

Article

Antifungal and Phytotoxic Activities of Essential Oils: In Vitro Assays and Their Potential Use in Crop Protection

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Abstract: (1) Background: The use of natural products based on essential oils (EO) is nowadays arousing great interest as an alternative method to control plant pathogens and weeds. However, EO possess low bioavailability and are highly volatile, and their encapsulation in hydroxypropyl- β -cyclodextrin (HP- β -CD) could be a means to enhance their stability and maintain their bioactivity. Thus, the current study aims at investigating, in the presence and the absence of HP- β -CD, the antifungal and phytotoxic activities of nine EO, distilled from plant species belonging to Alliaceae, Apiaceae, and Cupressaceae families, with considerations for their chemical composition. (2) Methods: EO antifungal activity was assessed by direct contact and volatility assays against *Fusarium culmorum*, a major phytopathogenic fungi, while phytotoxic effects were evaluated against lettuce (*Lactuca sativa* L.) and rye-grass (*Lolium perenne* L.), by seedling's emergence and growth assays. (3) Results: These EO inhibit fungal growth in both direct contact and volatility assays, with half-maximal inhibitory concentrations (IC₅₀) ranging from 0.01 to 4.2 g L⁻¹, and from 0.08 up to 25.6 g L⁻¹, respectively. Concerning phytotoxicity, these EO have shown great potential in inhibiting lettuce (IC₅₀ ranging from 0.0008 up to 0.3 g L⁻¹) and rye-grass (IC₅₀ ranging from 0.01 to 0.8 g L⁻¹) seedlings' emergence and growth. However, the EO encapsulation in HP- β -CD has not shown a significant improvement in EO biological properties in our experimental conditions. (4) Conclusion: All tested EO present antifungal and phytotoxic activities, with diverse efficacy regarding their chemical composition, whilst no increase of their biological effects was observed with HP- β -CD.

Keywords: essential oils; bioassays; *Fusarium culmorum*; *Lactuca sativa* L.; *Lolium perenne* L.

1. Introduction

In recent years, there has been a clear tendency towards the utilization of alternative methods in agriculture for pest and disease control, food preservation, and weed control that are less damaging to the environment and human health. Among these methods, the use of essential oils (EO) is

arousing great interest for their bactericidal, virucidal, fungicidal, antiparasitical and insecticidal applications [1,2]. EO are indeed complex oily volatile liquids characterized by a strong odor and synthesized by all aromatic plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and root) as secondary metabolites and stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. They can contain 20 to 60 active compounds, responsible for their biological activities [3].

Both in the fields and during postharvest storage, cereals, fruits, and vegetables are highly susceptible to fungal spoilage. Food safety from spoilers and pathogens is an important worldwide public health concern, and microbial contamination is a vital factor not only causing food deterioration and shelf life reduction, but also resulting in economic losses and diseases [4,5]. Furthermore, weeds are another major biological constraint in agriculture. Indeed, these plants compete with crops for natural resources and space and result in reduced crop yields and productivity. The control of both fungal diseases and weeds is currently achieved through various methods, with a predominance of synthetic pesticides. However, the inappropriate or overuse of pesticides is known to result in environmental issues (groundwater and soil contamination, presence of toxic residues in agricultural products), noxious effects on human health, as well as leading to the development of herbicide resistance in weeds [6–8]. So as to overcome these issues, EO-based products may represent a natural alternative to replace synthetic fungicides and herbicides and stand as potential bio-pesticides [3,9]. Nevertheless, EO have low aqueous solubility and bioavailability, which could be challenging for biological application. This drawback could be overcome by the use of cyclodextrins (CD), which are cyclic oligosaccharides possessing a hydrophilic surface and a hydrophobic cavity that allows the encapsulation of hydrophobic guests in aqueous solutions [10,11]. Hydroxypropyl- β -cyclodextrin (HP- β -CD-degree of substitution 5.6), a β -cyclodextrin derivative, is cited in the Food and Drug Administration's list of Inactive Pharmaceutical Ingredients [12]. In that matter, encapsulation in CD may be of interest to formulate oily substances into solid dosage, or to replace organic solvents or surfactants in liquid formulations [10], to avoid degradation and obtain a better persistence of EO's effects in time [13,14]. Notably, the encapsulation in CD of EO coming from clove (*Eugenia caryophyllata* L. Merr. and Perry) and Mexican oregano (*Lippia berlandieri* Schauer) was of interest in enhancing the antifungal activity against *Fusarium oxysporum* [15]. Similarly, encapsulated cinnamon leaf (*Cinnamomum verum* J. Pres) and oregano (*Origanum vulgare* L.) EO [16], as well as EO derivatives coming from cinnamon and clove, displayed higher efficiency against *Botrytis* sp. [17]. On another note, the inclusion of EO distilled from peppermint (*Mentha $\times$ piperita* L.), caraway (*Carum carvi* L.) and calamus (*Acorus calamus* L.) in CD exerted phytotoxic properties against maize (*Zea mays* L.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) and lambsquarters (*Chenopodium album* L.) [18].

Among fungal phytopathogens, *Fusarium* genus is widely studied not only for causing plant diseases such as Fusarium head blight and Fusarium crown rot—two major diseases occurring on wheat and responsible for considerable yield loss [19,20]—but also for producing highly toxic secondary metabolites, mycotoxins, that lead to the contamination of the grain harvested from infected ears and cause potential risk to human and animal health [21–23]. Most of the time, EO antimicrobial effects are evaluated by direct contact, with EO directly incorporated in the culture medium. Even so, several references have shown an interest of using volatility assays, where the accuracy of such assays has been demonstrated as more reliable and the efficiency of smaller compounds such as monoterpenes was greater [24–26]. In addition, the reliability of direct contact assays is sometimes compromised by the low solubility in agar media of EO compounds or by the addition of solvents [27].

Allium species such as garlic (*Allium sativum* L.), onion (*Allium cepa* L.), chive (*Allium schoenoprasum* L.), leek (*Allium porum* L.), and shallot (*Allium ascalonicum* L.) are cultivated and consumed in many Asian and European countries and known for their medicinal properties, especially from their organosulfur compounds [28]. In the same way, Apiaceae such as chervil (*Anthriscus cerefolium* L.) and angelica (*Angelica archangelica* L.) are used for flavoring and in folk medicine, while thuja (*Thuja plicata atrovirens* L.), a Cupressaceae species, is mainly used for cosmetic applications [29,30]. However, despite

food and cosmetics applications, little attention has been paid to the biological properties of these EO regarding their potential in crop protection and scarce are the studies targeting phytopathogenic species such as *Fusarium culmorum*. Moreover, to the best of our knowledge, despite the potential benefits of encapsulation, the biological activities of these EO encapsulated in HP- β -CD have not previously been investigated.

In this way, the present study aims to evaluate in vitro antifungal and phytotoxic activities of the nine EO distilled from different plant species belonging to Alliaceae, Apiaceae, and Cupressaceae families, with concerns for the potential of CD encapsulation.

