Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 07 | Issue :01 | Jan.-Mar.| 2017
Pages: 141-154

Analysis of Genetic Diversity of *Lavandula* Species using Taxonomic, Essential Oil and Molecular Genetic Markers

Heba M. M. Ibrahim[1], Azza M. Salama [2] and Omnia F. Abou El-Leel [3]

[1]*Genetics Department, Faculty of Agriculture, Cairo University, Egypt.*
[2]*Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt*
[3]*Medicinal and Aromatic Plants Department, Hort. Res. Inst., ARC, Giza, Egypt*

Received: 15 January 2017 / Accepted: 06 March 2017 / Publication date: 09 March 2017

ABSTRACT

The present investigation was conducted to study the differences in taxonomic characters, essential oil production and composition as well as the genetic relationship of three different *Lavandula species* that are widely grown in Egypt, wild type *L. pubescens* Decne., and two cultivated species, *L. angustifolia* Miller and *Lavandula* × *heterophylla* Viv**).** The essential oil study showed that the maximum volatile oil percent in the fresh leaves was observed in *L. pubescens* with 0.8%, while the minimum was observed in *L. heterophylla (Lavandula* × *heterophylla* Viv; 0.04%). Despite the noticed fact that the three *Lavandula* species under investigation were evaluated as the highest quality compared to other *Lavandula* species because of their content of linalool and linalyl acetate, it was obvious that the *L. angustifolia* volatile oil was distinctive due to containing unique components, α-fenchene and Camphene, which were absent in the other two species. The anatomical analysis revealed that the upper and lower surfaces of the leaves of *L. angustifolia* have epidermal hairs, which were absent in the other two species. In addition, the presence of capitates peltate glandular trichomes on upper and lower epidermis distinguished the other two species from *L. angustifolia* species. Results of the band-based approach came out to support the essential oil and anatomical studies by generating a dendrogram with two separate clusters, the first consists of *L. pubescens* and *L. heterophylla* in one clade with 79% bootstrap value, and the second consisted of *L. angustifolia* Miller in a distant clade. Based on these results, it could be concluded that *L. angustifolia* is distinguishable species that has distinct genetic composition, which could be due to the widespread and diverse exchange of the plant material during cultivation.

Key words: Lavandula, SEM, Anatomy, volatile oil, RAPD

Introduction

Lavender is a member of the family Lamiaceae, which has 30 species of small shrubs or herbs, native to France and the western Mediterranean. In the Mediterranean region, there are four different lavender species, *L. angustifolia*, *L. dentata*, *L. latifolia* and *L. intermedia.* While in Egypt, only two were found, *L. angustifolia*, *L. dentate (*Abdel-Hady *et al*., 2014). *Lavandula* species are mainly grown for their essential oils, which contain over 300 chemical compounds. Thus, it is cultivated worldwide for its contents of essential volatile oils, fragrance, craft material, and as ornamental plants as far north as Norway (Uremis *et al*., 2009). The dominant components are linalool, linalyl acetate, terpinen-4-ol, acetate lavandulol, ocimene, and cineole, which are used in perfumery, cosmetics, food processing and aromatherapy products, and ingredients in numerous cottage industry products. Certain types of lavender oil have antimicrobial and antifungal (Uremis *et al*., 2009). The dried flowers have been used for centuries in pillows and sachets to promote sleep and relaxation and the oil of spike lavender is used as an insect repellent (Verma *et al.*, 2010).
Lavender essential oil is characterized by high levels of linalool, and linalyl acetate, moderate levels of lavandulyl acetate, terpinen-4-ol and lavandulol. The amount of 1,8-cineole and camphor often varies from very low to moderate (Gimpsey and Porter,1999). Lavender oil typically contains more than 100 individual components (many minor ones often unidentified and/or not quantitated), each

Corresponding Author: Heba M. M. Ibrahim, Genetics Department, Faculty of Agriculture, Cairo University, Egypt.
E-Mail: heba.ibrahim@staff.cu.edu.eg

contributing to the chemical and sensory properties of the oils. The essential oils of *L. angustifolia* also = have phytotoxic effects on the seedling growth of *A. retroflexus* and *Portulaca oleracea* that infest tomato (*Lycopersicon esculentum*) and cotton (*Gossypium spp*.) crops **(**Argyropoulos *et al.,* 2008), as well as a negative effect on the germination of common cocklebur (*Xanthium strumarium* L.), sterile wild oat (*Avena sterilis* L), and short spiked canarygrass (*Phalaris brachystachys* L.; Uremis *et al*., 2009).

While phyto-chemical variations and mutations may occur naturally, in addition to the effect of the climate change, it is expected that these cultivars may aquire genetic modifications that may be reflected in the characteristics of the plant morphology and the composition of the essential oils of the *Lavandula* species (Nybom *et al*., 2014).

In addition to the common chemical and morphological methods used to study the different characteristics among plant genotypes, DNA fingerprinting offers a very robust and easy way to differentiate and classify the diversity and relatedness of different genotypes (Nybom *et al*., 2014). Random amplified polymorphic DNA (RAPD) is a DNA marker that has been widely used in genetic fingerprinting for genotype identification and heterogeneity detection in all different organisms (Olivier *et al*., 1999; Gaber *et al.*, 2007). In a RAPD reaction, random fragments of genomic loci are amplified without previous knowledge of the nucleotide sequences by using random pairs of 8-10 nucleotide long arbitrary primers (Olivier *et al*., 1999). This technique has been intensively used for genetic mapping of several plants and animal species (Olivier *et al*., 1999).

The objective of this study was to examine and attempt to correlate the morphological, anatomical and molecular taxonomy among three species of *Lavandula*; *L. angustifolia, L. heterophylla* and *L. pubescens*, to determine their relatedness, using RAPD molecular analysis, morphological/phenotypic and scanning electron microscopy of leaves.

Materials and Methods

Morphological and anatomical analysis

Mature plants were collected during the flowering stage to define the morphological and anatomical traits. Twenty fresh specimens of the collected species and the same as herbarium specimens were examined. The morphological/phenotypic traits examined are listed in Table 2. The anatomical examination was carried out according to Salama *et al*. (2016) The detailed leaf surface features were examined by using Scanning Electron Microscope (SEM) on leaves with different magnifications was carried out by JEOL-JSM-IT100 instrument (JEOL, MA, USA), at Central Laboratory, National Information and Documentation Center (NIDOC), Dokki, Giza, Egypt**.** Terminology of leaf surface sculptures followed Murley (1951) and Claugher (1990).

Investigation and identification criteria of the studied taxa were based on the authentic flora and taxonomic references (Hedge, 1992 and Harley *et al*., 1992). Lamina specimens were taken (at the 5th internodes). Specimens were fixed for at least 48 hrs. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The specimens were washed in 50 % ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56 ºC, sectioned to a thickness of 20 µm, double stained with crystal violet-erythrosine, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998).

