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ORIGINAL ARTICLE

Chemical composition, antioxidant, herbicidal and antifungal activities of leaf essential oils from three Tunisian *Eucalyptus* species

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Abstract

Medicinal plants produce several natural bioactive molecules and compounds called secondary metabolites that have very important biological properties. The objective of this work is to determine the chemical composition of essential oils (EO) obtained from the leaves of three Tunisian species of *Eucalyptus* (*E. oleosa*, *E. pimpiniana*, *E. polyanthemos*) and to evaluate their biological activities. As a result, 45 different compounds were identified: 26 from *E. pimpiniana*, 15 from *E. polyanthemos*, and 39 from *E. oleosa*, which represent 99.4%, 99.4% and 98.6%, respectively, of the entire essential oil constituents. The analyses showed that 1,8-cineole (35.3%) and β-eudesmol (25.5%) were the main components in *E. pimpiniana* essential oil, whereas 1,8-cineole (71.6%) and globulol (13.2%) characterized *E. polyanthemos* leaf oil. Also, 1,8-cineole (13.4%), spathulenol (11.9%), and β-eudesmol (8.5%) were found to be the main constituents of *E. oleosa* EO. Other compounds, such as phellandral, *p*-cymen-7-ol (syn. cumin alcohol), carvacrol, myrtenal, cumin aldehyde and cryptone, are specific to the EOs of *E. oleosa,* making it distinct from the other *Eucalyptus* species studied. The essential oils showed low antioxidant capacity, but significant antifungal activity against five *Fusarium* spp. Indeed, *E. oleosa* essential oil exhibited the highest level of antifungal activity. Additionally, herbicidal activity has only been proved in a preliminary in vitro test against 3 weed species (*Sinapis arvensis* L., and *Lepidium sativum*) of the same family. The greatest inhibition of seed germination was obtained with the *E. oleosa* essential oils even at low concentrations strongly suggesting that they could have application in agriculture, particularly as antagonists against *Fusarium* and other fungi and for weed control.

Keywords Antioxidant · Antifungal · Phytotoxic activity · Essential oil · *Eucalyptus* spp.

Introduction

Weeds are among the major threats to agricultural production (Mabrouk et al. 2007) because they compete with crops for natural resources, harbor pests, and reduce crop yields and quality (Brown et al. 2019; Ampong-Nyarko and De Data 1991), all of which increase costs. Chemical control remains the most common practice for decreasing weed growth, but an overdependence on chemical application has led to serious side effects, including damage to vegetation and non-target crops, persistence of chemical residues in water and soil, and serious concerns for the environment, human health, and biosafety in general (Arias-Estevez et al. 2008). In addition, the overuse of synthetic compounds can lead to the development of herbicide resistance (Heap and Le Baron 2001). To overcome these negative effects, research is actively moving toward the development of sustainable

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alternatives by using biological compounds that have phytotoxic potential (Mabrouk et al. 2016).

Phytopathogenic fungi cause severe crop yield losses, making them a major problem in global agriculture (Hemissi et al. 2013). To fight against these pathogens, farmers have intensified treatments that lead to numerous unwanted environmental problems, such as groundwater and soil contamination and the development of fungal resistance (Zubrod et al. 2019). For these reasons, researchers have investigated other means of disease control and plant growth promotion such as plant-derived essential oils for use as natural herbicides and fungicides to reduce the negative effects of exogenously added chemicals (Smith and Perfetti 2020; Steven et al. 1991). Essential oils are much less persistent than synthetic compounds, and they do not significantly affect the environment. Moreover, they employ very different modes of action to prevent the development of resistance in weeds or fungal species compared to synthetic compounds (Amri et al. 2023). Essential oils are formed by complex mixtures of hydrocarbons and their oxygenated derivatives. The active components are excreted or volatilized on the surface of plant organs, such as leaves, stems, flowers, fruits, and seeds, which gives them a fortuitous ecological location with respect to plants (Amri et al. 2011; Hazrati et al. 2018). Essential oils also have numerous biological effects including antiseptic, germicidal, antioxidant, phytotoxic and antifungal properties (Ootani et al. 2017; Amri et al. 2012, 2021). Many researchers have shown that essential oils are rich in compounds with allelopathic activity not only against weeds (Alipour et al. 2016), but also against crop pests (Amri et al. 2013; Hamrouni et al. 2014; Hanana et al. 2017).

The genus *Eucalyptus* belongs to the family *Myrtaceae* with about 900 species and subspecies (Tyagi and Malik 2011). It is a tree native to Australia and is characterized by evergreen and large single leaves. It was successfully introduced from Australia and planted worldwide and accounts for approximately 27% of total wood volume (Vázquez et al. 2008). In Tunisia, *Eucalyptus* species represent 5% of the total forest cover (Zaibet 2016). They are cultivated for two main reasons: wood production (40%) and forest protection (60%). Indeed, *Eucalyptus* species are important for providing combustible biomass and directly reducing carbon dioxide levels in the atmosphere (Barton 2000). In addition, their essential oils serve as pesticides (Barton 2000) and as antioxidants (Aragão et al. 2015; Loying et al. 2019), anticarcinogenic compounds (Ibáñez and Blázquez 2019), insecticides (Tolba et al. 2015), and antibacterial (Tolba et al. 2018), antiviral (Davies et al. 2019), and antifungal agents (Abril-Sánchez et al. 2019).

