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L-tryptophan affects the essential oil of navel orange under various growing regions

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ABSTRACT

Essential oil (EO) of navel orange had anticancer and antimicrobial activities. L-tryptophan and growing regions play important roles in physiological processes of navel orange EO. The application of L-tryptophan under different growing regions is one way of scientific research that has the potential to increase the EO production, so, the aim of this trial was to evaluate the EO isolated from young shoots and peels of navel orange trees under L-tryptophan treatments in West and Upper Egypt. The experiments were conducted at the two Citrus farms located in West and Upper Egypt. Selected trees were subjected to L-tryptophan doses at 0, 200 and 400 mg/L in both regions. The EO of young shoots and peels was isolated by hydro distillation method (HD), then, analyzed by GC and GC/MS equipments. Data were statistically analyzed using ANOVA-2. The EO percentages and constituents of young shoots or peels were significantly affected due to L-tryptophan doses in both regions. The major component of young shoot EO was sabinene while it was limonene of peel EO. The treatment of 400 mg/L (L-tryptophan) resulted in the greatest amounts of EO (%), sabinene and limonene in west and Upper Egypt. The application of L-tryptophan caused different variations in all chemical classes (MCH, MCHO, SCH and SCHO) of young shoots and peels EO in both regions. L-tryptophan levels produced positive variations on essential oil composition of navel orange trees grown in West and Upper Egypt.

1. Introduction

Recently, EO becomes a scientific interest and widely used as a natural source for several food and pharmaceutical industries (Velázquez-Nuñez et al., 2013). Food and Drug Administration (FDA) recommended that Citrus EO is a safe substance (Nannapaneni et al., 2009). It can be added to food products such as canned foods, beverages and cosmetics to prevent the growth of pathogens and spoiling microorganisms (Velázquez-Nuñez et al., 2013; Fisher and Phillips, 2006). Also, it can be used in medicine products due to its characteristic flavor, fragrance, and certain properties (Liu et al., 2012; Settanni et al., 2012). Navel orange "Washington" (*Citrus sinensis* L. Osbeck) belongs to family Rutaceae. Previous, investigators indicated that many useful natural products were identified in navel orange such as EO, flavonoids, carotenoids and vitamins (Bermejo et al., 2012; Eldahshan and Halim, 2016). It was found that navel orange EO isolated from peels resulted in positive effects on the inhibition of the proliferation of a human lung cancer cell line A549 and prostate cancer cell line 22RV-1, while, the EO of leaves and branches were highly effective as an antimicrobial agent (Eldahshan and Halim, 2016; Yang et al., 2017). Previous studies indicted that major constituent of navel orange EO extracted from peels was limonene while it was sabinene in the EO isolated from young shoots (El-Hawary et al., 2016; Eldahshan and Halim, 2016).

The quantitative and qualitative properties of Citrus EO are affected by several factors such species, varieties, plant organ, soil type, weather, irrigation, plant nutrition and biostimulants (Jing et al., 2014). There are several biostimulants that could be used to modify EO metabolites of aromatic plants; L-tryptophan is one of them (Bromke, 2013). L-tryptophan which known as an essential amino acid is very important source of organic nitrogen, carbon and energy for building the plant tissues. Also, it is a precursor of auxins in higher plants (Abd El-Aziz and Balbaa, 2007; Bromke, 2013). The EO content of geranium (*Pelargonium graveolens*) was gradually increased as L-tryptophan level increase (Talaat et al., 2005). Exogenous application of L-tryptophan improved the EO and its major constituents of thyme (*Thymus vulgaris*) aerial part (Orabi et al., 2014). Significant increments were found in the composition of *Philodendron erubescens* EO due to L-tryptophan application (Abd El-wahed et al., 2016).

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The EO constituents are reported to vary qualitatively and quantitatively due to growing regions which might produce changes in biological and pharmaceutical activities (Heywood, 2002; Lukas et al., 2015; Stefanaki et al., 2016); so, the effect of growing regions on the quantity and quality of EO is a great interest for the optimization of a reproducible production quality (Formisano et al., 2015). Important physiological roles were found in the processes of EO due to growing regions (Ciobanu et al., 2002; Zheljazkov et al., 2009; Teles et al., 2012). Growing regions resulted in highly significant changes in mint and *Bunium persicum* EO (Omidbaigi and Arvin, 2009; Teles et al., 2013). The EO compositions of lemon verbena herb were higher under the North region of Egypt than those produce under Upper Egypt (Ibrahim et al., 2014).