2. Materials and Methods

2.1. Essential Oils

Essential oils tested in the current study were kindly provided by Ferrant PHE (France), an EO producer company [31]. The purchase of the raw material and the distillation procedure were carried out by the EO producer. Flowering aerial parts of chervil (*A. cerefolium*) as well as bulbs of chive (*A. schoenoprasum*), garlic (*A. sativum*), leek (*A. porum*), onion (*A. cepa*) and shallots (*A. ascalonicum*), harvested at fruit maturity, were used as fresh material, and were purchased by the EO producer from food companies and farmers in the north of France (Hauts-de-France region). Prior to distillation, plant material was mechanically shredded into small pieces by blades, to facilitate the distillation process [28]. Distillation occurred through a water-steam entrainment process, on a Clevenger-type apparatus (3-h cycle, saturated water steam-0.3 bar).

Roots or seeds of angelica (*A. archangelica*) grown by the producer and harvested at flowering stage, were distilled through a similar process (steam-distillation unit, 14 m³ tank-saturated water steam, 0.3 bar), during a 3-h cycle, until no more EO was recovered. Thuja (*T. plicata atrovirens*) crushed twigs and fresh leaves were also distilled through the same process. No specific solvent was added in the hydrodistillation process.

2.2. Determination of the EO Chemical Composition

Previously diluted in ethyl acetate (ratio 1:200 (v/v)), EO were analyzed by gas chromatography (using FID as a detector) and by gas chromatography–mass spectrometry (Shimadzu QP 2010 Ultra). Similar operating conditions were employed as follows: the system was operated using helium as a carrier gas at a constant linear velocity (60 cm/s) and 0.2 μ L of the sample was injected in a split mode (split ratio 1:10, injector at 260 °C) in a Zebron ZB-5MS (5% phenyl-arylene/95% dimethylpolysiloxane-10 m length, 0.10 mm inner diameter, 0.10 μ m phase thickness) fused silica capillary column (Phenomenex). The column temperature was linearly programmed from 60 °C (held for 2 min) to 280 °C (held for 1 min) at a rate of 40 °C/min. The transfer line was heated at 280 °C. Mass spectra were acquired in the electronic impact mode (EI at 70 eV) in an m/z range of 35–350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in the literature [32] and supplemented by those listed in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley 275 computer libraries. The Kovats indices were determined from the retention times after co-injection with n-alkanes. Relative percentages of oil constituents were calculated from the GC peak areas. Analyses were led in triplicates.

2.3. Determination of the Retention Capacity of EO by HP- β -CD

The retention capacity of HP- β -CD was quantified by static headspace-gas chromatography (SH-GC), by comparing the sum of the chromatographic peak areas of EO components in the presence of CD with the blank experiments. This analysis was performed according to [10]. EO were added to 10 mL of either water or aqueous CD solutions, placed in 22 mL headspace glass vials. Vials were then sealed, using silicone septa and aluminum foil, and thermostated at 25 $\pm$ 0.1 °C. After the establishment of the equilibrium between aqueous and gaseous phases (30 min), 1 mL of vapor

present in the upper part of the vial, above the solution, was withdrawn from the vial by using a gas-tight syringe and injected directly into the chromatographic column via a transfer line (250 °C). All measurements were conducted with a headspace autosampler (Agilent, Les Ulis, France) and an Autosystem XL (Perkin Elmer, Courtaboeuf, France), equipped with a FID detector, using a DB624 column (6% cyanopropylphenyl/94% dimethyl-polysiloxane, bonded and crosslinked phase) (length (30 m), diameter (0.53 mm) and phase thickness (3 µm)). Temperature conditions were as follows: initial temperature of 50 °C for 2 min, increased to 200 °C at a constant rate of 5 °C/min. The total runtime for a sample was 32 min, with nitrogen used as a carrier gas. The percentage of retention (r) of EO by HP-β-CD was determined by SH-GC at 25 °C for a 10 mM CD solution and expressed as follows:

$$r (\%) = (1 - (\Sigma A_{CD}/\Sigma A_0)) \times 100 \quad (1)$$

where ΣA_0 and ΣA_{CD} stand for the sum of peak areas of each EO component in the absence and the presence of CD, respectively.

For each EO, in the presence or absence of HP-β-CD, measurements were done in triplicates.

2.4. Biological Activities of EO

2.4.1. EO Antifungal Activity

Antifungal activities of the different EO were tested against *F. culmorum* by using two in vitro complementary methods: direct contact (mycelial radial growth inhibition assay) and volatility assays in a completely randomized experimental design.

Direct contact assay: antifungal activity by direct contact was assessed according to [33,34], followed with slight modifications. Mycelial discs of *F. culmorum* (0.9 cm), from the edge of a 7-day-old culture fungal colony, were cut and placed at the center of a 9 cm Petri dish containing potato dextrose agar (PDA) culture medium (40 g L⁻¹). EO were mixed with PDA medium at 50 °C in order to obtain final concentrations ranging from 0.02 to 2.5% of EO in the medium. These latter were previously sterilized by filtration and prepared in 1% ethanolic solution. Direct contact assay was performed in the absence and the presence of HP-β-CD, at a final concentration of 10 mM, in the medium. Analyses were led in triplicates. Samples were incubated for 7 days at 20 °C, and subsequently, the radius of mycelium growth was measured. The inhibition rate (IR) was calculated following the formula:

$$IR (\%) = (X_0 - X_i)/X_0 \times 100 \quad (2)$$

where X_0 = average diameter of the fungal colony in control; X_i = average diameter of the fungal colony in treatment.

Aqueous solutions of ethanol 1% (v/v) or HP-β-CD (10 mM) were tested as negative controls. A commercial fungicide, Aviator XPro, was simultaneously evaluated as a positive one. Graphical interpolation analysis was used to calculate the half-maximal inhibitory concentration (IC₅₀) value.

Volatility assay: antifungal activity by volatility was assessed according to [35], followed with minor modifications. Mycelial discs of *F. culmorum* (0.9 cm), from the edge of a 7-day-old culture fungal colony, were cut and placed at the center of a 5.5 cm Petri dish culture medium plate with PDA. This plate was stuck with PDA into a 9 cm Petri dish that contained EO solutions. These latter were previously sterilized by filtration and prepared in both ethanolic solution and HP-β-CD (10 mM). EO concentrations used ranged from 0.01 to 5%. Analyses were led in triplicates. Samples were incubated for 5 days at 20 °C, and subsequently, the radius of mycelium growth was measured. The inhibition rate (IR) was calculated using Equation (2).

Aqueous solutions of ethanol 1% (v/v) or HP-β-CD (10 mM) were tested as negative controls. A commercial fungicide, Aviator XPro (Stolz, Wailly-Beaucamp, France), was simultaneously evaluated as a positive one. Graphical interpolation analysis was used to calculate the half-maximal inhibitory concentration (IC₅₀) value.

2.4.2. EO Phytotoxicity Assessment

Lolium perenne L. and *Lactuca sativa* L., one monocotyledon and one dicotyledon plant, respectively, commonly used in phytotoxicity assays and listed in the Organisation for Economic Co-operation and Development (OECD) guidelines (2003) for the assessment of chemicals, were used for an in vitro bioassay. The effects of the different EO were evaluated on seedlings' emergence and growth of the two plant species in a completely randomized experimental design, by using an adapted in vitro method [36–38].

