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ORIGINAL ARTICLE

Phytochemical Screening of Essential Oils and Methanol Extract Constituents of Wild *Foeniculum vulgare* Mill.: a Potential Natural Source for Bioactive Molecules

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Abstract

Purpose Weeds and fungi are serious pests of a wide range of agricultural crops. In addition, due to the negative environmental impacts of chemical pesticides, these pests are developing important resistance against them. Therefore, one alternative method to control pest is the use of plant derived active molecules; particularly essential oils and phenolic compounds have been an increasing interest in their safe and environmentally-friendly application to crops, as a powerful alternative to chemical pesticides.

Methods The present investigation studied the chemical composition of *Foeniculum vulgare* essential oil (Eos) and methanol extract (ME) respectively by GC/MS and LC/MS analysis. In second step, we evaluated their phytotoxic activity against *Sinapis arvensis*, *Trifolium campestre*, *Lolium rigidum* and *Lepidium sativum* and their antifungal potential was studied in vitro against five phytopathogenic fungal species (*Fusarium oxysporum* sp. *Lycopersici*, *F. culmorum, F. oxysporum* 184, *F. oxysporum mathioli* and *F. redolens*).

Results High amounts of monoterpenes were detected in Eos, consisting mainly of estragole (76.2%), α-thujone (9.6%) and limonene (8.6%). LC/MS analysis of ME allowed to the identification of 18 phenolic compounds distributed in eight phenolic acids, one non phenolic acid and nine flavonoids. Seed germination and seedling growth of weeds and crops were determined in Petri dish bioassay at different doses with reference to a negative control and a positive control (glyphosate). Fennel Eos and ME completely inhibited germination and seedling growth of tested crops and weeds in a dose-dependent manner. Data obtained with Fennel Eos and ME are similar to the activity of glyphosate. For the antifungal activity, the oils and the extract showed interesting variable activities depending on the dose and the strain tested. The best activities were obtained with Eos.

Conclusion Taken together, these results indicate that Fennel molecules have phytotoxic and antifungal properties that could be used as natural bio-pesticides for weeds and fungi management.

Keywords *Foeniculum vulgare* · Essential oils · LC/MS · Methanolic extract · Herbicidal activity · Antifungal potential

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1 Introduction

During the last years, the use of chemical control with synthetic pesticides is a common practice used to protect the crops from diseases and to control weeds, fungi, and pests [1]. However, this over-dependence on chemical products has led to the growing problems of resistance to pesticides and the persistence of chemical residues in crops and vegetation [2]. Such problems affect both environment and human health [3].

Consequently, as pesticides are failing to provide effective protection against plant diseases and the environment compatibility, a huge efforts and research achievements are being made to develop natural alternative products control with low effects on health and environment. Thus, the use of plant extracts appears as promising natural biopesticides to control agricultural and provide sustainable protection of the ecosystem [4].

Several plant extracts have been explored for their broad-spectrum bioactivities, as they are human health and environmentally -friendly. These bioactivities are ascribed to their richness in EOs and phenolic compounds which are of wide interest, particularly in foods and pharmaceutical industries and especially in agriculture.

Eos stored in secreted organs such as resin ducts, oil ducts, glands, or glandular hairs of plants, are a complex mixture of functionalized chemical classes including monoterpenes, sesquiterpenes and phenylpropanoids, amongst others [5]. Eos and their components are used in the nutritional and pharmaceutical fields for their reported antifungal, antibacterial, and antioxidant effects [6, 7]. They are also used in agricultural for their reported nematocidal, insecticidal, antifungal, and herbicidal properties [5, 8]. In fact, research conducted by Amri et al., on Tunisian Pine species reported the herbicidal effects of needles Eos on seed germination and seedling growth against a range of weeds. In addition, another study on *T. capitatus, Rosmarinus officinalis,* and *Pinus halepensis* essential oils exhibited a strong insecticidal activity against *Ectomyelois ceratoniae* Zeller, a pest that cause significant damage on various crops throughout the Mediterranean basin especially on dates [5]. Several reports exhibited that these activities depend on the composition and the action of the metabolites on the target organism [8].

Another aspect of Eos is their potential as therapeutic agents. Another interesting products of secondary metabolism of plants that has also gained special relevance are phenolic molecules. They have been found to exhibit antioxidant antiallergenic, anti-inflammatory, antimicrobial, and herbicidal properties [9–12]. Phenolic compounds are generally considered as one group of important compounds responsable for the antioxidant activity. Extensive phytochemical analysis has lead to the identification of major components of phenolics, particularly for their broad-spectrum activities, which can provide, alternative functional ingredients to preserve food products and ensure safety for consumers. Eos and phenolics, gained considerable interest as a promising natural substance to improve food quality and health care and to protect from many plant diseases.

Fennel, (*Foeniculum vulgare*) belonging to the Apiaceae family, is one of the well-known medicinal plants [12]. It grows wildly in the Mediterranean region, and it has become cultivated all over the world. Fennel is a highly aromatic plant with culinary use in Mediterranean cuisine. The bulb, stalks and foliage are used as vegetable dishes and in many traditional cooking. The seeds are used as spices for flavoring meat and fish dishes, cheese, baked goods, ice cream and as an herbal tea [6, 13]. As a folk medicinal plant, fennel seeds have been used for the treatment of a wide range of illness such as chronic coughs, kidney stones, bronchitis, and diabetes [11, 12]. The traditional uses of fennel are also supported by biological and pharmacological studies, such antioxidant, antimicrobial and anti-inflammatory activities [11, 12]. Recent studies on methanol extracts and Eos of *F. vulgare* seeds showed a strong antioxidant and antimicrobial activities against fungi and bacteria [9, 10]. Fennel Eos have been also reported for it antidiabetic activity and it was found to decrease plasma glucose levels in diabetic rats [12].

A great diversity of production of essential oils from *F. vulgare* has been described, a recent comparative study of essential oils from fennel growing in Egypt and China has been reported [14]. According to this study, Egyptian fennel essential oils were characterized by a chemotype: estragole (51%), limonene (11.4%), L-fenchone (8.2%) and trans-anethole (3.6%) however fennel oils from China have were characterized by a chemotype rich in trans anethole (54.4%), estragole (20.2%), L-fenchone (7.4%) and limonene (2.4%). Similarly, a variability in the richness of methanolic extracts in phenolic compounds has been demonstrated. Essential oils and methanolic extracts of fennel grown in Egypt and China have proven to be important and variable depending on the origin of the plant [14]. This could explain the variability and diversity of the production of metabolites synthesized by plants depending on their origins.

