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ESSENTIAL OILS AND MOLECULAR DIVERSITY ANALYSES IN SOME *MENTHA* SPECIES AND EGYPTIAN ECOTYPES

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ABSTRACT

Mentha which characterized by great morphological and chemical variations, are widely distributed herbaceous aromatic plant belongs to the Lamiaceae family. Since, the use of false materials as substitutes have become a major concern for pharmaceutical purpose the, authentication plants is very important. Recently, studies have assessed the genetic diversity in *Mentha* species suing RAPD, AFLP and SSR markers however, there is a broad lack of *Mentha*-specific molecular markers. Therefore, this study amid to extend our understanding of the chemical composition and molecular genetics of different identified *Mentha* genotypes and ecotypes plants grown on Egypt. The *M. piperita*, *M. piperita* (cv. Chocolate mint), *M. longifolia*, *M. suaveolens*, and *M. pulegium* and 5 Ecotypes *M. spicata* (Sohag, Behari, Aswan, Beni-suef and El-Minia) were collected, asexual propagated and adapted for the El-Minia governorate conditions. After 2 years individual cuttings were grown on plastic pots and cuts 3 times during the summer season. The biomass was significantly varied among the investigated *Mentha* species and ecotypes. Interestingly, the biomass of the Sohag and Beni-Suef ecotype plants exceeded that of the local ecotype by 166% and 148% respectively. The essential oils (EO) content of the *Mentha* species and ecotypes were significantly varied between 1.33% for *M. longifolia* and 0.47% for ecotype Aswan. The GC/MS analyses of the (EO) compositions

showed that the percentage of identified components was ranged between 100% (Aswan ecotype) and 89.42% (*M. pulegium*).Moreover, the EO composition were noticeably varied among the ecotypes. All primers were polymorphic conferring a 100% of polymorphism. The environmental and technical effect on *Mentha* genotypes could be negligible as all plants were cultured, isolated and analyzed under the same conditions and the main source of variance might be related to the genotypes. The dendrogram showed two main groups, the 1st clustering consisted of *M. longifolia*. The second group was separated into 2 sub-clusters, one of them included the 5 *M. spicata* ecotypes and *M. pulegium* species. However, *M. piperita* and *M. piperita* (cv. Chocolate mint) formed the second sub-cluster.

Key words: *Mint, Ecotype, Essential Oils composition, RAPD and ISSR*

INTRODUCTION

The genus *Mentha* which belongs to the Lamiaceae family is a fast growing perennial plant and generally tolerates a wide range of agro-climatic conditions (Brickell and Zuk, 1997). The economic value of *Mentha* plants, is evident as their essential oils, dried and fresh plant materials are widely used for cosmetics, beverages, confectionary, bakery, pharmaceuticals and pesticides products. Many of *Mentha* species are cultivated worldwide as official drugs in several pharmacopoeias (Shaikh *et al.*, 2014). The Egyptian flora contains many mint species; *M. spicata, M. longifolia, M. pulegium* and *M. microphylla* however, *M. spicata* is locally preferred for flavoring tea and culinary purposes (Edris *et al.*, 2003). Several studied; in particular, *M. spicata* and *M. piperita* showed differences in the metabolic pathways which result in differences in their pattern of essential oils composition. *M. suaveolens* (apple mint) and some new mint cultivars like *M. piperita* "chocolate" are common on other countries and recently introduced to Egypt. The *M. longifolia* (wild mint) which known locally as "Saudi mint" is grown in Egypt although it's not common as *M. spicata* or *M. piperita* (El Aansary and Ashmawy, 2013).

The taxonomy of *Mentha* genus has been in a changeable state, with more than 3000 published names since the commencement date of the modern plant nomenclature (Tucker and Naczi, 2006). This systematic complication is due to the natural interspecies hybridization, which occurring with high frequency in wild populations or even under cultivation (Harley, 1972 and Gobert *et al.*, 2002). For example *M. arvensis, M. aquatica, M. spicata, M. longifolia* and *M. suaveolens* have produced many natural hybrids. Since the heterozygosity and cytomixis of these species, many hybrids could

be generated following self-pollination. Nevertheless, most of these hybrids are infertile and asexually propagated using their highly invasive stolon system (Spencer *et al.*, 1993). Thus, complex hybrid populations may arise regardless the geographical or ecological factors (Gobert *et al.*, 2002 and Tucker and Naczi, 2006).

The botanical identification and essential oils compound content of different *Mentha* species have been thoroughly reviewed (Mimica-Dukić and Bozin, 2008 and European Pharmacopoeia, 2010). Different genotypes and chemotypes of *Mentha* genus which are specific to geographical locations show significant morphological, genetical and even pungency variations. Kapp (2015) stated that, the genus *Mentha* is consisting of 18 species, 11 hybrids and hundreds of subspecies, varieties and cultivar. Although, the essential oils composition of each *Mentha* plant is characteristic due to hybridization, the composition of *Mentha* plants may be resemble to other factor such as the environmental conditions and origin of the plants. In many cases, because of the similarity between genotypes, it is difficult to distinguish among them using morphological, physiological markers and even isozyme analyses (Reynders and Bollereau, 1994). Therefore, molecular markers could be employed as addition tool to estimate the genetic diversity as the DNA markers are liberated from the confusing effects of environmental factors. Moreover, these cheap and easy to apply genetic markers, can be used at any stage of plant development (Kabir *et al.*, 2014 and Kiełtyka-Dadasiewicz, 2017). Kapp (2015) raised a need for a proper taxonomical quality control for the industry purpose as the chemical analyses showed that some commercial *M. piperita* (peppermint) tea package contained *M. spicata* materials. However, it is known that the genetic structure of *Mentha* is a heterozygote (Campos-de-Quiroz and Ortega-Klose, 2001) and, it is difficult to distinguish between phenotypically similar cultivars using the classical methods.

A few studies have evaluated the genetic diversity in *Mentha* species based on RAPD (Khanuja *et al.*, 2000 and Shahbazi, 2004) and AFLP fingerprinting (Gobert *et al.*, 2002). Fenwick and Ward (2001) used RAPD markers to identify 17 accessions of *Mentha* belonging to 3 species grown widely in the USA and concluded that, *M. spicata* is more closely related to *M. gracilis* than to *M. piperita.* Simple sequence repeats (SSR) and inter-simple sequence repeats (ISSR) are also important tools for studying the genetic diversity. ISSRs considered as repeat-anchored primers to amplify DNA sequences between two inverted SSRs **(**Zietkiewicz *et al.*, 1994). Smolik *et al.* (2007) used 20 ISSR primers to study the genetic diversity of different *Mentha* species. The examined species were distinguished by 8 out of these primers. Rabia *et al.* (2015) referred that polymorphism could be used as an efficient tool for the

recognition, similarities and phylogenetic relations of the studied *Mentha* genotypes. However, it could be concluded that, there is a broad lack of *Mentha*-specific molecular markers to use in genetic studies and genetic improvement programs (Kumar *et al*., 2015).

