in addition to increasing risk of serious human diseases (Ataollahi *et al*., 2014; Ibáñez Jaime *et al.*, 2018; Verdeguer *et al*., 2020a). To overcome these problems, efforts are being made to reduce the reliance on chemical herbicides and to produce biological herbicides with environmental coexistence and desirable herbicidal action (Rassaeifar *et al*., 2013, Singh *et al*., 2003). Research on natural products as bioherbicides has greatly increased over the past few years, due to the shift in agricultural techniques to control weeds towards more sustainable ones, promoting integrated weed management (IWM) (Cordeau *et al.*, 2016; Andert and Gerowitt, 2020).

IWM is a combination of different weed control methods (cultural, physical, mechanical, biological, biotechnological and chemical) (Rassaeifar *et al*., 2013; Muñoz *et al.*, 2020; Verdeguer *et al*., 2020a). A major ecofriendly, cost-effective step to achieve weed management in a sustainable way would be the introduction of bioherbicides (Grichi *et al*., 2016; Verdeguer *et al*., 2020a).

1.2 European Union legislations on plant protection products

By adopting two main regulations, the European Parliament and Council supervise the use and marketing of pesticides in order to limit their risks. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of October 21, 2009, relating to the placement of plant protection products on the market. Simultaneously, because of being involved in and impacted by pesticide usage in general, including herbicides, society is becoming aware that new approaches are required to comply with the new regulations.

Therefore, attempts are made to develop compounds that degrade fast in the environment and have unique target sites. These two characteristics are present in natural plant products that perform a range of physiological functions, and so can be used to develop new herbicides (Jouini, 2020).

1.3 Allelopathy

Allelopathy was defined by the International Allelopathy Society as: “The science that studies any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence growth and development of agricultural and biological systems (excluding animals)” (Martínez, 2017; Jouini, 2020). Such secondary metabolites are known as “allelochemicals" and are of growing importance in sustainable weed management introducing “natural herbicides" (Verdeguer *et al*., 2009; Ataollahi *et al*., 2014; Flores-Macías *et al*., 2021). Essential oils (EO) of many aromatic plants are considered allelochemicals and interfere with the growth of other species (Batish *et al*., 2006 & 2007).

Allelochemicals exhibit their phytotoxicity from their ability to interfere with normal metabolic processes of the plant cell such as cell division, membrane permeability, photosynthesis, respiration, protein synthesis in addition to triggering reactive oxygen species (ROS) (Grichi *et al*., 2016).

1.4 Herbicidal Action *of Eucalyptus camaldulensis*

Eucalyptus camaldulensis Dehnh. (Family *Myrtaceae*) is a perennial tree native to Australia and one of the most widely spread tree species (Ataollahi *et al*., 2014; Martínez, 2017). Eucalyptus became cultivated in dry, temperate, and even tropical nations due to its morphological and genotypic variability, adapting to a wide variety of conditions (Martínez,

2017). It is one of the potential allelopathic plants having a variety of allelochemicals (El-Darier, 2002) and its essential oils possess pesticidal activity (Batish *et al*., 2006). Antibacterial, antifungal, and antiseptic, anticatarrhal, and anti-pneumonic properties of *E. camaldulensis* oil have been recognized for decades. (Batish *et al*., 2007; Verdeguer, 2011; Zhang *et al.*, 2012; Ibáñez Jaime *et al.*, 2018)

The allelopathic property of essential oil from Eucalyptus leaves increased its potential to be used as a natural herbicide being phytotoxic to many invasive weed species such as *Amaranthus viridis*, *Amaranthus retroflexus*, *Portulaca oleracea* and *Echinochloa crus-galli* (Zhang *et al.*, 2012; Verdeguer, 2020a). The herbicidal activity of *Eucalyptus globulus* essential oil against *Amaranthus blitoides*, *Cynodon doctylon* (Rassaeifar *et al*., 2013), and *Parthenium hysterophorus* (Kohli *et al*., 1998), of *E. citriodora* against *Cassia occidentalis*, *Echinochloa crus-galli*, *Phalaris minor, Parthenium hysterophorus* and *Amaranthus viridis* (Batish *et al*., 2006, 2007). Volatile terpenes, precisely, two highly toxic terpenes (cineol and alpha-pinene) were discovered in the volatile oil of *E. camaldulensis*, having allelopathic effect on the seeds and seedlings of other species (Ataollahi *et al*., 2014, Ibáñez Jaime 2018; Martínez, 2017; Flores-Macías *et al*., 2021). In laboratory, greenhouse, and field tests, these toxins were shown to inhibit the growth of many invasive weed species such as *Phalaris minor*, *Lolium rigidum*, *Hordeum glaucum*, *Echinochloa crus-galli*, *Sinapis arvensis*, *Erica vesicaria*, *Scorpiurus muricatus* and *Phaseolus vulgaris* (Batish *et al*., 2007; Wu *et al*., 2011; Vishwakarma and Mittal, 2014; Grichi *et al*., 2016).

1.5 Weeds

In this work, the two selected weeds for the study of the herbicide action of essential oil of *E. camaldulensis* are: *Amaranthus retroflexus* and *Echinochloa crus-galli*. Those were selected due to their impact on Mediterranean crops and herbicide resistance. (Heap, 2017).

1.5.1 *Amaranthus retroflexus* L.

Is an annual herbaceous plant belonging to the family *Amaranthaceae*, which can grow to a height of 100 cm (Martínez, 2017, Pagán, 2019). The plant has shallow roots, erect stems which can be simple or branched, alternate hairy dull green leaves, with rhombus-ovate blades, flowers are unisexual, numerous, small, green, clustered in dense spikes forming a terminal panicle, seeds are glossy dark brown and flattened (Pagán, 2019). It is considered a

serious invasive weed in summer crops, with cosmopolite distribution in the temperate and warm regions of the world including Spain (Martínez, 2017; Muñoz *et al.*, 2020).

Due to the ability of crossing between different species of *Amaranthus*, they have a wide genetic diversity, which increases their herbicidal resistance (Martínez, 2017). This plant is resistant to ALS (acetate-lactate synthase) inhibitors (Martínez, 2017) and atrazine (photosystem II inhibitor) (Pagán, 2019), ranking the 8th position worldwide with resistance to 5 modes of action of herbicides (Muñoz *et al.*, 2020).

1.5.2 *Echinocloa crus-galli* (L.) Beauv.

Echinocloa crus-galli (L.) Beauv**.** is an annual, herbaceous plant from the family *Poaceae*, which can grow to a height of 150 cm (Martínez, 2017). Holm *et al*. (1977) has reported *Echinochloa crus - galli* L. Cockspur or Cockspur Grass or Barnyard Grass as the worst weed of rice.

The plant has a fibrous root system, elongated, flat, hairless, rough leaves, flowers arranged in spikelets, pale green to dull purple color, seeds are very rounded with hairy lemmas (Cao *et al.*, 2021). *E. crus-galli* is native to *Eurasia* (Cao *et al*., 2021) with wide distribution around the world where it is considered a foreigner invasive weed species in Spain (Martínez, 2017).

E. crus-galli is well adapted to wet and submerged soils, found in moist areas like cultivated areas, swamps, marshes, shorelines, and ditches (Martínez, 2017, Cao *et al*., 2021). It is considered a summer weed with its seeds persistent in soil for 3 to 13 years (Cao et *al*., 2021). It is one of the world's worst weeds, causing forage crops to fail by absorbing up to 80% of usable nitrogen from the soil (Martínez, 2017). The plant is invasive to many crops such as wheat, corn, root crops, forage crops and pastures with rice on the top of the list (Vishwakarma and Mittal, 2014; Martínez, 2017).

Worldwide the plant has been reported to resist: ACCase inhibitors, synthetic auxins, microtubule inhibitors, long chain fatty acid inhibitors, cellulose inhibitors, PSII inhibitor (Ureas and amides) and DOXP (Deoxy xylulose- 5 Phosphate) inhibitors, while in Spain resistance to photosystem II and ALS inhibitors was reported (Martínez, 2017).

