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Essential oil and chemical composition of wild and cultivated fennel (*Foeniculum vulgare* Mill.): A comparative study

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ABSTRACT

Fennel (*Foeniculum vulgare Mill*) figures among medicinal and aromatic herbal species that grow wild in oasis environments. The goal of the current work is to study for the first time the impact of domestication on the yield, phytochemical profile and antiradical activity of essential oils drawn from wild and domesticated fennel seeds in Morocco. The main findings revealed that wild fennel had the highest seed yield (10.98±0.4 g/plant) compared to the cultivated plant (9.14±0.5 g/plant) and the yield of essential oils was not enhanced by domestication. Actually, wild fennel recorded the highest yield of essential oil (3.67 ± 0.13%), whereas cultivated fennel exhibited the lowest yield (2.13 ± 0.07%). Similarly, the wild fennel essential oil showed the highest phenolic content (222.24 μg/mL) and antioxidant power based on β-Carotene bleaching assay (IC_{50}= 0.694 mg/mL) and TBARS assay (IC_{50} = 1.193 mg/mL). The chromatography analysis showed that that estragole, anethole, and fenchone were the main compounds in both wild and cultivated fennel. However, cultivated fennel contained a high amount of estragole and a lower amount of anethole than that of wild fennel. Concerning the other compounds, the chemical profile was almost similar with minor quantitative variation. Regarding the antibacterial activity, cultivated fennel seeds essential oil recorded the strongest effect compared to that of the wild plant which might be related to the main compounds found in this essential oil.

1. Introduction

Herbal medicines have been employed for centuries by numerous civilizations worldwide for promoting health, anticipating and healing various disorders. The World Health Organization claimed that approximately 88% of the world population uses plants for health care (WHO, 2019). As a result, the demand for these plants has increased massively and their cultivation continues to grow (WHO, 2019). Wild fennel (*Foeniculum vulgare Miller*) figures among the well-valued aromatic and medicinal plants in the Mediterranean countries (Abdellaoui et al., 2017). Several pharmacological activities have been associated with fennel essential oils such as hepatoprotective, acaricidal, anti-inflammatory, antioxidant, antifungal, antithrombotic, anti-tumor, antidiabetic and antibacterial activities (Badgujar et al., 2014). These therapeutic potentials are due to the volatile components within fennel essential oil (Badgujar et al., 2014). Several works in the literature have shown that the yield, chemical profile and antioxidant activity of fennel essential oil are influenced by intrinsic and extrinsic factors in particular environmental conditions (Telci et al., 2009). In the same vein, Ehsanipour et al. (2012) have reported that the harvest of essential oil extracted from fennel seeds is affected by fertilizer application. Alternatively, various authors have reported that domestication enhances the production of essential oil (Pirbalouti et al., 2013; Julio et al., 2015). Nevertheless, it was demonstrated that drought drastically decreases fennel's growth, root mass, grain and dry mass yield while exogenous proline increases fennel's growth and grain yield (Gholami Zali et al., 2018). Moreover, Akbari-kharaji et al. (2020) have stated that meaningful positive associations were found between fennel seed yield and yield components as seeds/plant and certain physiological attributes, i.e., proline and total soluble carbohydrates concentrations, water potential, relative water content, and superoxide dismutase, catalase, and ascorbate peroxidase activities. The same authors have claimed that maximum seed and essential oil yields of plants grown under water-limited conditions were found in genotypes that benefited more from osmoregulative and antioxidative roles of proline and the antioxidative enzymes and, hence, were able to withstand better against water limitation. On the other hand, several researchers have found that cultivation did not have any significant effect on the amount of essential oil within plants (Economakis et al., 1999; El Bouzidi et al., 2013; Kasrati et al., 2013). In addition, Shahat et al. (2012) compared between the essential oils of the aerial parts of cultivated and wild

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fennel and found differences in the chemical composition but did not assess the effect of domestication. Furthermore, to the best of our knowledge, studies on the impacts of domestication on the contents of essential oil within fennel seeds and their biological activities are very scanty.

Hence, this work aims at evaluating the impacts of cultivation on the seed yield, essential oil yield, phytochemical profile, antioxidant and antibacterial activities by comparing the essential oils extracted from wild and domesticated fennel seeds in Morocco.

2. Material and methods

2.1. Plant materials

The seeds of wild fennel (*Foeniculum vulgare Mill*) were collected from 40 tufts (each tuft contains 6–25 individual) with an interspace in the range 5–30 m, belong to the natural population located in Gourrama, Southeastern Morocco (N 32°18'25.719" W 3°58'59.281"), at an altitude of 1089 m. The wild fennel seeds were collected twice on October 2016 for sowing and on October 2017 for analytical comparison. The plant material was authenticated by a qualified botanist at the Faculty of Sciences and Techniques- Errachidia (FST-E) and the voucher specimen (FST-E 061.19) was deposited at the herbarium of the faculty. The total rainfall recorded in the collection site during the period framing, from September 2016 until October 2017, of the crop cycle was 139 mm. the collected seeds were stored in a refrigerator at 4°C until use.