The adulteration and use of false materials as substitutes have become a major concern for users and industry purpose for reasons of safety and efficacy (Kapp, 2015). Therefore, authentication of medicinal plants is of highest importance. Morphological, anatomical, chemical and DNA markers could solve this problem by differentiating the genuine material from the adulterants, substitutes and fake drugs (Ganie *et al*., 2015). So that, this study aimed to broaden the knowledge of the essential oils composition of different identified genotypes and ecotypes of *Mentha* plants grown in Egypt via chymosystimatic and molecular markers.

MATERIALS AND METHODS

Plant materials

During summer 2014 different *Mentha* species and ecotypes were collected and adapted to the open field condition at Minia university farm. *M. spicata* L. ecotypes were collected from Behari (30.9157N and 30.3544E), Beni-Suef (28.9093N and 30.5918E), El-Minia (28.1244N and 30.3753E), Sohag (26.33N and 31.41E) and Aswan (22.4864N and 31.5072E). The *M. longifolia* L. which locally called “Saudi mint", *M. piperita L.*, *M. piperita* "cv. Chocolate" and *M. suaveolens* Ehrh (apple mint) were kindly provided by Prof Dr. Hosam El-Ansary, Faculty of Agriculture, Alexandria University however, *M. pulegium* was collected along streams from Minia university.

All plants were individually propagated by stem cuttings and kept as a mother plant. After two years stem cuttings were taken from the mother plants and transplanted into 30-cm in diameter plastic pot filled with clay and sand mixture (3:1 v/v) as a growth medium. Plants were grown, in a randomized complete block experimental design, with 6 plants from each genotype in each of the 4 replications. The above-ground biomass at 5 cm-height from the soil surface of each individual pot was harvested 3 times with 45 days intervals started on 15th May. For each cut the herb was weighted after air-drying.

Essential oils distillation and analyses

The herb of the 3 cuts was crushed and mixed together then the essential oils were extracted using a Clevenger apparatus for 2 hours using 20 g of material sample according to the (European Pharmacopoeia, 2010). The essential oils were then dried over anhydrous sodium sulfate and their content was expressed in ml/100 g dry weight. The identification of the essential oils compositions were determined by GC/MS which was carried out with Ds-Chrom 6200 Gas Chromatograph system (Donam Instrument, Korea). Identification of the essential oils

components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns.

The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30m x 0.25mm ID x 0.25µm film thickness. The injector temperature program ramp increases with a rate of 8˚C/min from 70 ˚C to 200 ˚C. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and air 330 ml/min. Injector and mass transfer line temperature were set at 250 and 300 ˚C, respectively. The relative percentage of each compound was calculated from the area of the peak corresponding to each compound.

Extraction of DNA

Total genomic DNA was extracted from *Mentha* fresh leaves as described by Doyle and Doyle (1987), with minor modifications. One gram of small cut pieces of Leaves was crushed using liquid nitrogen. Nearly 300 mg of fine powder were transferred to the 2 ml Ependorf tubes and 600 µl of freshly preheated 2.5X CTAB solutions with 0.8g Poly-vinyl-polypyrrolidone (PVP) were then added. The mixture was incubated for one hour at 65 ˚C in water bath with a constant stir at separate intervals of 10 min. After isolation and purification, the DNA concentration and purity titration was spectro-photometrically valued according to Sambrook *et al.* (1989).

RAPD and ISSR analyses

Five RAPD primers (OPT03, OPT05, OPT16, OPC05 and MPA04) and Ten ISSR primers (ISSR1, ISSR2, ISSR3, ISSR4, M2, M3, M7, M8, M12 and A1) were amplified for studying genetic variation and relationships of these amplicones occurring in Mentha taxa cultivated in Egypt. The sequences and melting temperatures of these primers are shown in Table (1).

PCR conditions for RAPD and ISSR analyses

The amplifications were carried out in a thermal cycler (Thermo Hybaid) programmed for initial preheating period in one step of 5 minutes at 94˚C subsequent PCR cycles of 3 steps in each, the 1st step was DNA denaturaion at 94˚C for 1 minute, followed by the 2nd one of primer annealing for 1 minute and then the 3rd one for primer extension at 72 ˚C for 2 minutes. Following the 3rd step of the PCR cycles, another one step of post extension at 72˚C for 7 minutes was used. The DNA annealing temperatures and number of cycles of each primer were shown in Table (1). Amplification products of RAPD and ISSR reactions were confirmed by electrophoresis in 2% agarose gels stained in ethidium bromide. Sizes of the amplified fragments were estimated according to the standard ladder of 100 bp.

Data analyses

The results of herb dry weight and essential oils content and yield were subjected to an analysis of variance (ANOVA), and the means

were compared using Duncen's test (p 0.05) between each pair of data (Clewer and Scarisbrick 2001). The analysis was performed using MSTAT program (version 4.0) edited in 1985 by the MSTAT development team, Michigan University and Agricultural University of Norway.

Data of RAPDs and ISSRs were scored for computer analyses on the basis of the presence (1) or absence (0) of the amplified products for each sample using GelAnalyzer3 (http://www.geocities.com/egygen, Gel Analyzer Version three, 2007). Hierarchical cluster analyses was conducted with the PAST software version 1.88 (Hammer *et al.*, 2009) based on Dice's (1945) similarity coefficient matrix within *Mentha* species and ecotypes.

Table (1): Nucleotide sequence and Tm ˚C (mealting tempreture) of five RAPD and ten ISSR primers used for PCR analyses and their references, while right two colums showed DNA annealing temperatures and number of cycles of both RAPD and ISSR primers used in the present study

Primer Name	Sequence (5′-3′)	Tm ˚C	References	Annealing temp. (ºC)	Number of cycles
OPT03	TCC ACT CCT G	32	Al-Rawashdeh (2011)	36	44
OPT05	GGG TTT GGC A	32	Al-Rawashdeh (2011)	36	44
OPT16	GGT GAA CGC T	32	Al-Rawashdeh (2011)	36	44
OPC05	GAT GAC CGC C	34	Al-Rawashdeh (2011)	40	44
MPA04	TGC GCG ATC G	34	Shasany *et al.* (2005)	36	44
ISSR1	(GA)9 T	55	Lal *et al*. (2014)	53	40
ISSR2	(TG)8 A	50	Smolik *et al*. (2007)	52	35
ISSR3	(CA)8 GC	56	Smolik *et al*. (2007)	52	35
ISSR4	(GACA)4	48	Smolik *et al*. (2007)	52	35
M2	(AC)8(C/T)G	54	Schanzer *et al*. (2012)	50	40
M3	(GA)8(C/T)C	54	Schanzer *et al*. (2012)	50	40
M7	(CAG)5	52	Schanzer *et al*. (2012)	50	40
M8	(GTG)5	52	Schanzer *et al*. (2012)	50	40
M12	(CA)6(A/G) (C/T)	38	Schanzer *et al*. (2012)	50	40
A1	(GAA)7	54	Devmurari and Mistry (2014)	54	40

