Acclimatization study of *Tagetes lucida* L. in Egypt and the chemical characterization of its essential oils

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ABSTRACT

Seeds of *Tagetes lucida* were imported to Egypt from Canada and propagated under greenhouse conditions in peat moss media. Soil was sandy in texture and the irrigation system was dripping irrigation. The growth parameters were determined at five successive plant ages, fresh and dry weights of herb were determined at three successive plant ages. The yield of aerial parts after 175 days, was about 7.5 Mg/ ha. The essential oil (EO) was extracted by hydro -distillation for three hours with a yield of about 0.5% (w/v). The EO of each sample was subjected to gas-chromatography/mass spectrometry analyses to study the chemical composition. The main component of the EO was identified as methyl chavicol which matched over 90% of the whole composition. Chlorophyll a and carotenes increased with increasing plant age in both sites and seasons. Flavonoids decreased with the development of plant age, while the opposite was true with coumarines content.

KEY WORDS
Tagetes lucida; methyl chavicol; essential oil; sand soil; growth parameters; GC–MS

1. Introduction

The *Tagetes lucida* L. (*T. lucida*) plant belongs to the family Asteraceae, and has several English and common names, such as Mexican Marigold, Mexican Mint Marigold, Mexican Tarragon and Sweet Marigold (Guzmán & Manjarrez 1962). The word Tagetes probably originated from the Greek word 'Tages', an Etruscan God (Bailey 1958). It is a half-hardy sub-shrub that grows

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46–76 cm tall (Linares et al. 1995). This plant is widespread throughout Mexico and south to Central America; in Mexico, it is known as ‘pericon’, ‘anisillo’, ‘hierba anis’, ‘hierba de Santa Maria’ and ‘hierba de San Juan’. Because *T. lucida* has yellow flowers, it is also known in Mexico as ‘hierba de San Juan’ (Guzmán & Manjarrez 1962). It is cultivated commercially in Costa Rica as a spice herb; it contains EO having an anise-like odour, furthermore the fresh aerial parts of this plant are sold in the supermarket as a substitute for tarragon, Artemisia dracunculus L. (Cicció 2004). *T. lucida* is cultivated in the United States, France and England as a flowering herb (Morton 1981) and it is used in the European countries as a culinary herb as well as a green spice.

In folk medicine, the decoction of the entire *T. lucida* is used as a malarial remedy, the ‘potion’ is taken internally against spitting blood and diarrhoea, it has also been referred in Mexican traditional medicine for the treatment of different central nervous system (CNS) diseases, mainly depression. *T. lucida* has been used to treat madness and epilepsy and its therapeutic applications are recorded in several documents and codices of the sixteenth century (De la Cruz & Badiano 1991; Sahagun 1999). An infusion of leaves and flowers is drunk to combat diarrhoea, rheumatism, asthma and cold (Cano 1997; Marquez et al. 1999).

Thirty compounds have been identified in the *T. lucida* EO, of which methyl chavicol (also known as estragole) was the major constituent and accounted for 95–97% (Cicció 2004). Similar results were reported for the EO from *T. lucida* collected in Colombia and Cuba (Regalado et al. 2011); Vera et al. 2014. Conversely Bicchi et al. (1997) reported that *T. lucida* EO from Peru had three main components: methyl chavicol (33.9%), E-Anethole (23.8%) and Methyleugenol (24.3%). The EO from aerial parts of *T. lucida* cultivated in Costa Rica has been examined by GC–MS: French chemists in 1938 identified the phenylpropanoids in high con-centration. Four flavonoid glycosides were identified from aerial parts of the plant collected in Argentina (Abdala 1999). Aquino et al. (2002) identified a new flavonol glycoside and two new phenolic acids from the air-dried leaves of the plant collected in Guatemala. More recently, Ibrahim and Mohamed (2016) isolated a new cytotoxic thiophene from *Tagetes minuta*.

Within the framework of our programme to enrich Egyptian cultivation with some medic-inal and aromatic plants that have a global market and medicinal uses in many foreign countries, the seeds of *T. lucida* (Mexican marigold) were imported from Canada. Here, we report our research on (i) the acclimatisation of *T. lucida* (ii) the study of its active components and (iii) the technological package for its production under the Egyptian environmental conditions. This study sheds light on EO composition, total flavonoids, pigments and cou-marins. It also sheds light on the behaviour of growth of *T. lucida* plants cultivated in two different locations in Egypt.