2.2. Experimental assay

The cultivation of wild fennel seeds was carried out in an experimental test installed on 21st January 2017 in an isolated plot, fallow for the previous 4 years which marks it a suitable place for organic manufacturing at the oasis of Oukhite, region of Errachidia (Morocco) (N 31 ° 27 '21′ '- W 4 ° 36′ 19 '') high of about 1107 m above sea level. In this experimental plot, wild fennel seeds were sown manually in line, with a seeding rate calculated to have 12 kg / ha. The cultivation of Fennel was performed in respect of organic farming method without recourse to chemical inputs. Thus, we used an organic authorized fertilization treatment (2.2 tonnes / ha) for use in organic farming compatible with the new European Union organic Regulation: (EC) No 834 / 2007 (Table 1). The cultivated fennel plants were subdivided into elementary plots, each consisting of six lines of 5 m in length with a space of 25 cm between the lines. The cultivated plants were irrigated by a drip system every two days using an irrigation dose of approximately 3750 m^3/ha/cycle. After the emergence stage which occurred on 08/02/2017, the thinning operation was performed after the plants reached 8 cm in height in order to set a distance of 30 cm between plants. Manual weeding was done whenever the weeds reached unacceptable threshold as a way to avoid the manipulation of herbicides. The domesticated fennel seeds were harvested in September 2017. The collection site and cultivation area are not far from each other and belong to the same oasian environment characterized by a sandy clay soil, total rainfall less than 150 mm, high temperatures and low humidity.

Table 1.
Characterization of the organic fertilizer used in the study (Italpollina, 2007)

Characterization of organic fertilizer	Value in (%)
-Azote total	5
- P_2O_5 (Phosphoric anhydride)	5
- K_2O (Potassium oxide)	8
- MgO (Magnesium oxide)	2
- CaO (Calcium oxide)	4
- Trace elements (Fe, B, Zn, Mn, Mo)	0.2
- Organic matter	60
- Humidity	8

2.3. Seed yield

The fennel seeds ripened on 20 October 2017. To avoid wasting seeds, they were harvested at their full maturity with a sickle in the early morning. The collected seeds were dried away from light and in a well-ventilated area during ten days. After then seeds were threshed prudently to avoid breaking them. Prior measurement, the production of seeds was depicted as the average of seed yield of ten plants and the results were expressed in gram per plant. The seeds were winnowed to eliminate impurities.

2.4. Extraction of essential oil

Steam distillation in a Clevenger-type apparatus was implemented to extract the essential oils following the method described by Bammou et al. (2019). Three distillations were performed for an average of three hours for each hundred gram of fennel seeds. The ratio between the volume of the essential oil obtained and the weight of the seeds used was considered the yield of essential oil. The anhydrous sodium sulfate was used to remove water from the collected essential oils and stored in small dark bottles at 4°C placed away from light prior analysis.

2.5. Phytochemical analysis

2.5.1. Quantification of total phenolic content

Total phenolic content (TPC) of the essential oils was assessed following the method depicted via Bouhlali et al. (2016) with minor adjustments. Briefly, an aliquot (100 μL) of essential oil diluted with methanol, was stirred consecutively with 500 μL of a 1/10 methanolic dilution of Folin–Ciocalteu's reagent, and then 400 μL of sodium carbonate solution (7.5% w/v). The resulting solution was incubated for 60 minutes at ambient temperature and then the turbidity was read at 765 nm. Gallic acid was implemented to construct the standard curve. The overall amount of phenolic components was depicted as μg Gallic acid equivalent per mL of essential oil.

2.5.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Clarus 600 GC-MS system (Perkin Elmer, USA) was implemented to analyze the essential oils following the procedure of Sellam et al., 2015. The compounds were separated in fused silica capillary Rtx-1 column (40 m × 0.18 mm × 0.4 μm, purchased from Restek, Germany). The temperature gradient over the column ranged from 60 °C to 250 °C at a speed of 4 °C/min; the injection amount was 1 μL; the mode of injection was split (split ratio of 1:50) and the temperature of the injector was 250 °C. The EI mechanism (70 eV) was implemented to ionize the essential oil compounds. Mass spectra and retention time (min) of the majority of the components were compared to those performed in similar GC-MS parameters following the mass-spectra library Wiley/NIST database (2014).