EO were first mixed with agar medium at 50 °C in order to obtain final concentrations ranging from 0.005 to 0.5% of EO in the medium. Seed samples were then placed on agar medium, in sealed Petri dishes and incubated for 8 days on a day/night cycle, with a 16-h photoperiod at 20 °C and an obscurity period of 8 h at 16 °C. After the incubation period, germination rates were evaluated by counting germinated seeds and root elongation was assessed through an imaging software (ImageJ), by measuring root length [38,39]. These in vitro assays were performed in the absence and the presence of HP- β -CD, at a final concentration of 10 mM, in an agar medium. Analyses were led in triplicates. Aqueous ethanolic and HP- β -CD solutions (10 mM) were tested as negative controls, whereas Glyphosate (isopropylamine salt) was used as a positive control.

Graphical interpolation analysis was used to calculate the half-maximal inhibitory concentration (IC₅₀) value, regarding both germination and root elongation parameters.

2.5. Statistical Analysis

Statistical analyses were performed using XLSTAT 2018.1.1 (Adinsoft, Paris, France) software and R 3.6.1 (R core Team, Vienna, Austria, 2019) [40]. Shapiro–Wilk and Bartlett tests were used prior to any statistical analysis to verify the normality of the distribution and the homoscedasticity, respectively. Non-normal data were “square-root” or “log₁₀” transformed. Statistical significance between retention percentages has been analyzed using one-way ANOVA, complemented with a post-hoc Tukey–HSD test. For both antifungal and phytotoxicity assays, the IC₅₀ resulted from non-linear regression analyses from triplicate trials and was expressed with mean values and standard deviations (mean $\pm$ SD). Statistical significance of the results was evaluated by two-way ANOVA (Analysis of Variance), with the absence or presence of CD and the “EO donor plant” as fixed factors, complemented with a post-hoc paired comparison (Tukey's HSD–honestly significant difference). Moreover, a principal component statistical analysis (PCA) based on the Pearson correlation matrix, has been carried out using XLSTAT, to identify the possibly existing correlations between the different EO and to investigate the separation between EO samples according to their main chemical constituents. Prior to the PCA, the variables were standardized for a normalized PCA. The PCA was then carried out on centered and reduced GC–MS data from all tested EO, using identified compounds with relative abundance higher than 0.1%.

3. Results

3.1. Determination of the EO Chemical Composition

The EO chemical composition revealed that EO from thuja and angelica (root and seed) contained mostly terpenes. Chervil EO was particularly rich in eugenol (52%) and estragole (42%). Garlic, leek, shallots, onion, and chive EO all mostly contained organosulfur compounds. On the one hand, garlic EO was dominated by allyl polysulfides, while, on the other hand, leek, shallots, onion and chive EO were characterized by propyl polysulfides (Table 1).

Table 1. Relative chemical composition of the 9 tested EO.

	A. Root ^a	A. Seed ^b	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja
α -Thujene	-	-	-	-	-	-	-	-	0.45 $\pm$ 0.006
Methyl propyl disulfide	-	-	-	3.5 $\pm$ 0.3	-	3.6 $\pm$ 0.02	2.8 $\pm$ 0.09	6.5 $\pm$ 0.1	-
α -Pinene	21.4 $\pm$ 1.1	11.4 $\pm$ 0.2	-	-	-	-	-	-	2.5 $\pm$ 0.06
2,5-dimethyl thiophene	-	-	-	-	-	-	-	1.7 $\pm$ 0.06	-
Camphene	0.8 $\pm$ 0.003	-	-	-	-	-	-	-	5.7 $\pm$ 0.3
Sabinene	5.4 $\pm$ 0.05	1.4 $\pm$ 0.01	-	-	-	-	-	-	-
Allyl propyl disulfide	-	-	-	-	-	-	-	6.5 $\pm$ 0.2	-
β -Pinene	1.2 $\pm$ 0.1	1.0 $\pm$ 0.3	0.1 $\pm$ 0.001	-	-	-	-	-	0.2 $\pm$ 0.001
β -Myrcene	4.0 $\pm$ 0.06	3.6 $\pm$ 0.09	-	-	-	-	-	-	0.8 $\pm$ 0.1
α -Phellandrene	8.9 $\pm$ 0.8	1.6 $\pm$ 0.05	-	-	-	-	-	-	-
δ -3-Carene	22.9 $\pm$ 1.0	-	-	-	-	-	-	-	0.6 $\pm$ 0.005
trans- β -Ocimene	0.9 $\pm$ 0.2	1.4 $\pm$ 0.02	-	-	-	-	-	-	1.8 $\pm$ 0.1
D-Limonene	7.5 $\pm$ 0.6	-	0.1 $\pm$ 0.003	-	-	-	-	-	2.1 $\pm$ 0.3
β -Phellandrene	16.5 $\pm$ 1.3	67.2 $\pm$ 1.6	-	-	-	-	-	-	-
cis- β -Ocimene	6.4 $\pm$ 0.3	4.9 $\pm$ 0.2	-	-	-	-	-	-	-
γ -Terpinene	1.4 $\pm$ 0.03	-	-	-	-	-	-	-	0.2 $\pm$ 0.001
Diallyl disulfide	-	-	-	-	29.3 $\pm$ 1.6	-	-	-	-
Terpilonene	1.0 $\pm$ 0.02	-	-	-	-	-	-	-	-
Undecane	-	-	3.62 $\pm$ 0.1	-	-	-	-	-	-
Dipropyl disulfide	-	-	-	43.4 $\pm$ 1.8	-	50.4 $\pm$ 1.9	35.5 $\pm$ 2.3	23.7 $\pm$ 0.9	-
3-Thujanone	-	-	-	-	-	-	-	-	71.5 $\pm$ 3.2
Disulfide, bis(1-methylethyl)	-	-	-	7.9 $\pm$ 0.8	-	4.5 $\pm$ 0.6	6.7 $\pm$ 1.5	6.8 $\pm$ 1.3	-
α -Thujone	-	-	-	-	-	-	-	-	10.6 $\pm$ 1.0
Allyl methyl disulfide	-	-	-	-	3.1 $\pm$ 0.05	-	-	-	-
Methyl propyl trisulfide	-	-	-	8.1 $\pm$ 1.1	-	6.4 $\pm$ 0.5	9.1 $\pm$ 0.8	20.5 $\pm$ 1.8	-
1,2-Dithiolane	-	-	-	-	2.9 $\pm$ 0.2	-	-	1.0 $\pm$ 0.1	-
Terpinen-4-ol	-	1.0 $\pm$ 0.1	-	-	-	-	-	-	2.9 $\pm$ 0.06
Isothujol	-	-	-	-	-	-	-	-	0.2 $\pm$ 0
Estragole	-	-	42.0 $\pm$ 0.3	-	-	-	-	-	-
Dimethyl tetrasulfide	-	-	-	-	-	-	-	1.5 $\pm$ 0.03	-
1-Propenyl propyl disulfide	-	-	-	-	-	0.4 $\pm$ 0.06	-	-	-
Camphanol acetate	0.8 $\pm$ 0.07	-	-	-	-	-	-	-	-
α -limonene diepoxide	-	-	0.2 $\pm$ 0.04	-	-	-	-	-	0.2 $\pm$ 0.005
Oxalic acid	-	-	0.3 $\pm$ 0.006	-	-	-	-	-	-
Diallyl trisulfide	-	-	-	-	24.2 $\pm$ 0.9	-	-	-	-

Table 1. Cont.