The application of L-tryptophan under different regions is one way of scientific research that has the potential to increase the EO production. In this trial, improve the EO composition of navel orange may increase the natural products using in food and pharmaceutical industries. Therefore the influences of L-tryptophan on navel orange EO were investigated in West and Upper Egypt.

2. Materials and methods

2.1. Plant materials and growing regions

The experiments were carried out at two Citrus farms located in two different growing regions during the seasons of 2017 and 2018. The first farm located in West of Egypt (135 km from Cairo). The second farm located in Upper Egypt (35 km south of Cairo). Soil analyses of the two experimental regions were conducted according to Carter and Gregorich (2008) & Margenot et al. (2017) (Table 1). Metrological data of both sites (means of both years) were obtained from Central Laboratory for Agricultural Climate (Fig. 1). Navel orange trees in both regions were divided into 3 groups. The first and second groups were exposed to L-tryptophan at 200 and 400 mg/L respectively. The third group was subjected to distilled water (as Control). L-tryptophan was applied to run-off to foliage at the end of January of both seasons.

2.2. Harvesting

During the first week of April and last week of September of both seasons, young shoots and fruits were collected. The fresh young shoots and peels of fruits (Fig. 2) were divided into small pieces by knife. Then, they were weighed to isolate EO.

Table 1
Soil analysis of different sites.

Items	Sites	
	West of Egypt	Upper Egypt
Sand	79.4%	19.9%
Silt	15.5%	35.8%
Clay	5.1%	44.3%
pH	8.8	7.7
EC (dS/m)	2.4	1.6
Organic matter	0.4%	1.1%
$CaCO_3$	7.4%	0.9%
Total N	0.2%	79.5%
Cations (mg/100 g Soil)		
P	0.5	21.8
K	0.4	22.9
Ca	2.5	77.3
Mg	3.9	18.9
Na	12.9	41.8
Anions (mg/100 g Soil)		
HCO_3	1.5	24.8
Cl	15.8	11.4
SO_4	1.9	22.9

2.3. EO isolation

The fresh young shoots and peels were collected from each treatment of both regions and then 250 g from each replicate (four replicates) of all treatments were subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928).

2.4. GC and GC–MS conditions

GC 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) (60 m × 0.25 mm i. d., film thickness 0.25 μm). Oven temperature was held at 50 °C for 5 min and then programmed to rise to 240 °C at a rate of 3 °C/min. The flame ionization detector (FID) temperature was 265 °C and injector temperature was 250 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors.

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

2.5. Identification of volatile components

The components of EO were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 1995). Mass spectra from the literature were also compared (Adams, 1995). Further identification was made by comparison of their mass spectra on both columns with those stored in NIST-98 and Wiley-5 Libraries. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

2.6. Statistical analysis

In this experiment, 2 factors were considered: L-tryptophan rates (0, 200 and 400 mg/L) and 2 sites (West and Upper Egypt). For each treatment there were 4 replicates, the experimental design followed a complete random block design. The averages data of both seasons were statistically analyzed using 2-way analysis of variance (ANOVA-2) (De-Smith, 2015). Significant values determined according to P values ($P < 0.05$ = significant, $P < 0.01$ = moderate significant and $P < 0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program version 10 (Statsoft, 2007).

3. Results

3.1. Effect of L-tryptophan x different regions on EO content

Data in Table 2 showed that application of L-tryptophan x various regions had significant variations ($P < 0.05$) in navel orange EO (%) of young shoots and peels. The trees treated with L-tryptophan resulted in slightly increase in EO percentages compared with untreated (control). Adding L-tryptophan at 400 mg/L produced the highest amounts (0.3%) of young shoots and peels EO in both regions.