RESULTS AND DISCUSSION

Plant dry weight

The *Mentha* plant dry weights were significantly varied among the investigated species and ecotypes (Table 2) with a slight difference compared with the fresh weights (data not presented). The dry weights of the 1st cut were ranged from0.705 to 1.483 g/plant for *M. piperita* and *M. spicata* "*Sohag*" respectively. The same trend was shown at the 2nd one (0.658 and 2.605 g/plant respectively). The first two cuts for the *M. suaveolens* plants exhibited the lowest fresh weight (0.705 and 0.658 g/plant), while ecotype "*Sohag*" had the highest plant dry weight at the three cuts. At the 1st cut, there were no significant differences of plant dry weight among Behari, Aswan, Beni-Suef and El-Minia ecotypes in

their dry weights. However, the local ecotype El-Minia ranked the 2nd one after Sohag ecotype at the second and third cuts with no significant difference with the Beni-Suef one. Interestingly, the dry weight of the Sohag and Beni-Suef ecotype plants exceeded that of the local ecotype at the 3rd by 166% and 148%, respectively (Table 2). Previously, Özgüven *et al.* (2002) observed a significant variation on plant dry weight among different *Mentha* species and ecotypes collected from Turkey after adaptation to new conditions. They concluded that *M. longifolia* and *M. piperita* ecotypes have been the most important types under the new condition. Similarly, the fresh and dry weights of three ecotypes of *Ocimum basilicum* were significantly varied after adaption to new growth condition (Khaliq *et al.* 2014).

Table (2): *Mentha* species and ecotypes dry weights (g/plant) at three cuts

Species and ecotypes	dry weights (g/plant)		
	1st cut	2nd Cut	3rd cut
Mentha piperita	0.895cd	1.275b	1.028f
M. piperita (cv. Chocolate mint)	1.125b	2.535a	2.660cd
M. longifolia	1.212b	0.853cd	1.095f
M. suaveolens (apple mint)	0.705d	0.658de	1.618e
M. pulegium	1.040 bc	0.659de	1.923e
M. spicata "*Sohag*"	1.483a	2.605a	4.988a
M. spicata "Behari"	0.903cd	1.143bc	2.650cd
M. spicata "Aswan"	1.035bc	0.760cde	2.445d
M. spicata "Beni-Suef"	1.010 bc	1.425b	3.463b
M. spicata "El-Minia"	1.087bc	1.067bc	2.995bc

The superfluity of some ecotypes such as Sohag over the others included the local type could be due to their ability to develop a good root system. Pigliucci (2005) thought that the extent of phenotypic plasticity is determined by the genotype, for instance, one ecotype of a plant species could be able to increase its root growth rate on a certain stimulus. Ecotypes characteristics often relate to some distinct and contiguous factors of the environmental conditions and plant response in terms of physiological and morphological differentiation (Jain, 1990). The genetic components which administrate traits in different ecotypes represent the outcome of adaptation retiring from the selection of those traits to achieve higher fitness than less well-adapted populations. Although, the local adaptation is common traits its genetic basis which is quantitative traits governed by multiple genes is still poorly understood (Trontin *et al.*, 2011 and Savolainen *et al.*, 2013). Our results have shown a significant variation among different ecotypes even after two years of adaptation to a new geographical region. That

achievement might suggest genetic variations among these ecotypes. This result confirm the previously suggested one by Ristova and Busch (2014) who refereed that the phenotypic differences are often caused by allelic variations at several loci, each of them making small contributions to the trait.

Essential oils content

Obtained results (Table 3) showed that essential oils content of either *Mentha* species or their ecotypes were varied between 1.33% (*M. longifolia*) and 0.467% (*M. spicata*, ecotype Aswan). However, there was no significant difference between *M. longifolia* and *M. piperita* (cv. Chocolate mint) which yielded 1.233%. The variation on the essential oils content was also, noticeable among the *M. spicata* ecotypes. The ecotype Sohag had the highest significant value (0.763%) followed by Beni-Suef (0.617%). However, there were no significant differences among Sohag, Behari and El-Minia ecotypes. On the other hand the Aswan ecotype yielded the lowest essential oils content (0.467%). When the total biomass of the 3 cuts were considered, the results showed that *M. piperita* cv. Chocolate had the highest essential oils yield over the other genotypes. These increments were 3.3 and 4.0 folds higher than those of both *M. pipertia* and *M. pulegium*, respectively. Table (3) also showed noticeable variations among the *M. spicata* ecotypes which ranged between 1.999 and 6.989 ml/plant for Aswan and Sohag ecotypes, respectively. Rather, Sohag ecotype had essential oils yield (359%) higher than that of the local ecotype (El-Minia) whereas; the Behari ecotype had only 69% of El-Minia essential oils yield.

Özgüven *et al*. (2002) on their comparative study of different *Mentha* species and ecotypes found that the highest content of essential oils were on the following order *M. longifolia, M. spicata, M. longifolia* (another ecotype), *M. spicata* (another ecotype) and *M. piperita*. Moreover, these genotypes had higher essential oil ratios than those of the other investigated ecotypes. There is no doubt that the biosynthesis of secondary metabolites is genetically controlled, however it's strongly affected by the environmental factors of a particular growing region (Abedi *et al*., 2015). Lawrence (2002) stated that the variation in essential oils production can be attributed to genetic constitute, environmental, the ontogenic factors and analytical methods. Meanwhile, in the present study, all *Mentha* species and ecotypes were cultured, isolated and analyzed under the same operating conditions. So that, the influence of the environmental and technical parameters could be negligible and the main source of variance might be related to the genotypes. So that, the variance that observed among these *Mentha* genotype on essential oil yields was closely related to the geographical origin of the ecotype.

Table (3): Essential oils production of different *Mentha* species and ecotypes

Species and ecotypes	Essential oils		
	Content (%)	(ml/plant)	% of El-Minia ecotype
Mentha piperita	0.727 cd	2.335	87
M. longifolia	1.330 a	4.203	216
M. piperita (cv. Chocolate)	1.233 a	7.774	290
M. suaveolens (apple mint)	0.883 b	2.623	71
M. pulegium	0.573ef	1.945	73
M. spicata "Sohag"	0.763 bc	6.989	359
M. spicata "Behari"	0.543 ef	2.536	69
M. spicata "Aswan"	0.467 f	1.993	74
M. spicata " Beni-Suef"	0.617 de	3.657	188
M. spicata "El-Minia"	0.517 ef	2.677	100

Essential oils constitute

The GC/MS study (Table 4) showed that the percentage of identified components of the essential oils was ranged between 100% (*M. spicata* Aswan ecotype) and 89.42% (*M. pulegium*). The least number of identified compounds (6) had been recorded for *M. spicata* (Aswan and Behari ecotypes) however, *M. piperita* (cv. Chocolate mint) had the highest number of identified compounds (13). Interestingly, there were 12 non-identified compounds on the essential oils of *M. spicata* ecotype Beni-Suef.