2. Result and discussion

Plant height increased with plant age to reach its maximum height after 175 days in both seasons and in both locations. The maximum active period for plant height change was observed between 75 and 105 days of plant age in Nobaria and 105–140 days in Bilbas in both seasons (Figure 1(a)). The same behaviour of plant height was observed with plant diameter in both locations (Figure 1(b)). The number of branches increased with the pro-gression of the plant age untill 140 days, when it tended to steady until the end of plant life in both seasons, as shown in Figure 1(c). Fresh and dry weight (g/plant) increased as the plant increased in age to reach the a maximum weight after 175 day and showed the active period of growth from 90 to 135 days as observed with the other growth parameters in the two

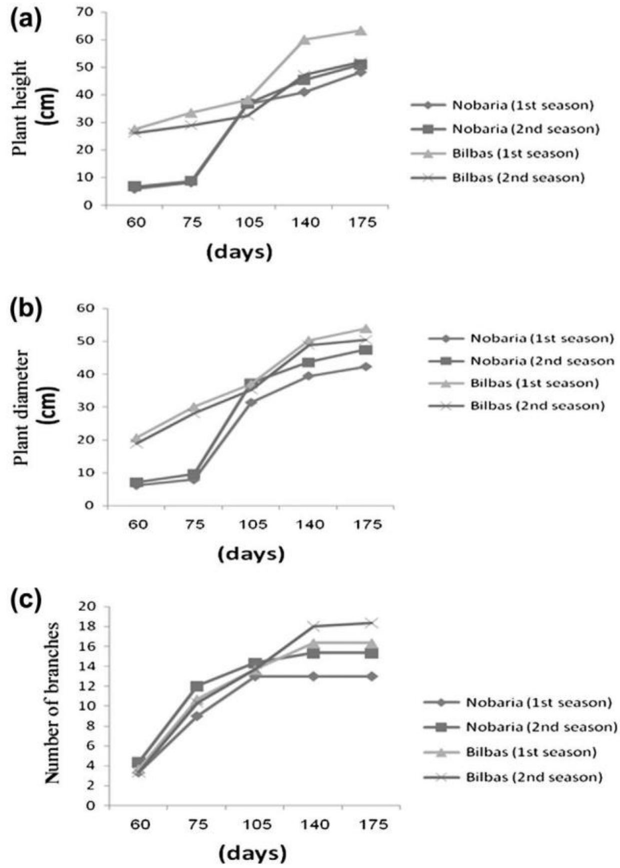

Figure 1. Seasonal variations in *Tagetes lucida* in the two locations during two seasons.

locations (Table 1). In this period, the rate of increasing in the fresh weight was 3.2 g/day, while the same value was 1.2 g/day during the last 40 days of plant age. The fresh yield (Mg/ ha) increased as the plant age increased and reached the maximum (8.5 Mg/ha.) after175 days from transplantation. The same trend was observed with dry yield (Mg/ha) in both seasons. This was true for plants cultivated in both localities, as shown in Table 1. Our results are somewhat lower than those obtained by Marotti et al. (2004) in a study of several *Tagetes* spp cultivated in Italy, where the authors reported collecting 41.9 Mg/ha of *T. lucida* leaves.

The flowers were collected after 140 days until the end of plant life and weighted to calculate the accumulated fresh weight of flowers per plant to reach 2.5, 5.5, 2.1 and 3.5 g per plant for plants cultivated in Nobaria and Bilbas in the first and second seasons, respec-tively. The fresh flower yield reached 66.3 and 147.0 kg/ha in Nobaria and 55.9 and 93.5 kg/ ha in Bilbas in the first and second seasons, respectively (Table 1). The corresponding dry values were 17.5 and 35.3 kg/ha for plants cultivated at Nobaria and 6.8 and 31.0 kg/ha for those cultivated in Bilbas. The fresh and dry weights of flowers in both seasons and sites increased as plant age increased which may be explained duo to the increase in the number and weigh of flowers.

Table 1. Fresh and dry weight and yield of *Tagetes lucida* plant cultivated at Nobaria and Bilbas in two seasons.