Essential oil analysis:

Essential oil percentages were determined in fresh leaves according to the method described in the British Pharmacopoeia (1963). Analysis of the essential oils by using capillary GLC-2010 plus Gas Chromatographs (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector; Shimadzu Corp., Kyoto, Japan)). The GLC system was equipped with a stable wax column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10 and the following temperature program: 40 ºC for 1 min; rising at 4.0 ºC/min to 150 ºC and held for 6 min; rising at 4 ºC/min to 210 ºC and held for 1min. The injector and detector were held at 210 and 250 ºC, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always injected. Most of the compounds were identified using GLC standards.

Genomic DNA isolation and DNA amplification

DNA was extracted from field-grown leaves using the CTAB protocol described by Porebski *et al.* (1997). Six different primers (C1, P13, N8, B12, H5 and P8; Table 1) were used for DNA amplification according to (Barcaccia *et al.,* 2006) The PCR reactions were carried out in 25 µl volume containing 50 ng of genomic DNA template, 30 pmoles/µl primers, 0.2 µM each of dATP, dCTP, dGTP and dTTP, 10 x buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, and 2 units of Taq polymerase (Thermo Fisher Scientific, CA, USA).

Table 1: The sequences of the six arbitrary primers used in the fingerprint analysis.

Primers		Sequences
1	C1	TTCGAGCCAG
2	P13	GGAGTGCCTC
3	N8	ACCTCAGCTC
4	B12	CCTTGACGCA
5	H5	AGTCGTCCCC
6	P8	GGAGCCCAG

PCR amplifications were carried out in an Applied Biosystem thermal cycler (Thermo Fisher Scientific, CA, USA) as follows: an initial strand separation at 94°C (5 min) followed by 40 cycles with the following temperature profile: 94°C (1 min), 36°C (1 min), 72°C (1.5 min) and a final extension at 72°C (7 min). Amplification products were electrophoresed with a 1 Kb molecular weight DNA ladder (Thermo Fisher Scientific, CA, USA) in 1.5% agarose gels stained with Ethidium Bromide then visualized and photographed with a SynGene gel documentation unit (SynGene, Cambridge, UK) the DNA banding patterns were scored.

The data was manually scored comparing with the pictures where a binary data was organized and only the polymorphic fragments were scored as present (1) and absent (0). Absent and present binary data of 8 individuals and106 polymorphic loci was used as the basis for the analysis. In the present study, based on (Bonin *et al*., 2007) the band-based approach was used for the analysis in the individual level and allele frequency-based approaches (for population level). Dice coefficient was used to calculate the similarity among 12 individuals (Dice, 1945). The cluster analysis was prepared through unweighed pair group method with arithmetic mean (UPGMA) based on Dice index (Nei 1978; Nei and Li, 1979). Bootstrap values (based on 1000 re-sampling) was used to estimate the reliability of the clustering pattern. This analysis was carried out in Free Tree (Hampl *et al*., 2001). The NTSYSpc version 2.20 was used to prepare Principal Co-ordinates Analysis (PCoA) of the correlations matrix to test the relationship among *Lavandula* individuals (Rohlf, 2005).

Results and Discussion

1- Leaf morphological characters

Leaves are simple and opposite with petiols in *L. pubescens*, while those of *L. angustifolia* and *L. heterophylla* are sessile. Blade shape lancelets in *L. angustifolia*, ovate to lancelet in *L. heterophylla*, pinnatifidum in *L. pubescens.* Blade margins are entire in *L. angustifolia*, dissected in *L. pubescens*, while pinnatifid in *L. heterophylla*. Obtuse leaf apex and truncated leaf base characterize all *Lavandula* species. Blade color is silver in *L. angustifolia*, gray in *L. heterophylla* and green in *L. pubescens* (Table 2).

2- The anatomical characteristics:

The anatomical measurements and counts of leaves are shown in (Table 3) and the transverse section of the middle part of the leaves was studied (Fig.1). The leaves of *L. heterophylla* were thin, and the lamina thickness was 362 µm, while the leaves of *L. angustifolia* and *L. pubescens* were 562 µm 595 µm, respectively. Collenchymatous cells were found around the vascular bundle in all species. the thickness of upper collenchymas was 350, 140 and 310 µm in *L. angustifolia***,** *L.*

pubescens and *L. heterophylla,* respectively. Lower collenchymas were 140 μm in *L. angustifolia* and *L. heterophylla*, and 72 μm in *L. pubescens*.

Table 2: Diagnostic characters of the studied *Lavandula* species.

Characters	*L. angustifolia*	*L. pubescens*	*L. heterophylla*
Leaf			
-Shape	Lanceolate	Pinnatifidum	Ovate -lanceolate
-Margin	Entire	Dissected	Pinnatifid
-Apex	Obtuse	Obtuse	Obtuse
-Base	Truncate	Truncate	Truncate
-Color	Silver	Green	Gray
- petiole	Sessile	Petiolate	Sessile
Upper epidermis:			
- Stomatal type			
- Stomatal level	Indistinct	Anomocytic	Paracytic
- Sculpture pattern	Indistinct	Deppraised	Deppraised
	Indistinct	Pusticulate	Ruminate
- Trichomes type			
	Capitate and peltate glandular trichomes which are modified epidermal hairs	Capitate Peltate glandular trichomes	Capitate Peltate glandular trichomes
Lower epidermis:	Indistinct		
	Indistinct		
-Stomatal type	Indistinct	Anomocytic	Paracytic
-Stomatal level		Deppraised	Semi deppraised
-Sculpture pattern	Capitate peltate glandular	Glebulate	Rugose
- Trichomes type	trichomes which are modified epidermal hairs	Capitate Peltate glandular trichomes	Capitate Peltate glandular trichomes

Table 3: Anatomical measurements (μm) and counts of different tissues of leaf lamina and petiole of eight studied species (average of 5 samples, 8 weeks old)**.**

Characters	*L. angustifolia*	*L. pubescens*	*L. heterophylla*
Lamina			
Upper epidermis	30	40	30
Lower epidermis	28	30	27
Upper collenchymas thickness	350	140	310
Lower collenchymas thickness	140	72	140
Lamina thickness	562	595	362
Palisade tissues thickness	145	130	148
Spongy tissues thickness	385	450	155
Main vein thickness	1060	825	1410
Vascular bundle dimension			
- Length	300	233	365
- width	395	245	450
Xylem thickness	187	120	210
Phloem thickness	77	65	125

The upper and lower epidermis consists of a single layer of rectangular cells in all species with different shapes and sizes. There were many multicellular trichomes on both epidermis. Mesophyll consists of the palisade and spongy parenchyma. In *L. angustifolia***,** *L. pubescens* and *L. heterophylla*,

the palisade tissue had loose cells with wide intercellular spaces were 145, 130 and 148 µm. Spongy tissue thickness were 385, 450 and 155 µm in *L. angustifolia***,** *L. pubescens* and *L. heterophylla*, respectively. Likewise, the main vascular bundle of the midvein was a collateral type, with the largest measurement in *L. heterophylla,* with a length and a width of 365 and 450 µm, respectively, due to the increased xylem (210 µm) and phloem (125 µm) volume.