This study aims to elucidate the chemical composition of the essential oils obtained from the leaves of three different *Eucalyptus* species growing in Tunisia and their in vitro antioxidant, antifungal, and herbicidal activities. The antifungal activity of the essential oils was assessed against five plant pathogenic *Fusarium* fungi (*F. oxysporum* (Schltdl.), *F. oxysporum f. matthioli* (K.F. Baker), *F. oxysporum* MN-2, *F. oxysporum* 184, and *F. redolens* (Wollenw), and their phytotoxic effects were tested by evaluating the extent of inhibition of seed germination and seedling growth of three test plants, *Sinapis arvensis* L., *Lepidium sativum* L., and *Raphanus sativus* L.

Material and methods

Plant material

Plant samples of about 2 kg each were randomly collected during December 2020 from the aerial parts of individual *Eucalyptus* species growing in the arboretum of Hinchir EnNaâme (Siliana). The collection site is characterized by the upper semi-arid bioclimatic stage, and it is located at longitude 9° 10′ E, latitude 36° 13′ N and at an altitude of 350 m.

Essential oil extraction

The volatile oils were obtained by hydro-distillation of the leaves for 4 h in a Clevenger type apparatus. Extracted oils were dried over anhydrous sodium sulfate and stored at 4 °C until analysis. Yields based on dried weight were calculated (w/w %).

Gas chromatography and mass spectrometry analysis

The chemical composition of essential oils was studied by gas chromatography/electron ionization-mass spectrometry (GC/EI-MS). The analysis was carried out using an Agilent 7890B gas chromatograph equipped with a quadrupole mass detector (Agilent 5977B) and an Agilent HP-5MS capillary column (coating thickness 0.25 µm; 0.25 mm × 30 m).

The analytical conditions were as follows: the oven temperature varied from 60 to 240 °C at 3 °C/min; transfer-line temperature 240 °C; injector temperature 220 °C, and the carrier gas was helium with a flow of 1 ml/min.

The acquisition parameters were as follows: full scan; scan time: 1.0 s; scan range: 35–300 m/*z*; threshold: 1 count. The identification of the components was made by comparing their linear retention indices (LRIs) relative to the series of *n*-alkanes and by the comparison of their mass spectra with those of ADAMS and NIST 14 (Adams 2007; NIST 2014) commercial libraries and from an in-house mass-spectral library.

Antioxidant potential of *Eucalyptus* oils

Radical scavenging activity

Radical scavenging activity was studied by using a solution of 1,1- diphenyl-2-picrylhydrazyl radical (DPPH) following the method of Hanato et al. (1988). One ml of the essential oils diluted in methanol at different concentrations was added to 1 ml of a 0.2 mmol/l DPPH methanolic solution.

The mixture was shaken and incubated in the dark and at room temperature for 30 min, after which the absorbance was measured at 517 nm. The antiradical capacity was expressed as IC50 (mg/ml), the concentration required to cause a 50% DPPH inhibition. The ability of the essential oils to reduce the DPPH radical was calculated according to the following equation:

$$\text{DPPH scavenging capacity } (\%) = \left[\frac{A0 - A1}{A0}\right] \times 100,$$

where $A0$: is the absorbance of the control (sample without oils) and $A1$: is the absorbance of the samples containing the oils.

All samples were analyzed in triplicate, and butylhydroxytoluene (BHT) was used as a positive control.

ABTS scavenging capacity

ABTS radical-scavenging capacity of the *Eucalyptus* volatile oils was assessed according to the method of Re et al. (1999). The ABTS radical cation was obtained by mixing 3.3 mg of 2.45 mM potassium persulfate solution and 19.2 mg of 7 mM ABTS solution, and the resulting emulsion was incubated at room temperature for 16 h in the dark. Subsequently, the ABTS solution was diluted with distilled water to an absorbance of 0.750 at 734 nm. Next, 160 µl of ABTS solution was added to 40 µl of the sample solution (essential oil in methanol) at different concentrations (6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml). All the assays were made in triplicate. The decrease in absorbance was determined 10 min after an initial mixing for all samples. BHT was used as an antioxidant standard. The inhibition capacity of each concentration was calculated relative to a blank absorbance following the equation:

$$\%\ \text{Inhibition} = \left[\left(A_{C(0)} - A_{A(t)}\right)/A_{C(0)}\right] \times 100$$

where $A_{C(0)}$ is the absorbance for the control; and $A_{A(t)}$ is the absorbance of the antioxidant (oils or BHT standard).

Herbicidal activity

Effects of *Eucalyptus* oils on seed germination and seedling growth.

Sinapis arvensis used in this work is important weed in Tunisian crops, with a wide distribution, many of which have developed herbicide resistant biotypes (Zargar et al. 2021). *Lepidium sativum* L., (garden cress) and *Sinapis arvensis* L., (field mustard) seeds were collected in a plot that was planted with field corn seed in Sidi Ismail, Beja, Tunisia, July 2020 (36° 35′ 58.6″ N 9° 06′ 24.1″ E). Seeds of *Raphanus sativus* L. (radish) seeds were purchased from AGRODIS company (Ben Arous 2013, Tunisia). These herbs were chosen for phytotoxicity assays because these species are widely used for herbicidal activities, and they are characterized by high and rapid germination rates (De Martino et al. 2010).

Before beginning the germination tests, the seeds were surface-sterilized for 5 min with 5% sodium hypochlorite and then, rinsed with distilled water. For each herb and oil tested, 20 seeds were placed in petri dishes (90 mm in diameter) between two layers of filter paper. Three replicates per treatment were made, and the operation was repeated three times. For each plant species and oil tested, 20 seeds were placed in petri dishes (90 mm in diameter) between two layers of filter paper, (three replicates per treatment), and the operation was repeated three times. After treatment with different concentrations (0, 1, 2, and 3 µl/ml) of volatile oil in a solution of Tween 20 (1%) or glyphosate (positive control), the Petri dishes were sealed with parafilm and incubated in a germination room (25 °C/16 h of light and 20 °C/8 h of darkness) (Tworkoski 2002). After 7 days, seed germination was assessed, and seedling shoot and root lengths were measured.