Plant age (days)	Fresh weight (g/plant)	Dry weight (g/plant)	Flowers fresh weight (g/plant)	Flowers dry weight (g/plant)	Fresh yield (Mg/ha)	Dry yield (Mg/ha)	Flowers fresh yield (kg/ha)	Flowers dry yield (kg/ha)
				First season Nobaria				
90	76.7± 6.65	24.3 ± 2.01			2.1 ± 0.07	0.6 ± 0.02		
135	221.1± 11.58	69.1 ± 4.55			5.9 ± 0.13	1.8 ± 0.05		
175	271.0± 20.30	86.9 ± 5.62	2.5 ± 0.131	0.7 ± 0.056	7.2 ± 0.23	2.3 ± 0.06	66.3 ± 5.23	17.5 ± 1.38
				Second season Nobaria				
90	77.00 ± 8.94	24.6 ± 2.37			2.0 ± 0.10	0.48 ± 0.03		
135	242.5± 9.12	80.5 ± 4.03			6.5 ± 0.10	2.1 ± 0.05		
175	317.7± 16.01	97.9 ± 5.35	5.5 ± 0.172	1.3 ± 0.058	8.5 ± 0.18	6.6 ± 0.06	147.0 ± 4.01	35.3 ± 0.96
				First season Bilbas				
90	60.8± 4.9	20.4 ± 1.7			1.6 ± 0.06	0.5 ± 0.02		
135	174.2± 21.3	55.0 ± 7.1			4.6 ± 0.24	1.5 ± 0.08		
175	285.9± 28.9	90.0 ± 13.6	2.1 ± 0.179	0.3 ± 0.025	7.6 ± 0.32	2.4 ± 0.15	55.9 ± 4.38	6.8 ± 0.51
				First season Bilbas				
90	63.8± 11.00	21.0 ± 2.8			8.3 ± 0.12	0.5 ± 0.03		
135	192.0± 26.1	64.7 ± 7.1			5.1 ± 0.29	1.7 ± 0.08		
175	276.8± 18.9	93.5 ± 8.5	3.5 ± 0.05	0.4 ± 0.037	7.3 ± 0.21	2.5 ± 11.05	93.5 ± 1.07	31.0 ± 0.358

Note: Results are expressed as mean of three measurements ± standard deviation.

Table 2. Chemical composition of *Tagetes lucida* plant cultivated at Bilbas and Nobaria in two seasons.

Plant age (days)	EO (%)	Oil (mL/plant)	Oil yield (l/ha)	Flavonoids content (mg/100 g)	Coumarins content (mg/100 g)	Chlorophyll (A) content (mg/g)	Chlorophyll (B) content (mg/g)	Carotene content (mg/g)
First season Bilbas								
90	0.3	0.2 ± 0.01	4.9 ± 0.17	840.8	1.0	1.4	0.3	0.6
135	0.4	0.6 ± 0.08	16.7 ± 0.86					
175	0.7	1.9 ± 0.19	51.2 ± 2.17	439.4	1.3	2.2	0.3	1.0
Second season Bilbas								
90	0.3	0.2 ± 0.03		1039.4	1.1	2.3	0.7	1.2
135	0.3	0.7 ± 0.09	17.9 ± 1.02					
175	0.5	1.4 ± 0.09	36.9 ± 1.06	757.8	1.7	3.4	0.4	2.0
First season Nobaria								
90	0.5	0.5 ± 0.26	14.2 ± 2.97	1990.5	0.8	1.2	0.1	0.7
135	0.5	1.2 ± 0.06	31.8 ± 0.70					
175	0.5	1.2 ± 0.09	32.5 ± 1.02	649.5	3.3	2.2	0.3	1.0
Second season Nobaria								
90	0.5	0.4 ± 0.05	14.2 ± 0.51	979.1	0.4	1.3	0.1	0.6
135	0.5	1.3 ± 0.05	35.5 ± 0.53					
175	0.4	1.3 ± 0.06	33.9 ± 0.72	728.9	3.3	2.6	0.7	2.3

Note: Essential oil results are expressed as mean of three measurements ± standard deviation.

Table 3. The main constituents of the essential oil of *Tagetes lucida* cultivated in Bilbas and Nubaria, results are expressed as percent area chromatogram.