	A. Root ^a	A. Seed ^b	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja
Dipropyl trisulfide	-	-	-	25.6 ± 1.3	-	20.1 ± 0.5	17.6 ± 0.6	13.9 ± 0.4	-
Allyl n-propyl sulfide	-	-	-	-	-	2.0 ± 0.05	3.3 ± 0.08	2.6 ± 0.07	-
trans-3,5-Diethyl-1,2,4-trithiolane	-	-	-	-	-	0.8 ± 0.004	2.3 ± 0.05	-	-
α-terpinyl acetate	-	0.6 ± 0.09	-	-	-	-	-	-	0.4 ± 0
1,2,4-Trithiolane, 3,5-diethyl-	-	-	-	-	-	2.1 ± 0.02	2.7 ± 0.06	2.4 ± 0.03	-
Allyl-propyl trisulfide	-	-	-	-	0.2 ± 0	-	-	-	-
Copaene	0.9 ± 0.05	2.0 ± 0.08	0.1 ± 0	-	-	-	-	-	-
Allyl-methyl trisulfide	-	-	-	-	2.13 ± 0.06	-	0.8 ± 0.07	-	-
Methyl 2-propenyl disulfide	-	-	-	-	1.5 ± 0.04	-	-	-	-
Methyl isoeugenol	-	-	1.1 ± 0.1	-	-	-	-	-	-
Methyl isopropyl disulfide	-	-	-	0.9 ± 0.05	-	0.6 ± 0.08	3.4 ± 0.1	6.1 ± 0.3	-
Methyleugenol	-	-	51.7 ± 1.8	-	-	-	-	-	-
cis-1-Propenyl propyl trisulfide	-	-	-	-	-	0.4 ± 0.2	-	-	-
Caryophyllene	-	-	0.2 ± 0	0.7 ± 0.05	-	0.6 ± 0.1	-	-	-
β-Germacrene	-	0.8 ± 0.04	-	-	-	-	-	-	-
δ-Germacrene	-	0.6 ± 0.03	-	-	-	-	-	-	-
Humulene	-	1.8 ± 0.2	-	-	-	-	-	-	-
β-Copaene	-	0.7 ± 0.1	-	-	-	-	-	-	-
Hexadecane	-	-	0.4 ± 0.03	-	-	-	-	-	-
Diallyl tetrasulfide	-	-	-	-	22.3 ± 0.5	-	-	-	-
Dipropyl tetrasulfide	-	-	-	5.8 ± 0.8	-	3.8 ± 0.1	11.0 ± 0.6	5.8 ± 0.3	-
Methyl propyl tetrasulfide	-	-	-	0.8 ± 0.2	-	-	-	-	-
1-Propyl-2-(4-thiohept-2-en-5-yl) disulfide	-	-	-	1.8 ± 0.09	0.9 ± 0.1	1.2 ± 0.1	0.94 ± 0.06	-	-
1-(1-Propenyl)-2-(4-thiohept-5-yl) disulfide	-	-	-	0.5 ± 0.06	-	0.4 ± 0.04	1.2 ± 0.03	-	-
8-Ethyl-4,5,6,7,9-pentathiadecane	-	-	-	-	-	0.3 ± 0.01	-	-	-
4-Methyl-1,2,3,5,6-pentathiepane	-	-	-	-	0.9 ± 0.07	-	-	-	-
2,4-Dimethyl-5,6-dithia-2,7-nonadienal	-	-	-	1.1 ± 0.03	-	0.7 ± 0.1	1.2 ± 0.05	1.0 ± 0.06	-
6-Ethyl-4,5,7,8-tetrathianonane	-	-	-	-	-	1.5 ± 0.03	1.7 ± 0.04	-	-
Diallyl sulfide	-	-	-	-	9.9 ± 0.5	-	-	-	-
Phytol	-	-	0.2 ± 0	-	-	-	-	-	-
(Z)-1-propenyl propyl tetrasulfide	-	-	-	-	-	0.3 ± 0	-	-	-
Cyclooctasulfur	-	-	-	-	2.7 ± 0.3	-	-	-	-

Values are presented as percentages and depicted as mean ± standard deviation. ^a A. Root: angelica root EO. ^b A. Seed: angelica seed EO.: not detected.

The PCA analysis results displayed a high correlation coefficient corresponding to EO having a highly similar chemical composition and vice versa. Our results have shown that chive, leek, onion and shallot EO are strongly correlated (correlation coefficient ranging from 0.814 to 0.982, data not shown). Garlic EO, which also consists of organosulfur compounds, is not correlated to the previously mentioned EO (correlation coefficients ranging from 0.066 to 0.095). Angelica seed and root EO are also significantly positively correlated, but with a lesser correlation coefficient (0.52). Finally, thuja and chervil EO are not correlated to any other EO (correlation coefficients respectively ranging from 0.021 to 0.059, and from 0.032 to 0.064).

These conclusions are also visible on the correlation circles obtained with the PCA analysis (Figure 1). The projection has been carried out on three different axes, due to the fact that the first two principal components, F1 and F2, represent 59% of the initial variability of the data. In order to avoid misinterpretation, the two additional projections were executed. The results have shown that chive, leek, onion, and shallot EO are strongly correlated, whatever the projection. Angelica seed and root EO are correlated, when projected on F1 and F2 axes (Figure 1A), but this observation is not visible on other correlation circles (Figure 1B, C).