There were considerable variations on the essential oils composition between *M. piperita* and *M. piperita* cv. Chocolate. The main components of the *M. piperita* were pulegone (24.00%) and menthone (18.63%) whereas, the *M. piperita* (cv. Chocolate) essential oils main constitutes were menthone (31.21%) and menthol (23.27%). Linalool (4.38%) was identified on *M. piperita* however, *M. piperita* cv. Chocolate contained 1,8-Cineole (2.00%), iso-menthone (4.71%) and germacrene-4-ol (4.29%). Similary, Aziz and Craker (2010) refered that menthol (34.29 %), isomenthyl acetate (30.47 %), and menthone (15.61 %) were the major components of the local Egyptian peppermint essential oils. Elansary and Ashmawy (2013) identified a high percentage of menthofuran (9.63%) in Chocolate mint essential oils. Menthofuran which is a metabolite of pulegone might be dangerous for human (Bertoli *et al.*, 2011 and Gershenzon *et al.*, 2000).

The genetic data have shown that the essential oils of *Mentha* species usually have either oxygenated monoterpene compounds such as carvone and related compounds or sesquterpene such as piperitenone, piperitone, pulegone, menthone which are oxygenated compounds but not both. The development of oxygenetaed monoterpene compounds is controlled by the dominant gene C; the recessive cc genotype allows the formation of sesquterpene oxygenated compounds (Hefendhel and Murray, 1976). Murray and Lincoln (1970) demonstrated that the dominant gene I allowed the accumulation of linalool (and/or

linalyl acetate), whereas the recessive gene i prevented or nearly prevented the formation of the cyclic ketones and their derivatives.

Our results in Table (4) showed that *M. longifolia* essential oils were abundent with menthone (30.74%), menthol (21.21%) and pulegone (12.15%). These components was simalir to those idintified on wild grown *M. longifolia* on Tunisia (Hajlaoui *et al.*, 2010); menthol (33%), menthone (21%), pulegone (18%). However, unlike the present study which estimated linalool by (10.45%) the Tunisia grown plant only had 0.20% of it. The main constitutes of *M. suaveolens* essential oils were carvone (69.36%) followed by limonene (13.04%). Hydrodistilled oils of cultivated *M. suaveolens* were categorized by carvone (50.59%) and limonene (31.25%) as the major ingredients (El-Kashoury *et al.*, 2014). However, Aziz and Craker (2010) found that the cultivated material from another place of Egypt showed dominance of linalool (35.32%), *p*-menth-1-en-8-ol (11.08%) and geranyl acetate (10.86%). Moreover, the wild material from the Alexandria-Cairo desert road (Egypt) showed piperitone oxide (35.14%), germacrene-D (22.65%), o-menth-8-ene (8.98%), trans-β-farnesene (6.92%), veridiflorol (7.67%) and L-limonene (5.89%) as the main constituents (Elansary and Ashmawy, 2013). *M. pulegium* did not contain carvone which is the predominant content of *M. spicata* and *M. suaveolens* but had a high concentration of piperitone (24.90%) which was missing from *M. spicata*, *M. suaveolens* and *M. longifolia* plants (Table 4). The other predominant compound on *M. pulegium* was cis-pulegol (20.05%) which did not detected on all other genotypes. Also, on contrast to all other investigated genotypes *M. pulegium* essential oils had iso-pulegone . El-Ghorab (2013) analyzed the essential oils of *M. pulegium* and classified it as pulegone chemotype. The other main ingredient of essential oils was piperitone (12.2 %).

All *M. spicata* ecotypes had carvone as a major constitute but its concentrations was varied between 60.93 and 74.37% for Beni-Suef and El-Minia respectively. The second main compound of the essential oils of *M. spicata* ecotypes were limonene (8.62, 9.97 and 13.27%) for Sohag, El-Minia and Behari ecotypes respectively, and α-terpnol (14.80 and 19.13%) for Aswan and Beni-Suef ecotypes respectively. Unlike other investigated genotypes included the five *M. spicata* ecotypes Behari ecotype did not contain α-pinene however, it is the only genotype contained caryophyllene oxide (2.82%). Moreover, this concentration of caryophyllene oxide was higher than that of *M. pulegium* plants (1.20%). Also, it was interesting to find that Aswan and Beni-Suef ecotypes contained a higher concentration of α-terpnol (14.80 and 19.13%, respectively) which was similar to *M. pulegium* (16.94%). Both of Behari and Aswan ecotypes did not contain 1,8-cineole .

Table (4): Essential oils chemical composition (expressed as %) of different *Mentha* species and ecotypes

Compound (%)	*Rt*	*M. piperita*	*M. piperita* (cv. Chocolate	*M. longifolia*	*M. suaveolens* (apple mint)	*M. pulegium*	*M. spicata* ecotype				
							Sohag	Behari	Aswan	Beni- Suef	El-Minia
Monoterpene hydrocarbons											
α-pinene	1.902	0.945	0.430	1.335	1.089	1.059	1.299	--	3.754	0.174	2.379
β-pinene	2.671	2.780	1.302	2.355	--	2.383	--	--	--	--	--
sabinene	2.771	--	--	3.472	1.307	--	2.981	1.989	1.655	1.230	1.093
Oxygenated monoterpenes											
1,8-Cineole	3.028	--	2.005	--	--	5.221	3.946	--	--	3.222	3.886
limonene	3.048	4.101	1.398	7.112	13.044	1.221	8.621	13.271	7.468	7.598	9.971
linalool	5.256	4.377	--	10.449	--	4.345	--	--	--	--	--
menthone	5.362	18.634	31.213	30.736	--	--	--	--	--	--	--
cis-sabinol	5.529	--	--	4.045	--	--	--	--	--	--	--
iso-pluegol	5.549	--	--	--	--	2.873	--	--	--	--	--
menthofuran,	5.570	7.187	2.324	--	--	--	--	--	--	--	--
iso-menthone	5.776	--	4.711	--	--	--	--	--	--	--	--
menthol	6.019	15.981	23.266	21.213	--	--	--	--	--	--	--
iso-menthol	6.323	6.501	2.236	0.462	--	--	--	--	--	--	--
pulegone	6.616	23.999	13.977	12.150		10.035	--	--	--	--	--
α-terpnol	6.972	--	--	--	2.756	16.941	5.743	4.975	14.795	19.132	4.888
piperitone	7.058	3.351	8.794	--	--	24.090	--	--	--	--	--
cis-pulegol	7.635	--	--	--	--	20.051	--	--	--	--	--
carvone	7.679	--	--	--	69.356	--	71.980	68.287	70.059	60.929	74.374
Sesquiterpene hydrocarbons											
germacrene-4-ol	7.311	--	4.292	--	--	--	--	--	--	--	--
germacrene D	7.848	5.998	1.340	--	--	--	--	--	--	--	--
b-caryophyllene,	8.640	--	--	0.871	3.357	--	1.643	1.158	2.269	1.553	1.825
caryophyllene oxide	9.805	--	--	--	--	1.196	--	2.816	--	--	--
Total compounds (%)		93.853	97.289	95.317	90.908	89.416	96.217	92.000	100.00	93.838	98.416

Rt retention time

Previous analyses of *M. spicata* essential oils had provided evidence of a chemical variability (Kokkini and Papageorgiou 1988) within the species. On another study Golparvar and Adelpoor (2013) indicated that the major components of *M. spicata* Iranian ecotypes were carvone (74.57%), 1,8-cineole (10.28%), limonene (8.41%) for Yasouj province ecotype, whereas C-Sakht province one had piperitenone oxide (53.19%), 1,8- cineole (27.47%), trans-caryophyllene (3.55%), and the main components of Bahram-Beigi province ecotype were 1,8-cineole (8.79%), carvone (79.6%), and lmonene (3.53%).