Antifungal activity

Fungal strains

Five fungal strains belonging to genus *Fusarium* were used for the tests. Four of the fungal strains (*F. oxysporum*, *F. oxysporum f. matthioli, F. oxysporum MN-2* and *F. oxysporum 184*) were obtained from the University of California Los Angeles (UCLA), whereas *F. redolens* was acquired from the laboratory of plant protection of the Tunisian National Institute of Agronomic Research (INRAT). All the fungi were stored on potato dextrose agar (PDA, Sigma) at 4 °C.

In vitro antifungal activities on mycelial growth The antifungal properties of the *Eucalyptus* oils were studied in Petri dishes using the contact assay method according to Cakir

et al. (2004). The oils were dissolved in a Tween 20 solution (0.1% v/v) and then, added to 20 ml of PDA (Potato Dextrose Agar medium) at 50 °C to provide the required concentrations (4–10 µl/ml). A mycelial disk of 6 mm in diameter from fresh cultures of the studied fungi was placed in a separate PDA plate (90 mm diameter) and incubated at 24 °C for 7 days. PDA plates that were treated only with Tween 20 (0.1%) were used as the negative control. The experiments were conducted with three replicates per treatment. The antifungal activity was determined as the percentage of inhibition (PI) of mycelia growth compared to the control according to the following formula:

$$\text{PI.}\,\% = \frac{(\text{dc} - \text{dt})}{\text{dc}} \times 100$$

where dc and dt are the mean diameter of control growth and oil-treated fungi, respectively.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). MIC is defined as the lowest dose at which there is complete inhibition of fungal growth. To establish the minimum fungicidal concentrations, the inhibited fungal disks (MICs) were inoculated into PDA Petri plates (without essential oil) and their growth was observed. After 3 days of incubation, the MFC was obtained as the lowest MIC at which no growth observed in the plates after culturing (Adjou et al. 2012).

Statistical analysis

The results were subjected to a one-way analysis of variance (ANOVA) using the SPSS 18.0 software package. Differences between means were tested through Student Newman–Keuls, and values with $P \leq 0.05$ were considered significantly different. Before performing the ANOVA, we verified two essential conditions for the analysis of variance: homoscedasticity and normality of the distribution. The verification of the homoscedasticity was performed by linear regression, whereas the verification of the normality of distribution was done by the computation of Skewness or the kurtosis *Z*-values. In both cases, these two conditions were verified.

Results

The results of the chemical analysis of the essential oils extracted from the aerial parts of the three Tunisian *Eucalyptus* species are listed in Table 1. A total of 45 different compounds were identified: 26 from *E. pimpiniana*, 15 from *E. polyanthemos*, and 39 from *E. oleosa*, representing 99.4%, 99.4%, and 98.6% of the entire essential oil constituents, respectively. These volatiles can be classified into 5 groups (Table 1), namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and non-terpene derivatives, and are listed by their LRI.

1,8-Cineole (35.3%) and β-eudesmol (25.5%) were the main components of the essential oil of *E. pimpiniana*, whereas 1,8-cineole (71.6%) and globulol (13.2%) characterized *E. polyanthemos* essential oil, 1,8-cineole (13.4%), spathulenol (11.9%) and β-eudesmol (8.5%) were the main constituents in the *E. oleosa* oil. Others important components of the EO of *E. oleosa* were phellandral, *p*-cymen-7-ol (syn. cumin alcohol), carvacrol, myrtenal, cumin aldehyde and cryptone, the combination of which allowed this oil to be distinguished from the EOs of the other two *Eucalyptus* species.

Antioxidant activity

The antioxidant activities of the three essential oils were determined using two test systems, namely the ABTS and DPPH assays (Fig. 1). The results revealed moderate antioxidant activities for the three essential oils. No significant differences between the essential oils were observed in the ABTS assay. Conversely, these differences were statistically significant in the DPPH assay. In the latter case, the essential oil of *E. oleosa* was the most active, with an IC_{50} value of 92.179 ± 2.484, followed by *E. pimpiniana* (163.593 ± 15.122) and *E. polyanthemos* (359.688 ± 2.778).

Herbicidal activity

The herbicidal activities of the three essential oils are presented in Tables 2, 3, 4. A significant inhibition was observed on the germination of the seeds of the tested plants in a dose-dependent way and on the type of essential oil tested. In summary, the essential oils of two species (*E. pimpiniana* and *E. oleosa*) completely inhibited the germination of *Sinapis arvensis* and *Raphanus sativus* at 1 µl/ml. Conversely, *E. polyanthemos* essential oils inhibited the germination of the seeds of the same species by 75 and 55%, respectively. However, the essential oil from *E. oleosa* was the most active regarding seed germination resulting in complete inhibition of *Sinapis arvensis* at 0.5 µl/ml. For *L. sativum*, only *E. oleosa* essential oil reduced seed germination by 20–30% at 0.75 and 1 µl/ml, respectively. In contrast, the essential oils of *E. pimpiniana* and *E. polyanthemos* had no effect on seed germination on *L. sativum* at all the tested concentrations.

A significant reduction in the length of *S. arvensis, L. sativum* and *R. sativus* seedlings caused by treatment with the essential oils was observed at 0.5 µl/ml (Table 3).

