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Changes in Bitter Fennel Essential Oils Exposed to Foliar Spray with L-Phenylalanine

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PRODUCERS in Egypt tend to use traditional methods of feeding medicinal and aromatic plants and separating the essential oils of bitter fennel from fruits only. However, they have not focused on other parts. This study was conducted to evaluate the essential oils of bitter fennel plants sprayed with L-phenylalanine and determine the possibility of using L-phenylalanine in the production of essential oils from bitter fennel parts. Bitter fennel exposed to different L-phenylalanine rates (0.0, 0.3, 0.6, 0.9, and 1.2g/L). Changes in essential oil sources (fresh and dry weights of herbs harvested at vegetative, flowering, and immature fruiting and dry weights of mature fruits) were recorded. Essential oils were extracted through hydrodistillation and analyzed through GC/MS. The greatest weights of plant parts and essential oil yields were recorded with 0.9 g/L. The main compounds were limonene and estragole. The highest values of limonene and monoterpene hydrocarbons were observed with the treatment of 0.6g/L L-phenylalanine in the flowering herb, whereas 0.3g/L L-phenylalanine produced the greatest value of estragole in mature fruits. The treatment of 0.9g/L L-phenylalanine produced the maximum value of oxygenated monoterpenes in mature fruits. The herb obtained in the immature fruiting period had the greatest value of sesquiterpene hydrocarbons under control treatment. Thus, this study could be used as a reference for selecting the suitable L-phenylalanine rate and bitter fennel part to produce essential oils based on the target constituents.

Keywords: Bitter fennel, Essential oil, L-phenylalanine, Plant parts.

Introduction

Medicinal and aromatic plants (MAPs) have been widely explored because of the increasing demand for their use in various purposes, such as food, medicine, and pharmaceuticals. Although MAPs contain different byproducts, they are economically important products and represent a major economic source that helps increase the national income (Kanel, 2000). The most important MAPs are essential oils (EOs; Dhifi et al., 2016). EOs have many biological characteristics because they have many applications, such as medicines, cosmetics, perfumes, flavor, fragrances, food additives, agrochemicals, and biopesticides (Bakkali et al., 2008). Scientific studies have proven that using EOs produced by MAPs in different purposes is better than using synthetic chemicals, but synthetic products are undesirable because of their carcinogenic effects, acute toxicity, and environmental hazard potential (Bakkali et al., 2008).

EOs from bitter fennel (*Foeniculum vulgare* Mill), which is a medicinal and spice plant belonging to Apiaceae (Omidbaigi, 2007), are used as a flavoring and aromatic agent in food products, aroma therapy, and pharmaceutical industries (Guillen & Manzanos, 1994).

EO production from plants is an important work because it is related to quantity and quality (Youssef & Talaat, 1998). Therefore, modern technology must be applied to achieve this goal because the

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use of traditional methods, such as fertilizers and pesticides, is no longer sufficient (Prins et al., 2010). Innovative methods that affect different components of plant EOs should be developed (Mehdizadeh & Moghaddam, 2020). One of the modern technology methods that influence their physiological processes and modify their growth and chemical contents is the treatment of aromatic plants with low values of natural biostimulants (Youssef & Talaat, 1998). Among biostimulants are amino acids, which play a fundamental role in the biosynthesis of organic components, such as pigments, vitamins, alkaloids, enzymes, EO, terpenoids, antioxidant, coenzymes, purine, and pyrimidine bases (Wu, 2009; Rodriguez, 2015). Amino acids are a source of energy, carbon, and nitrogen that constitute plant tissues and organs (Bromke, 2013). Previous studies reported that amino acids positively affect the production and components of EOs of aromatic plants. For example, geranium, thyme, philodendron, navel orange, hyssop, and lemon plants treated with amino acids have higher EO compositions than the untreated control (Talaat, 2005; Orabi et al., 2014; Abd El-wahed et al., 2016; Aghaei et al., 2019; Khalid et al., 2019, 2020). However, limited studies have been conducted on the aromatic amino acid L-phenylalanine (L-Phe), which is a key player in plant growth, development, and EO production (Pascual et al., 2016; Aghaei et al., 2019; Poorghadir, 2020). L-Phe is a precursor of different antioxidant substances, such as salicylate, flavonoids, anthocyanins, and tannins that protect plants from various stress conditions (Pascual et al., 2016). Studies have demonstrated that L-Phe is a nitrogen source, which plays an essential physiological role in plant growth and development (Jiao et al., 2017). Previous studies indicated that L-Phe improves the contents of EO and its constituents in hyssop, savory, and Khella plants (Talaat et al., 2014; Aghaei et al., 2019; Poorghadir, 2020).

Previous trials reported that changes in EOs in response to plant parts serve as references for selecting the suitable plant part to produce EOs because of the target constituents (Jing et al., 2014). However, producers focus on producing bitter fennel EOs from fruits only and disregard those generated from other plant parts. Furthermore, the response of bitter fennel EO (extracted from various aerial parts and mature fruits) to different doses of L-Phe has not been investigated in Egypt. Therefore, this trial was performed to evaluate the EOs of bitter fennel treated with L-Phe and determine the possibility of using L-Phe in the production of EO from bitter fennel parts.

Materials and Methods

Experimental

Experiments were carried out at the Experimental Farm of the Faculty of Agriculture, Cairo University in Giza, Egypt, in two successive seasons. The physical and chemical properties of the soil used in this study (Table 1) were determined in accordance with previously described methods (Jackson, 1973; Cottenie et al., 1982). Bitter fennel seeds were introduced from Fridal Company (6th of October City, Egypt) and sown in an open field during the first week of November of both seasons. A complete randomized block design with four replicates was utilized. The experimental area (plot) was 2m^2 (2m × 1m) containing four lines, and the distance between hills was 25 and 50cm apart. Three plants per hill were thinned 45 days after sowing. All agricultural practices other than the experimental treatments were performed in accordance with the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into five groups subjected to the foliar application of L-Phe with the control rates 0.0, 0.3, 0.6, 0.9, and 1.2g/L. Foliar L-Phe application was made twice. The first one was made after two weeks from thinning, and the second one was carried out after two weeks from the first one. Flood irrigation was used in these trials.