2.6. Antioxidant activity

2.6.1. Thiobarbituric acid reactive species assay (TBARS)

The essential oils were examined for their capacity to inhibit lipid peroxidation by using TBARS assay described by Bouhlali et al. (2020). Briefly, 500 μL of egg yolk homogenate (10% w/v in phosphate-buffered saline (pH 7.4)) was mixed with 100 μL of sample diluted in methanol and the volume was made up to 1000 μL with distilled water. Thereafter, the mixture was stirred with 50 μL of $FeSO_4$ (0.07 M) and allowed to stand at 37°C for 30 min to start fat peroxidation. Later on, the final solution received 50 μL of trichloroacetic acid (TCA) (20%), 1.5 mL of Thiobarbituric acid (TBA) (0.8% w/v

prepared in 1.1% sodium dodecyl sulphate) and 1.5 mL acetic acid (20%, pH 3.5), and then mixed, put on heat at 95°C for 1 hour. After cooling at room temperature, each tube received 6 mL of 1-butanol and the contents of the tubes were agitated and then centrifuged for 10 minutes at 3000 rpm to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was quantified against 3 mL butanol at 532 nm. Only, 100 μL of distilled water played the control. The prevention of fat peroxidation was calculated using the equation as follows:

$$\text{ILP}(\%) = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \text{x } 100$$

The ILP (%) plotted against the concentrations of samples or standards and IC_{50} values were determined (concentration of essential oil or standard to prevent 50% of lipid oxidation).

2.6.2. β-carotene bleaching assay

The β-carotene bleaching inhibition method was executed following the procedure of Bammou et al. (2019). Briefly, ten milliliter of chloroform was used to dissolve 2 mg of β-carotene, then, 4 mL of the resulting mixture was poured into a round-bottom flask, which contains 500 mg of Tween 40 and 40 mg of linoleic acid. The solvent was then withdrawn within vacuum at 40°C, and after that, oxygenated distilled water (100 mL) was poured into the flask and strongly vortexed to give a fresh emulsion. Afterwards, each test tube, containing 100 μL of the diluted essential oil, received 1 mL of the emulsion and incubated in a water bath at 50°C then the turbidity was read at 470 nm immediately (t = 0 min) and after 120 min of incubation against a blank which contained only the emulsion. BHT was the standard antioxidant. The β-carotene bleaching inhibition (%) of the analyzed solution was calculated via the equation as follows:

$$\beta \text{ carotene bleaching inhibition } (\%) = \frac{\beta \text{ carotene content after 2 h assay}}{\text{Initial } \beta \text{ carotene content}} \text{x } 100$$

The IC_{50} value was calculated using a calibration plot between the oil concentration and the percentage of β-carotene bleaching inhibition.

2.7. Antibacterial activity

2.7.1. Bacterial strains

The essential oils were investigated for their antimicrobial power against some targeted bacterial strains linked with numerous disorders, three Gram - bacteria: *Salmonella abony* NCTC 6017, *Pseudomonas aeruginosa* ATCC 27853 and *Echerichia coli* ATCC 25922 as well as three Gram + bacteria: *Bacillus cereus* ATCC 29213, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. The National Institute of Hygiene (Rabat) provided the bacterial strains.

2.7.2. Disc-diffusion method

The disc-diffusion test of fennel essential oil was executed following the procedure reported by Bammou et al. (2019) with minor adjustments. Summarily, Mueller Hinton agar media plates were used to spread over them 350 μL of bacterial cell suspension (concentration of 10^8 Colony Forming Unit (CFU)/mL prepared using an overnight culture growth incubated at 37°C) then a paper disc impregnated with essential oil (10 μL) was put on the top of the media. After incubating the Petri dishes for 24 hours at 37°C, the width of an inhibition zone following the perimeter of the discs was measured. Gentamicin (10 μg/disc) and Tetracycline (30 μg/disc) known antibiotic drugs played the positive control. A non-treated culture of bacterial cells was used as a negative control. All measurements were executed in triplicates. The widths of inhibition zone were assessed for discordance with standard antibiotics.

2.7.3. Minimum inhibitory concentration (MIC)

The fennel essential oils were also investigated for their antibacterial power, referring to the micro-dilution protocol of Bammou et al. (2019) with minor modification. The bacterial culture was sat up via an overnight dilution of test bacterium within 0.1% physiologic water to achieve 10^6 CFU/mL and resazurin solution was made by dissolving one tablet in sterile water. Afterwards, bacterial culture (100 μL) was poured into each numbered wells followed by resazurin solution (100 μL) and 100 μL of each serially diluted essential oil by dimethyl sulfoxide (DMSO). After incubation for 24 hours at 37°C under vigorous agitation, the blue colored solution in Microtiter plates indicates growth inhibition of bacteria. The negative control contained the sterile broth and resazurin solution, whereas broth culture, resazurin solution and DMSO played the positive control.