Table 1 Chemical composition of leaves essential oils of *Eucalyptus paniculata. Eucalyptus pimpiniana. Eucalyptus polyanthemos and Eucalyptus oleosa*

Compounds	RI	*Eucalyptus pimpiniana*	*Eucalyptus polyanthemos*	*Eucalyptus oleosa*
Monoterpene hydrocarbons		**3.4**	**1.4**	**10.7**
α-pinene	941	2.7	0.4	1.9
β-pinene	982			0.8
p-cymene	1028	0.4	1.0	7.2
Limonene	1032	0.3		0.8
Oxygenated monoterpenes		**46.8**	**79.5**	**37.9**
1.8-cineole	1034	35.3	71.6	13.4
Nopinone	1140			0.4
trans-pinocarveol	1141	6.6	3.4	4.5
Pinocarvone	1164	2.1	0.6	1.2
Borneol	1166	0.6		
4-terpineol	1179			1.0
p-mentha-1(7).8-dien-2-ol	1186	0.4	1.5	
α-terpineol	1191	0.7	0.5	1.2
Myrtenol	1193	0.2		
Myrtenal	1164			2.3
Verbenone	1206			0.5
trans-carveol	1220			0.3
cis-carveol	1228	0.5	1.3	
(Z)-tagetone (syn. *cis*-ocimenone)	1231	0.2	0.6	
cumin aldehyde	1241			2.9
Piperitone	1254			0.4
Phellandral	1275			4.8
p-cymen-7-ol (syn. cumin alcohol)	1290			2.2
Carvacrol	1298			2.8
α-terpinyl acetate	1352	0.2		
Sesquiterpene hydrocarbons		**1.9**	**0.4**	**2.3**
Aromadendrene	1440	1.0		1.0
α-vetispirene	1488			0.3
Bicyclogermacrene	1496			0.6
germacrene B	1557	0.9	0.4	0.4
Oxygenated sesquiterpenes		**47.3**	**18.1**	**39.4**
Ledol	1566	0.7		0.5
Spathulenol	1576	3.3	2.3	11.9
Globulol	1583	7.4	13.2	3.9
Viridiflorol	1591	1.7	1.5	1.1
Elemol	1592			0.5
Rosifoliol	1601	0.7	0.6	1.4
10-*epi*-α-eudesmol	1620	1.0	0.5	0.5
1-*epi*-cubenol	1629	2.1		1.9
γ-eudesmol	1631	2.0		1.9
Isospathulenol	1640			3.1
T-cadinol	1641			0.9
β-eudesmol	1650	25.5		8.5
α-eudesmol	1651	2.9		2.5
α-cadinol	1652			0.8
Non-terpene derivatives		**0.0**	**0.0**	**8.3**
Cryptone	1185			6.4
p-cumenol	1229			1.4
isoamyl benzoate	1439			0.5
Total		99.4	99.4	98.6

Compound identified according to MS and by comparison of RI with the literature (RI); RI: identification based on comparison of retention index with those of published data (Adams.2007)

Values in bold represent the compound contents for each chemical class for the different *Eucalyptus species*

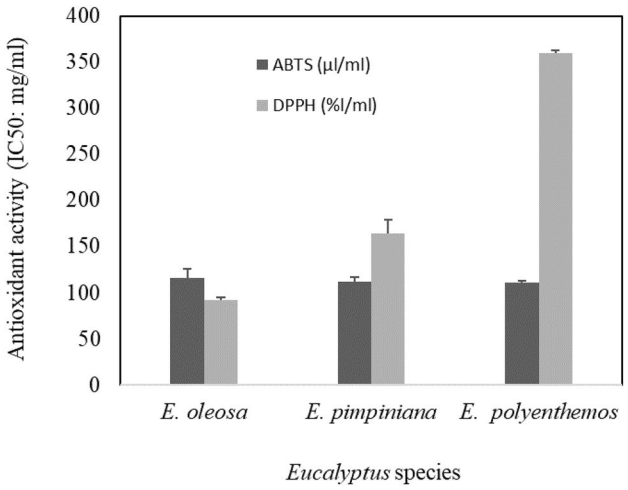

Fig. 1 Antioxidant activity (IC50 (mg/L) of the different Eucalyptus essential oil

Inhibition was observed for more than 50% of the treated seedlings. For *S. arvensis,* no growth was observed after treatment at 1 µl/ml for each of the three oils. At all concentrations, a partial reduction in shoot growth was noted for *R. sativus* treated with *E. polyanthemos* essential oil. Seedling root lengths in the three species were significantly shortened using1µl/ml of any of the essential oils. *E. pimpiniana* and *E. oleosa* essential oils affected root lengths more than did *E. polyanthemos* EOs. The greatest inhibition of root length was observed for the *E. oleosa* EOs.

Antifungal activity

In vitro inhibition of fungal mycelium growth by essential oils of the three Eucalyptus species.

The effects of increasing concentrations of the three essential oils on mycelium growth of the different fungal strains are shown in Fig. 2 and summarized in Tables 5 and 6. All the essential oils inhibited the growth of the tested five fungal strains in a dose-dependent manner. The highest inhibitory activity was exhibited by the *E. oleosa* essential oil, with 100% inhibition of mycelium growth at 6 µl/ml for all fungal strains (Table 5). The fungal strains *F. oxysporum* MN-2 and *F. oxysporum* 184 were the most sensitive to the three essential oils when used at 6 µl/ml.

Minimum inhibitory concentration and minimum fungicidal concentration.