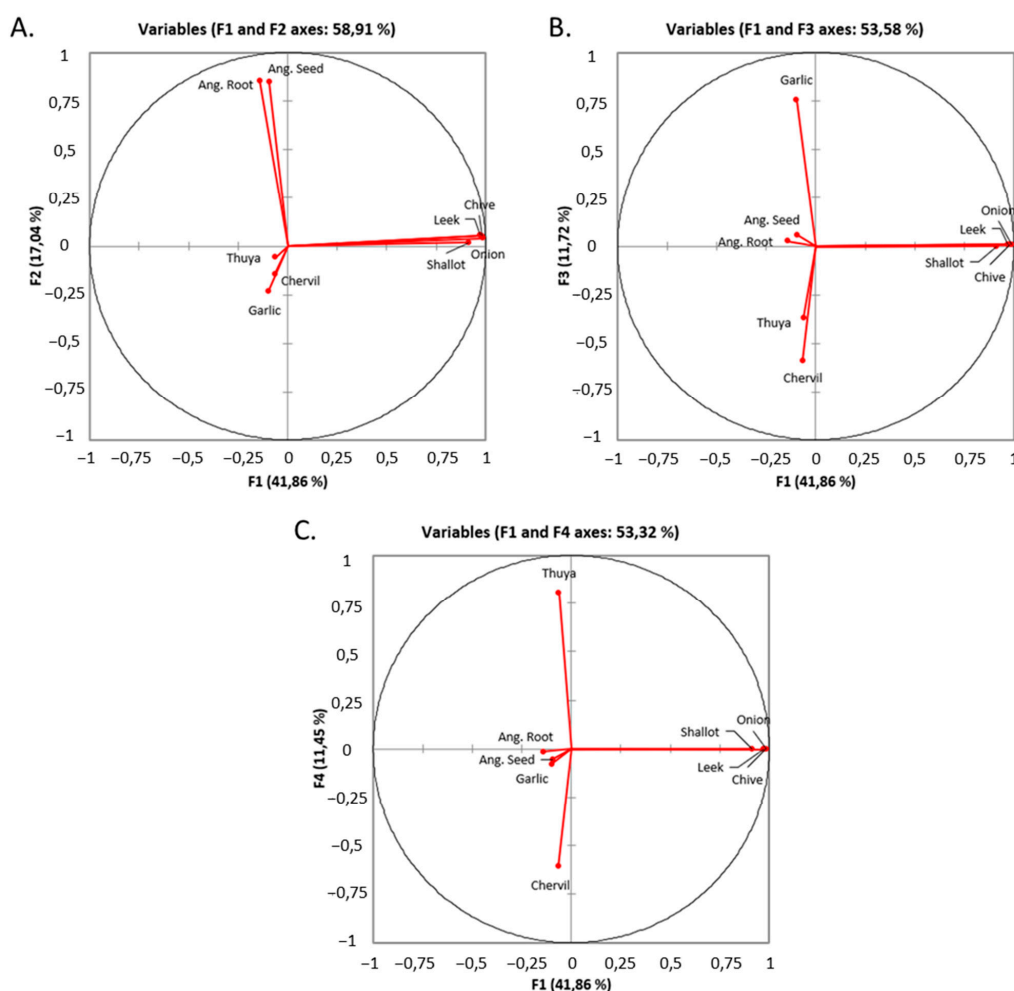


Figure 1. Correlation circles from the principal component statistical analyses (PCA) on the 9 tested essential oils (EO), with projections on F1 and F2 axes (A), on F1 and F3 axes (B) and on F1 and F4 axes (C).

3.2. Determination of the Retention Capacity of EO by HP- β -CD

All the EO evaluated in this study were able to be encapsulated in HP- β -CD. EO retention percentages by HP- β -CD are listed in Table 2.

Table 2. EO's percentages of retention in the presence of HP- β -CD.

	A. Root ^A	A. Seed ^B	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja
Retention percentage (%)	61 $\pm$ 1.9 b	27 $\pm$ 3.4 e	46 $\pm$ 2.1 cd	54 $\pm$ 1.7 c	78 $\pm$ 4.2 a	62 $\pm$ 5.7 b	71 $\pm$ 3.6 a	43 $\pm$ 2.8 d	60 $\pm$ 1.8 b

Values are depicted as mean $\pm$ standard deviation. ^A: A. Root: angelica root EO. ^B: A. Seed: angelica seed EO.

Among the retention percentages obtained, angelica seed's EO is the one with the lowest value. All the other EO have retention percentages ranging from 43 to 80%, with the highest retention value obtained for garlic EO. Essential oils are more efficiently retained by HP- β -CD when the retention percentage obtained is higher.

3.3. Biological Activities of EO

3.3.1. EO Antifungal Activity

The results presented in Table 3 have shown that all the tested EO presented antifungal properties against the phytopathogenic agent, *F. culmorum*, by both direct contact and volatility assays.

Direct contact assay: IC₅₀ obtained by direct contact varied from 0.01 to 4.2 g L⁻¹ for shallot EO and angelica root EO, respectively. Shallot EO has demonstrated a higher potential in terms of antifungal activity by direct contact assay, by presenting a significantly lower IC₅₀ in comparison with the other EO. The IC₅₀ are in the following order: IC₅₀ Shallot EO < IC₅₀ Onion EO < IC₅₀ A. seed EO < IC₅₀ Chive EO < IC₅₀ Leek EO < IC₅₀ Garlic EO < IC₅₀ Chervil EO < IC₅₀ Thuja EO < IC₅₀ A. root EO. On the other hand, no significant differences were observed between the IC₅₀ obtained in the presence and the absence of HP- β -CD. In the presence of HP- β -CD, shallot EO presented the lowest IC₅₀ (0.03 g L⁻¹) and angelica root EO the highest (5.8 g L⁻¹, Table 3). In comparison with the positive control, the obtained IC₅₀ for the tested EO are about 100 to 1000 times higher.

Volatility assay: IC₅₀ obtained by volatility assay ranged from 0.08 g L⁻¹ for shallot EO to 25.6 g L⁻¹ for leek EO. Once again, shallot EO has shown a greater potential antifungal activity by volatility assay in comparison with the other EO tested. The IC₅₀ increase in the following order: IC₅₀ Shallot EO < IC₅₀ A. seed EO < IC₅₀ A. root EO < IC₅₀ Onion EO < IC₅₀ Chervil EO < IC₅₀ Thuja EO < IC₅₀ Chive EO < IC₅₀ Garlic EO < IC₅₀ Leek EO. In the same way, HP- β -CD did not show any beneficial effect on obtained IC₅₀. IC₅₀ obtained by volatility assay, in the presence of HP- β -CD, ranged from 0.09 to 7.3 g L⁻¹ for shallots and angelica root EO, respectively. As for the raw EO, IC₅₀ obtained for the shallot EO, which is significantly lower, depicts a greater potential in terms of antifungal activity (Table 3). As the positive control is intended for use as a contact fungicide, the determination of an IC₅₀ regarding volatility effects was not possible.

3.3.2. EO Phytotoxicity Assessment

Various responses from the two plant species to the EO have been highlighted. Nevertheless, all tested EO have shown a phytotoxic activity, either by inhibiting seed germination or by affecting root elongation.

Seedlings' emergence assay: Regarding seedlings' emergence, most of the tested EO exerted a significant inhibitory effect (except for angelica root EO and thuja EO in the presence of HP- β -CD) on lettuce germination by suppressing 100% of the germination, at least at the highest tested concentration. In the same way, a significant germinative inhibitory effect of the tested EO has also been demonstrated on rye-grass, except for garlic and angelica root EO (data not shown). Indeed, angelica seed and onion EO have shown great potential in inhibiting lettuce seed germination, with a significant effect at the lowest EO concentration tested. In terms of IC₅₀, among the nine tested EO, only thuja and angelica root EO have shown a significantly lower anti-germinative potential (Table 4). The IC₅₀ increase in the following order: IC₅₀ A. seed EO < IC₅₀ Leek EO < IC₅₀ Shallot EO < IC₅₀ Onion EO < IC₅₀ Garlic EO < IC₅₀ Chervil EO < IC₅₀ Chive EO < IC₅₀ Thuja EO < IC₅₀ A. root EO.

Table 3. IC50 of antifungal activity of EO obtained by both direct contact assay and volatility assay.