TABLE 1. Physical and chemical properties of soil

Sand	15.6%
Silt	26.7%
Clay	57.7%
pH	7.5
Electrical conductivity (dS/m)	1.7
Organic matter	2.1%
$CaCO_3$	1.9%
Total nitrogen	71.9%
Phosphorous (mg)	22.7
Cations (mg/100 g soil)	
Potassium	11.6
Calcium	9.3
Magnesium	4.9
Sodium	16.8
Anions (mg/100 g soil)	
HCO_3	19.9
Cl	8.8
SO_4	13.6

Harvesting

Fresh herbs were collected from each treatment at the vegetative growth (60 days from sowing), flowering (120 days from sowing), and immature fruiting (150 days from sowing) stages. Mature fruits were harvested after 180 days from sowing. The fresh and dry weights of various herbs and the dry weights of fruits were recorded (g/plant).

EO isolation

Fresh herbs and air-dried fruits were collected, and 100 g from each replicate was subjected to hydrodistillation (HD) for 3h by using a Clevenger-type apparatus (Clevenger, 1928). For HD, a mixture of the divided samples and 1000mL of water were placed in a 2000mL round bottom flask. The process in the Clevenger-type apparatus was run for 3h (the time until no further EO could be isolated). The lighter EO settled above water and was collected. The collected EO was treated with anhydrous sodium sulfate to remove the traces of water present and stored in a sealed tube at 4°C until further use. The EO content was calculated as a relative percentage (v/w) and yield (ml/plant) based on dry weights.

Gas chromatography and gas chromatography–mass spectrometry conditions

Gas chromatography analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl)-fused silica column (J & W Scientific Corporation; 60m × 0.25mm i.d., 0.25µm film thickness). The oven temperature was held at 50°C for 5min and programmed to increase to 240°C at a rate of 3°C/min. The diluted samples (1/100, v/v, in n-pentane; 1µL) were injected. The flame ionization detector temperature and injector temperature were 265°C and 250°C, respectively. Helium was used as a carrier gas with a linear velocity of 32cm/s. The percentages of the compounds were calculated with the area normalization method without considering response factors.

Gas chromatography–mass spectrometry analyses were carried out in a Varian 3400 GC/MS system equipped with a DB-5-fused silica column (60m × 0.25mm i.d., 0.25mL film thickness). The oven temperature was set at 50°C–240°C at a rate of 4°C/min. Transfer line temperature 260°C, carrier gas, helium, with a linear velocity of 31.5cm/s, split ratio 1:60, ionization energy 70eV, scan time 1 s, and mass range 40–300 amu.

Identification of volatile components

The components of EO were identified by comparing their mass spectra with those in a computer library or with authentic compounds and confirmed by comparing their retention indices (RIs) either with those of authentic compounds or with data published in the literature (Adams, 1995). The mass spectra from the literature were also compared (Adams, 1995). The individual EO components were identified by comparing their retention times with those of standard substances and by matching the mass spectral data with MS libraries (NIST/NBS and Wiley 275.l) In addition, the available authentic sample of the identified compounds in GC or GC/MS was co-injected to confirm the assignment made. For the purpose of quantification was done by external standard method using calibration curves generated by running GC analysis of representative compounds. RIs were also determined for all constituents by injecting a homologous series of n-alkanes, C_8-C_{22}, into the chromatographic column and then compared with the values given in the literature to confirm the identification (Adams, 1995). Computer matching was conducted against commercially available data (Wiley GC/MS Library, Mass Finder 3 Library; Adams, 1995).

Statistical analysis

The experiment was performed in a complete randomized block design. The average data of both seasons were statistically analyzed through one-way ANOVA (Snedecor & Cochran, 1990). Significant values were determined on the basis of P values (P< 0.05= Significant, P< 0.01= Moderate significant, and P< 0.001= Highly significant). This technique was applied in accordance with STAT-ITCF version 10 (Statsoft, 2007). Means were compared via Duncan's multiple range tests at P< 0.05.

Results

Effect of L-Phe on EO sources

In general, EO sources, such as fresh and dry herbs (collected at vegetative, flowering, and immature fruiting stages) and mature fruits, varied because of the fluctuation in the L-Phe rates (Table 2). The greatest weights of various herbs and mature fruits were recorded with the plants treated with L-Phe at 0.9g/L,

with the values of 182.3 and 44.7g/plant; 397.3 and 112.2g/plant; 564.3 and 85.5; 90.3g/plant, respectively. Changes in the values of EO sources were highly significant (P< 0.001) at the given L-Phe rates, but the values of herbs harvested during the vegetative period were nonsignificant.

Effect of L-Phe on EO content and yield

The effects of various L-Phe rates on the EO content (%) and yield (mL/plant) are presented in Table 3. L-Phe application altered the EOs isolated from different plant parts, either from herbs (harvested at vegetative, flowering, and immature fruits) or mature fruits. The plants treated with 0.9g/L L-Phe had the maximum EO contents and yields of various herbs with the following values: 0.8% and 0.4mL/plant; 1.4% and 1.6mL/plant; and 5.3% and 4.5mL/plant, respectively. The changes in the EO contents of the vegetative parts were significant (P< 0.05), whereas the changes in the EO contents (%) of the flowering parts were nonsignificant. The EO yields of the flowering and immature fruiting parts had highly significant variations (P< 00.1). The plants treated with 1.2g/L L-Phe produced the highest value of EO (1.9%) of mature fruits, whereas those exposed to 0.9g/L gave the maximum EO yield (1.6%). The changes in EO (%) in mature fruits were nonsignificant, whereas the changes in the EO yield of mature fruits were significant (P< 0.05).