The minimum inhibitory concentration (MIC) is the lowest concentration capable of 100% inhibition of fungal mycelium growth (Table 6). The *E. polyanthemos* and *E. pimpiniana* oils MIC exhibited a range of 6–10 µl/ml for three of the five pathogens tested. To achieve a 100% inhibition of mycelial growth of *F. oxysporum* and *F. oxysporum f. matthioli*, a concentration of these oils greater than 10 µl/ml (Table 5) was required. For the *E. oleosa* oil, the MIC was 6 µl/ml for all the fungal strains. *E. oleosa* essential oil was shown to be the most lethal, with a minimum fungicidal concentration (MFC) between 6 and 8 µl/ml for the different pathogens. The second most toxic was *E. pimpiniana* oil, with an MFC between 6 and 10 µl/ml against

Table 2 Inhibitory effect of increasing doses of *Eucalyptus* essential oils on seeds germination of tested plants

Plant species	Dose (µl/ml)	*E. pimpiniana*	*E. polyanthemos*	*E. oleosa*	Control + (glyphosate)
Sinapis arvensis	0	83.3^{a}	83.33^{a}	83.33^{a}	83.33^{a}
	0.25	53.33^{b}	50^{b}	26.66^{b}	50^{b}
	0.5	46.66^{b}	23.33^{c}	0^{c}	70^{a}
	0.75	3.33^{c}	40^{bc}	3.33^{c}	80^{a}
	1	0 ± 0^{c}	20^{c}	6.66^{c}	83^{a}
Lepidium sativum	0	100 ± 0^{a}	100^{a}	100^{a}	100^{a}
	0.25	100 ± 0^{a}	96.66^{a}	93.33^{ab}	96.66^{a}
	0.5	100 ± 0^{a}	90^{a}	93.33^{ab}	1000^{a}
	0.75	93.33^{a}	100^{a}	80^{bc}	100^{a}
	1	93.33^{a}	100^{a}	70^{c}	96.66^{a}
Raphanus sativus	0	93.33^{a}	93.33^{a}	93.33^{a}	93.33^{a}
	0.25	36.66^{c}	63.33^{b}	63.33^{b}	70^{b}
	0.5	53.33^{b}	60^{b}	30^{c}	86.66^{ab}
	0.75	26.66^{c}	56.66^{b}	20^{c}	83.33^{ab}
	1	13.33^{d}	40^{c}	6.66^{d}	80^{ab}

Values are means ± standard deviations (n = 3); means followed by the same letter in are not significantly different by the Student_Newman_Keuls test ($P \leq 0.05$)

Table 3 Inhibitory effect of *Eucalyptus* essential oils on growth of plants shoots

Plant species	Dose (µl/ml)	*E. pimpiniana*	*E. polyanthemos*	*E. oleosa*	Control + (glyphosate)
Sinapis arvensis	0	3.73[a]	3.73 ± 0.7[a]	3.73 ± 0.7[a]	3.73 ± 0.7[a]
	0.25	2.97[ab]	2.79 ± 0.12[b]	1.99 ± 0.54[b]	0.72 ± 0.03[b]
	0.5	2.07[b]	1.58 ± 0.14[b]	0 ± 0[c]	0.79 ± 0.08[b]
	0.75	0[c]	2.22 ± 0.19[c]	0 ± 0[c]	0.7 ± 0.14[b]
	1	0[c]	0 ± 0[d]	0 ± 0[c]	0.63 ± 0.12[b]
Lepidium sativum	0	1.43[a]	1.43 ± 0.12[a]	1.43 ± 0.12[a]	1.43 ± 0.12[a]
	0.25	1.05[b]	1.1 ± 0.04[b]	0.83 ± 0.11[b]	0.76 ± 0.04[b]
	0.5	0.96[b]	1.02 ± 0.07[bc]	0.54 ± 0.03[c]	0.48 ± 0.05[c]
	0.75	0.58[c]	0.87 ± 0.03[c]	0.5 ± 0.05[c]	0.31 ± 0.02 cd
	1	0.35[d]	0.86 ± 0.01[c]	0 ± 0[d]	0.37 ± 0.02[d]
Raphanus sativus	0	2.05[a]	2.05 ± 0.25[a]	2.05 ± 0.25[a]	2.05 ± 0.25[a]
	0.25	2.12[a]	2.19 ± 0.11[a]	2.17 ± 0.02[a]	1.08 ± 0.19[b]
	0.5	1.72[a]	2.16 ± 0.45[a]	1.82 ± 0.42[a]	0.76 ± 0.1[c]
	0.75	1.77[a]	1.97 ± 0.52[a]	1.3 ± 0.1[b]	0.32 ± 0.08[d]
	1	0[b]	1.83 ± 0.03[a]	0.61 ± 0.05[c]	0.22 ± 0.11[d]

Values are means ± standard deviations ($n = 3$); means followed by the same letter in are not significantly different by the Student_Newman_Keuls test ($P \leq 0.05$)