		A. Root	A. Seed	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja	Positive Control
Direct Contact assay	IC50 (g L ⁻¹) without HP-β-CD	4.2 ± 0.1 b	0.3 ± 0.003 ij	3.1 ± 0.04 d	0.9 ± 0.007 g	1.6 ± 0.1 f	1.2 ± 0.03 g	0.1 ± 0.002 jkl	0.01 ± 0.0003 l	3.6 ± 0.02 c	0.0010 ± 0.0006 l
	IC50 (g L ⁻¹) with HP-β-CD	5.8 ± 0.1 a	0.2 ± 0.001 ij	1.9 ± 0.001 e	0.3 ± 0.009 ij	0.4 ± 0.01 hi	0.6 ± 0.001 h	0.2 ± 0.005 jk	0.03 ± 0.0003 kl	3.7 ± 0.02 c	
Volatility assay	IC50 (g L ⁻¹) without HP-β-CD	2.2 ± 0.01 ij'	0.8 ± 0.01 k'	5.5 ± 0.02 f'	16.9 ± 0.1 c'	22.6 ± 0.5 b'	25.6 ± 0.4 a'	2.3 ± 0.06 i'	0.08 ± 0.0005 k'	9.5 ± 0.08 d'	NC
	IC50 (g L ⁻¹) with HP-β-CD	7.3 ± 0.01 e'	0.7 ± 0.01 k'	6.7 ± 0.02 e'	2.4 ± 0.05 i'	3.2 ± 0.1 h'	4.4 ± 0.1 g'	1.4 ± 0.04 j'	0.09 ± 0.0002 k'	3.6 ± 0.02 gh'	

Values are depicted as mean ± standard deviation; IC50: inhibitory concentration; NC: not calculable; different lowercase letters indicate significant difference using two-way ANOVA, between results obtained in the presence or absence of HP-β-CD ($p < 0.05$) and displayed without and with apostrophe for direct contact and volatility assays, respectively. Results from direct contact and volatility assays have been analyzed separately. A. Root: angelica root EO. A. Seed: angelica seed EO.

Table 4. IC50 of EO's phytotoxic activity (inhibition of seedling's emergence) obtained by contact assay, against lettuce and rye-grass.

		A. Root	A. Seed	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja	Positive Control
Lettuce	IC50 (g L ⁻¹) without HP-β-CD	4.9 ± 0.9 c	0.01 ± 0.002 g	0.3 ± 0.05 g	0.9 ± 0.1 f	0.3 ± 0.05 g	0.1 ± 0.04 g	0.2 ± 0.04 g	0.2 ± 0.04 g	2.3 ± 0.4 d	0.01 ± 0.006 g
	IC50 (g L ⁻¹) with HP-β-CD	1.1 ± 0.3 f	NC a	1.7 ± 0.4 e	0.8 ± 0.2 f	5.4 ± 0.7 b	1.6 ± 0.5 e	0.083 ± 0.02 f	0.1 ± 0.03 g	NC a	
Rye-grass	IC50 (g L ⁻¹) without HP-β-CD	NC a'	0.6 ± 0.07 h'	1.7 ± 0.3 ef'	3.9 ± 0.5 c'	2.9 ± 0.5 d'	3.7 ± 0.5 c'	1.7 ± 0.3 ef'	0.7 ± 0.09 h'	2.1 ± 0.5 e'	0.04 ± 0.01 i'
	IC50 (g L ⁻¹) with HP-β-CD	NC a'	0.5 ± 0.07 h'	1.4 ± 0.3 fg'	1.2 ± 0.3 g'	NC a'	1.8 ± 0.3 ef'	0.4 ± 0.6 h'	1.3 ± 0.3 fg'	4.8 ± 0.9 b'	

Values are depicted as mean ± standard deviation; IC50: inhibitory concentration; NC: not calculable; different letters indicate significant difference using two-way ANOVA, between results obtained in the presence or absence of HP-β-CD ($p < 0.05$). Results for lettuce and rye-grass have been analyzed separately and statistical letters are displayed, respectively, without or with an apostrophe. A. Root: angelica root EO. A. Seed: angelica seed EO.

In the presence of HP- β -CD, no significant improvement has been demonstrated in our experimental conditions, with even significantly higher effects in the absence of HP- β -CD for angelica seed, leek, garlic, chervil, and thuja EO. Due to their limited efficiency at the tested concentrations, the IC₅₀ has not been calculated for thuja and angelica seed EO, as it would have resulted in particularly inaccurate and seemingly high values. In comparison with the positive control, the IC₅₀ are in the same range for the most efficient EO and more than 100 times higher for angelica root EO.

On the other hand, all the EO containing organo-sulfur compounds (garlic, chive, leek, onion, and shallot EO), as well as chervil, thuja and angelica seed EO, exerted an inhibitory effect on rye-grass seedlings' emergence, suppressing 70 to 100% of the germinative capacity at the highest tested concentration (data not shown). Angelica root EO, on the contrary, has shown a relatively limited anti-germinative capacity. In the presence of HP- β -CD, no significant improvement in terms of anti-germinative activity has been demonstrated for any of the tested EO (Table 4). As with lettuce, the IC₅₀ has not been calculated for angelica root and garlic EO. In comparison with the positive control, the tested EO displayed significantly higher IC₅₀ values (more than 10 times higher). The IC₅₀ are in the following order: IC₅₀ A.seed EO < IC₅₀ Shallot EO < IC₅₀ Chervil EO < IC₅₀ Onion EO < IC₅₀ Thuja EO < IC₅₀ Garlic EO < IC₅₀ Leek EO < IC₅₀ Chive EO < IC₅₀ A.root EO.

Seedlings' growth assay: IC₅₀ obtained for lettuce root elongation assessment varied from 0.0008 to 0.3 g L⁻¹ for angelica seed and thuja EO, respectively. Obtained IC₅₀ are listed in Table 5, where angelica seed, chervil, and leek EO displayed significantly lower IC₅₀ values (ranging from 0.0008 to 0.02 g L⁻¹). A significant improvement has been obtained in the presence of HP- β -CD for shallot EO only (Table 5). In the presence of HP- β -CD, shallot EO presented the lowest IC₅₀ (0.02 g L⁻¹) and angelica root EO the highest (3.2 g L⁻¹). IC₅₀ were increased as follows (Table 5): IC₅₀ Shallot EO ≤ IC₅₀ Garlic EO ≤ IC₅₀ Onion EO ≤ IC₅₀ A. seed EO ≤ IC₅₀ Chive EO ≤ IC₅₀ Leek EO ≤ IC₅₀ Chervil EO ≤ IC₅₀ Thuja EO ≤ IC₅₀ A. root EO.

On rye-grass, the IC₅₀ expressed ranged from 0.01 to 0.8 g L⁻¹ for angelica seed and thuja EO, respectively (Table 5).

No significant improvement has been demonstrated in the presence of HP- β -CD, whatever the EO. Furthermore, angelica seed EO, in the presence of HP- β -CD, presented the lowest IC₅₀ (0.06 g L⁻¹) and angelica root EO the highest (3.6 g L⁻¹). The IC₅₀ are in the following order: IC₅₀ A. seed EO ≤ IC₅₀ Shallot EO ≤ IC₅₀ Onion EO ≤ IC₅₀ Chervil EO ≤ IC₅₀ Chive EO ≤ IC₅₀ Leek EO ≤ IC₅₀ A. root EO ≤ IC₅₀ Garlic EO < IC₅₀ Thuja EO.