Effect of L-Phe on EO constituents

The herb samples (collected at vegetative, flowering, and immature fruiting stages) and mature fruits were examined through GC/MS to quantify the constituent contents. GC/MS analysis revealed 11 volatile components (Tables 4–7), which belonged to three chemical groups: monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons. Limonene and estragole were the major components. Various groups and major components in the L-Phe applications differed from those in the control.

The vegetative herb of the plants treated with 1.2g/L L-Phe resulted in the greatest values of limonene (40.5%), monoterpene hydrocarbons (47.9%), and sesquiterpene hydrocarbons (0.3%). The vegetative herb of the plants exposed to 0.6g/L L-Phe produced the highest values of estragole (69.6%) and oxygenated monoterpenes (72.5%). The statistical variations in different components and groups are presented in Table 4.

The flowering herb of the plants treated with 0.6g/L L-Phe gave the highest amounts of limonene (44.3%) and monoterpene hydrocarbons (51.1%), whereas those subjected to 0.9g/L L-Phe produced the greatest value of estragole (53.1%; Table 5). The greatest values of oxygenated monoterpenes (52.8%) and sesquiterpene hydrocarbons (1.3%) were obtained from the flowering herb of the plants exposed to the control (0.0g/L) and 1.2g/L L-Phe, respectively. The statistical differences in various constituents and groups are presented in Table 5.

The herbs harvested at the immature fruiting stage and produced by plants treated with 0.9 g/L L-Phe yielded the maximum values of limonene (36.9%) and monoterpene hydrocarbons (47.8%). By contrast, the herbs were subjected to 0.6g/L L-Phe resulted in the highest value of estragole (53.9%). The greatest values of oxygenated monoterpenes (57.4%) and sesquiterpene hydrocarbons (2.5%) were obtained from the treatments of 0.3 and 0.0g/L L-Phe, respectively (Table 6). The statistical variations in different components and groups are presented in Table 6.

The mature fruits from the control (0.0g/L L-Phe) plants resulted in the greatest values of limonene (12.1%) and monoterpene hydrocarbons (14.6%). Conversely, the mature fruits from the plants exposed to 0.3 g/L L-Phe had the maximum amounts of estragole and sesquiterpene hydrocarbons with values of 86.2% and 0.4%, respectively (Table 7). The maximum value of oxygenated monoterpenes (87.6%) was obtained from the mature fruits of the plants treated with 0.9g/L L-Phe. The statistical variations in different components and groups are shown in Table 7.

The highest values of limonene and monoterpene hydrocarbons were detected in the flowering herbs treated with 0.6g/L L-Phe (Figs. 1 and 3). By contrast, 0.3g/L L-Phe yielded the highest value of estragole in mature fruits (Fig. 2). The treatment of 0.9g/L L-Phe produced the maximum value of oxygenated monoterpenes in mature fruits (Fig. 4). The herb obtained at the immature fruiting stage resulted in the highest value of sesquiterpene hydrocarbons under the control treatment (Fig. 5).

TABLE 2. Effect of L-Phe on essential oil sources

L-Phe (g/L)	Essential oil sources						
	Aerial parts or herbs (g/plant)						Mature fruits (g/plant)
	Vegetative		Flowering		Immature fruiting		
	Fresh	Dry	Fresh	Dry	Fresh	Dry	
0.0	116.3 ± 8.9a	13.4 ± 0.9a	199.1 ± 4.6a	43.8 ± 2.7a	427.1 ± 7.5a	68.3 ± 3.5a	64.7 ± 3.6a
0.3	154.1 ± 12.7a	21.9 ± 1.1b	212.7 ± 5.3b	59.6 ± 3.5b	431.7 ± 8.4b	69.9 ± 4.6b	72.1 ± 4.4b
0.6	159.7 ± 12.6a	27.3 ± 2.4c	329.3 ± 6.9c	79.1 ± 4.3c	443.3 ± 8.9c	70.9 ± 5.4c	80.3 ± 4.9c
0.9	192.3 ± 13.1a	44.7 ± 2.9d	397.3 ± 7.8d	112.2 ± 5.4d	564.3 ± 9.8d	85.5 ± 6.1d	90.3 ± 5.1d
1.2	170.3 ± 11.3a	40.1 ± 3.7e	350.7 ± 7.6e	101.9 ± 4.7e	447.1 ± 8.7e	79.1 ± 7.3e	84.2 ± 4.8e
F values	1.1^{ns}	1599.1^{***}	98316.1^{***}	5865.2^{***}	71579.6^{***}	469.1^{***}	1736.4^{***}

L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); ns, nonsignificant.

All values are given as mean ± SD.

Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

TABLE 3. Effect of L-Phe on essential oil contents

L-Phe (g/L)	Essential oil % and yield (mL/plant)							
	Aerial parts or herbs						Mature fruits	
	Vegetative		Flowering		Immature fruiting			
	%	Yield	%	Yield	%	Yield	%	Yield
0.0	0.6 ± 0.2a	0.1 ± 0.0a	1.4 ± 0.3a	0.6 ± 0.2a	3.8 ± 0.3a	2.6 ± 0.5a	1.4 ± 0.3a	0.9 ± 0.2a
0.3	0.7 ± 0.2b	0.2 ± 0.0b	1.0 ± 0.2a	0.6 ± 0.2b	3.8 ± 0.4a	2.7 ± 0.4b	1.6 ± 0.4a	1.2 ± 0.3b
0.6	0.6 ± 0.2b	0.2 ± 0.0b	1.3 ± 0.4a	1.0 ± 0.3b	3.8 ± 0.3a	2.7 ± 0.4b	1.7 ± 0.4a	1.4 ± 0.5b
0.9	0.8 ± 0.2b	0.4 ± 0.1b	1.4 ± 0.3a	1.6 ± 0.2c	5.3 ± 0.5a	4.5 ± 0.5b	1.7 ± 0.4a	1.6 ± 0.6b
1.2	0.4 ± 0.1b	0.2 ± 0.0b	1.0 ± 0.2a	1.9 ± 0.3c	3.9 ± 0.4a	3.1 ± 0.4b	1.9 ± 0.3a	1.5 ± 0.4b
F values	3.1^{*}	4.5^{*}	1.3^{ns}	22.3^{***}	29.8^{ns}	19.8^{*}	1.2^{ns}	2.3^{*}

L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); ns, nonsignificant.