Reviewing the current literature nothing was reported concerning the phytotoxic effect of *E. camuldulensis* oil on germination of seeds / or the growth of seedling of *E.crus-galli* and *Amaranthus retroflexus* L. This study was designed to evaluate the effect of the essential oil on germination / seedling height, as well as plant height and damage level. For this purpose, the weeds were sprayed or irrigated with different doses of the oil emulsion in comparison with two reference standards. Additionally, less volume of water was applied per plant.

1.6 Aims and Objective

The aim of this work is to determine the phytotoxic activity of the essential oil of *E. camaldulensis* leaves. In order to achieve such aim, those objectives were set up:

1. Collection and extraction of the essential oil.
2. Determination of composition of the essential oil using gas chromatography and gas chromatography coupled to mass to determine which components have the herbicidal effect.
3. Evaluation of the inhibitory capability of the oil as applied in five different concentrations to two species of problematic weeds in comparison with two reference standards. This evaluation was made *in vitro and in vivo.*

2. Materials and Methods

2.1 Weed Seeds

Seeds of *A. retroflexus* were purchased from Herbiseed Company (Berkshire, United Kingdom) while seed of *E. crus-galli* were collected by the research group from cultivated fields of Valencia or purchased from Herbiseed Company (Figure 1).

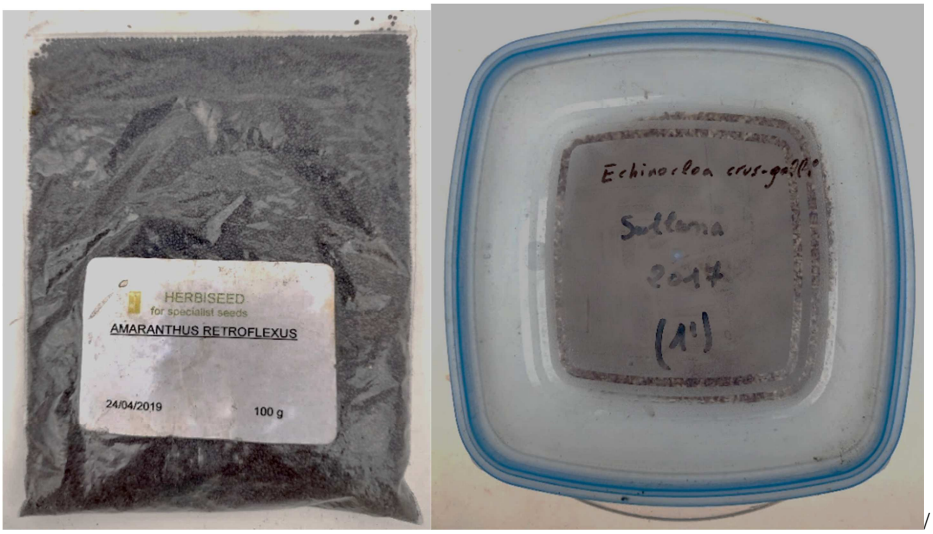


Figure 1: Seeds of *A. retroflexus* and *E. crus-galli*

2.2 Vegetative Material for essential oil:

Leaves of *E. camaldulensis* were collected from trees growing in the fields of Alcudia, Valencia province (Spain) on different dates starting from May till September 2021 (Figure 2, 3). It was observed that pest infestation and rainfall interfered with the quality and quantity of the final oil yield. The essential oil was stored at 4 C until analyzed (diluted at 1% v/v in diethyl ether) and the allelopathic potential was tested.

Figure (2) location of collection of Eucalyptus leaves

Figure (3) Photograph of *Eucalyptus camaldulensis* tree

2.3 Essential Oil Extraction

Two Clevenger apparatus were used together with round-bottom flasks of 2 and 4 liter volume to extract the oil (Figure 4). After the weighing of Eucalyptus fresh leaves, distilled water was added in a scale of 1000 ml for the 2 L flask and 2000 ml for the 4L flask. The essential oil was stored in the fridge at 4ºC.

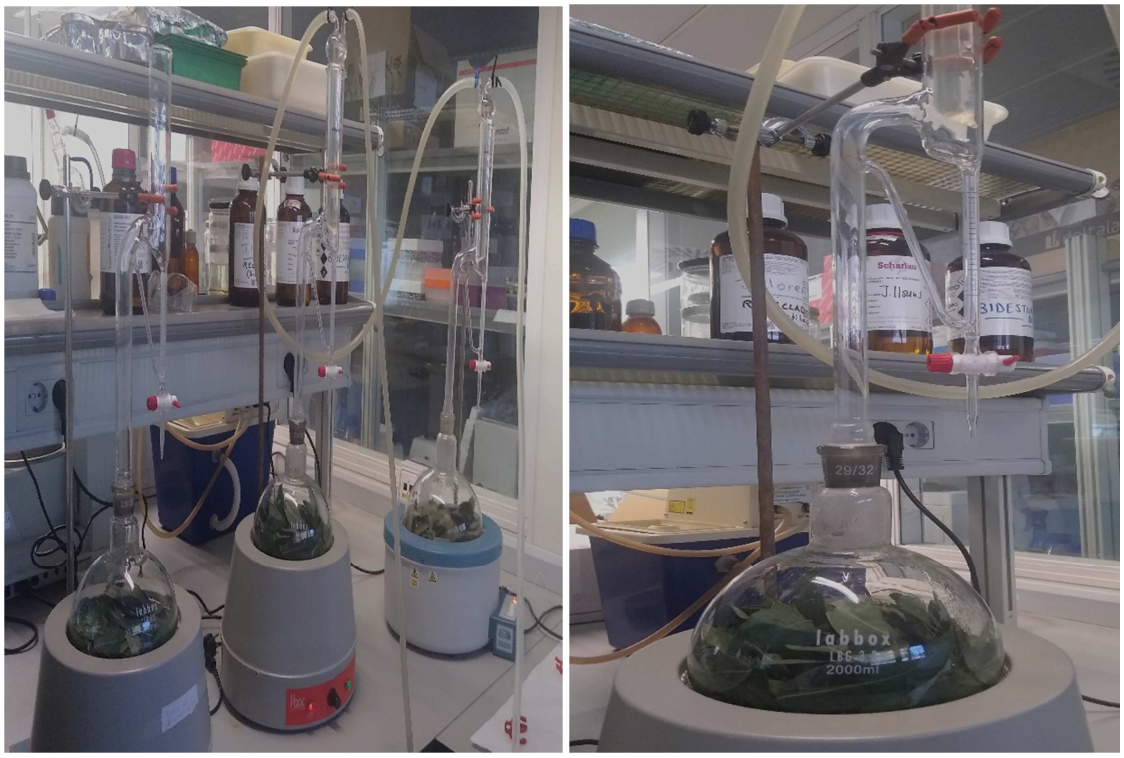


Figure (4) Extraction of Eucalyptus essential oil using Clevenger apparatus

2.4 Determination of composition of Essential Oil of *E. camaldulensis*

For chemical analysis, a sample of the essential oil (EO) was mixed with hexane at 10%. The quantitative composition was measured by GC and individual peaks identified by Kovats retention indices.

The analysis was performed using a chromatograph model Claurus 500GC Perkin-Elmer, equipped with a flame ionization detector (FID), one capillary column Hewlett-Packard HP-1 (methyl silicone) with 30 m long, 0.2 mm internal diameter and 0.33 μm film thickness. The GC temperature was set to 60ºC for 5 minutes, then was raised 3ºC/min till 180ºC, then 20ºC/min till 280ºC at which it was maintained for 10 min. The carrier gas used was helium with flux 1ml/min. The FID was kept at 250ºC and the injector at 220ºC (Verdeguer, 2020b).