Molecular analyses

M. suaveolens (apple mint) species was discarded from the molecular analyses because of its imprecise results of DNA isolation, purification and amplification (Fig. 1a). Out of five RAPD and 10 ISSR primers, only three RAPD and seven ISSR primers exhibited reproducible fragments with easily detectable bands. Number of monomorphic, unique, polymorphic (with & without unique bands), total number of amplified fragments, band frequency and the percentage of polymorphism obtained using three RAPD and seven ISSR markers are shown in Table (5) and Figure (1b-k). Data showed that no monomorphic bands were generated with the used RAPD and ISSR primers. All of the studied primers products were entirely (100%) polymorphic. The maximum numbers of bands (8) were produced using the ISSR 2 primer, whereas the minimum (2) was by primer M 2.

Table (5): Number of monomorphic, unique, polymorphic, total number of amplified fragments, band frequency and the percentage of polymorphism obtained using three RAPD and seven ISSR markers

Primers	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Mean of band frequency	Polymorphism (%)
			without Unique	with Unique			
OPT03	0	1	3	4	4	0.20	100
OPT05	0	0	5	5	5	0.54	100
OPT16	0	2	3	5	5	0.24	100
ISSR1	0	0	4	4	4	0.53	100
ISSR2	0	1	7	8	8	0.33	100
ISSR3	0	1	3	4	4	0.33	100
M 2	0	0	2	2	2	0.40	100
M 3	0	1	3	4	4	0.25	100
M 7	0	1	2	3	3	0.23	100
M 12	0	2	2	4	4	0.30	100

Data in Table (6) revealed that, one unique band of 297 bp was found in *M. spicata* "Behari" by using OPT03 primer (Fig. 1b).

Two unique bands of 278 and 403 bp were generated in *M. longifolia* by using OPT16 primer (Fig. 1d), while six unique bands were produced from the seven ISSR primers (Fig. 1e-k), one of them (234 bp) was detected in *M. longifolia* by using ISSR 2. The second one (676 bp) was generated in *M. piperita via* ISSR 3 primers. Two unique bands of 531 and 343 bp were detected in *M. piperita* (cv. Chocolate mint) by using M 3 and M 7 respectively. The last two unique bands of 1.355 and 1.744 bp were obtained in *M. longifolia* and *M. spicata* "Aswan ecotype" by using M 12 primer. Al-Rawashdeh (2011) studied the molecular taxonomy and genetic relationship between two *Mentha* species namely, *M. spicata* and *M. longifolia*, and *Ziziphora tenuior* using RAPD markers and stated that, the molecular analysis is considered one of the best methods for studying molecular taxonomy in order to classify and distinguish between *Mentha* species.

On the other hand, Schanzer *et al*. (2012) analyzed the genetic diversity among three populations of *M. aquatic* (collected from Usmanka River) using ISSR primers (M 2, M 3, M 7 and M 12) and reported that, the banding pattern for all samples seemed to be the same with all of the 8 primers. However, evident differences in banding patterns were found among samples from the river and among those from the bayou as well as from the other localities, including the outgroup specimens of *M. arvensis*. Likewise, Apostolova *et al*. (2015) examined the efficiency of ISSR markers for evaluating the genetic variability within genus *Mentha* and to make an attempt to discriminate the genotypes within species. They found 7 genotypes of 5 Bulgarian mint species which clustered into four clear groups. It could be concluded that, the ISSR technique had easily applied for assessing the genetic relationships between genotypes and ecotypes within the *Mentha* genus.

Table (6): Numbers and Molecular sizes of the polymorphic and unique bands generated by RAPD and ISSR primers

Primers	**Number of bands (Molecular weight Kb)**	
	Polymorphic bands without unique	Unique bands
OPT03	3 (1.436, 0.908 & 0.468)	1 (0.297)
OPT05	5 (1.040, 0.680, 0.455, 0.319 & 0.279)	0
OPT16	3 (1.461, 0.977 & 0.647)	2 (0.403 & 0.278)
ISSR1	4 (1.000, 0.482, 0.322 & 0.232)	0
ISSR2	7 (1.224, 0.848, 0.756, 0.547, 0.387, 0.340 & 0.264)	1 (0.234)
ISSR3	3 (1.051, 0.359 & 0.231)	1 (0.676)
M 2	2 (1.015 & 0.500)	0
M 3	3 (1.273, 0.836 & 0.363)	1 (0.531)
M 7	2 (0.723 & 0.241)	1 (0.343)
M 12	2 (0.772 & 0.453)	2 (1.744 & 1.355)