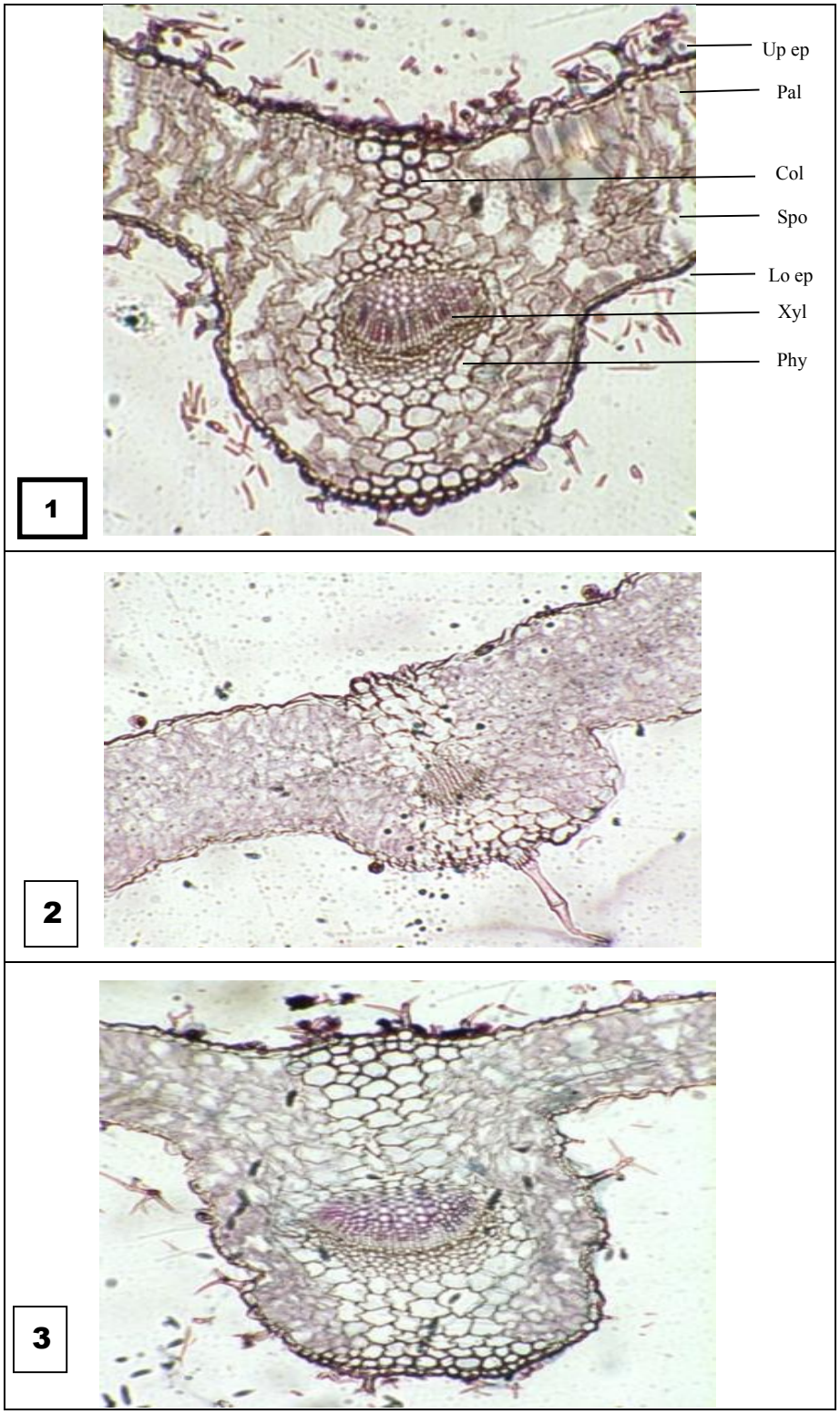

Fig. 1: Transverse section on the middle section of leaves of *L. angustifolia* (1)*, L. pubescens* (2) and *L. heterophylla* (3). up ep; upper epidermis, lo ep; lower epidermis, pal; palisade tissue, spo; spongy tissue, phl; phloem, xy; xylem.

3- Scanning electron microscopic

Leaf upper and lower surfaces are hairy (nonglandular, glandular). Capitate and peltate glandular trichomes were modified epidermal hairs in *L. angustifolia,* while in *L. pubescens* and *L. heterophylla* were capitates peltate glandular trichome on upper and lower epidermis.

Stomata in *L. angustifolia* on upper and lower epidermis were indistinct, with indistinct level on both surfaces. While, in *L. pubescens* stomata were anomocytic with appraised level on upper and lower surfaces, pusticulate sculpture of leaf upper surface, glebulate on lower one. *L. heterophylla* showed paracytic stomata with appraised level on upper epidermis and paracytic with semi appraised level on lower one, ruminate sculpture on upper surface, rugose sculpture on lower one.