In addition to their use in food and medicinal purposes, fennel bioactive compounds have been reported for their use in plant protection and for their application as natural products for agricultural control. In fact, fennel Eos exhibits important insecticidal and fungicidal effects against a wide range of insects and phytopathogenic fungi [13, 14].

Other investigations on fennel Eos have been reported for their allelopathic effects that restrain the development of weeds by inhibiting seed germination and growth [15]. Thus, allelopathy plays an important role in natural ecosystems

and presents a mode to protect the environment from the effects of synthetic pesticides [15, 16].

Recent studies investigated the herbicidal activity of fennel plant, Sabzi Nojadeh et al., tested the phytotoxic effect of *F. vulgare* Eos from Italy against *Convolvulus arvensis* [17]. Furthermore, the herbicidal activity of Turkish fennel seeds Eos has been studied against *Rumex nepalensis*, *Amaranthus retroflexus*, *Alcea pallida*, *Centaurea salsotitialis*, *Raphanus raphanistrum*, *Sinapis arvensis* and *Sonchus oleraceus* that grow in field crops [15].

According to the literature, the majority of studies have been carried out on Eos and little are the reports on the antifungal activity of fennel Eos against phytopatogenic fungi. However, no studies of biological activity like herbicidal and antifungal activity of the methanol extract. Even, the chemical composition of plant secondary metabolites throughout the world is very variable. For this, it is useful to characterize the volatile and non-volatile fraction of fennel which grows in Tunisia in order to link the biological activities of this species with the presence of chemical molecules and also in order to contribute to a valuation of this species which is the most abundant Apiaceae in Tunisia.

In Tunisia and to the best our knowledge, there are no studies conducted on the use of wild fennel for their phytotoxic and their antifungal effects. Therefore, the present study aims:

(i) to identify the chemical composition of wild fennel Eos and ME,
(ii) to investigate for the first time the herbicidal activity of fennel seeds Eos and ME against *S. arvensis* L., *Trifolium campestre* and *T. campestre* Schreb.
(iii) to test their antifungal activity against five phytopathogenic fungal strains: *Fusarium oxysporum* sp. *Lycopersici, F. culmorum, F. oxysporum* 184, *F. oxysporum mathioli* and *F. redolens*.

Such research can contribute to the development of biopesticide as alternative tool for sustainable management of weeds and fungi with low impact in the human health and environment.

2 Materials and Methods

2.1 Plant Material

Mature seeds of wild *F. vulgare* were collected in January 2021 from the Kef region: governorate located in the north of Tunisia. 10 individuals were harvested at distances exceeding 15 m to avoid the sampling of closely related individu and fennel seeds were then mixed and used for extraction and further analysis. The plant was identified by Dr AMRI Ismail, and a voucher specimen was submitted to the herbarium division of the National Center for Nuclear Sciences and Technologies.

To evaluate the phytotoxic effect, three seeds of annual weeds: *Lolium rigidum*, *T. campestre* and *S. arvensis*, and one cultivated species *Lepidium sativum* were harvested from crop fields. The data about plant material origin and the date of collection are listed in Table 1.

2.2 Essential Oils Extraction

Fennel Eos was extracted using a Clevenger apparatus. 200 g of powder seeds were extracted for 3 h, and the percentage yield was calculated after repeating the process three times. Obtained essential oils were dried over anhydrous Na_2SO_4 in dark vials at 4 °C.

2.3 Methanol Extract Preparation

Wild fennel seeds were cleaned with tap water and dried at room temperature before being crushed. About 200 g of dried powdered seeds were soaked in 2 L methanol for 24 h at room temperature, the extraction was repeated three times. Extraction step was followed by filtration using Whatman No. 4 filter paper and solvent removal was carried out using a rotary vacuum evaporator under reduced pressure and at a temperature of 40 °C. Obtained methanolic extract was kept at 4 °C until analysis.

Table 1 Data on plant material collection

Species	Preserved specimen	Part collected	Date of harvest	Harvest site
Foeniculum vulgare	FV2101	Seeds	January	Kef
Sinapis arvensis L.	SA2103		June	Sidi Ismail, Beja
Trifolium campestre Schreb	TC2101			
Lipidum. sativum	LC2101			
Lolium rigidum Gaudin	LR2105			

2.4 Gas Chromatography Analysis with MS Detection

Foeniculum vulgare Eos chemical composition were analyzed by GC and GC–MS using an Agilent 6890 gas chromatograph equipped with a fused silica capillary column type HP-5MS (30 m × 0.25 mm, and 0.25 µm thickness). Tested oils were dissolved in hexane (1 mg/mL). The injection of 1 µL of each sample was made with a split mode with a ratio of (1/100). Helium was used as the carrier gas at a rate of 1 mL/min. Temperature of injector was set at 250 °C and detector temperature was 280 °C. The oven temperature program varied as follow: 35–325 °C with a heating rate of 5 °C/min. The flow rate was equal to 1 mL/min and the equilibration time was 0.5 min. MS operating in electron impact was at a potential of 70 eV in a scan range between 50 and 550 m/z. Identification of the Fennel Eos compounds was based on their Kovats retention indices and those reported in the literature [18]. Furthermore confirmation was done by comparison of their mass spectra with those available in Wiley 275 mass spectral libraries and NIST 02 and by comparing their Kovats retention indices (KI) with those the literature. Kovats indices were calculated for all detected components by using a series of n alkanes (C8–C30). This identification was confirmed by co-injection of some authentic compounds. Quantification was made by an external standard method using calibration curves generated by running.

2.5 Liquid Chromatography with Mass Spectrometry (LC/MS)

LC–MS analysis of fennel ME was done by a Shimadzu ultra-fast liquid chromatography system, which consisted of: a CTO-20AC column oven, two LC-20ADXR pumps, a SIL-20ACXR auto-sampler, a SPD-M20A diode array detector with 13 mm flow cell and an online DGU-20AS degasser.

The LC separation was performed on a Discovery Bio C18 column (4.6 mm × 250 mm, 5 µm) by gradient elution using a mobile phase consisting of: solvent A: water (containing 0.1% formic acid) and solvent B: methanol (containing 0.1% formic acid) with the following gradient procedure conditions: 0–45 min with 10% B, 45–55 min with 100% B. The initial conditions were held for 10 min as a re-equilibration step. The injection volume was 5 µL, the column temperature was maintained at 40 °C and the flow rate of the mobile phase was 0.5 mL/min.

The LC system was equipped with a Shimadzu mass spectrometer coupled to an electrospray ionization source (ESI).

The mass spectrometer was used in negative ion mode with a capillary voltage of (− 3.5 V), a voltage detector of (1.35 V), a block source temperature of 400 °C, a nebulizing gas flow of 1.5 L/min a dissolving line temperature of (250 °C), the full scan spectra from (50–2000 Da) and a dry gas flow rate of (12 L/min).