Table 4 Inhibitory effect of Eucalyptus essential oils on growth of plants roots

Plant species	Dose (µl/ml)	*E. pimpiniana*	*E. polyanthemos*	*E. oleosa*	Control + (glyphosate)
Sinapis arvensis	C-	3.8 ± 0.72[a]	3.8 ± 0.72[a]	3.8 ± 0.72[a]	3.8 ± 0.72[a]
	0.25	2.67 ± 0.27[ab]	2.86 ± 0.12[b]	1.74 ± 0.11[b]	0.55 ± 0.05[b]
	0.5	2.21 ± 1.26[b]	2.41 ± 0.38[b]	0 ± 0[c]	0.5 ± 0.08[b]
	0.75	0.13 ± 0.23[c]	3.55 ± 0.09[a]	0 ± 0[c]	0.58 ± 0.08[b]
	1	0 ± 0[c]	1 ± 0[c]	0 ± 0[c]	0.46 ± 0.04[b]
Lepidium sativum	C-	4.12 ± 0.33[a]	4.12 ± 0.33[a]	4.12 ± 0.33[a]	4.12 ± 0.33[a]
	0.25	4.03 ± 0.46[a]	3.97 ± 0.21[a]	4.64 ± 0.65[a]	0.6 ± 0.05[b]
	0.5	4.05 ± 0.17[a]	3.69 ± 0.36[a]	3.25 ± 1.17[a]	2.06 ± 2.54[ab]
	0.75	1.19 ± 0.35[b]	2.5 ± 0.26[b]	0.85 ± 0.17[b]	0.39 ± 0.01[b]
	1	0.45 ± 0.05[c]	1.96 ± 0.39[b]	0.04 ± 0.07[b]	0.16 ± 0.03[b]
Raphanus sativus	C-	2.43 ± 0.11[a]	2.43 ± 0.11[a]	2.43 ± 0.11[a]	2.43 ± 0.11[a]
	0.25	2.4 ± 0.24[a]	3.14 ± 0.29[a]	2.41 ± 0.38[a]	0.53 ± 0.05[b]
	0.5	2.46 ± 0.3[a]	2.2 ± 0.13[b]	1.57 ± 0.57[ab]	0.43 ± 0.05[b]
	0.75	1.63 ± 0.11[b]	1.75 ± 0.35[b]	1.26 ± 0.25[bc]	0.24 ± 0.04[c]
	1	0 ± 0[c]	0.86 ± 0.68[c]	0.56 ± 0.6[c]	0.15 ± 0.05[c]

Values are means ± standard deviations ($n = 3$); means followed by the same letter in are not significantly different by the Student_Newman_Keuls test ($P \leq 0.05$)

the three out five fungal strains (Table 6). *Eucalyptus polyanthemos* oil exhibited a fungicidal activity on *F. oxysporum* 184 at the highest concentration (10 µl/ml). Overall, *F. oxysporum* and *F. oxysporum f. matthioli* were more tolerant of the three tested EOs.

Discussion

In this report, we describe the chemical composition of *E. pimpiniana* and *E. polyanthemos* essential oils and

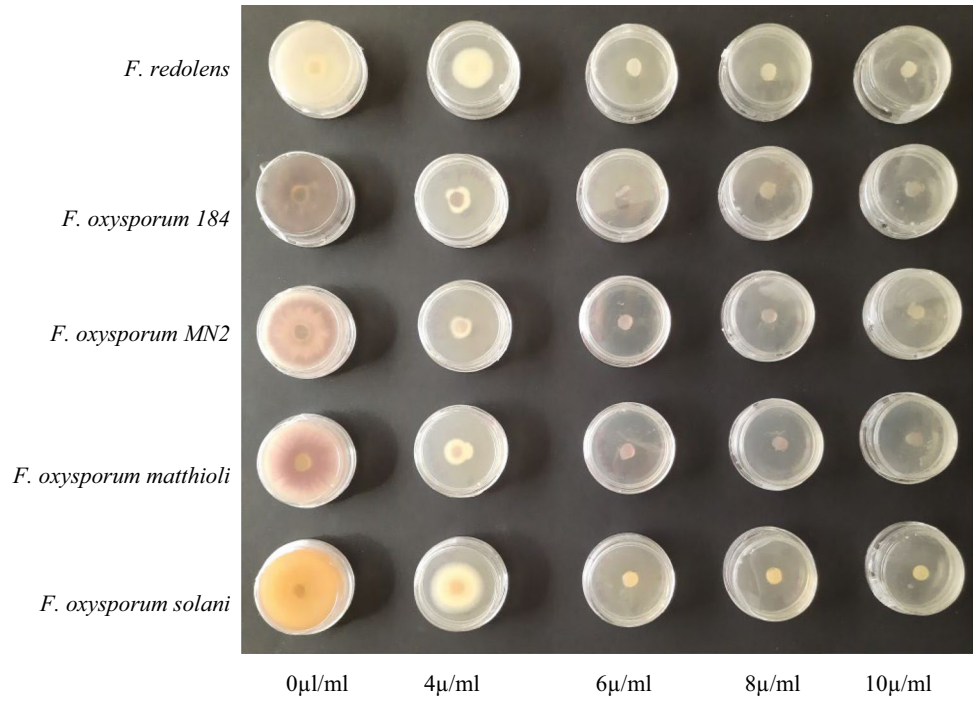

Fig. 2 Inhibition of fungal mycelia growth by *E. oleosa* essential oil at increasing concentrations (left to right) from 0 to 10 µl/mL after seven days of incubation

Table 5 Inhibition of fungal species in agar diffusion plate assay by essential oils from three *Eucalyptus* species

		Mycelial growth inhibition (%)				
Eucalyptus species	EOs (µl/ml)	*Fusarium oxysporum solani*	*Fusarium oxysporum matthioli*	*Fusarium oxysporum* MN-2	*Fusarium oxysporum* 184	*Fusarium redolens*
Eucalyptus polyanthemos	4	54.95^{cA}	63.06^{cA}	51.37^{bB}	64.22^{bA}	65.45^{cA}
	6	59.45^{bcB}	73.39^{bB}	100^{aA}	100^{aA}	70.90^{bB}
	8	64.86^{bB}	76.14^{abB}	100^{aA}	100^{aA}	73.63^{bB}
	10	72.97^{aB}	80.73^{aB}	100^{aA}	100^{aA}	100^{aA}
Eucalyptus pimpiniana	4	48.64^{bA}	41.46^{cB}	56.84 ± 0.32^{bB}	74.20^{bA}	52.64^{bA}
	6	59.45^{bB}	62.05^{bC}	100^{aA}	100^{aA}	66.36^{aB}
	8	89.18^{aA}	66.26^{bC}	100^{aA}	100^{aA}	71.81^{aB}
	10	100^{aA}	79.81^{aC}	100^{aA}	100^{aA}	73.63^{aB}
Eucalyptus Oleosa	4	45.04^{bA}	67.26^{bA}	89.08 ± 0.63^{aA}	80.15^{aA}	55.82^{bA}
	6	100^{aA}	100^{aA}	100^{aA}	100^{aA}	100^{aA}
	8	100^{aA}	100^{aA}	100^{aA}	100^{aA}	100^{aA}
	10	100^{aA}	100^{aA}	100^{aA}	100^{aA}	100^{aA}