For compound identification, Kovats retention indices were calculated using the hydrocarbon C_8-C_{32} which was chromatographed when the samples were analyzed. Once the retention times (minutes) was recorded for every compound of the essential oil, the Kovats was calculated using the following formula:

$K = 100 * (n^o\ C\ HC_{n-1} + [(\log TR\ X - \log TR\ HC_{n-1}) / (\log TR\ HC_{n+1} - \log TR_{\ HCn-1})])$

Given that:

$n^o\ C\ HC_{n-1}$: number of carbons of the hydrocarbon before the compound

$TR_{\ X}$: retention time of the compound

$TR\ HC_{n-1}$: retention time of the hydrocarbon before the compound

$TR\ HC_{n+1}$: retention time of the hydrocarbon before the compound

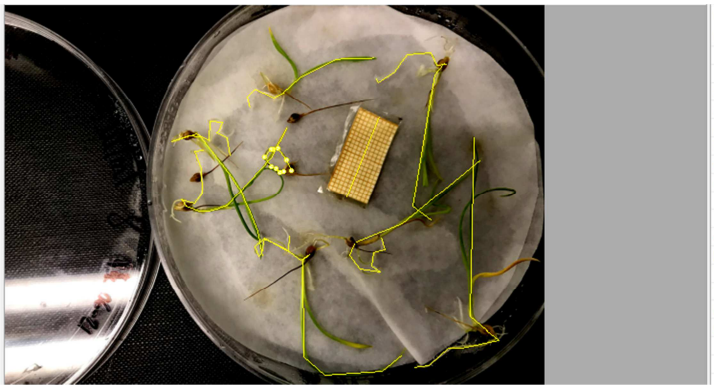
2.5 Phytotoxic activity assays

2.5.1 Pre-emergence assays

For the *in vitro* phytotoxicity tests, 20 seeds of *A. retroflexus*, and 10 of *E. crus-galli* were sown in two layers of filter paper (73 g/m^2) in Petri dishes (9 cm diameter) previously wetted with 5ml of distilled water, in case of *A. retroflexus* and 6 ml for *E. crus-galli*.

Five different concentrations of *E. camaldulensis* EO (0.125, 0.25, 0.5, 1, and 2 µl/ml) were prepared and loaded on the inner side of other two layers of filter paper placed above the seeds. The controls were prepared with the same quantities of distilled water. For each concentration, five replicates were conducted for the dicotyledon (*A. retroflexus)* and ten in the case of the monocotyledon (*E. crus-galli)* with a total of 100 seeds for each treatment.

All the Petri dishes were sealed with parafilm to reduce loss of moisture and release of EO, then incubated in a germination-growth chamber with the conditions 30°C 16h day and 20°C 8h night for 15 days. To evaluate the phytotoxic activity of the EO on germination and seedling length, data were recorded after 3, 5, 7, 10 and 14 days, by taking digital images of the Petri dishes, that were later processed with the software Digimizer v.4.6.1 to determine the seed germination percentage and the seedling length (Figure 5).



Measurement	Area	Perime...	Length	Angle	Radius	Unit
Unit			649.626			pix...
1 sh			2.341			cm
1 rt			1.231			cm
2 sh			3.964			cm
2 rt			2.130			cm
3 sh			3.474			cm
3 rt			2.260			cm
4 sh			4.654			cm
4 rt			2.501			cm
5 sh			4.992			cm
5 rt			3.184			cm
6 sh			3.429			cm
6 rt			3.008			cm
7 sh			3.298			cm
7 rt			2.099			cm
8 sh			0.021			cm
8 rt			1.585			cm

Figure (5) Phytotoxic activity assay *in vitro*

2.5.2 Post emergence assay in greenhouse

Nine treatments were applied on plants of the species *A. retroflexus* and. *E. crus-galli*. The substrate was a mix of ¾ peat and ¼ perlite. After germination (about 1 week), emerging seedlings were selected for uniformity in growth and were individually transplanted in square polypropylene pots (8×8×7 cm) previously filled with the same substrate.Ten repetitions of each treatment were prepared. The pots of the same treatment were placed in the same tray to avoid possible mixing of the treatments. The EO emulsions were prepared using 0.5 mL L^{-1} of the emulsifier Fitoil (Xeda, Italy). Two controls for each mode of application (irrigation and spraying) were established: one control irrigated only with water (CWI) and other irrigated with water plus Fitoil (CFI), then one control sprayed with water (CWS) and other sprayed with water plus Fitoil (CFS). Based on the results of previous studies (Verdeguer, 2011, Verdeguer, 2020a), five different concentrations were applied by irrigation and spraying: 2, 4, 8, 12 and 16 µL mL^{-1}. Also, two commercial references: biological reference pelargonic acid, Beloukha (Belchim, Belgium) and chemical reference glyphosate (Roundup) were used.

EO treatments were applied once when plants had reached the phenological stage of two to three true leaves for *E. crus-galli* and three to four true leaves for *A. retroflexus*. (Figure 6)

To evaluate any phytotoxic effect, images of the plants were registered 1, 3, 7, 15 and 30 days after treatment (DAA). At the end of the experiment (30 DAA), the entire plant from

each pot was reclaimed by dipping the root apparatus in water to remove any soil residues. The software Digimizer v.4.6.1 (MedCalc Software, Ostend, Belgium, 2005–2016) was used to process and analyze the images to determine the efficacy of the treatment, total (TL), root (RL) and aerial part (APL) length of the plants and also the damage level (DL).

The efficacy of a given EO was considered as its capacity to kill the plants and was assessed for each plant by attributing the value 0 if the plant was alive and 100 if the plant was dead. The damage level was assessed, developing a damage scale for each species. The scale range was from 0 (no damage) to 3 (death of the plant). Fresh (FW) and dry weights (at 60°C for 48 h; DW) were also determined.