LC–MS analysis of phenolic compounds in methanolic *F. vulgare* extract was determined by a validated method analysis using 33 standards as described by Mighri et al. [19].

2.6 Phytotoxic Activity

Three common very aggressive weeds and one cultivated species of agroecosystems in the Northern region of Tunisia were used as test herbs (*S. arvensis*, *T. campestre*, *L. rigidum* and *L. sativum*).

In the aim to assess the herbicidal effect of fennel Eos and ME on the germination of the selected species under laboratory conditions as per Ben Ghnaya et al. [20]. The bioassay was carried out in Petri dishes of 9 cm diameter lined with thin layer of cotton layer covered with Whatman No. 1 filter paper.

The seeds were sterilized with 5% sodium hypochlorite for 2 min and then were rinsed three times with distilled water. Then, 10 seeds were placed equidistantly in each Petri dishes. Further, each Petri dish were moistened with solution of 6 mL of EOs, ME or glyphosate at concentrations of (2.5, 5, 7.5 and 10 mg/mL) or distilled water (as negative control).

After that, immediately the Petri dishes were sealed with Parafilm to prevent water volatilization. Three replicates were maintained per each concentration and each tested sample. After 7 days, the effect of tested samples was assessed on the germination percentage and seedling growth length (cm).

2.7 Antifungal Activity

2.7.1 Fungal Strains

Five phytopathogenic fungal strains were used as follow: *F. oxysporum* f. sp. *Lycopersici,* causing tomato wilt; *F. culmorum* an important pathogen of *wheat* causing seedling blight, foot rot, and head blight these two strains were obtained were obtained from the Turkish Energy Nuclear and Mining Research Institute, whereas the strains *F. oxysporum* 184 and *F. oxysporum mathioli* were obtained from Dr Hirsch's Laboratory, University of California Los Angeles (UCLA) and the strain *F. redolens* responsible for chickpea wilt was obtained from the laboratory of plant protection of the Tunisian National Institute of Agronomic Research. All tested fungi were maintained and stored on potato dextrose agar at 4 °C.

2.7.2 In Vitro Antifungal Activities on Mycelial Growth

The antifungal properties of fennel tested samples were studied in Petri dishes using the direct contact method [20].

Tested samples were diluted in a Tween 20 solution (0.1% v/v) and then added to 10 ml of PDA at 40 °C to provide the required concentrations (2–10 mg/mL).

A mycelial disk of 6 mm in diameter was placed in each PDA plate, and then incubated at 24 °C for 5 days. PDA plates that contained only Tween 20 (0.1%) were used as negative control. All assays were done with three replicates per treatment. The fungicidal properties of fennel samples were determined as the percentage of inhibition (PI) of mycelia growth compared to the control following the formula:

$$\text{P.I.} = \frac{(\text{dc} - \text{dt})}{\text{dt}} \times 100$$

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

2.8 Statistical Analysis

Results were subjected to 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.

3 Result and Discussion

3.1 Phytochemical Studies on Fennel Eos and ME

Essential oil extracted from fennel seeds was found to yield a level of (2.04%) while methanol extract presented a yield of (9.7%). Khammassi et al., have reported similar results [6, 21].

Chemical composition of fennel seed essential oil was performed by GC/MS and identification was conducted according to it mass spectral data, retention time (RT) and retention indices (KI).

Results presented in Table 2 revealed the identification of 12 compounds accounting 97.3% of the total oil. Phenylpropanoids (76.2%) was the main subclass that constitute the analyzed oil followed by oxygenated monoterpene (10.6%) and monoterpene hydrocarbons (10.5%).

The compounds identified in concentrations greater than 5% were: Estragole as phenylpropanoids and as the main compound with a high percentage of 76.2, followed by α-thujone (9.6%) and limonene (8.6%).

According to the literature open review, several studies of *F. vulgare* Eos have been reported in several countries around the world, the main results are shown in Table 3.

Our results are in agreement with recent studies. Indeed, the majority of studies have shown that (*Z*)-Anethole and or Estragole as a major compounds (26.2–54.29%),

Table 2 Quantitative constituents identified in essential oil of *Foeniculum vulgare* seeds through the GC–MS analysis

N°	Compounds	RT	KI	% Area	Formula	Class
1	α-Pinene	8.156	939	0.7	$C_{10}H_{16}$	MH
2	Camphene	8.566	954	0.1	$C_{10}H_{16}$	MH
3	Sabinene	9.335	975	0.2	$C_{10}H_{16}$	MH
4	β-Myrcene	9.906	990	0.4	$C_{10}H_{16}$	MH
5	α-Phelladrene	10.257	1002	0.1	$C_{10}H_{16}$	MH
6	Limonene	11.055	1029	**8.6**	$C_{10}H_{16}$	MH
7	α-Terpinene	11.919	1059	0.4	$C_{10}H_{16}$	MH
8	(*Z*)-Thujan-4-Ol	12.227	1098	0.1	$C_{10}H_{18}O$	OM
9	α-Thujone	12.849	1102	**9.6**	$C_{10}H_{16}O$	OM
10	β-Fenchol	13.684	1121	0.6	$C_{10}H_{18}O$	OM
11	Camphor	14.431	1146	0.3	$C_{10}H_{16}O$	OM
12	Estragole	16.466	1252	**76.2**	$C_{10}H_{12}O$	PP
Total identification %				97.3		
Monoterpenes hydrocarbons %				10.5		
Oxygenated monoterpenes %				10.6		
Phenylpropanoids derivates %				76.2		

Compounds listed in order of elution from a HP-5 MS column

Values in bold indicate the major compounds characteristic of Fennel Eos

N° compounds number, *RT* retention time, *KI* Kovats Index [18], *MH* Monoterpenes hydrocarbon, *OM* Oxygenated monoterpenes, *PP* Phenylpropanoids derivates

Table 3 Comparative study on the chemical composition of *F. vulgare* from different countries

Origins	Used parts	Chemotype	References
Tunisia	Seeds	Estragole (66.1–85.2%), Fenchone (5.2–23.1%), Limonene (4.3–10.2%)	[6]
Egypt	Seeds	Estragole (51%), Limonene (11.4%), Fenchone (8.19%) and (*Z*)-Anethole (3.62%)	[14]
China	Seeds	Anethole (54.2%), Estragole (20.2%), Fenchone (7.3%), Limonene (2.4%)	[14]
Turkey	Seeds	(*E*)-Anethole (18.4–69.7%), Estragole (0.3–29.5%)	[22]
Italy	seeds	(*E*)-Anethole (34 and 89%), Fenchone (2–27%), α-Pinene (1–21%), Limonene (1–17%)	[23]
Serbia	Seeds	(*E*)-Anethole (64.8–69.9%)	[24]
Slovakia	Seeds	(*Z*)-Anethole (73.6%), Fenchone (6.0%), and Limonene (5.7%)	[25]
Iran	Seeds	(*Z*)-Anethole (1.2–88.5%), Estragole (0.2–59.1%), Fenchone (1.2–14.7%), Limonene (5.5–15.7%)	[26]
Portugal	Aerial parts	(*Z*)-Anethole (31–36%), α-Pinene (14–20%) and Limonene (11–13%)	[27]
	Seeds	Estragole (79–88%), Limonene (6.9–12.2%)	[27]

and other compounds such as Limonene, Fenchone and α-Pinene as compounds presented by significant amount.