3.2. Effect of L-tryptophan x different regions on EO constituents

The constituents of EO extracted from young shoots and peels under L-tryptophan levels in both locations were described in Tables (3 and 4). Sabinene and limonene were detected as the major components of EO in young shoots and peels respectively. The treatment of 400 mg/L (L-

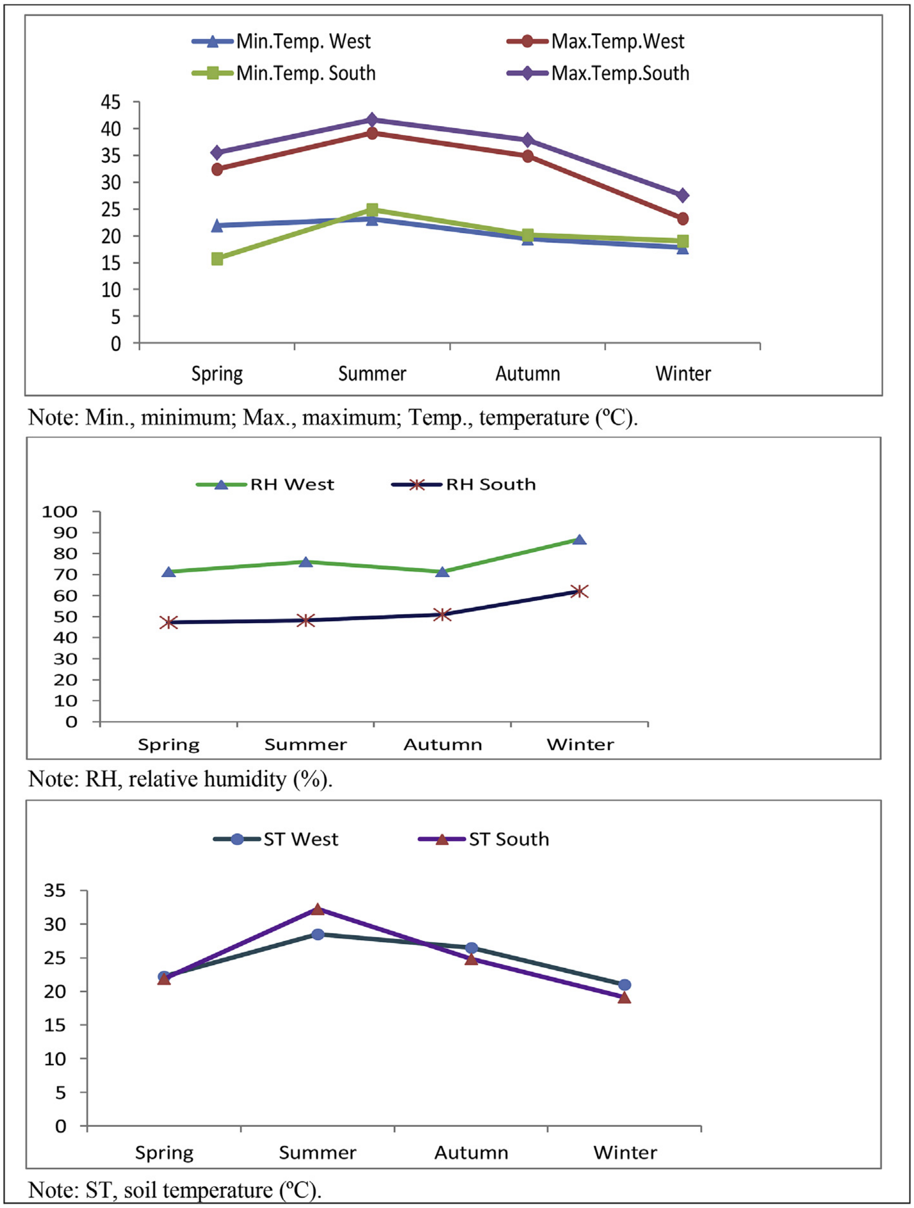

Fig. 1. Meteorological condition during various seasons.

tryptophan) resulted in the highest amounts of sabinene (43.1%) in West region while it gave the greatest value of limonene (81.7%) in Upper region. All identified components belonged to 4 chemical groups. The major groups were monoterpene hydrocarbons (MCH) and oxygenated monoterpenes (MCHO) while the sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) formed the minor classes. L-tryptophan doses and various locations caused different changes in EO components and various chemical groups (Tables 3 and

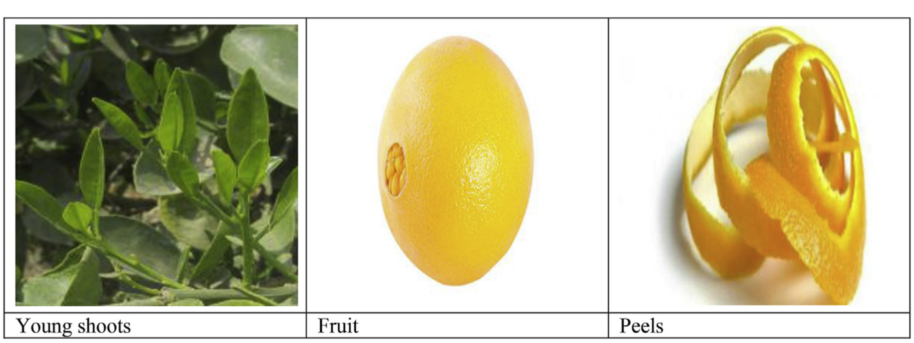

Fig. 2. Young shoots, fruit and peels of navel orange tree. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Effect of the interaction between L-tryptophan and various regions on EO content.