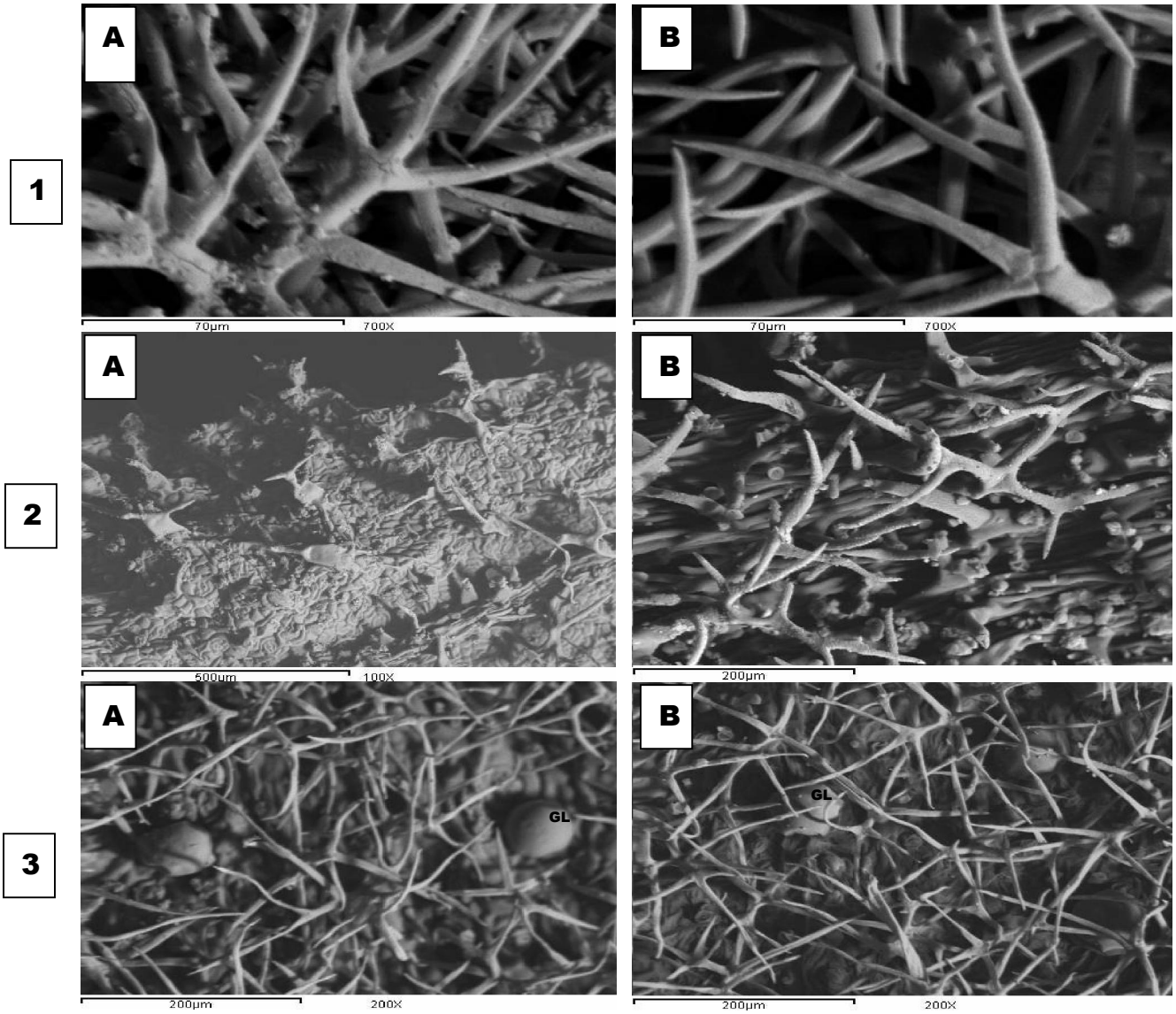

Fig. 2: Scanning electron micrographs of *L. angustifolia* (1), *L. pubescens* (2) *L. heterophylla* (3). A: Surface of upper epidermis, B: Surface of lower epidermis

Table 4: Volatile oil composition (%) of different *Lavandula* species. Values are means of three replicates

Peak No.	Components	Retention time (min)	Area %		
			L. angustifolia	*L. pubescens*	*L. heterophylla.*
1	α-Thujene	5.646	2.664	1.393	3.464
2	α-Pinene	5.760	0.168	0.397	0.931
3	α-fenchene	6.805	0.693	----------	----------
4	Camphene	8.095	2.340	----------	----------
5	β-pinene	8.566	0.850	1.832	3.257
6	Δ-3-carene	9.524	1.489	0.451	0.951
7	β -Myrcene	10.120	1.288	----------	0.765
8	o-cymene	11.273	0.255	1.573	0.155
9	α-Terpinene	12.025	66.652	4.136	61.341
0	1,8-Cineole	12.764	0.150	18.145	0.182
11	γ-terpinene	14.058	1.232	0.585	0.245
12	Terpinolene	14.601	--------	2.013	0.340
13	Linalyl oxide	20.863	0.530	0.952	0.507
14	Linalool	22.942	12.443	12.810	19.033
15	Octen-1-ol, acetate	24.544	0.232	14.122	0.355
16	Camphor	25.370	0.347	0.968	0.240
17	Borneol	25.509	0.478	1.051	0.146
18	Lavandulol	26.499	0.289	0.648	0.540
19	terpinen-4-ol	27.182	0.338	0.690	0.530
21	Cryptone	27.679	0.450	1.067	0.756
20	α-Terpineol	27.869	0.837	1.113	0.908
21	Verbenone	28.481	0.653	1.920	1.208
22	Linalyl acetate	28.622	1.897	1.381	0.846
23	Bornyl acetate	29.857	0.179	2.197	0.233
24	Lavandulyl acetate	31.598	0.255	0.355	0.182
25	β-caryophyllene	33.893	0.164	0.555	----------
26	Thymol	39.842	0.251	1.170	0.207
27	Carvacrol	47.400	0.857	18.528	----------

Table 5: The classification of volatile oil components of different of *Lavandula* species according to terpenoieds type. (Second year).

Components		Area %		
		L. angustifolia	*L. angustifolia*	*L. angustifolia*
1	Monoterpene hydrocarbons	76.938	12.38	71.449
2	Oxygenated monoterpenes	17.208	21.219	23.868
3	Sesquiterpene hydrocarbons	0.164	18.7	0.182
4	Oxygenated Sesquiterpene	0.232	14.122	0.355
5	Phenolic monoterpenes	1.108	19.698	0.207
6	Other oxygenated compounds	2.331	3.933	1.261
7	Total Identified	97.981	90.052	97.322
8	Unidentified	2.019	9.948	2.678

4-Essential oil composition

As for the essential oil, GLC analysis data (Fig. 3; Table 4 and 5) indicated that the maximum essential oil percent in fresh leaves was observed with the wild type L. pubescens (0.8%), while the minimum was observed in L. heterophylla (0.04%). In L. angustifolia it was 0.4%. The highest total identified compounds were 97.98% in L. angustifolia and 97.3% in L. heterophylla and the least were 90.052% in L. pubescens. The majority of compounds were the oxygenated monoterpenes (11 compounds) represented and ranged from 17.208% in L. angustifolia to 23.868% in L. heterophylla, while the monoterpene hydrocarbons were 9 compounds ranging from 12.38% in L. pubescens to 76.938% in L. angustifolia. The sesquiterpene hydrocarbons (two compounds) ranged from (0.164% to 18.7%) and oxygenated sesquiterpene (one compounds, octen-1-ol, acetate) ranged from (0.232%

to 14.122%) in L. angustifolia and L. pubescens, respectively. However Phenolic monoterpenes (two compounds, Thymol and Carvacrol) ranged from 0.207% in L. heterophylla to 19.698% in L. pubescens, respectively. In addition, other oxygenated compounds (Linalyl acetate, Bornyl acetate and Lavandulyl acetate) ranged from1.261% in L. heterophylla to 3.933%in L. pubescens. Lavandula species differed in these contents of monoterpenes and sesquiterpenes, and were characterized by high contents of oxygenated monoterpenes. The main constituents of L. angustifolia volatile oil were α-Terpinene (66.652%) and Linalool (12.443%). Volatile oil of L. pubescens major compounds were Carvacrol (18.528%), 1,8-Cineole (18.145%), Octen-1-ol, acetate (14.122) and Linalool (12.810%). The most abundant components observed in L. heterophylla were α-Terpinene (61.341%) and Linalool (19.033%).