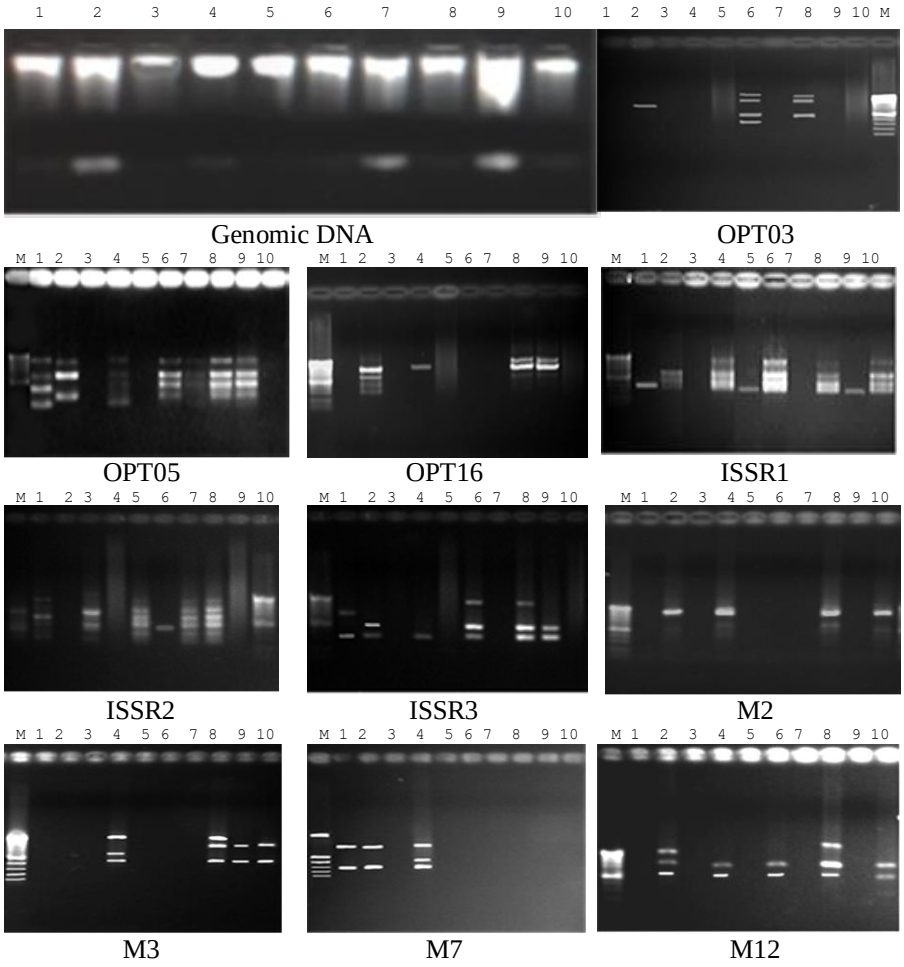

Figure (1): Electrophoretic profile of RAPD and ISSR primers used in the present study. M: 100 bp ladder marker and lanes 1 through 10 refer to: *Mentha piperita*, *M. longifolia*, *M. suaveolens* (apple mint), *M. piperita* (cv. Chocolate mint), *M. spicata* "*Sohag*", *M. spicata* "Behari", *M. spicata* "Aswan" , *M. spicata* "Beni-Suef", *M. spicata* "El-Minia" and *M. pulegium* respectively.

Cluster analyses

The dendrogram was constructed using the classified cluster analyses method with the average linkage between pairs from the matrix of Dices (1945) and similarity coefficient values (S) within *Mentha* species and ecotypes (Fig. 2). The dendrogram showed two main groups, the first was consisted of *M. longifolia*and and the second was separated into two sub-clusters, one of them included five ecotypes (*M. spicata* "*Sohag*", *M. spicata* "Behari", *M. spicata* "Aswan", *M. spicata* "Beni-Suef", *M. spicata* "El-Minia") in addition to *M. pulegium* species.

Mentha piperita and *M. piperita* (cv. Chocolate mint) formed the second sub-cluster. The present work in mint species and ecotypes of DNA profiling showed that it is possible to analyze the RAPD and ISSR patterns for adjusting similarity and distance between species and ecotypes by which it can be expected the origin of the species and cultivars **(**Khanuja *et al.*, 2000). Finding out a colinearity between molecular marker data, reported herein, and essential oils might not be crucial. So, further studies like those of bioinformatics of proteomes and transcriptomes may aid to detect and determine the genes involved in essential oil production of mint.

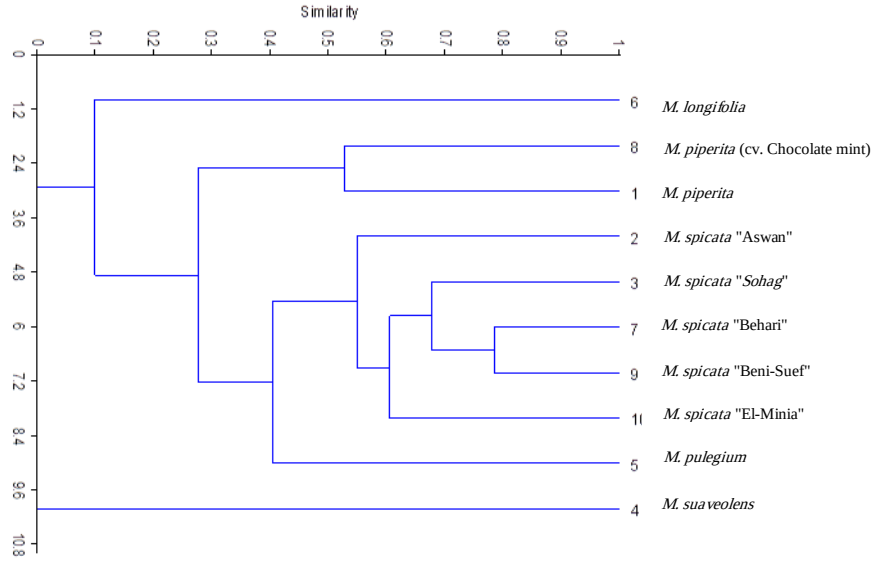

Figure (2): Dendrogram of the genetic distances among studied *Mentha* species and ecotypes based on the bands polymorphism generated by RAPD and ISSR -PCR primers

CONCLUSION

Previous analyses of essential oils in *Mentha* species and ecotypes from plants growing in different area provided evidence of a chemical variability. Molecular analysis is considered one of the best tools of studying molecular taxonomy to identify and differentiate between species and ecotypes. The present study offered adequate and reliable information for effective application of molecular markers in molecular and genetic characterization of the *Mentha* species and ecotypes. The used markers show high reproducibility and are useful for defining the profile of genetic relationships of the genus *Mentha*. Further molecular markers should be taken into consideration in the future.

REFERANCES

Abedi, R.; Golparvar, A. R. and Hadipanah, A. (2015): Identification of the essential oils composition from four ecotypes of *Mentha longifolia* (L.) Huds. growing wild in Isfahan province, *Iran. Journal of Bio Science and Biotechnology*, 4: 117-121.

Al-Rawashdeh, I. M. (2011): Molecular Taxonomy Among *Mentha spicata*, *Mentha longifolia* and *Ziziphora tenuior* Populations using the RAPD Technique. *Jordan Journal of Biological Sciences*, 4(2): 63-70.

Apostolova, L.G.; Zarow, C.; Biado, K.; Hurtz, S.; Boccardi, M.; Somme, J.; Honarpisheh, H.; Blanken, A. E.; Brook, J.; Tung, S., Lo, D.; Ng, D.; Alger, J. R.; Vinters, H. V.; Bocchetta, M.; Duvernoy, H.; Jack, C. R., and Frisoni G. B. (2015): Relationship between hippocampal atrophy and neuropathology markers: a 7T MRI validation study of the EADC-ADNI harmonized hippocampal segmentation protocol. *Alzheimers Dement*. 11:139-150.

Aziz, E. E. and Craker, L. E. (2010): Essential oils Constituents of Peppermint, Pennyroyal, and Apple Mint Grown in a Desert Agrosystem. *J. of Herbs, Spices and Medicinal Plants* 15: 361-367.