All values are given as mean ± SD.

Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

TABLE 4. Effect of L-Phe on the constituents of essential oil extracted from vegetative herb

No.	Components (%)	RT	RI^{C}	RI^{L}	L-Phe (g/L)					F values
					0.0	0.3	0.6	0.9	1.2	
1	α-Pinene	5.6	938	939	1.2 ± 0.3a	0.6 ± 0.2b	0.2 ± 0.0c	0.6 ± 0.2c	0.9 ± 0.3d	26.2^{***}
2	Camphene	7.9	955	953	0.4 ± 0.1a	0.5 ± 0.2a	0.1 ± 0.0b	0.2 ± 0.0b	0.1 ± 0.0b	16.5^{***}
3	β-Pinene	8.7	982	980	0.3 ± 0.1a	0.3 ± 0.1a	0.1 ± 0.0ab	0.1 ± 0.0b	0.2 ± 0.0b	5.0^{**}
4	α-Phellandrene	10.0	1006	1005	0.6 ± 0.2a	0.6 ± 0.2a	0.3 ± 0.1a	0.4 ± 0.1b	0.6 ± 0.2b	6.0^{***}
5	Limonene	11.5	1031	1031	35.3 ± 2.7a	34.5 ± 3.7b	21.8 ± 3.5b	26.3 ± 3.5c	40.5 ± 3.5d	640.4^{***}
6	γ-Terpinene	12.7	1062	1062	5.0 ± 0.5a	4.1 ± 0.8ab	4.6 ± 0.8b	4.1 ± 2.5b	5.6 ± 1.6b	5.8^{**}
7	Fenchone	14.4	1077	1075	2.1 ± 0.2a	2.9 ± 0.6ab	2.3 ± 0.4bc	1.8 ± 0.5bc	2.5 ± 0.6c	6.4^{**}
8	Terpinen-4-ol	21.8	1177	1177	0.5 ± 0.2a	0.7 ± 0.3b	0.4 ± 0.2c	0.3 ± 0.1d	0.3 ± 0.1d	5.3^{**}
9	Estragole	28.1	1196	1195	53.4 ± 4.7a	54.8 ± 4.9b	69.6 ± 5.8c	65.6 ± 5.7d	48.2 ± 3.9e	1211.4^{***}
10	cis-Carveol	28.9	1228	1229	0.4 ± 0.1a	0.5 ± 0.2a	0.2 ± 0.0b	0.4 ± 0.2b	0.5 ± 0.2c	4.5^{**}
11	Germacrene D	47.4	1490	1490	0.2 ± 0.0a	0.1 ± 0.0ab	0.2 ± 0.0ab	0.1 ± 0.0b	0.3 ± 0.1b	3.5^{**}
Monoterpene hydrocarbons (1-6).					42.8 ± 5.4a	40.6 ± 4.3b	27.1 ± 4.4c	31.7 ± 3.4d	47.9 ± 5.6e	3463.4^{***}
Oxygenated monoterpenes (7-10).					56.4 ± 6.6a	58.9 ± 5.9b	72.5 ± 6.6c	68.1 ± 7.6d	51.5 ± 6.9e	1639.6^{***}
Sesquiterpene hydrocarbons (11)					0.2 ± 0.0a	0.1 ± 0.0ab	0.2 ± 0.0ab	0.1 ± 0.0b	0.3 ± 0.1b	3.5^{**}
Identified compounds					99.4	99.6	99.8	99.9	99.7	
Unidentified compounds					0.6	0.4	0.2	0.1	0.3	

RT, retention time; RI^{C}, calculated retention index; RI^{L}, retention index from literature; L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); **, P < 0.01 (moderate significant).

All values are given as mean ± SD. Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

TABLE 5. Effect of L-Phe on the constituents of essential oil extracted from flowering herb

No.	Components	RT	RI	RI	L-Phe (g/L)					F values
					0.0	0.3	0.6	0.9	1.2	
1	α-Pinene	5.6	938	939	2.1 ± 0.5a	0.9 ± 0.3b	0.7 ± 0.2c	1.2 ± 0.3c	0.9 ± 0.2c	42.1***
2	Camphene	7.9	955	953	0.9 ± 0.3a	0.2 ± 0.0b	0.1 ± 0.0b	0.2 ± 0.0b	0.1 ± 0.0b	57.5***
3	β-Pinene	8.7	982	980	1.4 ± 0.5a	0.2 ± 0.0b	0.1 ± 0.0b	0.3 ± 0.1b	0.2 ± 0.0b	23.1***
4	α-Phellandrene	10.0	1006	1005	0.6 ± 0.2a	0.5 ± 0.2a	0.6 ± 0.2a	0.6 ± 0.2a	0.6 ± 0.2 a	0.6ns
5	Limonene	11.5	1031	1031	34.1 ± 3.6a	41.2 ± 4.6b	44.3 ± 4.5c	37.4 ± 3.3d	36.8 ± 3.7e	706.5***
6	γ-Terpinene	12.7	1062	1062	6.7 ± 0.7a	5.7 ± 0.9b	5.3 ± 0.8bc	4.2 ± 0.7c	5.1 ± 1.1d	36.9***
7	Fenchone	14.4	1077	1075	2.2 ± 0.4a	0.6 ± 0.2b	0.9 ± 0.2b	0.9 ± 0.2b	0.8 ± 0.2b	55.5***
8	Terpinen-4-ol	21.8	1177	1177	1.5 ± 0.3a	0.3 ± 0.1b	0.4 ± 0.1b	0.7 ± 0.2b	0.3 ± 0.1b	10.5***
9	Estragole	28.1	1196	1195	47.8 ± 4.6a	49.6 ± 4.8b	46.3 ± 4.5c	53.1 ± 5.3d	53.7 ± 5.5e	403.3***
10	cis-Carveol	28.9	1228	1229	1.3 ± 0.3a	0.4 ± 0.2b	0.7 ± 0.2bc	0.3 ± 0.1c	0.3 ± 0.0c	16.9***
11	Germacrene D	47.4	1490	1490	1.1 ± 0.3a	0.1 ± 0.0b	0.2 ± 0.0c	0.4 ± 0.2c	1.3 ± 0.3d	88.9***
Monoterpene hydrocarbons (1-6).					45.8 ± 4.5a	48.7 ± 5.4b	51.1 ± 6.2c	43.9 ± 4.6d	43.7 ± 4.6d	523.7***
Oxygenated monoterpenes (7-10).					52.8 ± 6.6a	50.9 ± 5.7a	48.3 ± 4.3b	55.0 ± 4.9c	55.1 ± 5.9d	108.5***
Sesquiterpene hydrocarbons (11).					1.1 ± 0.3a	0.1 ± 0.0b	0.2 ± 0.0b	0.4 ± 0.2c	1.3 ± 0.3c	88.9***
Identified compounds					99.7	99.7	99.6	99.3	99.3	
Unidentified compounds					0.3	0.3	0.4	0.7	0.1	