In comparison with the positive control, the tested EO displayed higher IC₅₀ values for all the tested EO, for both lettuce and rye-grass assays, displaying IC₅₀ values up to more than 1000 times higher for thuja EO.

Table 5. IC50 of EO's phytotoxic activity (inhibition of seedling's growth) obtained by contact assay, against lettuce and rye-grass.

		A. Root	A. Seed	Chervil	Chive	Garlic	Leek	Onion	Shallot	Thuja	Positive Control
Lettuce	IC50 (g L ⁻¹) without HP-β-CD	0.2 ± 0.04 cd	0.0008 ± 0.0004 e	0.02 ± 0.003 e	0.1 ± 0.04 de	0.2 ± 0.06 cd	0.03 ± 0.004 e	0.2 ± 0.04 cd	0.2 ± 0.04 cd	0.3 ± 0.05 c	0.0001 ± 0.0002 e
	IC50 (g L ⁻¹) with HP-β-CD	3.2 ± 0.9 a	0.04 ± 0.008 e	0.3 ± 0.1 c	0.08 ± 0.04 e	0.03 ± 0.007 e	0.08 ± 0.05 e	0.03 ± 0.02 e	0.02 ± 0.007 e	0.8 ± 0.1 b	
Rye-grass	IC50 (g L ⁻¹) without HP-β-CD	0.5 ± 0.06 e'	0.01 ± 0.003 h'	0.3 ± 0.06 de'	0.06 ± 0.02 gh'	0.3 ± 0.05 f'	0.2 ± 0.04 fg'	0.2 ± 0.03 fg'	0.2 ± 0.04 fg'	0.8 ± 0.1 d'	0.001 ± 0.0006 h'
	IC50 (g L ⁻¹) with HP-β-CD	1.3 ± 0.4 c'	0.06 ± 0.03 gh'	0.09 ± 0.03 gh'	0.1 ± 0.04 gh'	3.2 ± 0.5 b'	0.1 ± 0.04 gh'	0.08 ± 0.03 gh'	0.07 ± 0.02 gh'	3.6 ± 0.8 a'	

Values are depicted as mean ± standard deviation; IC50: inhibitory concentration; NC: not calculable; different letters indicate significant difference using two-way ANOVA, between results obtained in the presence or absence of HP-β-CD ($p < 0.05$). Results for lettuce and rye-grass have been analyzed separately and statistical letters are displayed, respectively, without or with an apostrophe. A. Root: angelica root EO. A. Seed: angelica seed EO.

4. Discussion

The use of biocontrol products and especially natural substances including essential oils (EO), in agricultural systems, is arousing great interest. They are indeed bio-sourced products, regarded as more ecological, and brought forward recently as alternative tools to replace synthetic pesticides, which show greater environmental and human health risks.

The results presented herein, based on in vitro antifungal activity against *F. culmorum*, by both direct contact and volatility assays, have shown that all tested EO are able to inhibit the growth of this phytopathogenic fungus, with higher efficiency for the direct contact method. Indeed, the inhibitory concentrations obtained by direct contact assay were significantly lower in comparison with those obtained with the volatility assay: the half-maximal inhibitory concentrations (IC₅₀) obtained by volatility assay ranged from 1.2 up to 23-fold higher than those obtained by direct contact assay. However, the efficiency of this kind of inhibition was strongly related to the type of EO used.

The antifungal activity of common synthetic antifungal agents has previously been reported. Among others, demethylation inhibitor (DMI), quinone outside inhibitor (QOI) fungicides [41], pimaricin [42] and tetraconazole [43] have been investigated for their fungicidal properties against *Fusarium graminearum* and *Fusarium spp.* The most efficient fungicide evaluated was pimaricin, with an IC₅₀ ranging from 2.5 up to 5 µg L⁻¹, whereas the IC₅₀ reported for tetraconazole was 16 µg L⁻¹ and the ones obtained with DMI and QOI were 0.01 mg L⁻¹ and 0.2 to 1.3 mg L⁻¹, respectively. The fungicide evaluated in the present study displayed an IC₅₀ value similar to the highest ones reported in the literature. Altogether, synthetic pesticides' inhibitory effects occurred at lower concentrations compared to EO. However, if the gap appears to be consistent, EO have shown themselves efficient, with lower noxious effects on both environment and human health and the occurrence of resistance phenomena has, to our knowledge, not been reported so far with the use of EO. Indeed, the use of EO distilled from *Mentha arvensis* L., *Mentha spicata* L., *Juniperus mexicana* Spreng, *Citrus sinensis* L., *Persicaria odorata* Lour., *Piper nigrum* L., *Canarium commune* L., *Cinnamomum zeylanicum* L., *Boswellia carterii* Birdw., *Cymbopogon flexuosus* L., *Litsea cubeba* Lour., *Artemisia herba-alba* Asso, *Cistus ladaniferus* L., *Copaifera* tree, *Ferula galbaniflua* Boiss. et Buhse., *Citrus aurantium* L. and *Schinus terebinthifolius* Raddi has been homologated and has shown promising results in agriculture, as reviewed in [3].

Onion and garlic EO were previously studied for their antifungal activity toward *Aspergillus niger*, *Aspergillus terreus*, *Penicillium cyclopium*, *Fusarium oxysporum*, *Candida albicans*, *Candida tropicalis*, *Rhodotorula glutinis*, *Monascus purpureus* [4,44–46]. However, scarce are the studies that evaluate the antifungal activities of the tested EO by both direct contact and volatility assays, against *F. culmorum*.

Besides antifungal properties, the tested EO revealed phytotoxic effects, being able to inhibit the seedlings' emergence of either one or both plant species tested in this in vitro bioassay, lettuce (*L. sativa*) and rye-grass (*L. perenne*). Our results have shown promising activities of the EO, especially the one distilled from angelica seeds, presenting the lowest IC₅₀ values (0.0008 and 0.01 g L⁻¹, respectively, on lettuce and rye-grass), notably due to the presence of a high amount of monoterpene compounds, such as beta-phellandrene [47–49]. The IC₅₀ obtained ranged from 0.0008 to 0.3 g L⁻¹, respectively, for angelica seed and angelica root EO used on lettuce, whereas the IC₅₀ expressed on rye-grass ranged from 0.01 to 0.8 g L⁻¹ for angelica seed and thuja EO. In terms of seedlings' emergence inhibition, most of the EO tested in this study have demonstrated an inhibitory effect, especially on *L. sativa*, with lower EO concentrations resulting in inhibition of seeds' germination. Overall, angelica seed EO has shown great potential in terms of inhibiting the growth of the studied plants but has been demonstrated as less efficient, especially on ryegrass, in terms of anti-germinative activity. By comparing our results with those obtained for glyphosate, used as a systemic herbicide in previous studies, and evaluated as a positive control in the present work, EO have demonstrated real potential. Indeed, previous studies have reported glyphosate's IC₅₀ ranging from 23–46.2 mg L⁻¹ [50] and 15.3 mg L⁻¹ [51], regarding ryegrass growth inhibition, whereas the IC₅₀ reported on lettuce ranged from 8.9 mg L⁻¹ [52] to 20 mg L⁻¹ [53]. The obtained IC₅₀ for glyphosate in the present work are similar to those previously

reported. Thus, the most efficient EO reported in terms of in vitro phytotoxic effects (especially angelica seed EO) exerted effects similar to those of glyphosate.