But, this present study confirms that the chemotype of Tunisian fennel is estragole, and the oils analyzed are distinguished from the literature by their richness in α-thujone (9.6%).

Indeed, Eos content can be mainly affected by environmental and genetic factors [6]. Moreover, it may be influenced by the method and the extraction conditions [7].

Liquid chromatography phytochemical profiling was carried out to reveal in *F. vulgare* methanolic extract 18 molecules represented with their chemical class, retention time, area and formula in Table 4 and their chemical structures were shown in Fig. 1.

The results of this investigation showed the presence of nine flavonoids in the extract, which was mainly presented by the abundance of cirsiliol as the major flavonoid however, epicatechin, rutin, quercetin-3-o-rhamonoside, naringin, apegenin-7-o-glucoside, quercetin, naringenin and apegenin were detected at remarkable levels. Eight phenolic acids were identified as follow: protocatchuic acid, chlorogenic acid, 4-O-caffeoylquinic acid, caffeic acid, syringic acid, p-coumaric acid, transfrulic acid and salviolinic acid and one compounds non phenolic acids: quinic acid was detected.

Khammassi et al. [21] obtained similar results in a study conducted on phenolic compounds of Tunisian wild fennel. Generally, the same major compounds were detected with a little variability in the order and content of compounds,

Table 4 Liquid chromatography-mass spectrometry analysis of methanolic extract of wild Fennel seeds

N°	Compounds	Class	RT	Concentration (ppm)	m/z	Formula
1	Quinic acid	NPA	2.028	4.699	191	$C_7H_{12}O_6$
2	Protocatchuic acid	PA	6.917	11.966	153	$C_7H_6O_4$
3	Chlorogenic acid	PA	9.396	1.845	353	$C_{16}H_{18}O_9$
4	4-O-caffeoylquinic acid	PA	11.982	1.097	353	$C_{16}H_{18}O_9$
5	Caffeic acid	PA	14.433	3.404	179	$C_9H_8O_4$
6	Syringic acid	PA	16.084	0.737	197	$C_9H_{10}O_5$
7	Epicatechin	F	16.274	0.495	289	$C_{15}H_{14}O_6$
8	p-coumaric acid	PA	20.872	4.238	163	$C_9H_8O_3$
9	Transfrulic acid	PA	23.043	4.094	193	$C_{10}H_{10}O_4$
10	Rutin	F	23.869	0.278	609	$C_{27}H_{30}O_{16}$
11	Quercetin-3-o-rhamonoside	F	26.639	2.47	447	$C_{21}H_{20}O_{11}$
12	Naringin	F	25.957	0.311	579	$C_{27}H_{32}O_{14}$
13	Apegenin-7-o-glucoside	F	26.844	0.585	431	$C_{21}H_{20}O_{10}$
14	Salviolinic acid	PA	28.314	0.554	717	$C_{36}H_{30}O_{16}$
15	Quercetin	F	31.782	0.855	301	$C_{15}H_{10}O_7$
16	Naringenin	F	33.865	0.231	271	$C_{15}H_{12}O_5$
17	Apegenin	F	34.446	1.16	269	$C_{15}H_{10}O_5$
18	Cirsiliol	F	35.533	27.018	329	$C_{17}H_{14}O_7$

F flavonoids compounds, *PA* phenolic acids, *NPA* non phenolic acids, *RT* retention time