RT, retention time; RI^C, calculated retention index; RI^L, retention index from literature; L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); **, P < 0.01 (moderate significant).
All values are given as mean ± SD. Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

TABLE 6. Effect of L-Phe on the constituents of essential oil extracted from immature fruiting herbs

No.	Components (%)	RT	RI	RI	L-Phe (g/L)					F values
					0.0	0.3	0.6	0.9	1.2	
1	α-Pinene	5.6	938	939	1.8 ± 0.4a	2.8 ± 0.6ab	1.5 ± 0.3bc	2.1 ± 0.4bc	2.3 ± 0.4c	8.5***
2	Camphene	7.9	955	953	1.1 ± 0.3a	2.1 ± 0.4a	1.1 ± 0.3b	2.2 ± 0.4c	1.4 ± 0.2c	18.7***
3	β-Pinene	8.7	982	980	1.4 ± 0.4a	3.5 ± 0.7b	0.4 ± 0.1c	2.3 ± 0.5d	0.3 ± 0.1d	52.4***
4	α-Phellandrene	10.0	1006	1005	1.8 ± 0.3a	4.8 ± 0.8b	0.7 ± 0.2c	0.7 ± 0.2c	1.9 ± 0.4d	156.5***
5	Limonene	11.5	1031	1031	31.2 ± 3.2a	23.9 ± 3.3b	34.7 ± 3.4c	36.9 ± 3.5c	31.0 ± 3.8d	322.7***
6	γ-Terpinene	12.7	1062	1062	9.1 ± 1.5b	3.2 ± 0.7b	3.9 ± 0.8c	3.6 ± 0.4cd	4.5 ± 0.6d	184.6***
7	Fenchone	14.4	1077	1075	2.7 ± 0.6a	3.1 ± 0.5ab	0.4 ± 0.2b	3.4 ± 0.3c	1.4 ± 0.3d	55.6***
8	Terpinen-4-ol	21.8	1177	1177	1.3 ± 0.5a	2.2 ± 0.4b	1.1 ± 0.3b	0.3 ± 0.1c	0.7 ± 0.2d	48.1***
9	Estragole	28.1	1196	1195	45.5 ± 4.6a	49.9 ± 4.9a	53.9 ± 5.7b	43.5 ± 5.3c	53.8 ± 5.4d	642.1***
10	cis-Carveol	28.9	1228	1229	1.4 ± 0.3a	2.2 ± 0.4a	0.2 ± 0.0b	2.4 ± 0.5c	1.2 ± 0.3d	28.2***
11	Germacrene D	47.4	1490	1490	2.5 ± 0.5a	2.2 ± 0.3ab	1.6 ± 0.4 b	2.2 ± 0.3bc	1.2 ± 0.3c	7.9**
Monoterpene hydrocarbons (1-6).					46.4 ± 5.4a	40.3 ± 4.1b	42.3 ± 4.2c	47.8 ± 4.3d	41.4 ± 5.7 e	234.4***
Oxygenated monoterpenes (7-10).					50.9 ± 6.1a	57.4 ± 6.5a	55.6 ± 5.9b	49.6 ± 5.8c	57.1 ± 6.8d	376.6***
Sesquiterpene hydrocarbons (11).					2.5 ± 0.5a	2.2 ± 0.3ab	1.6 ± 0.4ab	2.2 ± 0.3bc	1.2c ± 0.3	7.9**
Identified compounds					99.8	99.9	99.5	99.6	99.7	
Unidentified compounds					0.2	0.1	0.5	0.4	0.2	

RT, retention time; RI^C, calculated retention index; RI^L, retention index from literature; L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); **, P < 0.01 (moderate significant).
All values are given as mean ± SD. Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

TABLE 7. Effect of L-Phe on the constituents of essential oil extracted from mature fruits