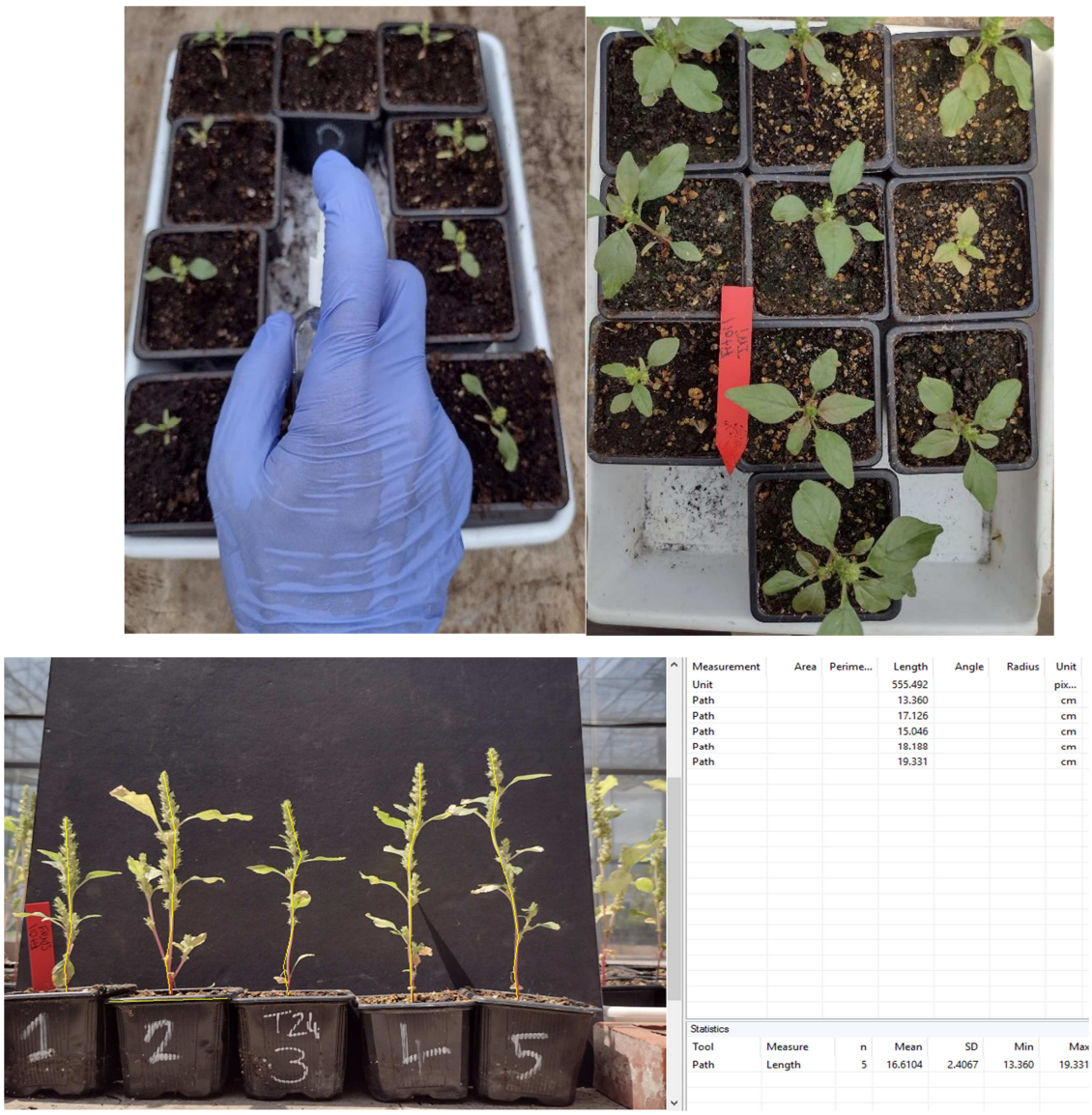


Figure (6) Phytotoxic activity assay in greenhouse A

2.6 Statistical Analysis of Data

The data were processed using the statistical package Statgraphics®Centurion XVI. A multifactorial analysis of variance (ANOVA) was applied. The ANOVA was performed using Fisher's multiple comparison test (LSD intervals, Least Significant Difference) for the separation of means, with a confidence level of 95% ($p \leq 0.05$). The significant differences between the different treatments have been indicated with different letters in the same column, in all the tables of results. In the presentation of the results, the means are accompanied, both in Tables and Figures, by the corresponding value of the standard error.

3. Results and Discussion

3.1 Composition of essential oil of *Eucalyptus camaldulensis* from Alcudia (Spain)

Hydrodistillation of the leaves yielded on average about: 1.1ml per 100 g of leaves (1.1%). It was noticed that the yield decreased to 0.5ml per 100 gr of leaves after periods of rainfall. GC analysis of *E.camaldulensis* essential oil led to the identification of 28 compounds, representing 90.66 % of total oil composition (Table 1). The oil was characterized by the predominance of oxygenated compounds as oxygenated sesquiterpenes and monoterpenes. The major component was Spathulenol (31,29%) a sesquiterpene alcohol , the second one was *ρ*- Cymene (20,36%) a monoterpene hydrocarbons and the third was cryptone (17,0%) from the group of oxygenated monoterpenes. These components were also the majority according to Verdeguer et *al*. (2009), Verdeguer et *al*. (2011) and Jouini (2020a). In this concern, phytotoxicity of *E. camaldulensis* was attributed mainly to the presence of monoterpenes as major constituents (Batish et *al*., 2006; Muñoz et *al*., 2020). The EO from *E. cladocalyx* cultivated in Morocco, which main compounds were spathulenol (21.6%) and 1,8-cineole (20.5%), followed by p-cymene (15.1%), showed herbicidal activity against *S. arvensis* (Fouad *et al*., 2015).

Table 1 : Results of GC analysis of *Eucalyptus camaldulensis* essential oil

Compound	R_t Sample a	KI b	% c
Monoterpene Hydrocarbons			**22.27**
α-Thujene	**5.65**	**931**	**0.41**
Thuja-2,4 (10) –diene	**6,13**	**947**	**0.11**
Sabinene	**7,42**	**979**	**0.08**
Myrcene	**7,86**	**991**	**0.09**
α-Phellandrene	**8,579**	**1006**	**0.13**
***ρ*-Cymene**	**9,50**	**1030**	**20.38**
Limonene	**9,68**	**1034**	**0.86**
γ-Terpinene	**10,93**	**1061**	**0.11**
ρ-Cymene	**12,24**	**1090**	**0.10**
Oxygenated monoterpenes			**33.76**
1,8-Cineole	**9.72**	**1035**	**2.31**
trans-Thujone	**13,55**	**1117**	**0.18**
Terpinen -4-ol	**16,40**	**1181**	**2.89**
Cryptone	**17,02**	**1191**	**17.00**
α-Terpineol	**17,30**	**1196**	**0.94**

m-Cumenol	**18.81**	**1231**	**0.45**
Cumin aldehyde	**19,423**	**1245**	**4.16**
Carvotanacetone	**19,69**	**1250**	**0.33**
p-Menth-1-in-7-al	**20,93**	**1279**	**2.86**
Thymol	**21,63**	**1292**	**1.14**
Carvacrol	**22,03**	**1300**	**1.50**
Sesquiterpene hydrocarbons	**0.16**		
allo-Aromadendrene	**28,48**	**1453**	**0.16**
Oxygenated sesquiterpenes	**34.47**		
Spathulenol	**33,22**	**1578**	**31.29**
Viridiflorol	**34,21**	**1606**	**0.54**
Spathulenol isomer	**34,43**	**1612**	**1.26**
Spathulenol isomer	**34,56**	**1616**	**0.03**
Isospathulenol	**35,34**	**1640**	**1.35**
Total Identified			**90.66**

3.2 Phytotoxic activity assays

3.2.1 Pre-emergence assays

In this point, the effect of *E. camaldulensis* oil (spraying) on seed germination of *E.crus-galli* and *A.retroflexus* was investigated. Results are displayed in Table 2.

Table 2: Effect of essential oil of Eucalyptus *camadulensis* on seed germination of E. *crus-galli* and *A. retroflexus* at the end of the assay (14 days after treatment).

Concentration (µl / ml)	E. *crus-galli* Germination (%) ± SE	A.*retroflexus* Germination (%) ± SE
0 (CTRL)	93.00 ± 4.40 [a]	77.00 ± 5.10 a
0.125	92.00 ± 4.60 [a]	4.00 ±1.90 b
0.25	83.00 ± 4.20 [ab]	0.00 ± 0.0 [c]
0.5	74.00 ± 7.90 [b]	0.00 ± 0.0 [c]
1	42.00 ± 14.00 [c]	0.00 ± 0.0 [c]
2	41.00 ± 13.20 [c]	0.00 ± 0.0 [c]

Data are means standard errors. Different letters in the same column indicate significant differences.

In the case of E. *crus-galli*, no significant differences were observed in the germination of the control seeds and those treated with lowest concentration of the oil (0.125 µl / ml). The four highest concentrations of the oil applied significantly inhibited germination with significant differences between them .The two highest concentrations 1 and 2 µl / ml reduced germination by 58 % and 59%, respectively.

In the case A.*retroflexus* the lowest concentration showed 96% inhibition and the four highest concentrations of the oil showed maximum inhibition 100%. From the above we conclude that *A.retroflexus* was the more sensitive species to the oil treatment.