Sites	L-tryptophan (mg/L)	EO (%)	
		Young shoots	Peels
West of Egypt	0	0.1 ± 0.0	0.1 ± 0.0
	200	0.2 ± 0.1	0.2 ± 0.1
	400	0.2 ± 0.1	0.3 ± 0.1
Upper Egypt	0	0.1 ± 0.0	0.1 ± 0.0
	200	0.2 ± 0.1	0.2 ± 0.1
	400	0.3 ± 0.1	0.2 ± 0.1
F values		4.0*	1.4*

Note: EO, essential oil *, P < 0.05; values are given as Mean ± SD.

4).

4. Discussion

In this study, obtained results indicated that treated navel orange trees by L-tryptophan in both regions resulted in positive effects on the chemical composition of navel orange EO; these effects may be due to L-tryptophan which improves plant growth characters and metabolism leading to improve the quantity of the secondary metabolites, i.e., EO (Abou Dahab and Abd El-Aziz, 2006). L-tryptophan is a precursor of auxins in various crops (Bromke, 2013). Auxin accelerated the differentiation of EO glands (Sudriá et al., 2004). Adding auxin to *Melissa officinalis* caused an increase in nerol and geraniol by 1.4 and 4.1 fold compared with control (Silva et al., 2005). Bano et al. (2016) indicated that auxin application enhanced some physiological and chemical processes which influenced the terpenoids pathway. The effects of L-tryptophan on EO composition were confirmed by previous investigators. The L-tryptophan caused an increase in *Pelargonium graveolens* EO (Talaat et al., 2005); the major components of thyme EO were significantly increased by L-tryptophan application (Orabi et al., 2014); the composition of *Philodendron erubescens* EO was significantly increased with L-tryptophan application (Abd El-wahed et al., 2016). Different variations were found in EO content and constituents under both regions. These results may be due to changes in environmental conditions in various growing regions that cause highly significant differences in secondary metabolites i. e EO of aromatic plants (Figueiredo et al., 2008). The soil variations in different regions can affect the EO production, for example, low soil pH resulted in high availability of soil nutrient solution that reflects an increase in plant growth and EO yield (Badawy et al., 2018). The meteorological variations in various sites are important factors in EO production. Sunlight, temperatures, relative humidity and altitudes lead plants to change their morphology, physiology and EO production (Ko and Fidis, 2008; Khalid and El-Gohary, 2014; Aissi et al., 2016). Growing region is one of important factors which make many variations in EO composition. It offers an opportunity to select EOs with preferential compounds for

Table 3
Effect of the interaction between L-tryptophan and various regions on the EO components of young shoots.

