IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 10 Ver. I (October 2018), PP 57-67
www.iosrjournals.org

Molecular Systematic And Productivity Evaluation Of Some Ecotypes Of Mentha Longfolia Under Different Ecological Conditions

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Abstract: *This work was carried out during the two successive seasons of 2015/2016 and 2016/2017 to assess the molecular systematic and genetic relationship between two Mentha longfolia ecotypes, which collected from South Sinai (St. Catharine) and Siwa Oasis in Egypt through ISSR molecular markers, Then evaluating the productivity of them under different ecological conditions. The experiment was conducted at two different locations in newly reclaimed lands as follows: Baloza (North Sinai Governorate) and Siwa Oasis (Matrouh Governorate). The results of molecular markers showed high similarity values between the studied ecotypes were 92%. ISSR analysis confirmed that the Mentha longfolia from Catharine and the Mentha longfolia from Siwa Oasis are genetically similar and have the same species.*

On the other hand, the results of productivity experiment indicated that all parameters under the study i.e. fresh and dry weight of herb/plant or feddan, essential oil percentage, essential oil yield /plant or feddan and essential oil constituents were varied according to location and ecotype. The best location for cultivation of these ecotypes was at Baloza location and the best productivity was for Siwa ecotype which recorded the highest quantity parameters in this respect. Also, GC-Ms analysis of volatile oil indicated that the major compounds in Siwa ecotype oil were 1,8 Cineole, 1-Menthone and Pulegone. While, Piperitone oxide, Piperitone, Piperitenone and 1,8 Cineole were major compounds in Catharine M. longfolia ecotype oil.

Key words: *Mentha, Molecular Systematic, ISSR, Productivity, essential oil, ecotypes*

Date of Submission: 12-10-2018 Date of acceptance: 27-10-2018

I. Introduction

Lamiaceae (Labiatae) is an important plant family that has been investigated for its medicinal properties due to its large quantity of phenolic acids, flavonoids and essential oil [1-2]**.** The family Lamiaceae is subdivided into two subfamilies Lamioideae and Nepetoideae. The genus *Mentha* belongs to the subfamily Nepetoideae. Genus *Mentha* is a member of the tribe Mentheae in the subfamily Nepetoideae. The tribe contains about 65 genera **[3,4,5].** The genus *Mentha* includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa **[6,7].** *Mentha* is represented in the Egyptian flora by three species; *M. spicata*, *M. pulegium* and *M. longifolia* **[8,9] .** The later species is widely distributed and includes two subspecies; *M. longifolia* subsp. *typhoides*, and *M. longifolia* subsp. *schemperei* **[9].**

Mentha longifolia L. (Habaq), grows extensively in different part of Iran and other regions such as Mediterranean, Europe, Australia, and North Africa **[10].** *M. longifolia* is used in the pharmaceutical, food and cosmetic industries. All of the plant parts including its leaves, flowers, stems, and seeds have been used widely in traditional medicine as antimicrobial, carminative, diuretic, antitussive, mucolytic, antispasmodic agent. Also, it is used for the treatment of various diseases such as headaches, digestive disorders, amenorrhea, gout, colds, increased micturition, and for skin diseases **[11,12].** The volatile oil of *M. longifolia* contain terpenoids compound like pulegone, isopiperiten-one and 1,8-cineole **[13].** The aromatic ingredients, lead to pleasant scent and taste which, encourages the manufacturer to add its different parts especially essential oil to drinking or eating products.

The geographical diversity of medicinal plants is an important parameter affecting their properties. It is because the morphological, Phonological and chemical differences among the native species, has made the cultivation and use of medicinal plants difficult. Although the use of wild populations of medicinal plants may be suitable for the production of medicines, their cultivation may be more beneficial because of their sustainable production and maintaining their genetic resources. The selection of the most efficient genotype is the first step, although the hardest and the most expensive one. Meanwhile, among the most important parameters, which may

affect the growth and the yield of medicinal plants is their geographical (ecotype) properties,**[14].** Moreover, evaluation of the productivity and quality of medicinal and aromatic plants at variable ecosystem locations in Egypt is considered a main target today, especially under current global climate changes problem and its negative impacts on agriculture **[15,16,17,18].**

ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. ISSRs segregate mostly as dominant markers following simple mendelian inheritance. However, they have also been shown to segregate as codominant markers in some cases thus enabling distinction between homozygotes and heterozygotes **[19] .** Inter Simple Sequence Repeat (ISSR) markers, developed by **[20]** based on the amplification of a single primer containing a microsatellite 'core' sequence anchored at the 5' or 3' end by a set of 2-4 purine or pyrimidine residues, offers ait high degree of reproducibility with the detection of the rich level of polymorphism in a relatively simple procedure. Hence, it has been widely used in assessment of genetic diversity and cultivar identification **[21] .**

ISSR has been successfully used to estimate the extent of the genetic diversity at inter and intra specific level in a wide range of crop species which include rice, wheat, fingermillet, Vigna, sweet potato and Plantago **[22].**

ISSR technique were used in identification of 4 species of mint : *Mentha aquatic L.*,*peppermint, Mentha spicata var crispa L.* , and avariety of *Mentha saveolens*. The results of the analysis confirmed genetic differences between compared mint species out of 20 primers used for the ISSR reaction distinct amplicons were generated in the reactions from 8 ones on the whole in the reactions carried out 134 ISSR products were amplified , of which 78 (52.8%) were monomorphic and 56 (41.8%) polymorphic **[23].**

Therefore, the aim of this work at first, studying the genetic relationships and molecular difference between two *Mentha longfolia* ecotypes from south Sinai (Cathrien) and siwa Oasis with ISSR molecular marker in order to achievement the systematic value and identification them. Then, studying productivity evaluation of them under different ecological conditions

II. Martials And Methods

This work was carried out to assess the genetic relationships and molecular difference between two *Mentha longfolia* ecotypes collected from Sant Catherine Protectorate of South Sinai, called (Catherine habaq) and from Siwa Oasis called (Siwa Habaq) in Egypt. After that, investigating the influence of the different planting environmental conditions at Baloza Research Station, Desert Research Center (DRC), North Sinai Governorate and Khemisa Research Station, (DRC), Siwa Oasis , Mersa Matrouh Governorate, on growth and productivity to both of them during two seasons 2015/2016 and 2016/2017.

Fresh leaves of two ecotypes of *Mentha longfolia* from South Sinai (St. Catherine) and Siwa Oasis in Egypt were collected, transported on ice and stored at -80 °C until subjected to grinding in liquid nitrogen for DNA extraction.

1. Molecular Genetic studies:

1.1. Extraction and purification of genomic DNA:

DNA was extracted from young leaves of two *Mentha* ecotypes by DNeasy Plant Mini Kit (Qiagen, Cat. No. 69104).

1.2. Estimation for the DNA concentration:

Run 2 µl of the parents DNA samples on 1% agarose gel in comparison to 10 µl of a DNA size marker (lambda DNA *Hind III* digest Phi X 174/HaeIII digest). To estimate DNA concentration, compare the degree of fluorescence of the DNA sample with the different bands in DNA size marker.

1.3. ISSR – PCR reaction:

Twelve ISSR primers were used in the detection of polymorphism among the two *Mentha* ecotypes.These primers were synthesized by Metabion Corp., Germany. The primers code and nucleotide sequences are presented in Table (1).

1.4. PCR amplification, ISSR-thermocycling profile and detection of the PCR products:

The PCR amplification reactions were carried out as mentioned by **[24].** Reactions were performed in 25 µl volume composed of 1x reaction buffer, 0.2 mM of dNTPs, 1.5 mM $MgCl_2$, 0.2 µM of primer, 0.5 unit of *Taq* polymerase (Qiagen Ltd., Germany) and 40 ng of template DNA, in sterile distilled water. PCR amplification of the DNA was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems). The temperature profile in the different cycles was as follows: an initial strand separation cycle at

94°C for 5 min followed by 40 cycles comprised of a denaturation step at 94°C for 1min, an annealing step at 45°C for 1 min and an extension step at 72°C for 1.5 min. The final cycle was a polymerization cycle for 7 min at 72°C.