In this study, we are reporting for the first time (according to our knowledge), the analysis of the leaf's volatile oil of these three-species cultivated in Egypt. This analysis revealed that the L. angustifolia and L. heterophylla contents have the highest value of α-Terpinene (66.652% and 61.341%) respectively, whereas Carvacrol (18.528%) and1,8-Cineole (18.145%) were the major compounds in L. pubescens. Uniquely, L. angustifolia's volatile oil contained some components comparable to the other two species. These components are: α-fenchene (0.693%), and camphene (2.340%). On the other hand, terpinolenes were not detected in L. angustifolia but existed in L. pubescens and L. heterophylla. β –myrcene was absent in L. pubescens volatile oil but present in the other two species. L. heterophylla volatile oil was poor in β-caryophyllene and carvacrol, and some other components that were found in the other two studied species .

As for the economic quality for lavender volatile oil, it is known that a good lavender species should contain a high proportion of linalyl acetate, linalool, and a low proportion of camphor (Kim and Lee, 2002). Linalyl acetate is known to have very low toxicity and skin irritation effect, in addition to high calming and spasmolytic properties (Kim and Lee, 2002). Likely, linalool has low toxicity in addition to antiviral and antibacterial properties (Ghelardini, *et al*., 1999). Therefore, the three *Lavandula* species in our study could be evaluated as the highest quality because of its linalool and linalyl acetate content.

5-DNA finger printing of Egyptian Lavender

Six random primers (C1, P13, N8, B12, H5, and P8), RAPD markers, were used for analysis of genomic DNA diversity among the three *Lavandula* field-grown cut leaf samples. RAPD markers profile and dendrograms results of *Lavandula* samples (Fig. 4 and 5; Table 6), respectively, revealed that 106 reproducibly scorable genomic DNA bands were generated by the six random primers. The amplified DNA fragments obtained in this study ranged from 3000 to 100 base pairs (bp). The number of amplified DNA fragments was scored for each primer with the highest number of amplified DNA fragments with 22 and 19 for primers N8 and B12, respectively. The other four primers amplified 65 DNA fragments, with an average of 16 amplicons per primer across the three *Lavandula* samples. The overall DNA patterns generated by primers OP-A3 and OP-M5 were five polymorphic bands. There were 6 polymorphic bands out of 9 scorable genomic DNA bands of the three lavender leaf samples amplified by primer OP-C5, while 7 polymorphic bands out of 10 reproducible genomic DNA bands generated by primers OP-D5 and OP-N4 (Table 6).

Genetic relationships of the three *Lavandula* species

The genetic similarities indices among the three Lavandula individuals were estimated based on the number of common fragments resulted from the six RAPD markers, in order to quantify the level of genetic similarity and/or distance of the studies species. The pairwise Jaccard's similarity indices similarity values among individuals ranged from 0.241 to 0.339 on the Dice index (Table 7). this values evidenced a closer genetic similarity between *L. heterophylla* and *L. pubescens* (0.339) than that between *L. pubescens* and *L. angustifolia* (0.241). The Jaccard's similarity matrix data was utilized in a cluster analysis to construct a dendrogram.

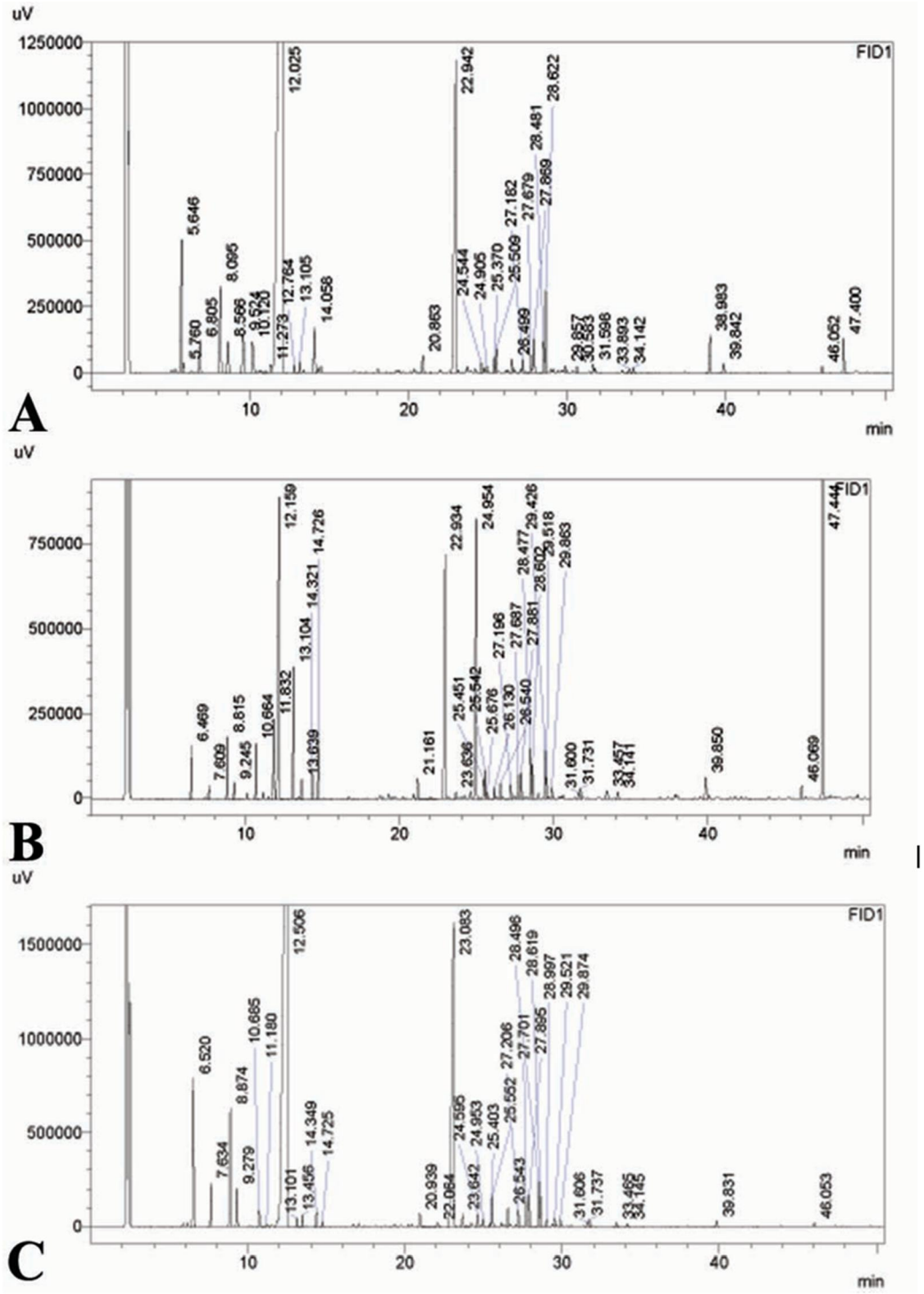

Fig. 3: The GLC Chromatographs for fresh leaves essential oil of *L. angustifolia* (A), *L. pubescens* (B) *L. heterophylla* (C).