Values are means ± standard deviations ($n = 3$); means followed by the same letter in are not significantly different by the Student Newman Keuls test ($P \leq 0.05$)

Table 6 Fungistatic and fungicidal activities of *Eucalyptus* essential oils on mycelia growth of 5 *Fusarium* strains

	Fusarium oxysporum solani		*Fusarium oxysporum matthioli*		*Fusarium oxysporum MN2*		*Fusarium oxysporum 184*		*Fusarium redolens*	
	CMI	CMF	CMI	CMF	CMI	CMF	CMI	CMF	CMI	CMF
Eucalyptus pimpiniana	10	10	> 10	> 10	6	6	6	6	> 10	> 10
Eucalyptus polyanthemos	> 10	> 10	> 10	> 10	6	> 10	6	> 10	10	> 10
Eucalyptus oleosa	6	8	6	8	6	6	6	6	6	8

compare them to *E. oleosa*, which grows in the same region. Few studies on *E. oleosa* essential oil exist in the literature, and only one study has been published that is comparable to our results (Ben Marzoug et al. 2011). These authors identified 38 compounds accounting for 99.1% of the essential oil isolated from the leaves of *E. oleosa*. Their results further showed that the oil contained about twice the amount of sesquiterpenes compared to monoterpenes (44.3% and 28.7%, respectively). The main oxygenated sesquiterpenes were spathulenol (16.1%) and γ-eudesmol (25.0%), followed by the monoterpenes *p*-cymene (10.6%), 1,8-cineole (8.7%), *p*-cymen-8-ol (4.4%), *cis*-sabinol (4.2%), *p*-cymen-7-ol (4.0%) and verbenone (3.7%). In our study, the oil contains the same compounds, but in very different proportions. These differences depend on various factors, such as geographical origin, soil conditions, harvesting period, and the physiological state of the plant.

According to the DPPH and ABTS assay results, moderate antioxidant activities were observed for the three essential oils, with some differences depending on the species. *E. oleosa* EO exhibited the highest DPPH antiradical activity ($IC_{50} = 92.179 \pm 2.484$ mg/ml), which is lower than the reference antioxidant, vitamin C. Its antioxidant activity has been reported to be related to the monoterpene hydrocarbon content (Marzoug et al. 2010). The essential oil of *E. oleosa* leaves contains the highest level of monoterpenoids (10.7%), indicating a potential for having the highest antioxidant activity.

We found that *Eucalyptus* oils exhibited herbicidal activity for all three plants tested. Our results are consistent with previous work showing herbicidal effects of *Eucalyptus* essential oils against several weeds and crop plants (Batish et al. 2006; Singh et al. 2005). Thus, the compounds contained in *Eucalyptus* oils are strong candidates for use as natural herbicides. Nevertheless, the phytotoxic activity of *Eucalyptus* oils results in damage to some crops (Zengh and Li 1997). It is therefore essential to maximize the herbicidal activity of *Eucalyptus* oils against weeds while minimizing the negative impacts on crop growth. All the three studied species of *Eucalyptus* showed contain appreciable percentages of 1,8-cineole, a monoterpene with phytotoxic properties (Singh et al. 2006; Zhang et al. 2012; Romagni et al. 2000). However, based on our data, this compound alone cannot explain the differences in the inhibition of seed germination, root, and aerial part growth observed among the three different essential oils. In fact, no correlation was observed between 1,8-cineole contents and the inhibition of these plant-associated parameters. The greatest inhibition was obtained using *E. oleosa* essential oil, which has the lowest level of this compound, confirming that the observed herbicidal activities do not primarily depend on 1,8-cineole and further suggests that other minor compounds may be involved. In previous reports (De Martino et al. 2010; Nishida et al. 2005), many other monoterpene hydrocarbons earlier identified in eucalypt essential oils, such as α-pinene, β-pinene, and limonene, possess herbicidal activity. Recently and for the first time, the compound *trans*-pinocarveol was described by Li et al. (2020) for its herbicidal effect. In our study, these compounds are more abundant or present only in the eucalypt species that have the most active essential oils. In addition, we noted remarkable levels of oxygenated sesquiterpenes such as α-, β- and γ-eudesmol in both *E. oleosa* and *E. pimpiniana,* whereas they are absent in *E. polyanthemos.* These results strongly suggest that these constituents may confer herbicidal activities to essential oils. Moreover, the results of this study showed that the more active essential oil (*E. oleosa*) contains distinctive compounds, such as isospathulenol and cryptone, which are also known for their herbicidal effects (Fouad et al. 2015). All these findings confirm a synergy between the various constituents of essential oils for the observed phytotoxic effect.

To explain the mode of action of essential oils on the inhibition of germination and as reported in the literature, different physiological and biochemical mechanisms may be explained by the fact that these monoterpenes affect physiological processes, such as cell viability, enzyme activity, and organelle reduction following membrane rupture (Mahdavikia et al. 2017; Fagodia et al. 2017). Other studies have reported that essential oils cause reduced germination and seedling growth, as well as several metabolic alterations: e.g., altering the assimilation of nitrogen into amino acids, which in turn affects glutamine metabolism and, consequently, results in an excess of toxic ammonia that accumulates in the leaves. These symptoms are associated with oxidative stress and damage and decrease the efficiency of the photosynthetic apparatus and alter the photorespiratory pathway (Araniti et al. 2018).