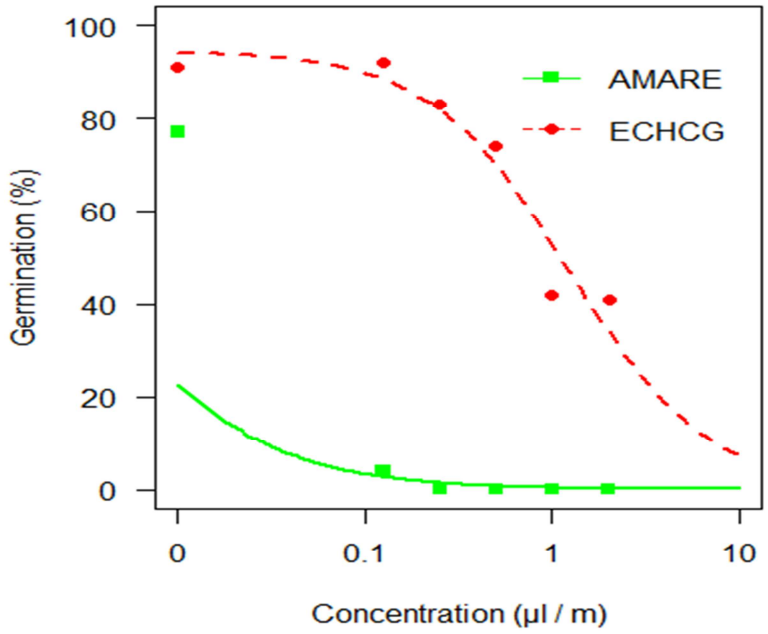


Figure 7: The effect of different concentration of *E. camaldulensis* oil on the germination rate of *A.retroflexus* and *E. crus galli*

All concentrations were effective in inhibiting germination of both weeds, showing significant differences between the control and the treated seeds with the different concentrations of the oil (Figure 7). Decrements in seed germination % with increasing doses of EO of *E. camaldulensis* were also reported by Verdeguer et *al*. (2009) and (2020) on the control of *Amaranthus hybridus*, *Portulaca oleracea* and *Erigeron bonariensis*, respectively. Similarly, Jouini (2020a) reported an inhibition in seed germination of *A.retroflexus* and *E. crus galli* in response to application of *E. camaldulensis* EO with *E. crus galli* being more resistant.

Table 3: Effect of essential oil of Eucalyptus *camadulensis* on seedling length of *E. crus-galli* and Amaranthus *retroflexus* seedlings at the end of the assay (14 days after treatment)

Concentration (µl / ml)	E. *crus-galli* Length (mm) ± SE	A.*retroflexus* Length (mm) ± SE
0 (CTRL)	8.05 ± 0.18 [a]	3.63±0.13 [a]
0.125	6.70 ± 0.19 [b]	1.21±0.00 [b]
0.25	6.24 ± 0.19 [c]	0.00±0.00 [b]
0.5	4.70 ± 0.16 [d]	0.00±0.00 [b]
1	3.57 ± 0.14 [e]	0.00±0.00 b
2	2.45 ± 0.10 [e]	0.00±0.00 b

Data are mean ± standard errors. Different letters in the same column indicate significant differences.

Regarding the growth of *E. crus galli*, treatment with the oil induced a reduction in the length of the seedling varying from 16.8 to 70% (Table 3). There were significant differences between all concentration and control. The lowest concentration (0.125 µl/ml**)** exhibited a 66.7% reduction while the four highest concentrations of the oil showed a maximum 100% reduction. From the above we conclude that *A.retroflexus* was more sensitive to the oil

treatment

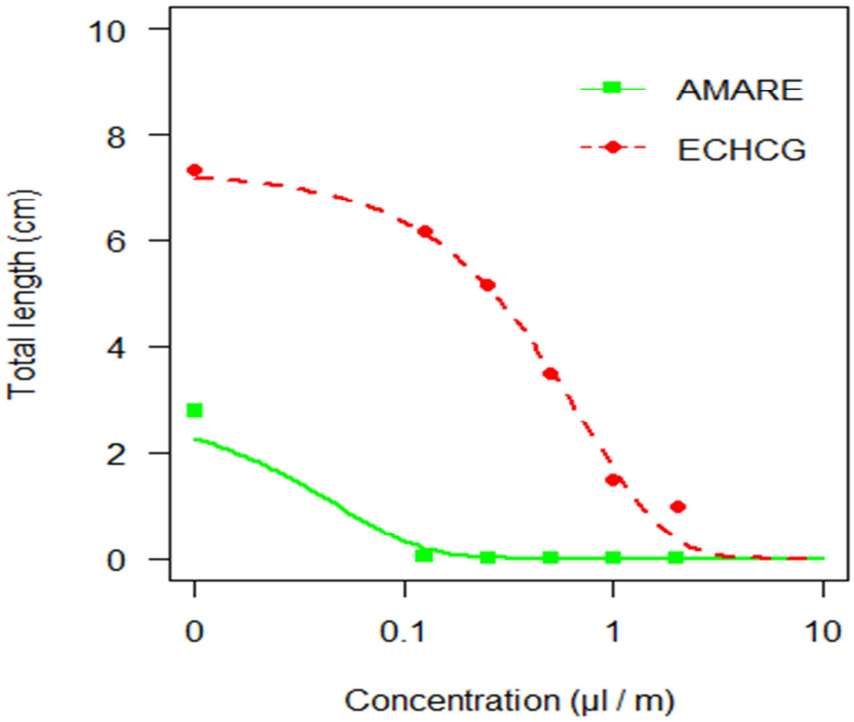


Figure 8: The effect of different concentrations of E. camaldulensis oil on the seedling length of *A.retroflexus* and *E. crus galli*

About the growth of A.*retroflexus*, all the concentrations showed an effect with respect to the control, with significant differences in the length of the seedlings treated with the two lowest concentrations and the two highest concentrations of the oil. The maximum growth reduction was 100% (Figure 8). This essential oil showed highest inhibition against the growth of seedlings of A.*retroflexus.*The oil significantly inhibited the growth of seedlings in case *E. crus galli* at all concentrations tested, from 16.8 to 70%, showing differences between the control and the highest concentrations (Figure 8). Inhibition in seedling length of *Amaranthus viridis and Echinochloa crus-galli* was also reported by Batish et *al*. (2006) while decrements in seedling length of A.*retroflexus* and *Echinochloa crus-galli* with increasing doses of EO of *E. camaldulensis* were found by Jouini (2020a).

3.2.2 Post- emergence Assays

In the following part of the experiment, the herbicidal activity of *E. camaldulensis* in post emergence assays was evaluated, by spraying different concentrations of the essential oil when the plant was already germinated and in the optimum phenological state for herbicide application. The efficacy and damage level were recorded at the beginning (Day 1) and at end of the assay (Day 30).

Table 6: Overall efficacy of E. *camadulensis* on *A.retroflexus* per treatment: Analysis of Variance for Efficacy- Sum of Squares type III

Source	Sum of Squares	DF	Mean Squares	F-value	*p*-value
Main Effects					
A: Treatment	**271031**	**9**	**30114.5**	**131.18**	**0.0000**
B: DAY	**557656**	**5**	**11151.2**	**48.58**	**0.0000**
INTERACTIONS					
AB	**197938**	**45**	**4398.61**	**19.16**	**0.0000**
RESIDUES	123964	540	229.562		
TOTAL (corrected)	648688	599			

Multiple range Test for efficacy X treatment (Method: 95.0 percent LSD)

TREATEMENT[a]	Cases	LS Mean	LS Sigma	Homogenous Groups
T7	**60**	**0**	**1.95603**	**D**
T6	**60**	**0**	**1.95603**	**D**
T2	**60**	**0**	**1.95603**	**D**
T5	**60**	**0**	**1.95603**	**D**
T1	**60**	**0**	**1.95603**	**D**
T8	**60**	**1.66667**	**1.95603**	**D**
T9	**60**	**3.36667**	**1.95603**	**D**
T10	**60**	**11.6667**	**1.95603**	**C**
T4	**60**	**43.3333**	**1.95603**	**B**
T3	**60**	**63.3333**	**1.95603**	**A**

[a]Treatments were: Control (T1 only water, , T2 Water + Fitoil only water, nor EO application)) , Reference standards (T3 Beloukha, T4 Glyphosate) , EO (T5 , T6, T7, T8, T9, T10: 2,4,8,12,16, and 32 µL mL−1 doses, respectively). The efficacy (E) was considered as its capacity to kill the plants and was assessed by attributing the value 0 if the plant was alive and 100 if the plant was dead (it is dimensionless). Data are arithmetic mean of 10 replicates.