2.8. Statistical analysis

Data were analyzed using SPSS 23 software. The experiments were carried out according to a fully randomized plan (completely randomized design (C.R.D)). Three repetitions were performed and the results were expressed as means (n = 3) and standard deviation (SD). One way ANOVA was performed followed by *post-hoc* Fisher PLSD tests in order to point out discrepancies among the analyzed

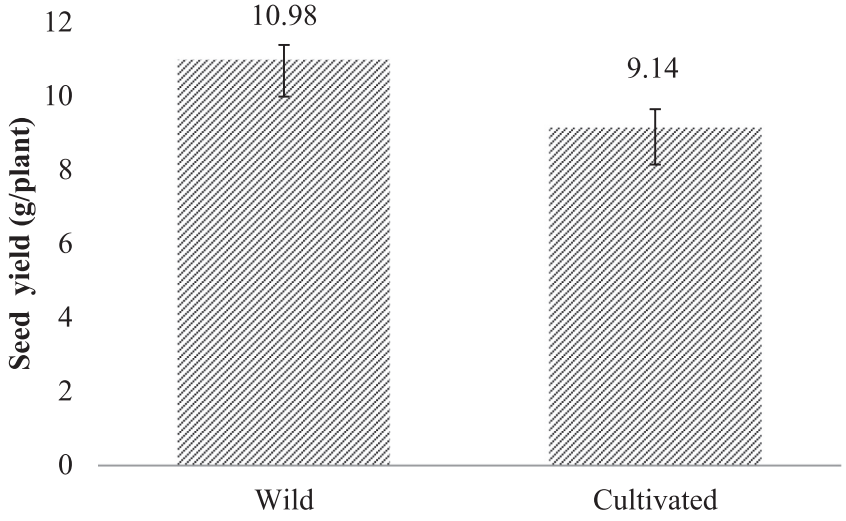

Fig. 1. Seed yield of fennel.

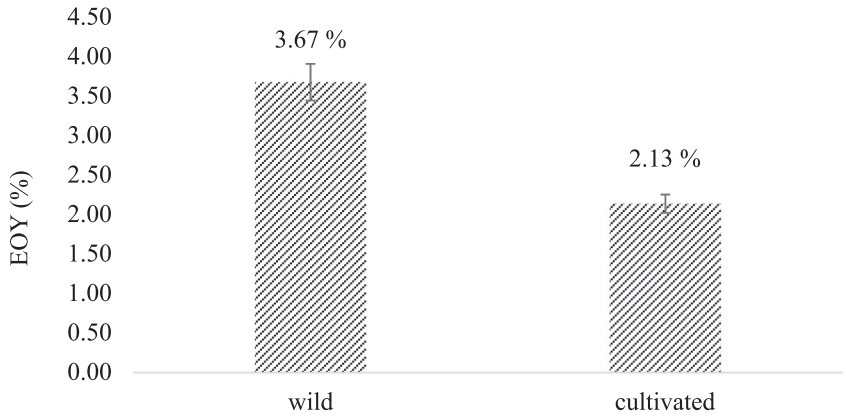

Fig. 2. Essential oil yield (EOY) of fennel

groups. Differences at $p < 0.05$ were confirmed as statistically different.

3. Results and discussion

3.1. Seed yield

The first major difference between wild and cultivated fennel seeds was the seed yield as illustrated in Fig. 1. Wild fennel seeds exhibited the highest seed yield (10.98±0.4 g/plant) compared to the domesticated plant (9.14±0.5 g/plant). Our findings are higher than those found by Ehsanipour et al. (2012) on fennel from Iran (3.68–10.37 g/plant). Moreover, during seed collection, we have observed that the rate of seed production in wild condition was higher than in cultivated state with a difference of at least 15 days. In line with our result, Senatore et al. (2013) have reported that plants yield reproductive tissues (seeds) more rapidly under abiotic stress conditions favored in the wild more than in the cultivated state. Moreover, Malek et al. (2012) and Bakal et al. (2017) have found that domestication decreases seed yield of different varieties of Bangladeshi carrot and soybean, respectively. Gholami Zali et al. (2018) have claimed that foliar-applied proline enhances fennel seed yield while drought decreases the plant seed yield. In addition, Akbarikharaji et al. (2020) have reported that maximum seed yields of fennel plants grown under water-limited conditions were found in genotypes that benefited more from osmoregulative and antioxidative roles of proline and the antioxidative enzymes and, hence, were able to withstand better against water limitation. As a result, given that the collection and cultivation sites belong to the same oasian environment, the discordances found in our results may be ascribed to the growing conditions related to the domestication of wild fennel seeds.