No.	Components (%)	RT	RI	RI	L-Phe (g/L)					F values
					0.0	0.3	0.6	0.9	1.2	
1	α-Pinene	5.6	938	939	1.1 ± 0.4a	0.4 ± 0.1ab	1.6 ± 0.5b	0.9 ± 0.0b	1.2 ± 0.4c	12.6***
2	Camphene	7.9	955	953	0.1 ± 0.0a	0.2 ± 0.0b	0.7 ± 0.3bc	0.2 ± 0.0bc	0.4 ± 0.2c	12.2***
3	β-Pinene	8.7	982	980	0.3 ± 0.1a	0.2 ± 0.0b	0.5 ± 0.2bc	0.2 ± 0.0bc	0.3 ± 0.1c	5.1*
4	α-Phellandrene	10.0	1006	1005	0.2 ± 0.0a	0.3 ± 0.1ab	0.9 ± 0.3bc	0.5 ± 0.2cd	0.7 ± 0.2d	15.4***
5	Limonene	11.5	1031	1031	12.1 ± 1.9a	10.5 ± 2.5b	10.1 ± 2.4bc	9.4 ± 2.5c	10.9 ± 2.4d	34.4***
6	γ-Terpinene	12.7	1062	1062	0.8 ± 0.2a	0.5 ± 0.2b	3.4 ± 0.9c	0.7 ± 0.3b	0.8 ± 0.3b	76.2***
7	Fenchone	14.4	1077	1075	1.8 ± 0.4a	0.8 ± 0.3b	2.1 ± 0.7b	1.9 ± 0.5b	4.3 ± 1.2c	130.8***
8	Terpinen-4-ol	21.8	1177	1177	0.3 ± 0.1a	0.2 ± 0.0ab	0.1 ± 0.0ab	0.1 ± 0.0b	0.2 ± 0.0b	3.5*
9	Estragole	28.1	1196	1195	81.7 ± 7.7a	86.2 ± 7.7a	79.8 ± 8.8b	85.5 ± 9.8b	80.6 ± 8.7b	20.2***
10	cis-Carveol	28.9	1228	1229	1.1 ± 0.3a	0.1 ± 0.0c	0.3 ± 0.1b	0.1 ± 0.0c	0.1 ± 0.0c	141.1***
11	Germacrene D	47.4	1490	1490	0.3 ± 0.1a	0.4 ± 0.1a	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.1a	2.1ns
Monoterpene hydrocarbons (1-6).					14.6 ± 2.2a	12.1 ± 0.9b	17.2 ± 2.6b	11.9 ± 3.6c	14.3 ± 2.9c	226.3***
Oxygenated monoterpenes (7-10).					84.9 ± 8.6a	87.3 ± 7.1b	82.3 ± 9.8b	87.6 ± 9.7c	85.2 ± 9.9c	130.8***
Sesquiterpene hydrocarbons (11).					0.3 ± 0.1a	0.4 ± 0.1a	0.2 ± 0.0b	0.2 ± 0.0b	0.3 ± 0.1c	2.1ns
Identified compounds					99.8	99.8	99.7	99.7	99.8	
Unidentified compounds					0.2	0.2	0.3	0.3	0.2	

RT, retention time; RI^C, calculated retention index; RI^L, retention index from literature; L-Phe, L-phenylalanine; ***, P< 0.001 (highly significant); **, P < 0.01 (moderate significant).
All values are given as mean ± SD. Different letters mean significant differences according to Duncan's multiple range tests at P< 0.05.

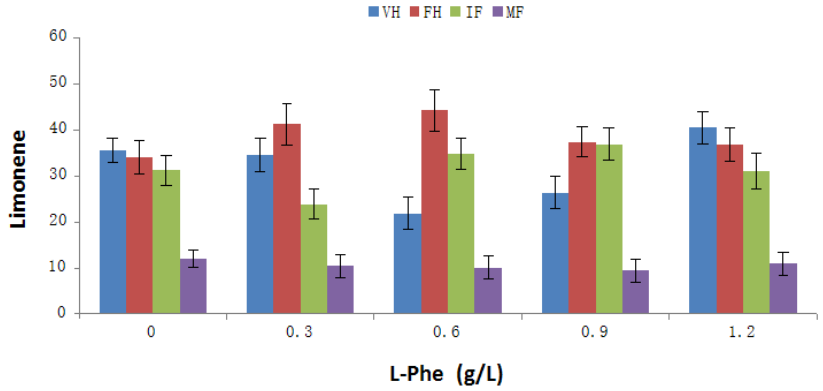

Fig. 1. Effect of L-Phe on limonene contents in different plant organs [L-Phe, L-phenylalanine; VH, vegetative herb; FH, flowering herb; IF, immature fruiting herb; MF, mature fruits]

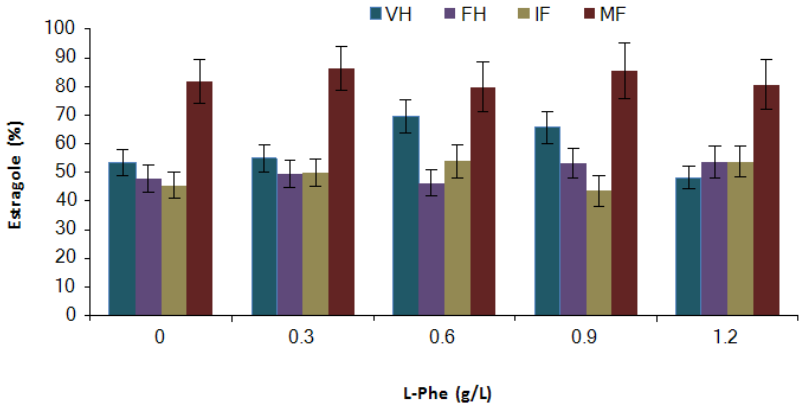

Fig. 2. Effect of L-Phe on estragole contents in different plant organs [L-Phe, L-phenylalanine; VH, vegetative herb; FH, flowering herb; IF, immature fruiting herb; MF, mature fruits]

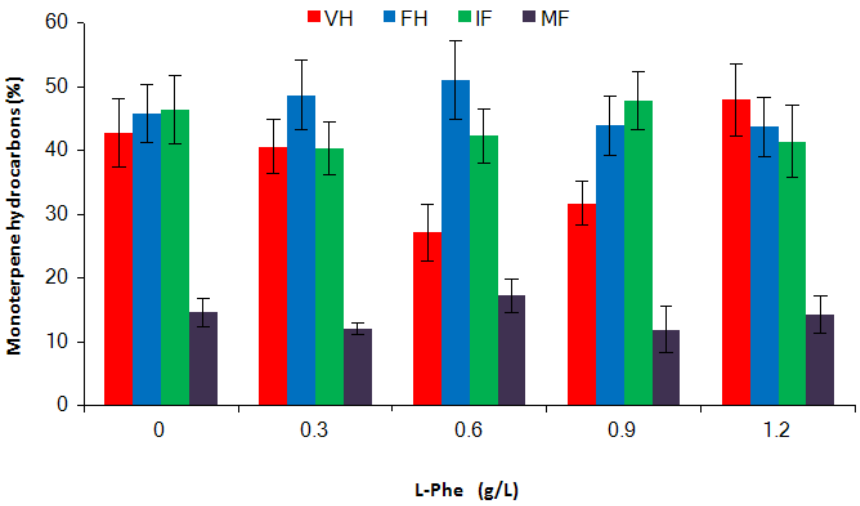

Fig. 3. Effect of L-Phe on monoterpene hydrocarbons in different plant organs [L-Phe, L-phenylalanine; VH, vegetative herb; FH, flowering herb; IF, immature fruiting herb; MF, mature fruits]