A multi-factor analysis of variance (Multi-Factor ANOVA) was used to study the different influences that the factors have in the different variables (Table 6). Finally, looking at the multiple range test: efficacy x treatment; it was observed that treatment 10 was the most effective treatment of essential oil, followed by treatment 9 and 8. There were no significant differences between control and fitoil, indicating that fitoil possesses no herbicidal effect on *A.retroflexus.*

Table 7 : Efficacy and damage level of EO *E. camadulensis* on *A.retroflexus* applied by spraying (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL , 32 µL/mL,) and two references at the beginning and end of the assay (30 days after treatment)

Concentration (µl / ml)	***Efficacy (%) ± SE**		**Damage Level ± SE**	
	Day 1	Day 30	Day 1	Day 30
Control	0.0 0.0 [a]	0.00 ± 0.0 [c]	0.00 ±0.00 [a]	0.00 ±0.00 [e]
Control + Fitoil	0.00 ± 0.0 [a]	0.00 ± 0.00 [c]	0.00 ± 0.00 [a]	0.00 ± 0.00 [e]
Pelargonic acid	0.00 ± 0.0 [a]	90.00 ± 10.00 [a]	0.00 ± 0.00 [a]	2.90 ± 0.10 [a]
Glyphosate	0.00 ± 0.0 [a]	80.00±13.33 [ab]	0.00 ± 0.00 [a]	2.80 ± 0.13 [a]
Dose 2	0.00 ± 0.0 a	0.00 ± 0.00 c	0.00 ± 0.00 a	0.00 ± 0.00 e
Dose 4	0.00 ± 0.0 a	0.00 ± 0.00 c	0.00 ± 0.00 a	0.00 ±0.00 e
Dose 8	0.00 ± 0.0 a	0.00 ± 0.00 c	0.00 ± 0.00 [a]	0.70 ± 0.15 e
Dose 12	0.00 ± 0.0 a	10.00 ± 10.00 c	0.00 ± 0.00 a	1.40 ± 0.22 d
Dose 16	0.00 ± 0.0 a	10.00± 10.00c	0.00 ± 0.00 a	1.90 ± 0.18 c
Dose 32	0.00 ± 0.0 a	60.00 ± 16.33 b	0.00 ± 0.0 a	2.60 ± 0.16 a

Data are mean ± standard errors. Different letters in the same column indicate statistical differences ($p < 0.05$) using Fisher's least significant difference (LSD) test. *Efficacy goes from 0 to 100 and the damage level was assessed on a scale from 1 to 3.

Over 30 days, there is a gradual increase in the level of damage to more severe levels with significant differences between the different doses after applying the treatments (Table 7). All the treatments (except the lowest two Dose 2, Dose 4) managed to control the species with different levels of damage, the results of the treatments are statistically significant when compared with the control. The treatments managed to control *A.retroflexus* with the greatest efficacy given to the references pelargonic acid ,Glyphosate and Dose 32 of essential oil. The active ingredient of commercial Beloukha is pelargonic acid, a saturated fatty acid with a direct rapid bio-herbicidal effect on weeds as they affect lipid content of cell membrane causing leakage and eventually cell and plant necrosis (Muñoz et *al*., 2020; Ogbangwor, 2021). On the other hand, the second reference glyphosate is a chemical herbicide, whose mode of action depends on the inhibition of 5-enolpyruvylyshikimate-3-phosphate enzyme essential for protein, cell wall and secondary plant product synthesis in weed species. However, the effect of glyphosate in weed control is reduced compared to pelargonic acid owing to its slower translocation in weed root and shoot systems, needing longer time to show efficiency (Muñoz et *al*., 2020, Andert and Gerowitt, 2020). Higher efficacy of pelargonic acid and glyphosate in weed control is supported by Muñoz et *al*. (2020) on some weed species including *A. retroflexus*. Results of the current study reported that higher doses of EO is required to induce similar effects to biological and chemical references. Increased efficiency of *E. camadulensis* EO in weed control was reported by Verdeguer et *al*. (2020a)

In this experiment the total plant length of *A.retroflexus* after spraying with essential oil of *Eucalyptus camaldulensis* was measured at the end of the assay, as well as fresh and dry weight. Results are displayed in Table 8

Table 8 :Plant length (cm), fresh weight (g) and dry weight (g) of *A.retroflexus* after spraying with essential oil of *Eucalyptus camaldulensis* (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL , 32 µL/mL,) at the beginning and end of the assay (30 days after treatment).

Concentration (µl / ml)	Plant length(cm) ± SE	Fresh weight (g) ± SE	Dry weight (g) ± SE
Control	6.60 ± 0.40 [b]	0.34 ±0,03 ab	0.04±0.00 ab
Fitoil	7.49 ± 0.27 [ab]	0.25 ±0.02 b	0.02±0.00 ab
Pelargonic acid	0.00 ± 0.00 [d]	0.00±0.00 [d]	0.00±0.00 [d]
Glyphosate	0.00 ± 0.00 d	0.00±0.00 [d]	0.00±0.00 [d]
Dose 2	8.49 ±0.54 a	0.38±0.05 a	0.04±0.01 [ab]
Dose 4	7.35 ± 0.43 ab	0.34±0.03 [ab]	0.05±0.01 [a]
Dose 8	6.16 ± .019 b	0.35±0.03 ab	0.05±0.00 [a]
Dose 12	5.67 ± 0.72 b	0.26±0.07 [b]	0.03±0.01 [bc]
Dose 16	5.64 ± 0.72 b	0.15±0.05 c	0.02±0.00 [c]
Dose 32	2.20 ± 0.91 c	0.02±0.01 [d]	0.01±0.00 [d]

Regarding the phytotoxic effects on the plant, both references Pelargonic acid and Glyphosate showed maximum length reduction 100%, while Fitoil didn't show any significant activity. Only the highest dose (32 µL/mL) exhibited a significant reduction in plant length and the plants were 66.7% shorter as compared to control. While reference biological and chemical herbicides exhibit the highest plant length reduction, higher doses of EO of *E. camaldulensis* could provide similar reduction as reported by Martínez (2017) on *A.retroflexus* and *E. crus-galli*. The same author did not report any sound effects of Fitoil. Decrements in shoot length of *Solanum elaeagnifolium* in response to EO of four different species of eucalyptus was also reported by Zhang *et al.* (2012) While EO of *E. tereticornis* decreased shoot length of *E. crus-galli* (Vishwkarma and Mittal, 2014).

Regarding plant weight (dry/ fresh) showed comparable results. Both references Beloukha and Glyphosate showed maximum weight reduction (100%). The highest two doses 16 & 32 showed significant differences between them compared to control, while Fitoil didn't show any significant activity on plant weight reduction. In the same manner, the reference herbicides had the best reduction effect on plant weight followed by the higher doses of *E. camaldulensis* EO. Such effect of eucalyptus EO on weight reduction matches with that found by Batish *et al.* (2007) using *Eucalyptus citriodora* EO against *Phalaris minor*. Dry weight of *Solanum nigrum* also exhibited a significant decrease in response to EO of *Eucalyptus globulus* (Ataollahi *et al.*, 2014).

Table 9: Efficacy and damage level of EO *E. camadulensis* on *E. crus-galli* applied by spraying (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL) and two references at the beginning and end of the first assay (30 days after treatment)

E. *crus-galli*				
Concentration (µl / ml)	**Spraying Damage level ± SE**		**Spraying Efficacy ± SE**	
	Day 1	**Day 30**	**Day 1**	**Day 30**
Control	**0.00±0.00 e**	**1.20±0.69 bc**	**0.00±0.00 c**	**40.00±0.49 bcd**
Control + Fitoil	**0.00±0.00 e**	**2.10±0.65 ab**	**0.00±0.00 c**	**70.00±0.46 ab**
Pelargonic acid	**3.00±0.00 a**	**3.00±0.00 a**	**100.00±0.00 a**	**100.00±0.00 a**
Glyphosate	**0.00±0.00 e**	**3.00±0.00 a**	**10.00±10.00 c**	**100.00±0.00 a**
Dose 2	**0.00±0.00 e**	**0.60±0.56 c**	**0.00±0.00 c**	**20.00±0.40 d**
Dose 4	**0.00±0.00 de**	**0.60±0.56 c**	**0.00±0.00 bc**	**20.00±0.40 d**
Dose 8	**0.00±0.00 c**	**1.50±0.70 bc**	**10.00±0.00 c**	**50.00±0.50 bcd**
Dose 12	**0.00±0.00 cd**	**1.90±0.65 ab**	**20.00±10.00 b**	**60.00±0.46 bc**
Dose 16	**0.00±0.00 b**	**2.10±0.65 ab**	**20.00±13.30 b**	**70.00±0.46 ab**

Data are mean ± standard errors. Different letters in the same column indicate statistical differences ($p < 0.05$) using Fisher's least significant difference (LSD) test. Efficacy goes from 0 to 100.The damage level was assessed on a scale from 1 to 3.