Table 6: Total number of amplicons, monomorphic and polymorphic amplicons as revealed by RAPD primers among the three *Lavandula* plants.

Primer	Total Number of Amplicons	Polymorphic amplicons	Monomorphic amplicons	% of Polymorphism
C1	16.0	16.0	0.0	100%
P13	17.0	17.0	0.0	100%
N8	22.0	22.0	0.0	100%
B12	19.0	19.0	0.0	75%
H5	16.0	12.0	4.0	100%
P8	16.0	16.0	0.0	100%
Total	**106**	**102**	**4**	**95.8%**

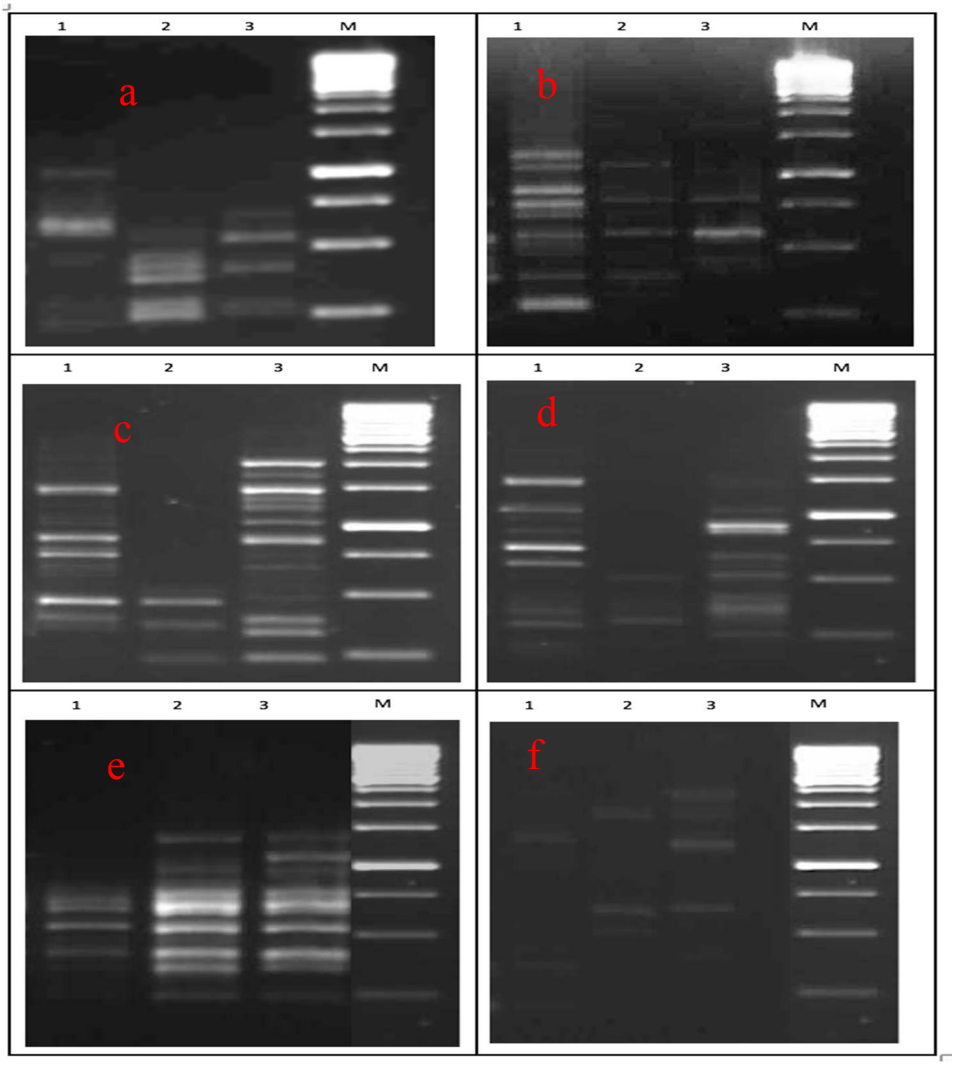

Fig. 4: RAPD profile for the three Lavandula species as detected with primers C1 (a), P13(b), N8 (c), B12 (d), H5 (e) and P8 (f). 1- *L. angustifolia Miller,* 2- *L. pubescens Decne,* 3- *L. heterophylla.* M: 1 kb DNA Ladder

Table 7: Genetic similarity indices for the three individuals of *Lavandula* species based on RAPD-PCR data using Nei (1972) method.

	L. angustifolia Miller	***L. pubescens* Decne**	***L. heterophylla***
***L. angustifolia* Miller**	1.0000		
***L. pubescens* Decne**	0.2414	1.0000	
L. heterophylla	0.2714	0.3390	1.0000

The cluster analysis using the numerical analysis technique "NTSYS-PC" version1.5 program was followed (Rohlf, 2005). Dendrogram based on unweighed pair group method of arithmetic means (UPGMA) were generated to represent the levels of similarity distance between studied species by classifying the three *Lavandula* species into main clusters (Fig. 5a and b).