3.2. Yield of essential oil

The essential oil extracted from these seeds consisted of a transparent liquid of light-yellow color with a specific flavor of fennel. The average yields were depicted as milliliter per 100 g of seeds (Fig. 2). Statistical calculations of the production of essential oil evidenced very high significant differences ($p < 0.001$). Wild fennel (3.67 ± 0.13%) showed higher amount of volatile fraction, whereas the domesticated plant (2.13 ± 0.07%) recorded lower amount. This is probably attributed to other causes like high altitude and low temperatures, which provide the best conditions for high levels of essential oil (Pirbalouti et al., 2013). The yields found in the current work were higher than those observed by other authors, 1.1% and 1.6% for the respective fennel seeds from China and Egypt (Ahmed et al., 2019) and from 1.75% to 2.97% by Sabzi Nojadeh et al. (2020). However, our results are lower than those reported by Bettaieb Rebey et al. (2019) (18.72 - 20.02%) as well as those found in some Tunisian (3.24 – 5.26%) and French (3.81 – 4.12%) fennel seeds essential oils as reported by Kalleli et al. (2019). These discrepancies could be ascribed to genetic, geographic and environmental factors (Bahmani et al., 2015), phenological stage (Telci et al., 2009), distillation time (Moser et al., 2014) and cultivation practices (Mona et al., 2008). Our findings demonstrated that cultivation did not enhance the yield of essential oil. However, it was demonstrated that some cultivation practices are needed to improve the amounts of essential oil within fennel (Ehsanipour et al., 2012). Nevertheless, Economakis et al. (1999), El Bouzidi et al. (2013) and Kasrati et al. (2013), who worked on oregano (*Origanum dictamnus*), thyme (*Thymus sp*) and mint (*Mentha Suaveolens* Subsp "Timija" (*Briq*)), respectively, have reported that cultivation had no marked effect on the content of essential oil. On the other hand, Pirbalouti et al. (2013) and Julio et al. (2015) have found that the domestication improves the production of essential oil by thyme (*Thymus daenensis Celak*) and absinthe (*Artemisia absinthium*), respectively. Alternatively, Bettaieb Rebey et al. (2019) have concluded that salinity enhances the production of essential oil by *Foeniculum vulgare* and induced marked changes on the essential oil quality. Moreover, Zali et al. (2018) have reported that proline treatment improves the production of essential oil by different varieties of fennel while drought decreases the quantity and quality of this essential oil.

3.3. Phytochemical analysis

3.3.1. Determination of total phenolic content

Knowing that polyphenols have antioxidant properties, El Ouariachi et al. (2014) mentioned that the wild fennel from Morocco can be a potential source of natural antioxidants which need to be identified and quantified in order to assess the differences between the essential oils extracted from the wild and cultivated fennel seeds. The results in Table 2 showed that the essential oil of wild

Table 2.
Phenolic content and antioxidant activities of fennel seeds essential oil.

	IC_{50} (TBARS)	IC_{50} (βCBA)	TPC (μg GAE/mL)
Wild fennel (mg/mL)	1.193 ± 0.137	0.634 ± 0.027	222.24 ±13.53
Cultivated fennel (mg/mL)	2.346 ± 0.129	0.872 ± 0.031	139.68 ± 14.25
BHT (μg/mL)	1.045 ± 0.112	0.915 ± 0.042	–––

Values are displayed as average (n = 3) ± Standard Deviation (SD). Averages, in the same column, with different letters are significantly different using post hoc Fisher LSD tests ($p < 0.05$). Abreviations: TBARS – Thiobarbituric acid reactive substances, βCBA – Beta carotene bleaching assay, TPC – Total phenolic content, GAE- Gallic acid equivalent.