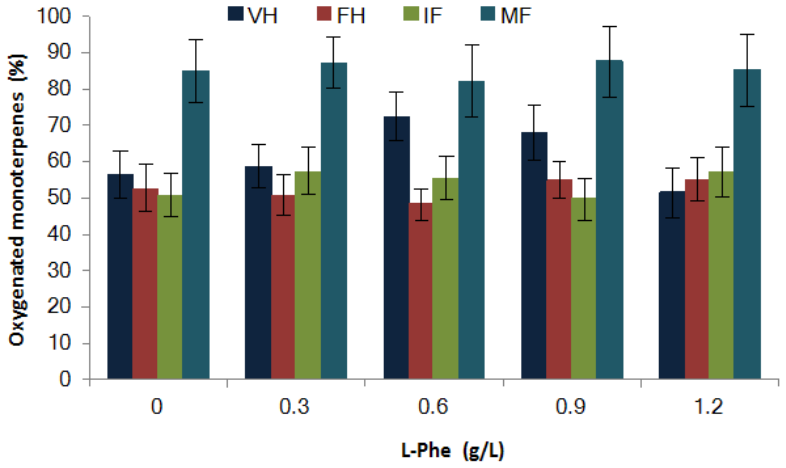

Fig. 4. Effect of L-Phe on oxygenated monoterpenes in different plant organs [L-Phe, L-phenylalanine; VH, vegetative herb; FH, flowering herb; IF, immature fruiting herb; MF, mature fruits]

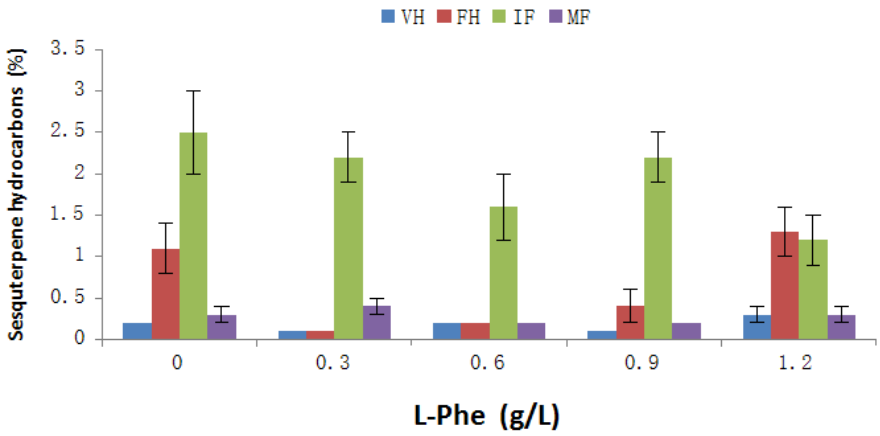

Fig. 5. Effect of L-Phe on sesquiterpene hydrocarbons in different plant organs [L-Phe, L-phenylalanine; VH, vegetative herb; FH, flowering herb; IF, immature fruiting herb; MF, mature fruits]

Discussion

This study showed that L-Phe application led to various modifications in the sources, contents, and components of bitter fennel EO because L-Phe, as an amino acid, has a physiological role in plant development and changes in chemical contents. Amino acids are compounds involved in the biosynthesis of enzymes and other components with nitrogen; L-Phe works to tolerate the plant and adapt it under inappropriate conditions (Tegeder & Ward, 2012). L-Phe is a precursor of several components, such as phenylpropanoids, flavonoids, anthocyanins, lignin, tannins, and salicylate, which are involved in plant growth, reproduction, and defense against abiotic and biotic stresses, because L-Phe is considered a key role in lignin formation (Pascual et al., 2016). Phe ammonia lyase catalyzes the conversion of L-Phe to ammonium and trans-cinnamic acid for reuse in amino acid synthesis (Shi et al., 2013). L-Phe application significantly increased the water content and promoted nutrient absorption, consequently enhancing the vegetative growth, photosynthetic pigments, and EO of pepper and hyssop plants (Rashad et al., 2002; Dahab & El-Aziz, 2006). L-Phe spraying modified the EO composition and various constituents possibly because it acted as a potent inducer to improve the biosynthesis of secondary metabolites. Amino acids, such as L-Phe, function in the synthesis of terpenoids and volatile constituents. Many biosynthetic routes participate in the conversion of phenylalanine into plant volatiles (Vogt, 2010; Gonda et al., 2018). A savory plant treated with L-Phe produces higher values of herbs, EO yields, and EO constituents (carvacrol, p-cymen, α-pinene, and γ-terpinene) than those treated with distilled water (Poorghadir et al., 2020).