The amplification products were separated in 1.5% agarose gels containing ethidium bromide (0.5 mg/ml) in 1 x TBE buffer at 120 volts. A 100bp DNA ladder was used as molecular size standard. PCR products were visualized under UV light and documented using a ™XR+ Gel Documentation System (Bio-Rad 2000)

Data Analysis:

The amplified products were scored as 1 for presence and 0 for absence respectively. All computations were carried out using the NTSYS- pc, version 2.2 package **[25]** The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient. A dendrogram based on similarity coefficients was generated using the Unweighted Pair Group Method of Arithmetic means (UPGMA) by NTSYS software.

Table (1): ISSR primers names and sequences.

Primer name	Sequence
ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR- 9	5'-GATAGATAGATAGATAGC-3'
ISSR- 10	5'-GACAGACAGACAGACAAT-3'
ISSR- 11	5'-ACACACACACACACACYA-3'
ISSR- 12	5'-ACACACACACACACACYC-3'
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR- 19	5'-HVHTCCTCCTCCTCCTCC-3'
ISSR- 20	5'-HVHTGTGTGTGTGTGTGT-3'

A: Adenine, T: Thymine, G: Guanine and C: Cytosine

2. Productivity evaluation studies:

The seedlings of the two ecotypes of *Mentha Longifolia* L. collected originally from St. Catherine Protectorate of South Sinai and from Siwa Oasis were transplanted successfully in the open field at the different locations on March 2016 and 2017 for the first and second seasons, respectively. In each season, a split plot design with three replicates was used. The main plots assigned to the different locations and the sub plots deviated to the different ecotypes of plant. Planting was carried out under drip irrigation system; the spaces between rows were 50 cm. In all locations, compost at a rate of 10 m3/feddan and Calcium superphosphate (15.5% P2O5) at a rate 100 kg/fed were added during soil preparation. All agricultural practices of growing *Mentha* plants were done when ever needed. All treatments were fertilized with 200 kg/fed ammonium sulphate (20.5% N) and 50 kg/fed potassium sulphate (48% K_2O). Nitrogen and potassium fertilizers were applied in two equal doses in the season. The first dose was added one month after transplanting, the second dose was added one week after cut of plants.

The plants were harvested two times per season, i.e. on September and December at Baloza and Siwa Oasis locations. Harvesting was done by cutting the vegetative parts of plants 5 cm above the soil surface leaving two branches for regrowth.

The following data were recorded:

1. Geographical Information about the Different Locations

1.1. GPS data

The latitude and longitude data for the different locations were recorded and are presented in table (2).

Table (2): The GPS data for the different locations.

Locations	Latitude (N)	Longitude (E)
Baloza	31° 3\	32° 36\
Siwa Oasis	29 ° 21\	25 ° 40\

1.2. Soil analyses

At the beginning of the experiment, soil samples were collected from the soil surface layer (30 cm) to determine the mechanical and chemical soil properties for the different locations. All soil samples were analyzed at the laboratories of Desert Research Center (DRC). The results are shown in tables (3 and 4).

Table (3): The mechanical analysis of the experimental soil at the different locations.

Locations	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
Baloza	0-30	90	5	5	Sandy
Siwa Oasis	0-30	92.91	5.21	1.88	Sandy

Table (4):The chemical analysis of the experimental soil at the different locations.

Locations	pH	E.C.	O.M	Soluble anions (meq/l)				Soluble cations (meq/l)			
		(ds/m)	(%)	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
Baloza	8.17	5.99	0.17	-	2.46	40.71	17.17	19.53	13.25	26.91	0.65
SiwaOasis	7.50	4.10	0.50	-	3.60	31.30	6.10	8.60	7.50	0.20	24.70

1.3. Irrigation water analyses

Irrigation water samples were analyzed at the laboratories of (DRC). The results are illustrated in table (5).

Table (5): The chemical analysis of irrigation water at the different locations.

Locations	pH	E.C.	Soluble anions (meq/l)				Soluble cations (meq/l)			
		(ds/m)	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
Baloza	7.10	1512	0.50	3.81	3.69	8.20	3.25	3.05	9.50	0.40
SiwaOasis	4.32	2710	----	2.17	22.20	15.77	9.47	7.75	21.75	0.99

1.4. Meteorological data

The average of meteorological data of two seasons of 2016 and 2017 for the different locations are shown in table (6).