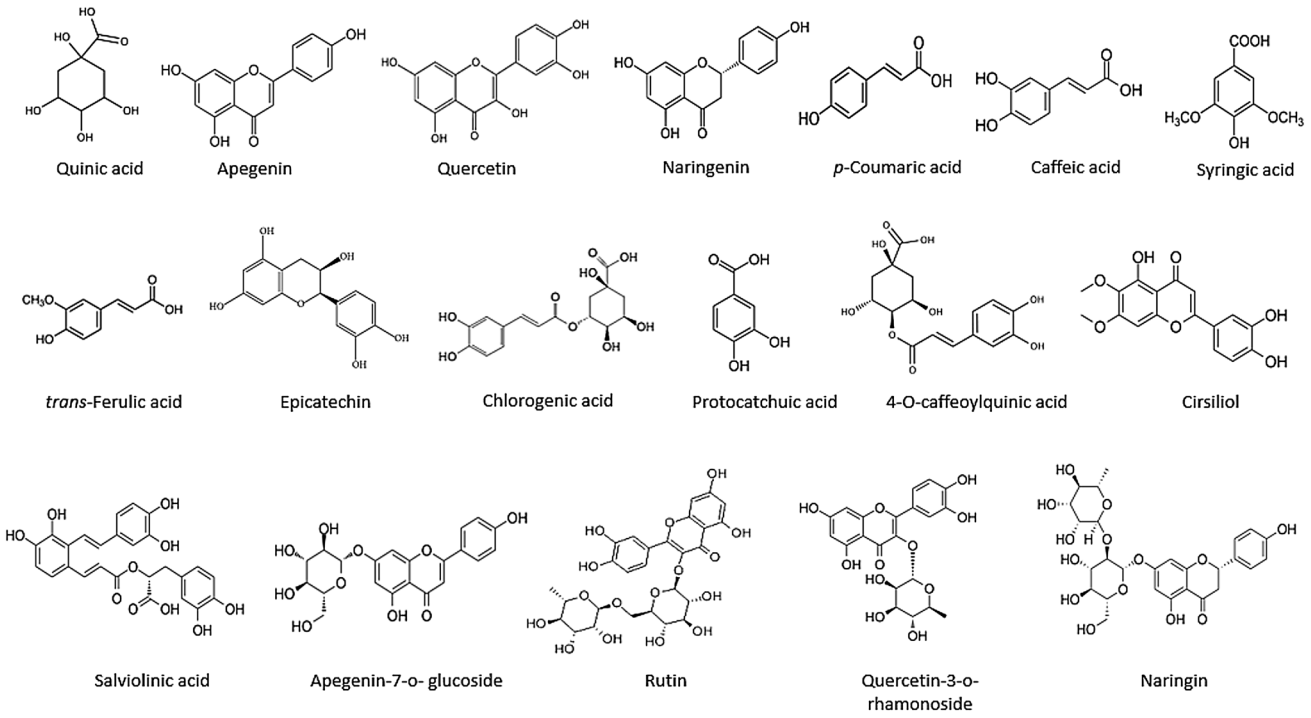

Fig. 1 Major components of Fennel seeds methanolic extract

which can be related to the sampling conditions, namely the date and the provenance of even the conditions of preparation of the plant material and of extraction. According to the literature, studies of the chemical composition of fennel crude extracts were very limited throughout the world. However, there are few studies on several species member of *Apiaceae* family. Previous results obtained with the species *Oenanthe fistulosa* L. have detected several compounds in common with those of fennel such as chlorogenic, trans ferulic and p-coumaric acid, rutin, quercitrin and quinic acid [28].

3.2 Herbicidal Activity

The ME and the Eos obtained from the seeds of *F. vulgare* were tested for their possible herbicidal potential against germination and growth of the aerial and root parts of *S. arvensis*, *T. campestre* (dicots) and *L. rigidum* and *L. sativum* (monocots).

Herbicidal activity was tested at different doses ranged between (0, 2.5, 5, 7.5 and 10 mg/mL) with reference to a negative control and a positive control (glyphosate).

The main results of the dose–response effect of the germination-inhibiting activity of the different herbs tested weeds, the growth-inhibiting activity of the aerial parts and the inhibition of roots growth are presented respectively in Tables 5, 6 and 7. Similarly, the dose–response effect of fennel tested ME and Eos on the germination and growth of aerial parts and roots is represented in Figs. 2 and 3.

According to Table 5, the ME and the Eos inhibited the germination of all the tested species in a dose-dependent manner ($p \leq 0.05$), the same for glyphosate.

According to statistical analysis, the tested herbs showed varying degrees of sensitivity to ME, Eos and glyphosate.

Indeed, *S. arvensis* showed a high sensitivity to the action of ME and Eos, a total inhibition at the dose of 5 mg/mL, however, at the same dose, this inhibition is partial with glyphosate (23%).

Similarly, for *L. rigidum*, total inhibition of germination by ME and Eos was obtained at a dose of 5 mg/mL, however this inhibition is partial with glyphosate (50%).

So, the ME and Eos of *F. vulgare* showed a great antigerminative capacity against *S. arvensis* and *L. rigidum*, this herbicidal activity exceeds that of glyphosate. However, for *L. sativum*, a similar activity was obtained for Eos and glyphosate (total inhibition of germination at 5 mg/mL). Whereas, at the same dose, this inhibition is partial with ME (10%).

For *T. campestre*, this weed showed significant resistance to the action of ME and Eos, indeed at a dose of 7.5 mg/ml, inhibition of germination and total with glyphosate. EOs completely inhibits germination at doses (10 mg/mL), however at the same dose, germination in the presence of ME is in the order of (56.66%). On the other hand, the action of ME and Eos is exerted not only on germination but also

Table 5 Phytotoxic activity of Fennel essential oils and methanolic extract on seeds germination (%) of tested herbs (cm)

Tested herbs	Germination (%)			
	Doses (mg/mL)	Essential oils	Methanol extract	Glyphosate
T. campestre	0	96.7 ± 0.6^{a}	96.7 ± 0.6^{a}	96.7 ± 0.6^{a}
	2.5	93.4 ± 0.57^{a}	93.4 ± 0.57^{a}	80 ± 2.9^{b}
	5	83.33 ± 0.57^{a}	60 ± 0^{b}	60 ± 0^{c}
	7.5	70 ± 2.9^{b}	60 ± 0^{b}	0 ± 0^{d}
	10	0 ± 0^{c}	56.66 ± 0.57^{b}	0 ± 0^{d}
S. arvensis	0	90 ± 0^{a}	90 ± 0^{a}	90 ± 0^{a}
	2.5	10 ± 0^{b}	26.66 ± 0.57^{b}	26.66 ± 2.51^{b}
	5	6.66 ± 0.57^{b}	0 ± 0^{c}	23.33 ± 0.57^{b}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
L. rigidum	0	90 ± 0.57^{a}	90 ± 0.57^{a}	90 ± 0.57^{a}
	2.5	20 ± 0^{b}	36.66 ± 0.57^{b}	81.7 ± 1.7^{b}
	5	0 ± 0^{c}	0 ± 0^{c}	50 ± 2.9^{c}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{d}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{d}
L. sativum	0	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	2.5	26.66 ± 0^{b}	100 ± 0^{a}	36.66 ± 0.57^{b}
	5	0 ± 0^{c}	10 ± 0^{b}	0 ± 0^{c}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}

Results are reported as the mean ± SD of three experiments. The superscripts are used to compare the dose effect for each tested samples and on each tested herbs. Means followed by the same letter are not significantly different by the Student–Newman–Keuls test ($p \leq 0.05$)

Table 6 Phytotoxic activity of Fennel essential oils and methanolic extract on shoots growth of tested herbs (cm)

Tested herbs	Shoots growth (cm)			
	Doses (mg/mL)	Essential oils	Methanol extract	Glyphosate
T. campestre	0	2.3 ± 0.16^{a}	2.3 ± 0.16^{a}	2.3 ± 0.16^{a}
	2.5	0.58 ± 0.05^{b}	0.7 ± 0.08^{b}	0.18 ± 0.01^{b}
	5	0.3 ± 0.1^{c}	0.56 ± 0.06^{bc}	0.15 ± 0.01^{b}
	7.5	0 ± 0^{c}	0.46 ± 0.06^{bc}	0 ± 0^{c}
	10	0 ± 0^{c}	0.40 ± 0.9^{c}	0 ± 0^{c}
S. arvensis	0	2.4 ± 0.23^{a}	2.4 ± 0.23^{a}	2.4 ± 0.23^{a}
	2.5	0.5 ± 0^{b}	1.7 ± 0.25^{b}	0.5 ± 0.1^{b}
	5	0.3 ± 0.28^{bc}	0 ± 0^{c}	0.26 ± 0.12^{b}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}
L. rigidum	0	4.5 ± 0.97^{a}	4.5 ± 0.97^{a}	4.5 ± 0.97^{a}
	2.5	0.9 ± 0.17^{b}	1.5 ± 0.3^{b}	0 ± 0^{b}
	5	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}
	7.5	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}
	10	$0 \pm 0b$	0 ± 0^{b}	0 ± 0^{b}
L. sativum	0	2 ± 0.1^{a}	2 ± 0.1^{a}	2 ± 0.1^{a}
	2.5	1.8 ± 0.26^{a}	1.16 ± 0.03^{a}	0.27 ± 0.23^{b}
	5	0 ± 0^{b}	0 ± 0^{c}	0.15 ± 0.04^{b}
	7.5	$0 \pm 0b$	0 ± 0^{c}	0 ± 0^{c}
	10	0 ± 0^{b}	0 ± 0^{c}	0 ± 0^{c}