Table 10: Efficacy and damage level of essential oil Eucalyptus *camaldulensis* on *E. crus-galli* applied by spraying (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL) at the beginning and end of the second assay (30 days after treatment)

Concentration (µl / ml)	Spraying Damage level ± SE		Spraying Efficacy ± SE	
	Day 1	Day 30	Day 1	Day 30
Control	0.00±0.00 c	0.00±0.00 b	0.00±0.00 c	0.00±0.00 b
Control + Fitoil	0.00±0.00 c	0.00±0.00 b	0.00±0.00 c	0.00±0.00 b
Pelargonic acid	3.00±0.00 a	2.70±0.42 a	100.00±0.00 a	90.00±10.00 a
Glyphosate	0.00±0.00 c	2.70±0.42 a	0.00±0.00 c	90.00±10.00 a
Dose 2	0.00±0.00 c	0.00±0.00 b	0.00±0.00 c	0.00±0.00 b
Dose 4	0.00±0.00 c	0.00±0.00 b	0.00±0.00 c	0.00±0.00 b
Dose 8	1.10±0.14 b	0.00±0.00 b	0.00±0.00 c	0.00±0.00 b
Dose 12	1.30±0.22 b	0.00±0.00 b	10.00±10.00 bc	0.00±0.00 b
Dose 16	1.30±0.22 b	0.00±0.00 b	20.00±13.33 b	0.00±.00.0 b

Data are mean ± standard errors. Different letters in the same column indicate statistical differences ($p < 0.05$) using Fisher's least significant difference (LSD) test. Efficacy goes from 0 to 100.The damage level was assessed on a scale from 1 to 3.

In the first assay, using EO via spraying, there is a gradual increase of the level of damage to more severe levels over 30 days with significant differences between the different doses after applying the treatments (Table 9). All the treatments (except the highest doses 12, 16) were not significant. The results of the treatments were statistically significant when compared with the control. The treatments managed to control with the greatest efficacy achieved by the references Pelargonic and Glyphosate. Some plants died or became dry because *Echinochloa* plants suffered from stress in the greenhouse. Reporting results of spraying in the second assay. It is important to mention that we reduced the water volume used in this assay from 20 ml to 5 ml. The data showed no significant difference between the treatments of essential oil applied by spraying (Table 10). The doses tested in this assay did not show any effect. On the other hand, only the references Beloukha (pelargonic acid) and Glyphosate gave maximum damage level and efficacy at the end of the assay.

Similar results reporting superiority of pelargonic acid followed by glyphosate effects on *E. crus-galli* and other weed species were found by Ogbangwor (2021). On the other hand, Andert and Gerowitt (2020), on some weed species including *E. crus-galli*, reported rapid effect of Beloukha as pelargonic acid on the short run (due to higher biodegradability) and a more persistent effect of glyphosate on the long run (due to slower translocation in weed plant tissues). Similair to *A. retroflexus*, a higher dose of EO of *E. camaldulensis* is required to induce the same effect of the biological and chemical references. Similar effect EO of *E. camaldulensis* on some weed species using spraying or irrigation methods was reported by Verdeguer et *al*. (2020a).

Table 11: Efficacy and damage level of essential oil Eucalyptus *camaldulensis* on *E. crus-galli* applied by irrigation (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL) at the beginning and end of the first assay (30 days after treatment)

E. crus-galli Irrigation				
Concentration (µl / ml)	**Damage level ± SE**		**Efficacy ± SE**	
	Day 1	**Day 30**	**Day 1**	**Day 30**
Control	**0.00±0.00 a**	**0.90±0.65 ab**	**0.00±0.00 a**	**30.00±16.32 a**
Control + Fitoil	**0.00±0.00 a**	**1.50±0.70 ab**	**0.00±0.00 a**	**50.00±16.67 a**
Dose 2	**0.00±0.00 a**	**0.60±0.57 c**	**0.00±0.00 a**	**20.00±13.33 a**
Dose 4	**0.00±0.00 a**	**0.60±0.57 c**	**10.00±0.00 a**	**20.00±13.31 a**
Dose 8	**0.00±0.00 a**	**1.30±1.50 ab**	**0.00±0.00 a**	**40.00±13.33 a**
Dose 12	**0.00±0.00 a**	**1.80±1.32 ab**	**0.00±0.00 a**	**50.00±13.31a**
Dose 16	**0.00±0.00 a**	**2.00±1.33 a**	**0.00±0.00 a**	**60.00±16.32 a**

Data are mean ± standard errors. Different letters in the same column indicate statistical differences ($p < 0.05$) using Fisher's least significant difference (LSD) test . Efficacy goes from 0 to 100.The damage level was assessed on a scale from 1 to 3.

Table 12: Efficacy and damage level of essential oil Eucalyptus *camadulensis* on *E. crus-galli* applied by irrigation (Control, Fitoil control, 2 µL/mL, 4 µL/mL, 8 µL/mL, 12 µL/mL, 16 µL/mL) at the beginning and end of the second assay (30 days after treatment)

Concentration (µl / ml)	Irrigation Damage level ± SE		Irrigation Efficay ± SE	
	Day 1	Day 30	Day 1	Day 30
Control	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Control + Fitoil	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Dose 2	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Dose 4	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Dose 8	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Dose 12	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a
Dose 16	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a	0.0±0.00 a

Data are mean ± standard errors. Different letters in the same column indicate statistical differences ($p < 0.05$) using Fisher's least significant difference (LSD) test . Efficacy goes from 0 to 100.The damage level was assessed on a scale from 1 to 3.

According to Table 11 and 12 the data showed no significant difference between the treatments applied by irrigation in either assay 1 or 2. Water volume used for irrigation was reduced in assay 2, however, the doses of EO tested in both assays did not show any effect. On the other hand, (Verdeguer *et al*., 2020a) found the opposite, E. *camaldulensis* EO was the second most effective treatment in controlling *Erigeron bonariensis* but only when administered by watering. It is important to highlight that the herbicidal activity of the EOs tested depend on the way they were administered.

4. Conclusion

In conclusion, it is interesting to note the results obtained with *Eucalyptus camadulensis* essential oil: with the oxygenated sesquiterpene spathulenol as the main compound. It showed great selectivity against *A.retroflexus* . We conclude at the end that even the highest doses were *Echinochloa crus-galli* in both assays. It is important to highlight that the herbicidal activity of the EOs tested depended on the way they were applied. So, it is necessary to develop adequate formulations to enhance the activity of the EOs.

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

Summary

In search for novel compounds to be used as herbicides, particularly natural plant products, with potential herbicidal activity as essential oil. We investigated the herbicidal potential of the essential oil of *Eucalyptus camaldulensis* eg. efficacy and damage level in the greenhouse against two weeds in the Mediterranean region.: *Echinochloa crus-galli* and *Amaranthus retroflexus* in pre- and post-emergence trials. For this purpose, the EO with spathulenol as the main component was applied in different concentrations by spraying and irrigation with pelargonic acid and glyphosate as reference. The results showed that the essential oil was effective in every dose for inhibition of germination in pre- emergence assays. The oil was more effective when applied by spraying and *Amaranthus retroflexus* species was more sensitive than *Echinochloa crus-galli*. The results suggest the possible use of the oil as natural herbicide. Further research is needed to determine the effective doses for different species.