L-Phe is a source of N (Jiao et al., 2017). N is one of the elements essential for plant growth and development because it affects many enzymes that control plant physiological processes, such as EO formation and production; it is also found in all plant cells, plant proteins, hormones, and chlorophyll (Khalid, 2012). N affects the production of terpenes through carbon metabolism and acetyl-CoA formation via the mevalonate pathway (Dewick, 1997). Different increments were observed in the growth, yield, and EO composition of thyme, black cumin, coriander, and parsley because of N treatments (Baranauskiene et al., 2003; Ashraf et al., 2006; Akbarinia et al., 2007; Khalid, 2018a). Anise hyssop plants treated with N (100kg/ha) produce the maximum herb yield, EO content, and major constituents of EO (Omidbaigi et al., 2008a). The treatment of 200kg/ha of N resulted in the highest yield values, EO (%), p-cymene, limonene, dihydrotagetone, E-tagetone, Z-tagetone, Z-ocimenone, and E-ocimenone of Tagetes minuta (Omidbaigi et al., 2008b). The greatest biomass and EO composition of lemon grass herb were detected at a rate of 50kg (N)/hectare (Rashmi & Singh, 2008). Davana plants exposed to 94kg (N)/ha have enhanced growth and higher yields and EOs than those of the untreated control (Kumar et al., 2009). The maximum vegetative characters and EO yield of dill plants are obtained with the treatment of 100kg N/ha (Hellal et al., 2011). A highly significant increase in growth characters, EOs and their major components, and chemical groups, such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes, has been found in anise, coriander, and sweet fennel (Khalid, 2012, 2014). In Brazilian piper (Pinto et al., 2016), various rates of N (6, 36, 60, 84, and 114kg/ha) produced higher mass production, EO yield, α-pinene, limonene, sabinene, β-pinene, α-copaen, and Z-zalvene than those at the control rate. Ginger plants subjected to N at 120kg/ha have a greater mass production than untreated plants (Singh et al., 2016). The maximum EO yield, p-cymene, and α-thujene have also been obtained from black cumin plants treated with N at 100kg/ha (Khalid, 2018b).

Bitter fennel EO and its constituents in various plant parts differed. These variations may be due to the effect of plant parts on the enzyme activity and metabolism of EO production (Burbott & Loomis, 1969). These results were also confirmed by previous trials. The flowering herb of thyme yielded higher contents of EO, thymol, and carvacrol than those of green herb (Jordan et al., 2006; Nejad-Ebrahimi et al., 2008; Omidbaigi et al., 2010). The EO isolated from the rosette, vegetative, flowering, and fruiting herbs of Tarhana plant (Sanli et al., 2016) significantly varied in terms of the EO content, methyl eugenol, and α-phellandrene in response to various plant parts. The amount of EO in the apple of Sodom fruits is greater than those of leaves, stems, and flowers; the values of major components (E-phytol, myristicin, myristic acid, and E,E-farnesyl acetone) and different chemical classes (mono and diterpenes) are related to plant parts (Wahba & Khalid, 2018). The EO (%) and its composition (major components, monoterpenes, and sesquiterpenes) in lantana also differ in various

plant parts (vegetative, flowering, and fruiting herbs; Khalid, 2019). Similar constituents were identified in bitter fennel EO under Egyptian conditions (Shalaby & Hendawy, 2011). Other factors affect EO production in conjunction with current treatments, such as stress conditions (Khalid & Shedeed, 2014, 2016; Ahmed et al., 2017; Khalid & Ahmed, 2017), fertilization (Khalid, 2012), location (Khalid et al., 2018), and distillation methods (Khalid et al., 2009). This trial recommended treating bitter fennel plants with L-Phe to produce EO and served as a reference for selecting a suitable plant part to produce EO based on the target constituents.

Conclusion

This study indicated that bitter fennel EO production was affected by L-Phe application and related to plant organs. The EO content (% or yield), main constituents, and chemical classes of various parts were changed by L-Phe rates in different plant parts. Thus, producers could yield bitter fennel EOs upon L-Phe application and select the source of plant parts for EO production.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: AAAE, carried out the experiments in the field. AEE, participated in the design of the study. AMAA carried out the chemical studies and performed the statistical analysis. KAK drafted the manuscript and participated in the sequence alignment. All authors share in every step of this work, and all of them contribute to writing the manuscript. The authors read and approved the final manuscript.

Ethical approval: Not applicable

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التغيرات الحادثه في الزيت العطري للشمر المر المعرض للرش الورقي بالفينيل الانين

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يميل المنتجون في مصر إلى استخدام الأساليب التقليدية لتغذية النباتات الطبية والعطرية وفصل الزيوت العطريه للشمر المر من الثمار فقط ولم يركزوا على الأجزاء الأخرى. أجريت هذه الدراسة لتقييم الزيوت العطريه لنبات الشمر المر المعامل بالفينيل الانين وتحديد إمكانية استخدام الفينيل الانين في إنتاج الزيوت العطريه من أجزاء الشمر المر. تعرض الشمر المر لمعدلات مختلفه من الفينيل الانين (صفر، 0.3، 0.6، 0.9، 1.2جم / لتر). تم تسجيل التغيرات في مصادر الزيوت العطريه (الأوزان الطازجة والجافة للأعشاب المحصودة في مرحلة النمو الخضري والازهار والثمارغير الناضجة والأوزان الجافة للثمار الناضجة). تم استخراج الزيوت العطريه من خلال التقطير المائي وتحليلها بالغاز الكروماتوجرافي. تم تسجيل أكبر أوزان لأجزاء النبات وإنتاج الزيت العطري عند مستوي 0.9 جم / لتر. كانت المركبات الرئيسية هي الليمونين والإستراجول. اعلى قيم في الليمونين والهيدوكربونات الاحاديه تم تسجيلها في العشب المزهر عند مستوي 0.6 جم / لتر، في حين اعطى المستوى 0.3 جم / لتر اعلي قيمه للاستراجول في الثمار الناضجه. أنتجت المعامله 0.9 جم / لتر اعلى قيمه من التربينات الاحاديه الاكسيجينيه في الثمار الناضجه. اعلى قيمه للسيسكوتربينات الهيدروكربونيه تم الحصول عليها في العشب المحصود في فترة الثمار غير الناضجة تحت معاملة الكنترول. وبالتالي، يمكن استخدام هذه الدراسة كمرجع لاختيار معدل الفينيل الانين المناسب وجزء الشمر المر لإنتاج الزيوت العطريه على أساس المكونات المستهدفة.