Results are reported as the mean ± SD of three experiments. The superscripts are used to compare the dose effect for each tested samples and on each tested herbs. Means followed by the same letter are not significantly different by the Student–Newman–Keuls test ($p \leq 0.05$)

on the growth of the aerial and root parts (Tables 5 and 6). According to the statistical analysis, the tested samples inhibit seedling growth in a dose-dependent manner. The growth inhibitory potential of ME and Eos is comparable to that of glyphosate. In the same way as the anti-germinative activity, the phytotoxic effects are variable depending on the used herb and tested sample, whether for the growth of the roots or the aerial parts. Similarly, *S. arvensis* is the most sensitive to the action of the samples tested and *T. campestre* is the most resistant.

In summary, the two tested samples showed herbicidal activities on the germination and growth of the tested weeds and cultivated species, these activities are similar for the two samples and are also comparable with that of the synthetic herbicide, without noting any selectivity between the monocot and dicots.

On the other hand, it is very important to assess the herbicidal activities recorded with ME, indeed this study is the first to describe the phytotoxic activity of fennel ME. From a practical point of view and exploitation of wild fennel, the yield of ME is around 9.7%, saying 5 times higher than that of essential oils (2.04%), which could explain the importance of the exploitation of wild fennel ME for their application as potential sources of molecules with herbicidal properties. The results of this study are in agreement with the literature; indeed, several studies have shown the herbicidal potential, and the key role played by Eos and crude extracts in the phenomenon of allelopathy [8, 29, 30]. For the methanolic extract of fennel, several families of compounds co-exist in this extract. According to Table 4, 18 molecules between phenolic acid and flavonoids have been highlighted. This type of molecules, namely flavonoids or phenolic acids, are known for their herbicidal and phytotoxic properties [31, 32]. Similar results of the aqueous extract of *Adenosma buchneroides* were obtained by Wang et al. [33], in fact, a specific richness of this species in flavonoids and phenolic acids was described, as well as a great herbicidal potential exerted by the aqueous extract on the germination and growth of the aerial and root parts of three weeds (*Paspalum thunbergii*, *Bidens pilosa*, and *Bromus japonicas*)

Table 7 Phytotoxic activity of Fennel essential oils and methanol extract on roots growth of tested herbs (cm)

Tested herbs	Roots growth (cm)			
	Doses (mg/mL)	Essential oils	Methanol extract	Glyphosate
T. campestre	0	4.4 ± 0.22^{a}	4.4 ± 0.22^{a}	4.4 ± 0.22^{a}
	2.5	1.4 ± 0.58^{b}	4.1 ± 0.08^{a}	0.58 ± 0.06^{b}
	5	0.51 ± 0.1^{c}	2.8 ± 0.03^{b}	0.47 ± 0.02^{b}
	7.5	0.4 ± 0.02^{c}	2.7 ± 0.02^{b}	0 ± 0^{b}
	10	0 ± 0^{c}	1.2 ± 0.07^{c}	0 ± 0^{b}
S. arvensis	0	3.5 ± 0.22^{a}	3.5 ± 0.22^{a}	3.5 ± 0.22^{a}
	2.5	1.6 ± 0.75^{b}	1.9 ± 0.2^{b}	0.56 ± 0.25^{b}
	5	0 ± 0^{c}	0 ± 0^{c}	0.5 ± 0^{b}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
L. rigidum	0	6.5 ± 0.3^{a}	6.5 ± 0.3^{a}	6.5 ± 0.3^{a}
	2.5	0.5 ± 0^{b}	1.5 ± 0.3^{b}	0 ± 0^{b}
	5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
	7.5	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
	10	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
L. sativum	0	9.2 ± 0.08^{a}	9.2 ± 0.08^{a}	9.2 ± 0.08^{a}
	2.5	3.2 ± 0.3^{b}	5.7 ± 0.13^{b}	0.62 ± 0.06^{b}
	5	1 ± 0.28^{c}	0 ± 0^{c}	0.42 ± 0.02^{b}
	7.5	0 ± 0^{d}	0 ± 0^{c}	0 ± 0^{b}
	10	0 ± 0^{d}	0 ± 0^{c}	0 ± 0^{b}

Results are reported as the mean ± SD of three experiments. The superscripts are used to compare the dose effect for each tested samples and on each tested herbs. Means followed by the same letter are not significantly different by the Student–Newman–Keuls test ($p \leq 0.05$)

and tow cultivated crops: *Oryza sativa* and *Zea mays*. Which is in agreement with our study. On the other hand, several phenolic acids have been described in the aqueous extract of *Artemisia vulgaris* including chlorogenic, caffeic, and dicaffeoylquinic acids which have been described as the ultimate bioherbicide against *A. retroflexus* L. in Maize in the pre- and post-emergence stage [34], these compounds exist in the ME of fennel, which proves the herbicidal potential described in this present study. Among the flavonoids of fennel ME myricitrin, naringenin and quercetin were detected in appreciate amount, these phenolic compounds were reported in recent study conducted in Tunisia as the main active phytotoxic compounds in *Cynara cardunculus* [32].

The Eos of *F. vulgare* have been reported to have a phytotoxic potential on the germination and growth of weeds, however, according to our extensive knowledge, no studies on the non-volatile fraction represented in our study by the methanolic extract.

Previous study conducted on the Eos from *F. vulgare* growing in Turkey [15], Iran and Germany [17] showed phytotoxic potential on the germination and growth of *A. pallida*, *A. retroflexus*, *C. salsotitialis*, *S. arvensis*, *S. oleraceus*, *R. raphanistrum*, *R. nepalensis* and *C. arvensis* [15, 17].

Apiaceae is the family of the mostly aromatic plants that contain nearly 3780 species [35]. Several studies carried out on plants belonging to this family proved their allelopathic properties. *Eryngium triquetrum* and *Smyrnium olusatrum* Eos showed high phytotoxic effect against *Lepidium, sativum* [36]. Similarly, Eos of *Carum carvi* exhibited significant herbicidal activity against germination and seedling growth of *Echinochloa crus-galli* as a very aggressive weeds in maize fields [37].