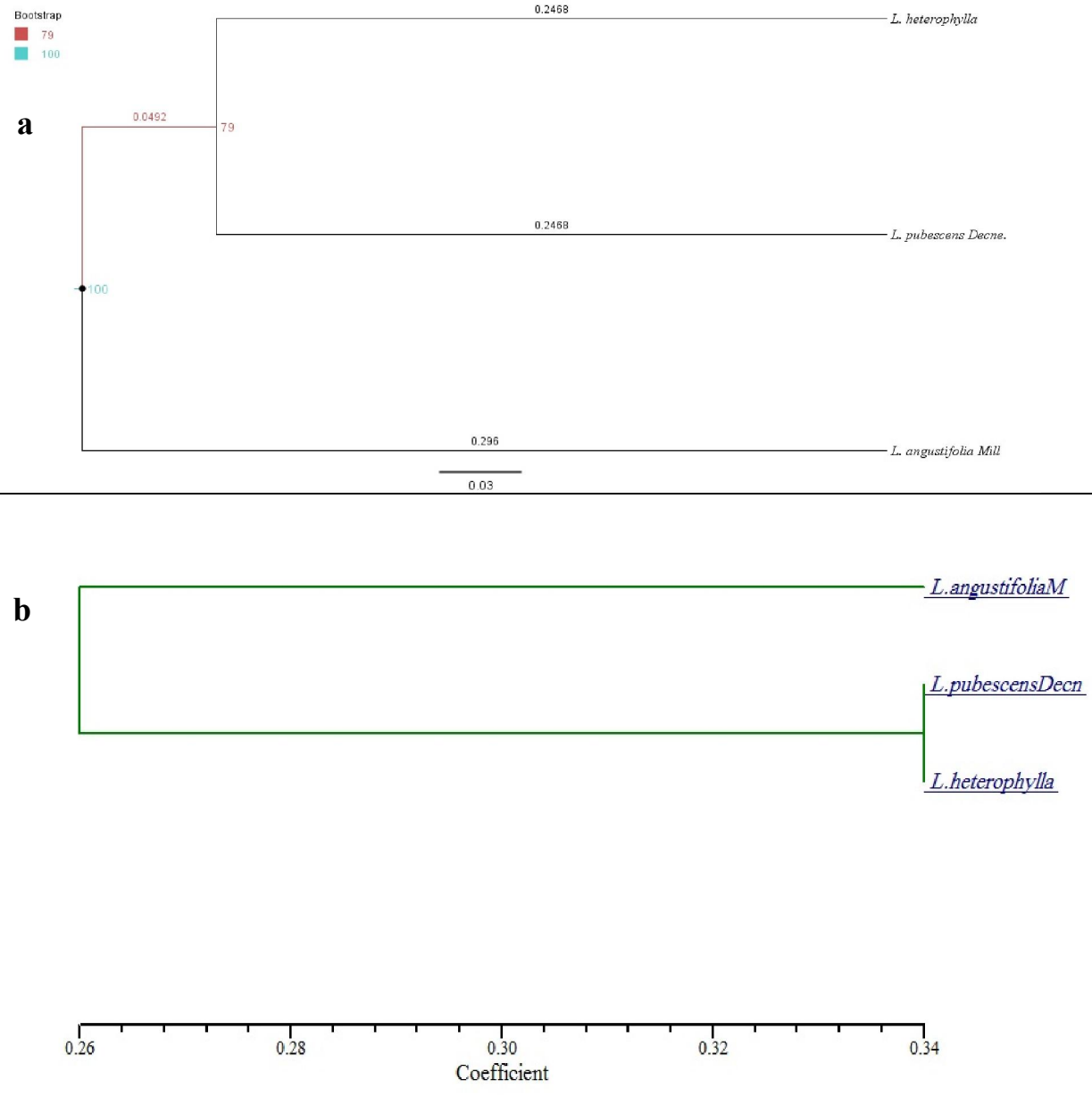

Fig. 5: UPGMA-based dendrogram of closely related genera of *Lavandula* generated from106 RAPD markers. (a) Confidence limits for the dendrogram based on 1000 bootstrap replications. Boot strap values were cut off at 50% and presented at the nodes. Dendrogram was assessed by Free Tree (Hampl *et al*., 2001). (b) UPGMA dendrogram based on Nei's (1978). Bar on the bottom indicates similarity index based on S.M. coefficients.

The dendrogram in Fig. (5a) revealed that *Lavandula* species were divided into two distinct sub-groups, *L. pubescens,* and *L. heterophylla* in a sub-group (79 % bootstrap value), and *L. angustifolia* in a separate sub-group. This data were also supported by generating a similar UPGMA dendrogram using a different multivariate statistics and data analysis software based on Nei's (1978; Fig 5b) which produced the same results. These clustering patterns were further supported by two-dimensional principal coordinate analysis generated by NTSYSpc,2.20 software (Fig. 6; Rohlf, 2005), based on the Jaccard's similarity coefficients.

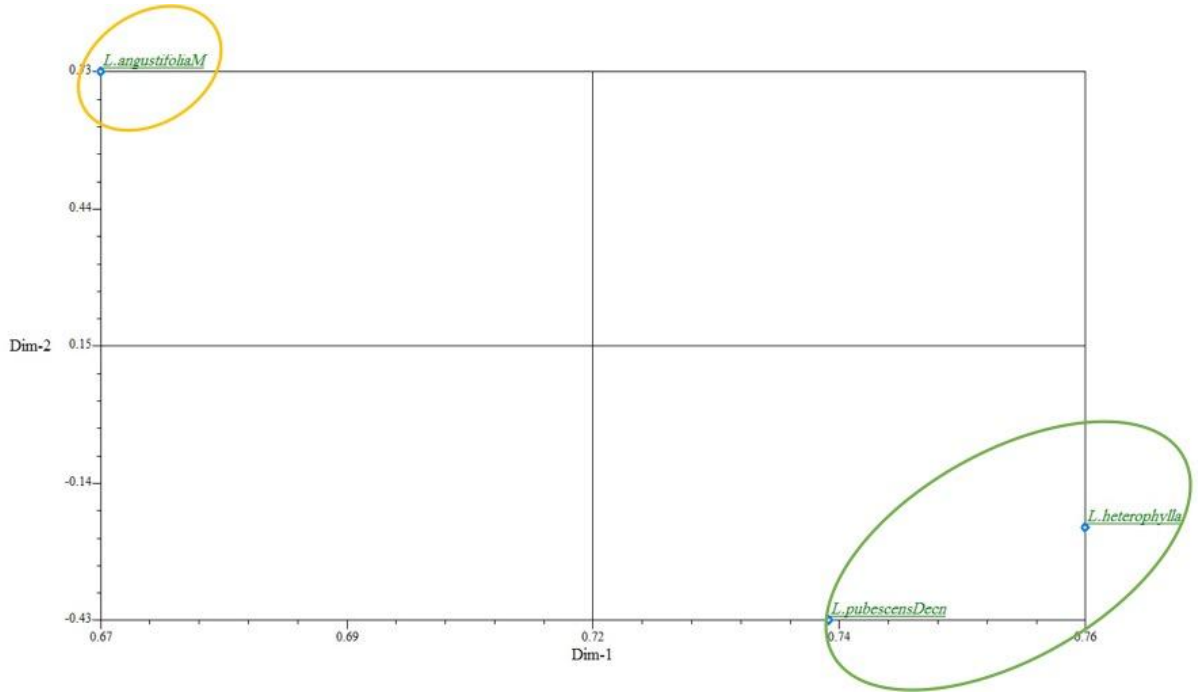

Fig. 6: Principal coordinate analysis, PCoA (2-D View) of the three *Lavandula* species, based on the Jaccard's similarity coefficients using RAPD banding pattern. The matrix plot is processed by NTSYSpc,2.20 (Rohlf, 2005)