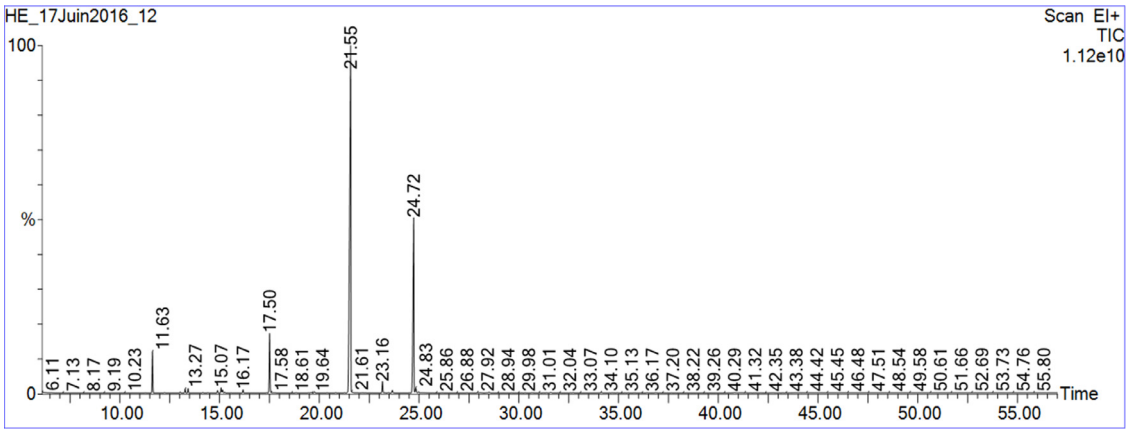

Fig. 3. Chromatogram GC-MS of seeds essential oil of cultivated fennel.

fennel seeds recorded the highest TPC (222.24 μg/mL) compared to that of cultivated fennel (139.68 μg/mL). Our findings corroborate with the results obtained by Marín et al. (2016) who found a TPC of 262.59 GAE mg/L in organic fennel essential oil. However, our results were slightly higher than those found within Egyptian fennel (146.51 mg/L) (Viuda-Martos et al., 2011). The differences in phenolic content between wild and cultivated fennel seeds essential oils could be explained mainly by growing conditions related to the domestication of wild fennel seeds. In fact, plants produce secondary metabolites (including phenolic compounds) to defend themselves against various natural stressors including abiotic factors which are present abundantly in the wild state more than in the cultivated condition (Senatore et al., 2013). Our data are much lower than those reported for other aromatic and medicinal plants widely present within Mediterranean diet, such as clove (898.89 GAE mg/L) thyme (783.81 GAE mg/L), and oregano (763.97 GAE mg/L) but very close to those reported for rosemary (225.08 GAE mg/L) and sage (122.98 GAE mg/L) (Viuda-Martos et al., 2010).

3.3.2. Chemical composition

Essential oils drawn from fennel seeds were investigated qualitatively and quantitatively using GC-MS, which have allowed identifying 21 compounds representing 98.42% and 97.42% amongst the overall constituents of wild and domesticated fennel respectively (Fig. 3; Table 3). These oils are characterized by the dominance of aromatic compounds derived from phenylpropenes (82.54% - 87.92%) followed by oxygenated monoterpenes (8.66% - 5.65%) and hydrocarbon monoterpenes (6.22% - 4.85%) (Table 3). The predominant compounds were estragole (60.01% - 35.33%), anethole (22.15% - 52.27%) and fenchone (6.50% - 4.32%). The same compounds have been reported frequently by several authors, but in different percentages (Viuda-Martos et al., 2011; Moser et al., 2014). However, Rahmani (1986) has reported that wild fennel seeds essential oil from the Azrou region (Middle Atlas, Morocco) did not contain anethole yet, they were rich in hydrocarbon monoterpenes with a predominance of α-phellandrene (39%), limonene (21.4%) and α-pinene (17.6%). Moreover, Shahat et al. (2012), who have analyzed the

Table 3.
Chemical composition of essential oils obtained from the seeds of wild and cultivated fennel.

N°	Retention time (min)	Compound	Cultivated fennel %	Wild fennel %
1	11.629	α-pinène	3.85	2.01
2	12.24	Camphène	0.06	0.32
3	13.017	Sabinène	0.12	0.29
4	13.267	β-Pinene	0.38	0.03
5	13.414	β-Myrcene	0.43	0.06
6	14.154	α- Phellandrène	0.06	0.04
7	14.891	p-cymène	0.25	0.23
8	15.072	Limonène	0.41	0.40
9	15.148	3-carène	0.33	0.86
10	15.255	Eucalyptol	0.10	0.13
11	16.169	Y-terpinène	0.33	0.61
12	17.5	Fenchone	6.50	4.32
13	19.578	Carveol	0.07	0.05
14	19.755	Camphor	0.15	0.13
15	20.815	4-Terpineol	0,05	0,03
16	21.559	Estragole	60.01	35.33
17	23.157	p-cumic aldehyide	1.24	0.5
18	23.457	Cis-Anethole	0.06	0.05
19	23.654	para-Anisaldehyde	0.32	0.27
20	24.724	Anethole	22.15	52.27
21	24.828	α-Terpinen-7-ol	0.55	0.49
Phenylpropenes derivatives			**82.54**	**87.92**
Hydrocarbon monoterpenes			**6.22**	**4.85**
Oxygenated monoterpenes			**8.66**	**5.65**
Total			**97.42 %**	**98.42 %**