From the point of view of correlation between the chemical composition of the tested samples and their herbicidal potential, looking at Table 2, the chemical composition of essential oils is characterized by a great richness in hydrocarbonated monoterpenes like limonene (8.6%) and oxygenated monoterpenes, in particular derivatives of phenylpropanoids: estragole (76.2%) and α-thujone (9.6%), these compounds are known to have herbicidal activities which could explain the obtained results in this study [38, 39].

About the mechanism of action of allelochemicals, several studies indicated that the mode of action of plant secondary metabolites is related to their ability to penetrate through cell membranes and inducing alteration of membrane integrity. Lins et al., indicated that allelochemicals are able to cross the cell to interact with the plant plasma membrane [40].

In addition, the phenolic compounds such as phenolic acids and flavonoids identified in *F. vulgare* methanolic extract were known to be involved in alteration of several physiological activities, in fact, these molecules like: quercetin-3-o-rhamonoside, rutin, myricitrin, naringenin and quercitin inhibit germination [41], induce disruption of membrane integrity, inhibition of photosynthesis and protein synthesis, blockage of mineral uptake alteration, stomatal closure, alteration of various enzyme activities and induction of water stress [42].

Similarly, Maloney et al., who showed that flavonoids can influence auxin transport and inducing inhibition of seedling roots growth due to the breakage of cell homeostasis leading to ultimate allelopathic stress [43].

Moreover, seedling growth inhibition (Tables 6 and 7) in response to fennel ME and Eos was caused by oxidative stress potentially due to the interaction of phenolic compounds with various hormones transport that auxin, cytokinin, and gibberellin [31, 44].

In accordance with our study, Franco et al. found that exogenous phenolic compounds, inhibit the growth of primary root and improve the development of lateral roots which explain the inhibition of roots growth of tested species in our current study [44] (Table 8).

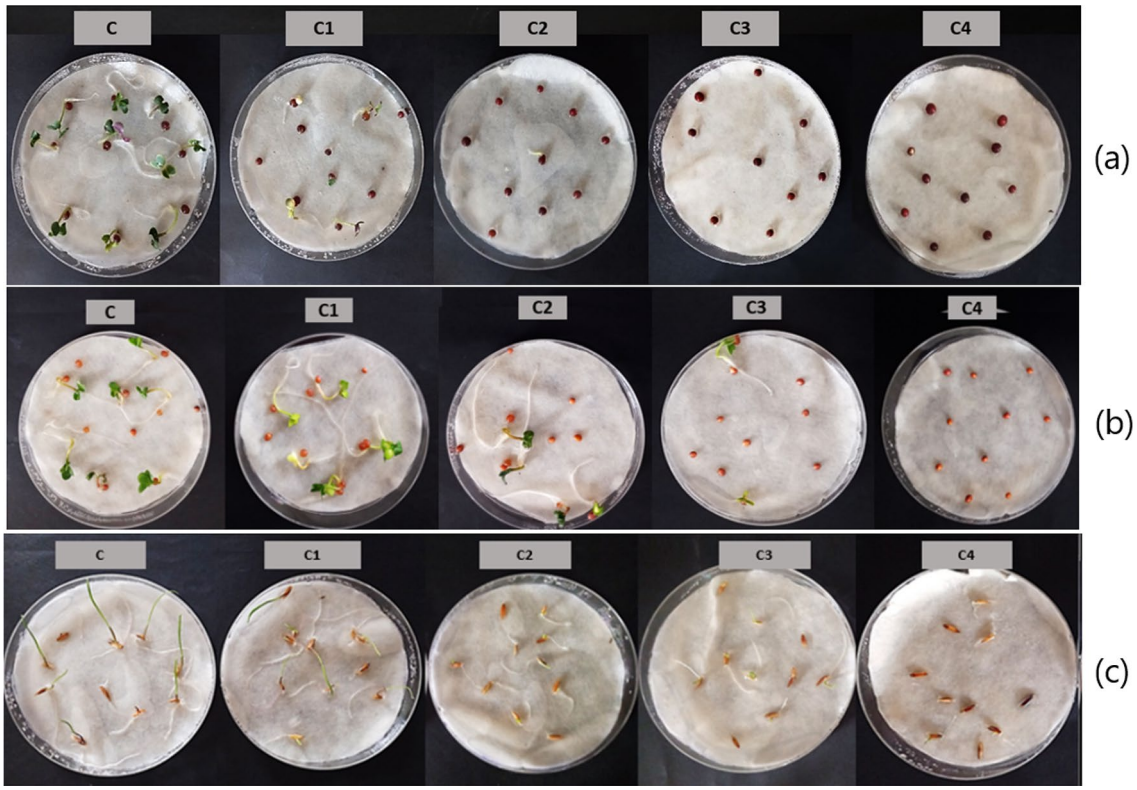

Fig. 2 Antigerminative activity of Fennel EOs on *S. arvensis* (**a**), *T. campestre* (**b**) and *L. rigidum* (**c**)

Similarly, terpenes, phenolic acids and flavonoids have been reported to induce the production of phenoxyl radicals, directly linked to lipid peroxidation and reactive oxygen species accumulation in the cell causing damage DNA, lipids, and other biological functions and consequently stimulates the oxidative defense system of the plant by activation of glutathione reductase, guaiacol peroxidase and superoxide dismutase [31, 41, 45].

In addition, plant allelochemicals that quercetin and rutin have been described to induce an alteration in the energy metabolism of several weeds, these metabolites may interfere in mitochondrial respiration and ATP production by the uncoupling between the synthesis of ATP and the oxidative phosphorylation and inhibiting the electron transport chain. [46, 47].

3.3 Antifungal Activity

The evaluation of the antifungal properties of Eos and ME of fennel was carried out against the growth of five strains of phytopathogenic fungi which are very harmful to the fields of different crops and which are responsible for the contamination of seedlings, root rot, ear and stalk and other diseases of cereals and tomato vascular wilt. The results obtained, made it possible to observe and notice the inhibitory activities of the growth of the different strains tested.

These activities are variable depending on the nature of the extract evaluated (oil or extract), the dose applied and especially the strain of fungus tested. Both tested samples showed significant and remarkable antifungal effects. A dose–response effect was recorded for the oils or methanolic extract ($p \leq 0.05$). With the rates of inhibition of fungal growth are high, total inhibition was obtained at a dose of 10 mg/ml for all strains except *F. culmorum* where we obtained inhibition of the order 94% at 10 mg/mL.