Compound	Formula	M.W.	Bilbas (%)	Nubaria (%)	RI	ID
α-pinene	$C_{10}H_{16}$	136	0.02	0.01	938	RI, MS, STD
Sabinene	$C_{10}H_{16}$	136	0.12	0.10	971	RI, MS, STD
β-pinene	$C_{10}H_{16}$	136	0.01	0.15	974	RI, MS, STD
Myrcene	$C_{10}H_{16}$	136	1.65	0.35	993	RI, MS, STD
Phellandrene	$C_{10}H_{16}$	136	0.09	0.04	1003	RI, MS, STD
α-terpinene	$C_{10}H_{16}$	136	0.19	0.05	1017	RI, MS, STD
γ-Terpinene	$C_{10}H_{16}$	136	0.14	0.11	1060	RI, MS, STD
β-E-Ocimene	$C_{10}H_{16}$	136	0.01	0.26	1061	RI, MS
Cis-sabinene hydrate	$C_{10}H_{18}O$	154	0.01	0.07	1070	RI, MS
Terpinolene	$C_{10}H_{16}$	136	0.03	n.d.	1108	RI, MS, STD
Linalool	$C_{10}H_{18}O$	154	2.00	1.29	1138	RI, MS, STD
Methyl chavicol	$C_{10}H_{12}O$	148	93.18	94.33	1201	RI, MS
Methyl eugenol	$C_{11}H_{14}O$	178	0.83	0.63	1408	RI, MS
Caryophyllene (E)	$C_{15}H_{24}$	204	0.11	0.16	1412	RI, MS, STD
Germacrene(D)	$C_{15}H_{24}$	204	0.07	0.08	1480	RI, MS
(E)α-farnesene	$C_{15}H_{24}$	204	0.01	0.03	1502	RI, MS
Germacrene-D-4-ol	C H O	222	0.04	n.d.	1569	RI, MS
Spathulenol	$C_{15}^{15}H_{24}^{26}O$	220	0.06	n.d.	1575	RI, MS
Caryophyllene oxide	$C_{15}H_{24}O$	220	0.10	0.02	1584	RI, MS, STD
Epi-α-cadinol	$C_{15}H_{26}O$	222	0.02	n.d.	1635	RI, MS
α-cadinol	$C_{15}H_{26}O$	222	0.03	n.d.	1655	RI, MS
Valeranone	$C_{15}H_{26}O$	222	0.26	0.1	1675	RI, MS
Eudesma-4(15),7-dien-1-beta-ol	$C_{15}H_{24}O$	220	0.03	n.d.	1682	RI, MS
Total of non oxygenated compounds			2.42	1.41		
Total of oxygenated compounds			96.58	96.37		
Not identified peaks			1.00	2.32		

Note: RI, Retention index; ID, Identification method; MS, Comparison of mass fragmentation pattern with libraries; STD, pure standard coinjection; n.d., not detected.

The chemical composition of *T. lucida* plants cultivated in Nobaria in the two seasons is shown in Table 2. EO content in the fresh herb ranged from 0.4 to 0.5% with a maximum content 135 days after transplantation; the minimum content was observed after 175 days after transplantation, although the differences were not great. EO content increased according to plant age to reach a maximum (1.2 and 1.3 mL/plant in the first and second seasons, respectively). The same trend was observed with EO yield in both seasons for which 32.5 and 33.9 L/ha were recorded for plants of the first and second seasons in Nobaria. Total flavonoids and total coumarins were determined at two dates (90 and 175 days after transplantation) to show that total flavonoids accounted for 199 and 64.9 mg/g while coumarins reached 0.4 and 1.6 mg/g dry weight for the first and second dates, respectively, in the first season. The equivalent values in the second season were 97.9 and 72.9 mg/g for flavonoids and 0.22 and 1.65 mg/g for coumarins. Chlorophylls and carotenes were also determined in the fresh herb after 125 and 155 days from transplanting in both seasons. Their values in the 1st season were 1.2 and 2.2 mg/g for chlorophyll a and 0.1 and 0.3 for chlorophyll b and 0.7 and 1.0 for carotenes, respectively. In the second season, the corresponding values were 1.3 and 2.6 for chlorophyll a, 0.1 and 0.7 for chlorophyll b and 0.6 and 2.3 mg/g for carotenes, respectively (Table 2). Chlorophyll a and carotenes increased with increasing plant age in both sites and seasons. Flavonoids decreased with the development of plant age, while the opposite was true with coumarines content. These differences may be explained as result of day length and temperature as stated by Tang and Vincent (2000) and (Kazaz et al. 2010). They mentioned that both carotenes and Chlorophyll a content increased significantly with increasing temperature. However, carotenes rose more slowly with temperature than Chlorophyll a.