References

Andert S, B Gerowitt (2020). Controlling arable weeds with natural substances as bio-based herbicides. *Julius-Kühn-Archiv* 464: 407-414. DOI: 10.5073/jka.2020.464.061

Ataollahi R, M Dejam, SS Khaleghi (2014). Phytotoxic effects of *Eucalyptus globulus* leaf extract on *Solanum nigrum*. *South Western Journal of Horticulture Biology and Environment* 5 (1): 43-53.

Batish DR, HP Singh, N Setia, RK Kohli, S Kaur, SS Yadav (2007). Alternative control of little seed canary grass using eucalypt oil. *Agronomy for Sustainable Development* 27 (3): 171-177. DOI: 10.1051/agro:2007008.

Batish DR, HP Singh, N Setia, S Kaur, RK Kohli (2006). Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. *Zeitschrift für Naturforschung C – A Journal of Biosciences* 61 (7-8): 465-471. DOI: 10.1515/znc-2006-7-801

Cao L., L Berent, A Fusaro (2021). *Echinochloa crus-galli* (L.) P. Beauv.: U.S. Geological Survey, non-indigenous Aquatic Species Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI, URL: https://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx , accessed on 17.1.2022.

Cordeau S, M Triolet, S Wayman, C Steinberg, JP Guillemin (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. *Crop Protection 87*: 44-49. DOI :10.1016/ j.cropro .2016.04.016

El-Darier SM, (2002) Allelopathic Effects of *Eucalyptus rostrata* on Growth, Nutrient Uptake and Metabolite Accumulation of *Vicia faba* L. and *Zea mays* L. *Pakistan Journal of Biological Sciences* 5: 6-11.

Flores-macías A, GG Reyes-zarate, CAG Da Camara, R López-ordaz, JC Guillén, MÁ Ramos-lópez (2021). Chemical composition and phytotoxic potential of *Eucalyptus globulus* essential oil against *Lactuca sativa* and two herbicide-resistant weeds: *Avena fatua* and

Amaranthus hybridus. *TIP Revista Especializada en Ciencias Químico-Biológicas* 24: 1-8. DOI: 10.22201/fesz.23958723e.2021.303

Fouad, R.; Bousta, D.; Lalami, A.E.; Chahdi, F.O.; Amri, I.; Jamoussi, B.; Greche, H. Chemical composition and herbicidal effects of essential oils of *Cymbopogon citratus* (DC) Stapf, Eucalyptus cladocalyx, *Origanum vulgare* L and Artemisia absinthium L cultivated in Morocco. J. Essent. Oil Bear. Plants 2015, 18, 112–123.

Grichi A, Z Nasr, ML Khouja (2016). Phytotoxic effects of essential oil from *Eucalyptus cinerea* and its physiological mechanisms. *Journal of New Sciences, Agriculture and Biotechnology* (13): 1289-1296.

Heap I, (2017). Herbicide Resistant Weeds in Spring Barley Globally. URL: http://www.weedscience.org/Summary/Crop.aspx, accessed on 4.11.2021.

Holm LG, DL Plucknett, JV Pancho, JP Herberger (1977). The world's worst weeds - distribution and biology. University Press of Hawaii. Honolulu. 621 pp.

Ibáñez Jaime MD, MA Blázquez Ferrer (2018). Post-emergent herbicidal activity of *Eucalyptus globulus* Labill. essential oil. *Nereis* 2 (10): 25-36. DOI: 10.3390/mol2net-04-05374

Jouini A, (2020a). Herbicidal activity of Mediterranean essential oils and their effects on soil bioindicators. PhD thesis. Universitat Politècnica de València & Universita Degli Studi di Palermo.

Jouini A, M Verdeguer, S Pinton, F Araniti, E Palazzolo, L Badalucco, VA Laudicina (2020b). Potential effects of essential oils extracted from Mediterranean aromatic plants on target weeds and soil microorganisms. *Plants* 9 (10): 1-24. DOI: 10.3390/plants9101289

Kohli RK, DR Batish, & HP Singh, (1998). Eucalypt oils for the control of Parthenium (*Parthenium hysterophorus* L.). *Crop Protection* 17 (2): 119-122. DOI:10.1016/s0261-2194(97)00095-1

Martínez A, (2017). Determination of *Eucalyptus camaldulensis Dehnh*. herbicidal activity against *Amaranthus retroflexus L.*, *Chenopodium album L.*, *Echinochloa crus-galli (L.) P.Beauv.* and *Avena fatua L*. PhD thesis. Universitat Politècnica de València.

Muñoz M, N Torres-Pagán, R Peiró, R Guijarro, AM Sánchez-Moreiras, M Verdeguer (2020). Phytotoxic effects of three natural compounds: Pelargonic acid, carvacrol, and cinnamic aldehyde, against problematic weeds in Mediterranean crops. *Agronomy* 10 (6): 791. DOI: 10.3390/agronomy10060791

Ogbangwor N, (2021). Studies on the effect of the herbicide pelargonic acid. MSc thesis. Georg August University of Göttingen

Pagán N, (2019). Study of the herbicidal activity of three natural compounds on important weeds in Mediterranean crops for their development as a commercial product. PhD thesis. Universitat Politècnica de València.

Rassaeifar M, N Hosseini, N Asl, P Zandi, A Aghdam (2013). Allelopathic effect of *Eucalyptus globulus* essential oil on seed germination and seedling establishment of *Amaranthus blitoides* and *Cyndon dactylon*. *Trakia Journal of Sciences* 11 (1): 73-81.

Singh HP., RB Daizy, RK Kohli (2003). Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. *Critical Reviews in Plant Sciences* 22 (3-4): 239-311. DOI: 10.1080/713610858

Verdeguer M. (2011). Fitotoxicidad de aceites esenciales y extractos acuosos de plantas Mediterráneas para el Control de Arvenses. PhD thesis. Universitat Politècnica de València.

Verdeguer M, D García-Rellán, H Boira, E Pérez, S Gandolfo, MA Blázquez (2011). Herbicidal activity of *Peumus boldus* and *Drimys winterii* essential oils from Chile. *Molecules* 16 (1): 403-411. DOI: 10.3390/molecules16010403

Verdeguer M, LG Castañeda, N Torres-Pagan, JA Llorens-Molina, A Carrubba (2020a). Control of *Erigeron bonariensis* with *Thymbra capitata*, *Mentha piperita*, *Eucalyptus*

camaldulensis, and *Santolina chamaecyparissus* essential oils. *Molecules* 25 (3): 562. DOI: 10.3390/molecules25030562

Verdeguer M, MA Blázquez, H Boira (2009). Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology* 37 (4): 362-369. DOI: 10.1016/j.bse.2009.06.003

Verdeguer M, N Torres-Pagan, M Muñoz, A Jouini, S García-Plasencia, P Chinchilla, M Berbegal, A Salamone, S Agnello, A Carrubba, L Cabeiras-Freijanes, L Regueira-Marcos, AM Sánchez-Moreiras, MA Blázquez (2020b). Herbicidal activity of *thymbra capitata* (L.) Cav. essential oil. *Molecules* 25 (12): 2832. DOI: 10.3390/molecules25122832

Vishwakarma GS, S Mittal (2014). Bioherbicidal potential of essential oil from leaves of *Eucalyptus tereticornis* against *Echinochloa crus-galli L. Journal of Biopesticides 7*: 47-53.

Wu H, J Zhang, R Stanton, M An, DL Liu, Dgrass and barley grass. *Allelopathy Journal* 28 (1): 87-94.

Zhang J, M An, H Wu, DL Liu, R Stanton (2012). Chemical composition of essential oils of four *Eucalyptus* species and their phytotoxicity on silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia. *Plant Growth Regulation* 68 (2): 231-237. DOI: 10.1007/s10725-012-9711-5

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DECLARATION

I hereby declare that I wrote this thesis paper independently, without assistance from external parties, and without use of other resources than those indicated. All information taken from other publications or sources in text or in meaning are duly acknowledged in the text. The written and electronic forms of the thesis paper are the same. I give my consent to have this thesis checked by plagiarism software.

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