For ME, the inhibition is always partial, even at a dose of 10 mg/mL. Similarly for ME, the two strains *F. culmorum* and *F. oxysporum mathioli* are the two strains most resistant to the action of ME. These results are in agreement with recent work on the antimicrobial potential of oils [6, 48], and extracts from *F. vulgare*. However, the majority of activities have been carried out on bacteria and especially clinical strains. Similarly, no studies on the antifungal potential of the ME of fennel in the literature. The study of the chemical composition of fennel oils and extracts could explain the observed activities. In particular, fennel oils showed richness in phenylpropanoides (estragole as major compounds 76%), and limonene as monoterpene hydrocarbons, these two compounds are known to have antimicrobial potential [49, 50]. In agreement with the results of this present study, the study of antimicrobial activity of estragole have been reported, it

Fig. 3 Phytotoxic effects of Fennel EOs on seedling growth of *L. rigidum* (**a**), *T. campestre* (**b**) and *S. arvensis* (**c**)

Table 8 Antifungal activity of Fennel essential oils and methanol extract on growth of tested strains

Tested fraction	Doses (mg/mL)	Fungal strains growth inhibition (%)				
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Essential oils	2	43.1 ± 2.4^{a}	28.9 ± 3.8^{a}	18.3 ± 3.7^{a}	14.5 ± 2.5^{a}	31.9 ± 3.6^{a}
	4	73.6 ± 6.4^{b}	91.1 ± 7.7^{b}	52.7 ± 16.2^{b}	69.5 ± 11.5^{b}	70.2 ± 7.4^{b}
	6	83.3 ± 8.3^{b}	86.7 ± 0^{b}	80.6 ± 11.2^{c}	88.5 ± 5^{c}	80.8 ± 6.4^{c}
	8	95.8 ± 7.2^{c}	86.7 ± 0^{b}	91.4 ± 7.4^{c}	88.5 ± 5^{c}	87.2 ± 0^{c}
	10	100 ± 0^{c}	100 ± 0^{c}	100 ± 0^{c}	94.2 ± 5^{c}	100 ± 0^{d}
Methanol extract	2	69.4 ± 2.4^{b}	71.1 ± 7.7^{a}	31.18 ± 7.4^{a}	14.5 ± 10^{a}	53.2 ± 3.7^{a}
	4	81.9 ± 4.8^{a}	77.8 ± 3.8^{b}	35.4 ± 0.1^{bc}	33.3 ± 2.5^{b}	51 ± 3.6^{a}
	6	55.5 ± 2.4^{c}	82.2 ± 3.8^{b}	39.7 ± 3.7^{bc}	42 ± 6.6^{cd}	63.8 ± 3.7^{b}
	8	59.7 ± 2.4^{c}	64.4 ± 7.7^{a}	44 ± 3.7^{c}	52.2 ± 4.3^{d}	66 ± 3.6^{b}
	10	66.6 ± 9.7^{b}	82.1 ± 3.8^{b}	42 ± 0^{c}	47.8 ± 0^{d}	68.1 ± 0^{b}

Strain 1: *Fusarium oxysporum* sp. *Lycopersici*, Strain 2: *F. redolens,* Strain 3: *F. oxysporum mathioli,* Strain 4: *F. culmorum*, Strain 5: *F. oxysporum* 184

Results are reported as the mean ± SD of three experiments. The superscripts are used to compare the dose effect for each tested samples and on each tested fungi. Means followed by the same letter are not significantly different by the Student–Newman–Keuls test ($p \leq 0.05$)

has been shown to carry a significant antibacterial potential [49]. Similarly, the antimicrobial potential of estragole was tested against *Pseudomonas syringae* pv. *actinidiae* that causes bacterial canker disease in kiwi fruit, it was found that this compound has antimicrobial potential when tested by the vapor diffusion and liquid culture assays [51], this could explain the activities observed in our present study. Moreover, the antifungal activity of limonene has been reported in the literature [50, 51]. In a recent study conducted by Weisany et al., the content of limonene was enhanced in volatile seed oils of plants colonized by *Funneliformis mosseae*: arbuscular mycorrhizal fungi [51]. This explains that this monoterpene present in the oil obtained from seed of Fennel is involved in the plant's defense mechanism particularly against fungi. In addition, the antifungal properties of limonene were tested against *Trichophyton rubrum*. Obtained result showed a remarkable fungicidal effect linked to the presence of this compound [51].

Which could explain the antimicrobial potential of tested oils in the present study and it allows us to attribute the antimicrobial properties of fennel EOs to their major compounds: limonene and estragole. Similarly, for the methanolic extract, several molecules detected have been described to have antifungal activities, in particular: Caffeic acid, Epicatechin and Quercetin have been described effective against the growth of several fungi such as *T. rubrum* and *Candida albicans* [52]. In the same way, The 4 hydroxybenzoic acid, cisic acid, rozmarinic acid, rutin, vanillic acid, gallic acid, cafeic acid, ferulic acid, quercetin and gentisic acid as phenolic compounds obtained from ME of *Muscari aucheri* were tested for their antifungal activities against several fungal strains: *Alternaria solani*, *Rhizoctonia solani*, *Botrytis cinerea*, *Verticillium dahliane*, and *F. oxysporum* f. sp. *Cucumerium*. Obtained data showed 100% inhibition were observed with all tested strains at 10 and 20 mg/mL doses [53].

The mechanisms of action of these compounds have been described from Inhibits isocitrate lyase enzyme activity [54], and Reduces ergosterol levels and modulates the expression of FAS1 and ERG6 in fungi cells [55]. Generally, Eos components like terpenoids and phenylpropanoids derivatives, phenolic acids and flavonoids were reported to exert a pronounced antifungal effects. In fact, recent studies evidence that extracts/compounds can affect fungal cells through interaction with the lipid bilayer of membrane inducing an alteration of membrane integrity and by intercalation in the cell wall and DNA [56].

Nevertheless, further studies are needed not only to assess the antifungal potential of molecules involved in antifungal potential, but also to elucidate the modes of action involved in the potential of fennel phenolic and terpene compounds against phytopathogenic fungi, as well as to validate results in field studies.

4 Conclusion

The utilization of fennel EOs and ME in the agricultural and agro-industrial applications, as a source of functional molecules to use in ingredients and bio-pesticide formulations against weeds and fungal pathogens, is a promising field. The observed herbicidal and antifungal properties of fennel oils and ME made them attractive for several applications. In this way, the obtained data in this study show that the biological properties of Fennel EOs and ME can be exploited as bio-pesticides for fungi and weeds control.

Data availability statement The data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest Authors declare no conflicts of interest relevant to the content of this work.

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