The chemical composition of *T. lucida* plants cultivated in Bilbas in the two seasons is shown in Table (2). The EO per cent in the fresh herb ranged from 0.3 to 0.7% in the two seasons and showed a maximum content 175 days after transplantation in the first season, while the minimum content was recorded 90 days after transplantation in the second season. EO content increased with increasing plant age to reach a maximum (1.9 and 1.4 mL/plant in the first and second seasons, respectively) after 175 days. This increase could be attributed to the increase in plant fresh weight and EO per cent. The same trend was observed with EO yield in both seasons in which 51.2 and 36.9 L/ha were recorded for plants of the first and second seasons, respectively. Total flavonoids and total coumarins were determined at two plant ages (90 and 175 days after transplantations) and show that total flavonoids were 84.1 and 43.9 mg/g, while coumarins reached 1.0 and 1.3 mg/100 g dry weight for the two ages, respectively, in the first season. The equivalent values were 103.9 and 757.8 mg/g for flavonoids and 1.1 and 1.7 mg/100 g for coumarins in the second season. Chlorophylls and carotenes were also determined in the fresh herb 90 and 175 days after transplanting in both seasons. Their values in the 1st season were 1.4 and 2.2 (mg/g) for chlorophyll a and 0.3 and 0.3 (mg/g) for chlorophyll b and 0.6 and 1.0 (mg/g) for carotenes, respectively. In the second season, the corresponding values were 2.3 and 3.4 (mg/g) for chlorophyll a and 1.2 and 0.4 (mg/g) for chlorophyll b and 0. 7 and 2.0 (mg/g) for carotenes, respectively. The main constituents of the EO of *T. lucida* cultivated in two locations, as separated and identified with GC, are shown in Table 3. The major compound was identified as methyl chavicol (estragole) with a relative proportion of 93.2 and 94.3% of the total identified compounds in EO of plants cultivated in Bilbas and Nobaria, respectively. Compounds with phenolic or aromatic moieties like estragole are powerful scavenging compound with antioxidant properties (Petretto et al. 2016). The predominance of estragole in the EO confirms that the shikimic acid biosintetic pathway is predominant in this plant. The other main compounds were found to be linalool with relative content of 2.0 and 1.3% and myrcene with relative content of 1.6 and 0.3% in the EO of Bilbas and Nobaria plants, respectively. Methyl eugenol was identified in the EO with relative content of 0.8 and 0.6% then valeranone with 0.3 and 0.1% in the EO resulted from Bilbas and Nobaria plants, respectively. β-E-ocimene reached 0.3% in the EO of plants cultivated in Nobaria but only in trace amounts in those cultivated in Bilbas. Six compounds were detected in plants cultivated in Bilbas and were absent in those cultivated in Nobaria, although their relative proportions are very small. These compounds are Terpinolene, Germacrene-D-4-ol, Spathulenol, Epi-α-cadinol, α-cadinol and Eudesma-4(15)-7-dien-1-betal-ol. The total percentages of oxygenated compounds were 96.6 and 96.4% in the EO of Nobaria and Bilbas plants, respectively. The corresponding values of non-oxygenated compounds were 2.4 and 1.3%, respectively. The chemical composition of EOs from Egyptian *T. lucida* shows several similarities with literature data. In particular, examples of high content estragole chemotype have been reported by several authors (Cicció 2004; Regalado et al. 2011 and Vera et al. 2014) where estragole represented over 90% of the whole EO composition.

Bicchi et al. (1997) reported a different chemotype of *T. lucida* originating in Guatemala, where the EO was dominated by phenylpropanoid compounds like estragole. By contrast, the chemical composition of *T. lucida* differs significantly from that of other *Tagetes* spp. (Reddy et al. 2016).

3. Conclusion

Seeds of *T. lucida* were imported from Canada and successfully domesticated and cultivated in two locations in Egypt. Similar growth parameters were recorded in both Nobaria and Bilbas: plant heights at 175 days were similar at about 50 cm, while plant diameters ranged between 43 and 53 cm. The fresh herb yield was 7.8 and 7.5 Mg/ha in Nobaria and Bilbas respectively. The Cumarins content was somewhat higher in Nobaria plants (3.3 mg/100 g) than in Bilbas plants (1.5 mg/100 g). Conversely the EO yield, obtained by steam distillation, was slightly higher in Bilbas plants (44 L/ha) than in Nobaria plants (33 L/ha).

It is clear from our results that no meaningful changes are observed in the main constituents of the EO of *T. lucida* arising from the site of cultivation. This may be attributed to the fact that there is no significant change in the temperature or the humidity between the two sites and that the soils of the two locations are both sandy in texture or it may be that the constituents of the EO are genetically controlled. This suggests that it may be worth studying the impact of soil types, latitude and altitude on the main constituents of the EO of *T. lucida* if it is to be used as a new source of methyl chavicol in Egyptian cultivation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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