In the present investigation, morphological, anatomical, essential oil composition, and genetic similarity studies were conducted in order to elucidate the genetic relation of three *Lavandula* species growing in Egypt (*L. pubescens*, *L. heterophylla* and *L. angustifolia*). Data of the studied characters of the leaf morphological, anatomical and surface scan descriptions, and essential oil composition distinguished the three different *Lavandula* species from one another, though it was not conclusive. Morphological parameters provided a greater discrimination along the spectrum of taxonomic differences among *Lavandula* species, and were also more sensitive in the delimitation of the studied taxa. In addition, the study revealed more detailed information on the level of relationship within the genus *Lavandula*. For instance, it is obvious from the essential oil composition study that *L. angustifolia* volatile oil was uniquely characterized among the other two tested *Lavandula* species because of its contents of some other chemical components. However, in this investigation we can conclude that the three *Lavandula* species had high quality of desired volatile oil content, linalool and linalyl acetate. In this investigation, the RAPD markers did not only elucidate the genetic relationship in the genus Lavandula for the three studies species, but also identified species specific RAPD markers with all the six RAPD primers used. The RAPD data showed 95.8% polymorphism among the three *Lavandula* species. The high value of variation may be due to the fact that *Lavandula* are out-breeding plant species. From the above-mentioned results, it is worthy to note that the two species *L. pubescens* and *L. heterophylla* join the rest of the lately studied species at the highest similarity level. The reason for that may be because these species have many morphological and surface scan features similar to each other and have similar morphological characters (leaf shape, margin and

color) and essential oil compassion. According to the obtained dendrogram of RAPD results, *L. angustifolia* existed alone in subgroup, which may be explain that *L. angustifolia* volatile oils were different from the other two species because it contained some components which were absent in the other two species. In addition, leaf upper and lower surfaces have modified epidermal hairs in *L. angustifolia*, while in *L. pubescens* and *L. heterophylla* were capitates peltate glandular trichome on upper and lower epidermis.

In a conclusion, these results showed a similarity between *Lavandula* plants, which suggest that the exiting lavender species (*L. pubescens* and *L. heterophylla)* are genetically related and originated from the same ancestor. Furthermore, our data indicated that neither the phenotypic, nor the oil contents studies stand separately or collectively to provide a definite distinction between the three-studied species. And, the molecular markers examinations are the determining factor in studying the genetic relationships between species. This information could be useful in breeding programs and proper management and expansion of *Lavandula* germplasm.

Acknowledgment:

The authors thank Mr. Abd El-Hay Gaber, Cairo University Research Park, for his excellent technical support.

References

Abdel-Hady N. M., Gamil M. Abdallah and Nagi F. Idris, 2014. Phytochemical studies and in vivo antioxidant activity of two Lavandula species (Lamiaceae) against streptozotocin induced oxidative stress in albino rats. Journal of Biomedical and Pharmaceutical Research, Volume 3, Issue 4, 2014, 30-40.

Argyropoulos, E. I., I. G. Eleftherohorinos, , and D. Vokou, 2008. In vitro evaluation of essential oils from Mediterranean aromatic plants of the Lamiaceae for weed control in tomato and cotton crops. Allelopathy J. 22:69–78.

Barcaccia, G., F. Arzenton, T. F. Sharbal, S. Vatotto, P. Parrini, and M. Lucchin, 2006. Genetic diversity and reproductive biology in ecotypes of the facultative apomicts *Hypericum perforatum* L. Heredity, 96: 322–334.

Bonin, A., D. Ehrich and S. Manel, 2007. Statistical analysis of amplified fragment length polymorphism data: a tool book for molecular ecologists and evolutionists. Molecular Ecology 16:3737–3758.

British Pharmacopoeia, 1963. Determination of volatile oil in drugs. The pharmaceutical press 17 Bloomsburg, square. W.C.I. London.

Claugher, D., 1990. Scanning Electron Microscopy in Taxonomy and Functional Morphology. The Systematics Association Special Vol. No. 41: 70-94.

Dice, L. R., 1945. Measures of the amount of ecologic association between species. Ecology 26, 297–302.

Gaber, A., H. M. M. El-Maragh, M. A. M. Aly, Rashed, N. A. and A. Y. El-Din, 2007. Induction of somatic embryogenesis and DNA fingerprinting of jojoba. Arab Journal of Biotechnology, 10: 341-354.

Ghelardini, C., N. Galeotti, G. Salvatore and G. Mazzanti, 1999. Local Anaesthetic Activity of the Essential Oil of Lavandula angustifolia. Planta Med 1999; 65(8): 700-703

Hampl, V., A. Pavlicek and J. Flegr, 2001. Construction and bootstrap analysis of DNA finger print in g based phylo-genetic trees with the free ware program Free Tree: application to *Trichomonad* parasites. International Journal of Systematic Evolutionary Microbiology, 51,731–735.

Harley, M.M., A. J. Paton, R. M. Harley and P.G. Cade, 1992. Pollen morphological studies in tribe *Ocimeae* (*Nepetoideae*: Labiatae): I. *Ocimum* L. *Grana* 31: 161–176.

Hedge, C., 1992. A global survey of the biogeography of the Labiatae. In Harley RM & Reynolds T. (eds) *Advances in Labiatae Science*. Royal Botanic Gardens, Kew 7–17.

Kim, N. and D. Lee, 2002.Comparison of different extraction methods for the analysis of fragrances from *Lavandula* species by gas chromatography–mass spectrometry. J. of Chromatography A, 982: 31–47

McGimpsey, J.A. and N.G. Porter, 1999. Lavender. A growers guide for commercial production, New Zealand Institute for Crop and Food Research Limited, Christchurch, New Zealand,

Murley, M.R., 1951. Seed of the Cruciferae. Amer. Midl. Nat., 46: 1-18.

Nassar, M. A. and K. F. El- Sahhar, 1998. Botanical Preparations and Microscopy (Microtechnique). Academic Bookshop, 219-224. (In Arabic))

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endo nucleases. Proceedings of the National Academy of Sciences USA 76:5269–5273.

Nybom H., K. Weising, and B. Rotter, 2014. DNA fingerprinting in botany: past, present, future. Investig. Genet. 2014; 5: 1.

Olivie M. R., M. A. Meehl and G. Lust. 1999. Random Amplified Polymorphic DNA (RAPD) Sequences as markers for canine genetic studies. The American genetic association. 90: 78-82

Porebski, S., L. G. Bailey and B. R. Baum, 1997. Modification of a CTAB extraction protocol for plants containing high polysaccharide and phenol components. Plant Mol. Biol. Reporter, 15 (1): 8-15.

Rohlf, F. J., 2005. Numerical Taxonomy and Multivariate Analysis System. Version 2.2. Exeter software, New York, NY.

Salama A. M., H. M. M. Ibrahim, Hanan S. Abd-El Maksoud, and Mohamed S. Boghdady, 2016. Genetic identification and taxonomic studies on six species of *Pelargonium* in Egypt. Asian Journal of Plant Science and Research, 2016, 6(3):55-70.

Uremis, I., M. Arsilan and M. K. Sangun, 2009. Herbicidal activity of essential oils on the germination of some problem weeds. Asian J. Chem. 21:3199–3210.

Verma R. S., L. U. Rahman, C. S. Chanotiya, R. K. Verma, A. Chauhan, A. Yadav, A. Singh and A. K. Yadav. 2010. Essential oil composition of *Lavandula angustifolia* Mill.cultivated in the mid hills of Uttarakhand, India. *J. Serb. Chem. Soc. 75* (3) 343–348.