Moreover, *L. sativa* has shown a higher sensibility to EO (in both evaluation methods, herbicidal or anti-germinative activities) compared to *L. perenne*, which is in line with the literature, reporting a higher sensitivity of lettuce to chemicals and environmental pollutants than many other plants [37,51,52,54,55], possibly because of the plant metabolism. Although there are frequent mentions of this sensitivity to chemicals [56–60], there is, to our knowledge, no clear evidence of the mechanisms and biological pathways involved. Besides, the EO tested in this study have, to the best of our knowledge, not previously been reported for their herbicidal properties, in terms of either seedlings' emergence or growth inhibition.

As reported above, all EO were efficient regarding all of the biological activities assessed. Nevertheless, some EO, such as those coming from shallot, onion and angelica seed, presented high efficiency for both antifungal and phytotoxicity assays, since lower concentrations were required to obtain a total inhibitory effect. This may be imputed to the difference in the nature and the abundance of the major active principles of EO.

Indeed, the EO tested in this study may be classified into three different groups, regarding their chemical compositions. Angelica (root and seed) and thuja EO are mostly composed of terpene compounds, chervil EO is rich in phenylpropanoids whereas chive, garlic, leek, onion, and shallot EO contain organosulfur compounds. The balance between the active principles of EO among the same subgroup might be the cause of the wide scale of efficiency rates that have been spotlighted in this study. Leek and onion EO, rich in organosulfur compounds, respectively contain mainly dipropyl disulfide (50.4 and 35.5%), dipropyl trisulfide (20.1 and 17.6%), dipropyl tetrasulfide (3.8 and 11.0%), methyl propyl trisulfide (6.4 and 9.1%), bis(1-methyl ethyl)disulfide (4.5 and 6.7%), for instance.

These findings are in line with the literature, as it has been demonstrated that the major principles of EO, belonging to different classes of organic compounds, such as terpenes, flavonoids and isoflavonoids, coumarins, phenolic compounds, and organic acids, are biologically active and that some of them are likely to act synergistically to exert a stronger biological activity [61–63]. Additionally, one should note that, besides major compounds, the contribution of the compounds present in smaller amounts should not be neglected regarding biological effects. In fact, previous studies reported that the efficiency of the EO was in most cases greater than those of the major compounds studied separately [64,65]. In particular, EO may play a role in inhibiting fungal cell wall formation, disturbing cell membrane and fungal mitochondria function, as well as inhibiting cell proliferation, DNA, RNA, and protein synthesis [66,67]. These mechanisms of action are principally due to their major active principles such as terpenes (carvone, limonene), and phenolic compounds (eugenol, apiol) [66].

However, concerning the herbicidal assay, it is surprising to find EO from the same subgroup (terpene-containing compounds) in terms of composition, showing very different rates of efficiency, presenting either the lowest or the highest IC₅₀ values. This might be attributed to the relative abundance of major compounds. Indeed, the main differences between angelica seed and root EO reside in the balance between major compounds, especially in mono-terpenes: Beta-phellandrene (resp. 67.2 and 16.5%), alpha-phellandrene (resp. 1.6 and 8.9%), alpha-pinene (resp. 11.4 and 21.4%), limonene (resp. not detected and 7.49%) and cis-beta-ocimene (resp. 4.92 and 6.36%). In addition, the results obtained from the PCA have shown a positive correlation between angelica root and seed EO, showing that the chemical compositions are similar, but the correlation factor is close to 0.5, meaning that the correlation is not optimal. Altogether, we found that EO from shallot, chive, leek and onion were closely related in terms of composition, which, most of the time, is also the case concerning their biological activities. On the contrary, EO from angelica seed and root have very different efficiency rates, despite their seemingly close compositions.

On another note, EO encapsulation in HP- β -CD did not provide a significant difference in the IC₅₀ of EO in all the tested biological activities with respect to free-HP- β -CD treatment. In the presence of HP- β -CD, the IC₅₀ of antifungal activity by direct contact assay varied from 0.03 g L⁻¹ for shallot EO

to 5.8 g L⁻¹ for angelica root EO, while IC50 ranged from 0.09 to 7.3 g L⁻¹ for shallot EO and angelica root EO, respectively, by volatility assay.

In the same way, the IC50 of herbicidal activity in the presence of HP- β -CD were not significantly altered, with shallots and angelica root EO presenting the lowest IC50 (16.6 mg L⁻¹; 62 mg L⁻¹ on lettuce and rye-grass, respectively) and angelica root EO the highest (3.2 g L⁻¹; 3.6 g L⁻¹ on lettuce and rye-grass, respectively).

In the presence of HP- β -CD, whatever the biological property assessed, no significant effect of HP- β -CD has been demonstrated. This raises the issue of EO retention by HP- β -CD. It is well known that HP- β -CD can form inclusion complexes with EO components. This phenomenon was highlighted by the decrease in the peak areas of EO in the presence of HP- β -CD. This effect has already been described in previous studies [11,68,69]. For the EO listed above, retention percentage values have shown that EO were efficiently retained by HP- β -CD (except for angelica seed's EO, presenting a relatively low retention percentage). These results suggest that HP- β -CD could efficiently retain EO and that way avoid EO evaporation and degradation. Additionally, our results have not demonstrated that a high retention percentage significantly reduces the EO activity and vice versa, in the case of volatility assay. Finally, HP- β -CD might be considered as an efficient material to encapsulate EO and improve the release efficiency and persistence in time of volatile compounds, enlarging the applications of EO from an agricultural perspective.

5. Conclusions

In conclusion, altogether our findings have shown that EO, especially those distilled from shallot, onion, and angelica seed, possessed both interesting antifungal and phytotoxic activities. This may be attributable to the nature of the chemical compounds, their richness, and their relative abundance. One should note that even minor compounds may contribute to the efficiency of the EO. In particular, these EO presented a great potential to control *F. culmorum* growth by both direct contact and volatility assays. They have also shown phytotoxic effects against mono- and dicotyledon species, displaying inhibitory effects in the same range as common herbicides. Such in vitro tests give an indication of the potential role of EO used as biopesticides to control phytopathogen infection. Afterwards, it would be interesting to carry out in planta assays, using individual or combined EO, to validate EO-based biopesticides' efficiency. Although this efficacy was strongly related to the EO used and its composition, all EO tested in the current study could potentially be used as natural and eco-friendly pesticides. Additionally, it should be noted, that in our experimental conditions, the encapsulation of the tested EO did not significantly improve the EO efficiency, yet reduced EO volatility. Further analyses should be conducted in glasshouse or field conditions to evaluate the efficiency of the controlled release of EO.

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