No	Compounds (%)	RI[a]	RI[b]	L-tryptophan (mg/L)						F values
				West of Egypt			Upper Egypt			
				0.0	200	400	0.0	200	400	
1	α-Thujene	931	931	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	1.8ns
2	α-Pinene	939	939	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	12.0***
3	Sabinene	977	976	42.4 ± 0.3	42.6 ± 0.4	43.1 ± 0.5	41.8 ± 0.6	42.9 ± 0.6	42.3 ± 0.7	0.5 ns
4	β-Pinene	981	980	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.2	0.4 ± 0.1	0.2 ± 0.0	40.8***
5	Myrcene	990	991	4.5 ± 0.2	4.7 ± 0.3	4.8 ± 0.6	4.1 ± 0.3	4.4 ± 0.4	4.2 ± 0.2	2.3 ns
6	ʒ-Carene	1010	1011	11.7 ± 0.3	11.9 ± 0.4	12.0 ± 0.6	11.5 ± 0.7	11.8 ± 0.6	11.7 ± 0.3	0.1 ns
7	α-Terpinene	1017	1018	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	0.3 ± 0.1	25.6***
8	*p*-Cymene	1026	1026	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	4.6*
9	Limonene	1031	1031	7.9 ± 0.2	8.0 ± 0.5	8.1 ± 0.5	7.4 ± 0.5	7.8 ± 0.6	7.6 ± 0.4	3.6*
10	*cis*-β-Ocimene	1041	1040	0.6 ± 0.2	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.0	0.5 ± 0.1	6.5***
11	*trans*-β-Ocimene	1052	1050	3.7 ± 0.2	3.9 ± 0.3	4.0 ± 0.1	3.6 ± 0.3	3.9 ± 0.4	3.7 ± 0.2	0.3 ns
12	γ-Terpinene	1061	1059	3.3 ± 0.2	3.5 ± 0.2	4.1 ± 0.3	3.1 ± 0.4	3.8 ± 0.5	3.4 ± 0.3	1.3 ns
13	α-Terpinolene	1090	1088	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	9.7***
14	*cis*-Sabinene hydrate	1097	1097	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	6.2***
15	Linalool	1099	1098	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	2.0 ns
16	Citronellal	1152	1153	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	1.8 ns
17	Terpinen-4-ol	1178	1177	19.8 ± 0.3	20.9 ± 0.6	21.4 ± 0.7	19.5 ± 0.5	21.1 ± 0.4	20.6 ± 0.3	16.1***
18	α-Terpineol	1190	1189	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.2	5.3**
19	*cis*-Piperitol	1193	1193	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	15.8***
20	Citronellol	1229	1228	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	18.4***
21	Neral	1241	1240	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	5.9**
22	Geraniol	1255	1255	0.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	12.6***
23	Geranial	1270	1270	0.6 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	15.6***
24	Neryl acetate	1365	1365	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2	0.2 ± 0.0	0.5 ± 0.2	23.4***
25	β-Elemene	1376	1375	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	4.0*
26	Β-Caryophyllene	1419	1418	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.2	3.6*
27	β-Humulene	1440	1440	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	2.5*
28	β-Sinensal	1696	1696	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.4*
29	α-Sinensal	1752	1752	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	3.0 ns
MCH				75.5 ± 0.4	76.2 ± 0.7	76.9 ± 0.8	75.0 ± 0.7	76.1 ± 0.5	74.8 ± 0.7	0.5 ns
MCHO				23.0 ± 0.3	22.6 ± 0.4	22.5 ± 0.3	23.6 ± 0.4	22.8 ± 0.4	23.6 ± 0.2	0.9 ns
SCH				0.8 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.8 ± 0.3	0.4 ± 0.1	0.8 ± 0.2	6.9***
SCHO				0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.9 ns
Total identified				99.5	99.5	99.9	99.6	99.6	99.5	

Note: EO, essential oil; [a]RI, retention index calculated; [b]RI, retention index from literature; MCH, monoterpene hydrocarbons; MCHO, oxygenated monoterpenes; SCH, sesquiterpene hydrocarbons; SCHO, oxygenated sesquiterpenes; ns, non significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001; values are given as Mean ± SD.

Table 4
Effect of the interaction between L-tryptophan and various regions on the EO components of peels.