Bertoli, A.; Leonardi, M.; Krzyzanowska, J.; Oleszek, W. and Pistelli, L. (2011): Mentha longifolia in vitro cultures as safe source of flavouring ingredients. *Acta Biochemica Polonica*. 58: 581-587.

Brickell, C. and Zuk, J. D. (1997): The American Horticultural Society: A-Z Encyclopedia of Garden Plants. New York: DK Publishing Inc.

Campos-de-Qurioz, H. and Ortega-Klose, F. (2001): Genetic variability among elite red clover (*Trifolium pretense* L.) parents used in chile as revealed by RAPD markers. *Euphytica*, 122: 61-67.

Clewer, A.G. and Scarisbrick D.H. (2001): Practical statistics and experimental design for plant and crop science. John Wiley and Sons, Ltd., Chichester.

Devmurari, L. S. and Mistry, K. N. (2014): Genetic diversity analyses of different varieties of *Mentha piperita* from central and South Gujarat (India) through RAPD, ISSR and SSR markers. International Journal of Biology, *Pharmacy and Allied Sciences (IJBPAS)*, 3(1): 63-77.

Dice, L.R. (1945): Measures of the amount of ecologic association between species. *Ecology*, 26: 297-302.

Doyle, J.J. and Doyle J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*., 19: 11-15.

Edris, A. E.; Shalaby, A. S.; Fadel, H. M. and Abdel-Wahab, M. A. (2003): Evaluation of a

chemotype of spearmint (*Mentha spicata* L.) grown in Siwa Oasis, Egypt. *European Food Research and Technology*, 218: 74-78.

Elansary, H. O. and Ashmawy, N. A. (2013): Essential Oils of Mint between Benefits and Hazards. *J. of Essential Oil-Bearing Plants*, 16: 429-438.

El-Ghorab, A. (2013): The Chemical Composition of the *M. pulegium* L. Essential oils from Egypt and its Antioxidant Activity. *J. of Essential oils Bearing Plants*, 92: 183-195.

El-Kashoury, E. A.; El-Askary, H. I.; Kandi, Z. A., Ezzat, S. M.; Salem, M. A. and Sleem, A. A. (2014): Chemical and biological study of *Mentha suaveolens* Ehrh. *cultivated in Egypt. J. Med. Plants Res.,* 8: 747-755.

European Pharmacopoeia (2010): 7th edition., Vol. 1. Strasbourg: Council of Europe, Fatiha.

Fenwick, A. L. and Ward, S. M. (2001): Use of random amplified polymorphic DNA markers for cultivar identification in mint. *HortScience*, 36:761-764.

Ganie, S. H.; Upadhyay, P.; Das, S. and Sharma, P. M. (2015): Authentication of medicinal plants by DNA markers. *Plant Gene*, *4*: 83–99.

GelAnalyzer, Version three, (2007): Gel Analyzer Ver.3 program software for windows. www.geocities. Com/egygene.

Gershenzon, J.; McConkey, M. E. and Croteau, R. B. (2000): Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology*, 122: 205-213.

Gobert, V.; Moja, S.; Colson, M. and Taberlet, P. (2002): Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. *Am. J. of Botany*, 89: 2017-2023.

Golparvar, A. R. and Adelpoor, M. J. (2013). Chemical composition of essential oils of three ecotypes of *Mentha spicata* L. from Kohgiluyeh va Boyer-Ahmad Province, Iran. *J. Herbal Drugs*, 4(3): 143-146.

Hajlaoui, H.; Mighri, H.; Noumi, E.; Snoussi, M.; Trabelsi, N.; Ksouri, R. and Bakhrouf, A. (2010): Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against Vibrio spp. strains. *Food Chem. Toxicol.,* 48: 2186-2192.

Hammer, Ø.; Harper, D. A. T. and Ryan, P. D. (2009): PAST: PAlaeontological Statistics, ver. 1.88. User's manual.

Harley, R. M. (1972): *Mentha*. In Tutin T.G. and Heywood V.H. (Eds.), Flora Europaea, 3 (pp. 183-186). Cambridge: Cambridge University Press.

Hefendehl, F.W. and Murray, M. J. (1976): Genetic aspects of the biosynthesis of natural odors. Lloydia, 39:39-52.

Jain, S. K. (1990): Variation and Selection in Plant Populations. In: Viewpoints, E., Heidelberg S.B., York N., Paris L., and Hong T. Population Biology Ecological and Evolutionary Viewpoints. K. Wohrmann S.K. Jain (Eds.), 199-238, Springer-Verlag

Kabir, S.; Tahira, R.; Khan, M. F.; Naeemullah, M. and Jamal, A. (2014): Molecular characterization of genetic diversity of mint gene pool through RAPD markers. Int. *J. Agric. Innov. Res.*, 2: 2319–1473.

Kapp, K. (2015): Polyphenolic and Essential oils Composition of *Mentha* and Their Antimicrobial Effect Polyphenolic and essential oils composition of *Mentha* and their antimicrobial effect. PhD. Thesis, University of Helsinki Finland

Khaliq, S.; Zafar, Z. U.; Athar, H. R. and Khaliq, R. (2014): Physiological and biochemical basis of salt tolerance in *Ocimum basilicum* L. *J. of Medicinal Plants Studies*, 2: 18-27.

Khanuja, S. P. S.; Shasany, A. K.; Srivastava, A. and Kumar, S. (2000): Assessment of genetic relationships in *Mentha* species. *Euphytica* 111:121-125.

Kiełtyka-Dadasiewicz, A. (2017): Morphological and Genetic Diversity Among Peppermint (*Mentha* × *Piperita* L.) Cultivars. *Acta Scientiarum Polonorum Hortorum Cultus*, 16: 151-161.

Kokkini, S. and Papageorgiou, V. P. (1988): Constituents of essential oils from *Mentha longifolia* growing wild in Greece. *Planta Med.*, 54:59-60.

Kumar, B.; Kumar, U. and Yadav, H. K. (2015): Identification of EST–SSRs and molecular diversity analyses in Mentha piperita. *Crop Journal*, *3*: 335–342.

Lal, S.; Devmurari, A. and Mistry, K. N. (2014): Genetic diversity analyses of different varieties of *Mentha piperita* from central and south Gujarat (India) through RAPD, ISSR and SSR markers. *IJBPAS*, 3: 63-77.

Lawrence, B. M. (2002): From the sensation to the synthesis , in Advances in Flavours and Fragrances (Special Publication 277) , ed. by Swift KAD . *Royal Society of Chemistry, Cambridge;* 57 - 83.

Mimica-Dukić, N. and Božin, B. (2008): *Mentha* L. species (Lamiaceae) as promising sources of bioactive secondary metabolites. *Curr. Pharm. Des.*, 14: 3141-3150.

Murray, M. J. and Lincoln, D. E. (1970): The genetic basis of acyclic constituents in *Mentha citrata* Ehrh. *Genetics*, 65: 457-471.