phytochemical profile of wild and cultivated fennel, found that cultivated plants showed higher percentages of α-pinene (32.82%), ß-pinene (2.09%), fenchone (5.91%), estragol (15.33%), myrcene (1.57%) and camphene (0.51%) while the wild plants showed much higher level of limonene (84.49%). These variations may be related to the combined effect of many agents comprising genetic factors and geographic origin as reported by several studies (Bahmani et al., 2015; Gholami Zali et al., 2018). The phytochemical profile of the essential oils extracted from wild and domesticated fennel seeds displayed quantitative rather than qualitative differences. Moreover, the nature and the total number of phytochemicals identified were not affected by the cultivation. On this side, the essential oil extracted from the cultivated plant showed high levels of estragole (60.01%) followed by anethole (22.15%), fenchone (6.5%) and α-pinene (3.85%). On the other side, the wild fennel recorded high amounts of anethole (52.27%) followed by estragol (35.33%), fenchone (4.32%) and α-pinene (2.01%). This chemical variation could be attributed mainly to the impacts of domestication on the biosynthesis of the main constituents. In order to reduce the effect of climatic and geographic conditions, the domestication of fennel seeds was done in an area not very far from the area where the wild fennel seeds have been collected which share the same climatic (yearly rainfall, temperature and humidity, ...) and geographic conditions (altitude (1089 m in collection site and 1107 m in the cultivation site). In the current work, it appears that the cultivation of wild fennel under oasis conditions induced an elevation of estragole amount compared to anethole. However, concerning other phytochemicals, they were almost similar between wild and cultivated fennel seeds with minor quantitative variation. Although estragole is exploited as a food flavoring agent and in certain liqueurs, it is suspected of being a carcinogen at high doses (Ishii et al. 2011). It was demonstrated that estragole has been associated with the growth of malignant tumors in rodents (Paini et al., 2010). Hence, the European Union Scientific Committee for Food (SCF) has fixed a new legal limit of estragol (10 mg /Kg) in soft drinks (Zeller and Rychlik, 2006).

3.4. Antioxidant activity

The inhibition of β-carotene bleaching assay and TBARS formation were used to evaluate the antioxidant activity of the essential oils. The concentrations that scavenged 50% of free radical in each test (IC_{50} values), presented in Table 2, showed that both of the analyzed volatile fractions of fennel seeds exhibited powerful antioxidant activity, but remaining lower than that of BHT. In both assays the antioxidant effects varied in a dose dependent manner. Moreover, they attained a plateau above which higher amounts of essential oil had no effect on the antioxidant activity. The strongest antioxidant power was recorded by wild fennel based on β-carotene bleaching assay (IC_{50}= 0.634 mg/mL) and TBARS assay (IC_{50}= 1.193 mg/mL). However, cultivated fennel exhibited the lowest antioxidant capacity. Given the fact that phenolic compounds are known antioxidants, these results appear normal since the wild fennel which exhibited higher phenolic content showed the highest antioxidant activity. In fact, in wild condition, plants are more exposed to natural stresses; especially abiotic factors such as drought, nutritional starvation among others, compared to the cultivated condition, which causes them to release secondary metabolites and increase their antioxidative power in order to defend themselves, hence, the wild fennel scored the highest levels of these compounds (Senatore et al., 2013). Our results are very lower compared to those of Mata et al. (2007) using ß-Carotene bleaching test (IC_{50} = 32.32 μg/mL). However, they remain more effective than those reported by Shahat et al. (2011) for Egyptian fennel using DPPH test (IC_{50} = 15.33 mg/mL) and TBARS assay (IC_{50} = 30.51 mg/mL). These findings back up our former work in which we have found a high antioxidant capacity of fennel seeds essential oil which was higher in wild fennel using DPPH free radical scavenging ability and iron reducing capacity (Abdellaoui et al., 2017). Based on these results, the essential oil obtained from fennel seeds may limit oxidative stress damage occurring in the human body.

3.5. Antibacterial activity

Antibiotics are widely used to manage infectious diseases, however not only the treatment is expensive, antimicrobials were demonstrated to show deleterious effects on gut micro-biota, acidity and burning sensation (Bammou et al., 2019). Essential oils appear to be most relevant in order to treat infections in a natural and cost effective way. The investigation of antibacterial power retained by wild and cultivated fennel seeds essential oils against *B. subtilis, S. abony, P. aeruginosa, E. coli, B. cereus and S. aureus* is revealed in Table 4. The antimicrobial potential of the samples was quantified by means of the MIC. The two fennel seeds essential oils showed an antibacterial effect that varied between the samples and also between the experimental bacteria with a statistical difference only towards the inhibition of *S. aureus* (Table 4). Cultivated fennel exhibited the highest antibacterial activity especially against *E. coli* which was the most sensitive among other bacteria recording the largest inhibition zone (18.35 mm) and the weakest MIC value of 150 μg/mL (Table 5). *P. aeruginosa* and *B. cereus* were the resistant bacterial strains with MIC values $\geq$ 1000 μg/mL. The wild and cultivated fennel exhibited strong antibacterial power but remaining lower than that of Gentamicine (10 μg/ disc) and Tetracycline (30 μg/ disc) used as standard antibiotics. Generally, cultivated fennel recorded lower MIC values

Table 4.
Antibacterial activity of cultivated and wild fennel essential oil against the bacterial strains based on disc diffusion method.