No	Compounds (%)	RI[a]	RI[b]	L-tryptophan (mg/L)						F values
				West of Egypt			Upper Egypt			
				0.0	200	400	0.0	200	400	
1	α-Pinene	939	939	1.4 ± 0.2	0.4 ± 0.2	1.4 ± 0.2	0.3 ± 0.0	0.5 ± 0.2	0.4 ± 0.2	13.5***
2	Sabinene	977	976	0.9 ± 0.4	0.2 ± 0.0	0.9 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	24.9***
3	Myrcene	990	991	4.5 ± 0.6	4.9 ± 0.4	4.7 ± 0.4	4.1 ± 0.4	4.4 ± 0.6	4.8 ± 0.6	2.8*
4	Octanal	1002	1001	0.9 ± 0.2	1.2 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	38.4***
5	ȝ-Carene	1010	1011	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.1 ± 0.0	0.5 ± 0.2	0.2 ± 0.0	28.5***
6	Limonene	1031	1031	74.5 ± 0.8	79.1 ± 0.9	78.5 ± 0.8	80.8 ± 0.8	81.6 ± 0.9	81.7 ± 0.8	159.1***
7	*cis*-β-Ocimene	1041	1040	0.3 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	5.0*
8	γ-Terpinene	1061	1059	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	1.7 ns
9	α-Terpinolene	1090	1088	0.2 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.1 ± 0.0	0.5 ± 0.2	0.4 ± 0.2	8.5***
10	Linalool	1099	1098	4.0 ± 0.3	5.3 ± 0.5	4.8 ± 0.7	3.5 ± 0.2	3.7 ± 0.6	4.1 ± 0.7	5.7***
11	Nonanal	1103	1103	0.2 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.2	6.6***
12	Citronellal	1152	1153	0.3 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	1.8 ± 0.3	0.2 ± 0.0	0.1 ± 0.0	1.3 ns
13	Terpinen-4-ol	1178	1177	1.0 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.2 ± 0.1	0.9 ± 0.6	0.2 ± 0.0	9.8***
14	α-Terpineol	1190	1189	2.6 ± 0.3	0.1 ± 0.0	0.8 ± 0.3	1.5 ± 0.4	0.4 ± 0.2	0.3 ± 0.2	34.4***
15	Nerol	1229	1228	1.2 ± 0.4	0.6 ± 0.2	0.4 ± 0.2	0.2 ± 0.0	0.5 ± 0.2	0.3 ± 0.1	25.3***
16	Citral	1240	1240	2.2 ± 0.5	1.2 ± 0.4	0.5 ± 0.2	2.0 ± 0.5	0.9 ± 0.3	1.1 ± 0.7	7.0***
17	Geraniol	1255	1255	0.4 ± 0.2	0.1 ± 0.0	0.7 ± 0.2	0.7 ± 0.4	0.9 ± 0.2	0.6 ± 0.2	12.9***
18	β-Elemene	1376	1375	1.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	18.9***
19	α-Copaene	1377	1376	0.8 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.3 ± 0.1	8.8***
20	Decanal	1378	1377	0.6 ± 0.2	0.1 ± 0.0	0.8 ± 0.2	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	14.8***
21	Geranyl acetate	1384	1383	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	4.9*
22	β-Caryophyllene	1419	1418	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	6.7***
23	Germacrene D	1480	1480	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	1.2 ± 0.9	21.4***
24	*E-E*-α-Farnesene	1509	1508	0.4 ± 0.2	0.5 ± 0.3	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	7.8***
MCH				83.0 ± 0.8	87.2 ± 0.7	87.9 ± 0.8	86.4 ± 0.7	88.6 ± 0.9	88.8 ± 0.7	8.8***
MCHO				13.4 ± 0.4	10.5 ± 0.8	9.9 ± 0.7	10.8 ± 0.6	8.1 ± 0.6	8.0 ± 0.5	41.9***
SCH				3.3 ± 0.4	1.7 ± 0.5	1.8 ± 0.2	1.7 ± 0.3	2.1 ± 0.2	2.6 ± 0.2	15.3***
SCHO				0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	7.8***
Total identified				99.8	99.6	99.8	99.4	99.1	99.8	

Note: EO, essential oil; [a]RI, retention index calculated; [b]RI, retention index from literature; MCH, monoterpenes hydrocarbons; MCHO, oxygenated monoterpenes; SCH, sesquiterpene hydrocarbons; SCHO, oxygenated sesquiterpenes; ns, non significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001; values are given as Mean ± SD.

pharmaceutical, drugs, perfumes and food industries (Aboukhalid et al., 2017). The obtained results are in agreement with previous studies; they reported that different variations were found in EO of some aromatic plants with different growing regions (Omidbaigi and Arvin, 2009; Teles et al., 2013; Ibrahim et al., 2014; Khalid et al., 2018). Previously, there were no research papers on the evaluation of the EO of navel orange under the interaction between L-tryptophan and geographical regions in Egypt. So this investigation may increase using the EO of navel orange as a source of natural products.

5. Conclusion

It may be concluded that treated the trees of navel orange with L-tryptophan x different regions produce significant variation in EOs isolated from young shoots and peels. The treatment of 400 mg/L resulted in the highest amounts and its major constituents of both young shoots and peels EOs under the regions of West and Upper Egypt. The effect of L-tryptophan with various locations of Egypt on the navel orange EO has not been investigated before. This study discovered that production of EO with L-tryptophan x different regions is required because the application of L-tryptophan produced significant variation in the EO composition of navel orange and this study helps the farmers and pharmaceutical companies to increase the yield and major constituents of the EO extracted from navel orange as a natural source of drug industries.

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Appendix A. Supplementary data

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