The essential oils of the three *Eucalyptus* species resulted in antifungal activity for all five of the *Fusarium* strains tested: *F. oxysporum*, *F. oxysporum f. matthioli*, *F. oxysporum MN-2*, *F. oxysporum* 184, and *F. redolens*. Mycelial growth inhibition is known to be dependent on essential oil concentrations. The results of our study are consistent with those of previous studies that evaluated the antifungal activity of against various phytopathogenic fungi of essential oils of eucalyptus species with different chemical profiles. In earlier results, Katooli et al. (2011) evaluated whether *E. camaldulensis* EOs had a suppressive action on the growth of the mycelia of some postharvest pathogenic fungi and soil pathogenic fungi and reported complete inhibition of mycelial growth in *Pythium ultimum* and *Rhizoctonia solani* (J.G. Kühn) by all concentrations tested (25, 50, 75 and 100%). After an additional 30 days of incubation, complete inhibition of *Bipolaris sorokiniana* (Shoemaker) and *Colletotrichum gloeosporioides* (Penz. & Sacc.) occurred, but no inhibition was recorded for *Penicillium*

digitatum (Sacc.) and *Aspergillus flavus* (Link). Studies on *E. camaldulensis* EO antifungal activity against *Fusarium graminearum* (Schwabe) and *Fusarium sporotrichioides* (Sherb.) demonstrated a dose-dependent activity ranging between 0 and 34.1% for *F. sporotrichioides* and between 29.1 and 41.8% for *F. graminearum* (Mehani et al. 2014). In another study, the inhibitory activity of *E. camaldulensis* EO against several strains of species of phytopathogenic fungi showed mycelial growth inhibition up to 100% at concentration of 5 mg/ml against *F. oxysporum* (Schltdl.) and *Thanatephorus cucumeris* (A.B. Frank) and against *Chaetomium globosum* (Kunze) at 10 mg/ml Siramon et al. 2013). More recent work on eucalyptus species in Tunisia has shown that essential oils inhibit the growth of 8 phytopathogenic fungi and inhibition can be complete at a concentration of 4 µl/ml (Amri et al. 2023). Another study showed that *E. camaldulensis* EOs inhibited fungal growth with MICs ranging from 7 to 12 µl/ml depending on the fungal strain (Abo Elgat et al. 2020).

In this study, only *E. oleosa* showed fungicidal activity against all five *Fusarium* strains tested, whereas EOs obtained from *E. pimpiniana* were fungicidal only against two of the five fungi, *F. oxysporum* MN-2 and *F. oxysporum* 184. The EOs of *E. polyanthemos* and *E. pimpiniana,* at the four different concentrations studied, did not exhibit fungicidal activity toward *F. oxysporum f. matthioli* and *F. redolens*. Consequently, higher concentrations should be tested to determine the MFC of these oils against these two fungi. Disk bioassays revealed that the three essential oils inhibited fungal growth in a dose-dependent manner. There are many reports in the literature on the concentration-dependent antifungal activity of essential oil (Rana et al. 2011; Aguiar et al. 2014).

Based on the chemical composition of the different EOs isolated, and based on the literature, antifungal activity is ascribed to α-pinene, *trans*-pinocarveol, pinocarvone, aromadendrene, globulol, 1-epicubenol, eudesmols (α-, β- and γ-), and spathulenol. Many researchers have described these components as antifungal agents (Tan et al. 2016). They are mainly found in *E. oleosa* and *E. pimpiniana* which, accordingly, showed the highest antifungal activities. Compounds such as 4-terpineol, myrtenal, cumin aldehyde, phellandral, *p*-cymene, carvacrol, 1-epicubenol, γ-eudesmol, isospathulenol, cryptone, *p*-cumenol and isoamyl benzoate were found only in *E. oleosa* and could explain the significant antifungal effect observed for its EOs. However, synergistic effects with other compounds cannot be ignored.

Conclusion

This work reports to our knowledge for the first time the antifungal and potential herbicidal effects of essential oils obtained from the leaves of three different *Eucalyptus* species, *E. oleosa*, *E. pimpiniana*, and *E. polyanthemos* were tested against five phytopathogenic fungi and one weed and two cultivated species belonging to the same family. The essential oil of *E. oleosa,* in particular, was found to the most active against the tested fungal pathogens and reduced seed germination rate and inhibited seedling growth. This EO also had moderate antioxidant activity. The main constituents of the essential oil of *E. oleosa* are the terpenes 4-terpineol, myrtenal, cumin aldehyde, phellandral, *p*-cymene, carvacrol, 1-epicubenol, γ-eudesmol, isospathulenol and the non-terpene derivatives cryptone, *p*-cumenol and isoamyl benzoate. Hence, the possibility of using these essential oils as ingredients in the formulation of sustainable herbicides to reduce the use of potentially environmentally harmful synthetic pesticides should be pursued further. Essential oils as well as plant extracts have proven their antimicrobial powers in order to use them as a bioherbicide it would be interesting to verify their effects on the environment, cultivated crops, and the beneficial microorganisms of the soil. Employing novel biomolecules in biopesticide formulations will provide a less polluting approach and more sustainable weed and fungus management alternatives for growing food for the world's population.

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Author contributions IA and YM conceived and designed the experiments. HK, MS, YP and GF performed experiments and statistical analysis. IA, MS, HK and GF contributed reagents/material tools. HK, YM, IA, YP and GF analyzed the data. HK, IA, YM, AMH, and GF contributed to manuscript preparation and revision.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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