Bacterial strains	Cultivated fennel	Wild fennel	Gentamicine (10 μg/ disc)	Tetracycline (30 μg/ disc)
Gram-negative bacteria				
P.a ATCC 27853	14.64 ± 0.51	12.27 ± 0.74	17.84 ± 0.39	29.54 ± 1.02
E.c ATCC 25922	18.35 ± 0.54	15.62 ± 0.33	19.00 ± 0.00	28.32 ± 0.68
S.ab NCTC 6017	15.37 ± 0.73	13.41 ± 0.69	18.66 ± 0.22	20.84 ± 0.76
Gram-positive bacteria				
S.a ATCC 25923	12.84 ± 0.54[a]	13.73 ± 0.72[a]	20.33 ± 0.22	32.24 ± 0.72
B.c ATCC 29213	14.68 ± 0.72	12.19 ± 0.56	19.66 ± 0.33	24.94 ± 1.12
B.s ATCC 6633	13.26 ± 0.68	15.83 ± 0.57	18.00 ± 0.22	21.61 ± 0.71

Values are mean ± SD. Different letters in each column represent the significance difference at 5% level of error among samples.
P.a: *Pseudomonas aeruginosa*; E.c: *Escherichia coli*; S.ab: *Salmonella abony*; S.a: *Staphylococcus aureus*; B.c: *Bacillus cereus*; B.s: *Bacillus subtilis*.

Table 5.
The MIC (μg/mL) values of cultivated and wild fennel essential oil against the bacterial strains tested.

Bacterial strains	Cultivated fennel	Wild fennel
Gram-negative bacteria		
P.a ATCC 27853	1000	> 1000
E.c ATCC 25922	125	250
S.ab NCTC 6017	250	500
Gram-positive bacteria		
S.a ATCC 25923	250	500
B.c ATCC 29213	750	> 1000
B.s ATCC 6633	250	500

P.a: Pseudomonas aeruginosa; E.c: Escherichia coli; S.ab: Salmonella abony; S.a: Staphylococcus aureus; B.c: Bacillus cereus; B.s: Bacillus subtilis.

compared to those of the wild plant (Table 5). These discordances could be attributed to domestication which was already implicated in the change of chemical composition, thus in biological activities. Our results are in agreement with the study declaring that monoterpenes hydrocarbons plus their oxygenated derivatives which are the principle chemicals of essential oil, display potent antimicrobial activity (Cakir et al., 2004). Many authors have reported that fennel seeds essential oil revealed antibacterial power against food-borne and pathogenic bacteria like *Listeria innocua, Streptococcus haemolyticus, Klebsiella* species and *Staphylococcus epidermis* (Mohsenzadeh, 2007; Marin et al., 2016). Essential oils are known for their hydrophobicity which is accountable for the destruction of bacterial macromolecules (Man et al., 2019). Furthermore, volatile components have different antimicrobial mechanisms including cell membrane permeability and role dysfunction, cell wall and plasmic membrane breakage leading to cytoplasm coagulation and also their capacity to diffuse through the membrane lipid bilayer (Man et al., 2019). On the basis of these results, essential oils extracted from wild and domesticated fennel seeds are rich in antimicrobials effective against a wide spectrum of bacteria. This efficiency could be due to their different chemical composition which might be enhanced by domestication related factors as demonstrated in the current work.

4. Conclusion

The finding of the current work depicted that growing conditions related to the domestication of wild fennel seeds reduced significantly the seed yield, seed essential oil yield as well as phenolic content and antioxidant activity along with changing the phytochemical profile as compared to the essential oil extracted from cultivated fennel seeds. This variation was marked essentially by a considerable elevation of estragol and a decrease in anethole levels. However, the antibacterial activity was increased in cultivated fennel seeds essential oil which may depend on their phytochemical composition. Yet, studies are in progress to evaluate the effects of certain environmental factors and cultural practices on agronomic parameters, phytochemical composition and some other biological activities of essential oil obtained from *Foeniculum vulgare Mill* in the oasis environments.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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