Özgüven, M.; Kirici, S. and Ayanoğlu, F., (2002): Domestication and determination of yield and quality aspects of wild Mentha

species growing in southern Turkey. Biodiversity: biomolecular aspects of biodiversity and innovative utilization, 227-245.

Pigliucci, M. (2005): Evolution of phenotypic plasticity: where are we going now *Trends Ecol Evol*., 20: 481- 486.

Rabia, K.; Muhammad, A.; Yamin, B.; Saira, A. and Sunbal, K. C. (2015): Evaluation of Ethnopharmacological and Antioxidant Potential of *Zanthoxylum armatum* . *DC. J. Chem.*, 1-8.

Reynders, S. and Bollereau, P. (1994): Characterization of genetic diversity in Rosa by randomly amplified polymorphic DNA. *Acta Hort.* 424: 253-260.

Ristova, D. and Busch, W. (2014): Natural Variation of Root Traits: From development to Nutrient Uptake. *Plant Physiology*, 166: 518-527.

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989): Molecular cloning: A Laboratory Manual Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Savolainen, O.; Lascoux, M. and Merilä, J. (2013): Ecological genomics of local adaptation. *Nat Rev Genet*., 14: 807–820.

Schanzer, I. A.; Maria, V. S.; Olga, V. S. and Tatyana, V. V. (2012): Genetic diversity and natural hybridization in populations of clonal plants of *Mentha aquatica* L. (Lamiaceae), *Wulfenia* 19: 131-139.

Shaikh, S.; Yaacob, H. B. and Rahim, Z. H. A. (2014): Prospective role in treatment of major illnesses and potential benefits as a safe insecticide and natural food preservative of mint (*Mentha* spp.): a review. *Asian J. Biomed. Pharm. Sci*., 4: 1-12.

Shasany, A. K.; Darokar, M. P.; Dhawan, S.; Gupta, A. K.; Gupta, S.; Shukla, A. K.; Patra, N. K. and Khanuja, S. P. S. (2005): Use of RAPD and AFLP markers to identify inter-and intraspecific hybrids of *Mentha. J. of Heredity*, 96**:** 542-549.

Shahbazi, S. H. (2004): Investigation and analyses of the components and elements of Ardabil's Jome Mosque. Tabriz, Sadra publication.

Smolik, M.; Rzepka-Plevens, D.; Jadczak, D. and Sękowska, A. (2007): Morphological and genetic variability of chosen *Mentha* species. *Herba Pol.* 53: 90-97.

Spencer, A.; Hamill, J. D. and Rhodes, M. J. C. (1993): Transformation in *Mentha* species (Mint). In Bajaj Y.P.S. (Ed.) Biotechnology in agriculture and forestry 22. Plant Protoplasts and Genetic Engineering III (pp. 278-293). Berlin, Heidelberg: Springer-Verlag.

Trontin, C.; Tisné, S.; Bach, L. and Loudet, O. (2011): What does Arabidopsis natural variation teach us *(and does not teach us) about adaptation in plants. Curr Opin Plant Biol.*, 14: 225-231.

Tucker, A. O. and Naczi, R. F. C. (2006): Mentha: An overview of its classification and relationships. In Lawrence B.M. (Ed.) Mint. The genus *Mentha*. Medical and Aromatic Plants – Industrial Profiles (pp. 3-35). Boca Raton, London, New York: CRC Press, Taylor and Francis Group.

Zietkiewicz, E.; Rafalaki, A. and Labuda, D. (1994): Genome fingerprinting by simple sequence repeats (SSR)-anchored polymerase chain reaction amplification, *Genomics*, 20: 1176-183.

تحليل التنوع الجزيئى والزيوت الطيارة فى بعض انواع وسلالات النعناع المصرى

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يعتبر النعناع (التابع للعائلة الشفوية) والذى يتصف بالتباينات الكبيرة من الناحية الظاهرية والكيميائية من النباتات العشبية العطرية المنتشرة بكثرة. وقد اصبح استعمال المواد النباتية الموثوقة من الاعتبارات المهمة لصناعة العقاقير لتفادى الغش ببدائل غير حقيقية. وقد تم حديثا استعمال واسمات RAPD, AFLP and SSR لدراسة التباين الوراثى فى جنس النعناع. لكن تشير الدراسات إلى نقص واضح فى الواسمات الجزئية فى مثل هذه الدراسات. ولذا فإن هدف هذه الدراسة هو زيادة فهمنا للمركبات الكيميائية والتركيب الجزيئى لبعض الطرز الوراثية المعرفة من النعناع وكذا بعض الطرز البيئية المزروعة فى مصر. تم تجميع لخمس أنواع من النعناع [*Mentha piperita, M. longifolia, M. suaveolens* (apple mint), *M. piperita* (cv. Chocolate mint) and *M. pulegium*] وخمس طرز بيئية مزروعة في مصر تنتمى إلى *M. spicata* وهى من سوهاج، والبحيرة، وأسوان، و بنى سويف، والمنيا. تم الإكثار الخضرى واقلمة هذه الطرز الوراثية للظروف البيئية لمحافظة المنيا لمدة عامين. وبعد هذه الفترة تم زراعة عقل فردية من النباتات فى أصص بلاستيكية وتم حش النباتات ثلاث مرات خلال موسم النمو الصيفى وتقدير الوزن الجاف للنباتات. قد اظهرت النتائج تباين معنوى فى الوزن الجاف للنباتات التابعة للطرز الوراثية المختلفة محل الدراسة. ومن الجدير بالذكر ان الوزن الجاف للطرز البيئية من سوهاج وبنى سويف قد تجاوز الطرز البيئى المحلى بنسب 166، 148% على الترتيب. اظهرت الطرز الوراثية محل الدراسة اختلافات معنوية فى محتوى الزيوت الطيارة والتى تباينت من 1.33% لنباتات *M. longifolia* إلى 0.47% للطرز البيئي الأسوانى. وبتحليل الـ GC/MS

للزيوت الطيارة اظهرت النتائج تباين نسبة المكونات التى تم التعرف عليها بين 100% للطرز البيئى الأسوانى الى 89.42% للنوع *M. pulegium*. وعلاوة على ذلك بينت الدراسة تباين كبير فى مكونات الزيت الطيار بين الأنواع والطرز البيئة المختلفة. اظهرت نتائج الدراسة الجزيئية ان كل البادئات المستخدمة اظهرت نسبة عالية من التباين (100%). اظهرت نتائج العلاقات التطورية انقسام الانواع والطرز البيئة الى مجوعتين اساسيتين، احتوت الاولى على النوع *M. longifolia* في حين انقسمت المجموعة الثانية إلى مجموعتين فرعيتين احتوت أحداهما على الخمس طرز البيئة المنزرعة في مصر (سوهاج- البحيرة- أسوان- بنى سويف والمنيا) الى جانب النوع *M. pulegium* في حين كون النعناع *Mentha piperita* and *M. piperita* (cv. Chocolate mint) المجموعة الفرعية الثانية.