Table (6) : The average of meteorological data at Baloza and Siwa Oasis locations

Locations	**Baloza**				**Siwa Oasis**			
Parameters / **Months**	**Temperature at 2 Meters (C°)**		**Daylight Hours (hours)**	**Wind Speed Range at 10 Meters (m/s)**	**Temperature at 2 Meters (C°)**		**Daylight Hours (hours)**	**Wind Speed Range at 10 Meters (m/s)**
	Max.	**Min.**			**Max.**	**Min.**		
January	16.90	11.06	9.02	3.97	17.39	6.07	10.52	3.19
February	18.00	12.40	9.60	4.31	19.04	6.50	11.18	3.71
March	20.03	15.16	10.60	4.90	23.07	9.02	11.98	3.95
April	22.60	16.90	11.89	5.01	28.37	13.09	12.87	4.12
May	26.45	20.50	13.04	4.68	32.77	17.25	13.60	4.10
June	28.80	23.40	15.04	4.50	36.30	20.21	13.97	3.91
July	31.09	26.04	14.6	4.08	37.12	21.43	13.82	3.82
August	31.20	26.16	14.40	3.86	36.81	21.90	13.18	3.73
September	29.4	25.06	13.47	3.82	34.42	20.45	12.37	3.60
October	26.60	21.70	12.18	3.93	29.39	16.62	11.48	3.23
November	22.90	18.06	10.31	3.40	23.46	11.84	10.73	3.04
December	20.45	15.45	8.16	3.31	18.69	7.71	10.32	3.11

2. Productivity Parameters of *Menthe longfolia*

2.1. Fresh weight of herb/cm^2 (g).

2.2. Fresh weight of herb/fed (kg).

2.3. Dry weight of herb/cm^2 (g).

2.4. Dry weight of herb/fed (kg).

2.5. Essential oil percentage was determined in the air dried herb by Hydro distillation for 3 hours using a Clevenger type apparatus. The essential oil (%) was calculated as a relative percentage (v/w) **[26].**

2.6. Essential oil yield / m^2 was calculated as follows:

$$\frac{\text{oil percentage} \times \text{herb dry weight (g/m}^2\text{)}}{100}$$

2.7. Essential oil yield per feddan (L)was calculated as follows :

Essential oil yield / $m^2 \times 4000\ m^2$(feddan).

2.8. Essential oil chemical constituents: The GC-MS analysis of essential oils was conducted in the second season using Gas Chromatography-Mass Spectrometry instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt with the following specifications. Instrument: a TRACE GC

Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min at a split ratio of 1:10 and the following temperature program: 80ºC for 1 min; rising at 4ºC/min to 300ºC and held for 1 min. The injector and detector were held at 220 and 200ºC, respectively. Diluted samples (1:10 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published.

Statistical analysis:

Data of the productivity evaluation studies were statically analyzed and the differences between the means of the treatments were considered significant when they were more than the least significant differences (L.S.D) at the 5% level, respectively by using computer program of Statistix version 9,**[27]**.

III. Results And Discussion

1. Molecular systematic studies

From 24 initially applied ISSR primers, only 12 showed reproducible fragments with easily recordable bands. The total number of bands, the number of polymorphic bands, and the number of monomorphic bands along with the percentage of polymorphism are shown in (Table 7). A total of 248 fragments were consistently recognized, of which 18 were polymorphic in the two tested samples. There is low percentage of polymorphism ranged from 0.09 to 0.20% with high percentage of monomorphic bands reached to 100% monomorphic in five primers profile. The table (8) shows the similarity matrix between the two studied samples, they have 92% percentage of similarity, and we can say that the two studied ecotypes are genetically similar.

These results agree with morphological characters for the two studied sample, which they are morphologically similar, except few represented morphologic marker that can we see by normal eyes, which the ecotype of Siwa Oasis is slightly long and wide than Cathrien ecotype 2-4 mm more and the ecotype of Siwa Oasis has deep green colour and odour than Cathrien ecotype. The ISSR markers confirmed that the two studied ecotypes are the same species *Mentha longfolia* L.

Also, *Menthe longfolia* in Egypt is widely distributed and includes two subspecies; *M. longifolia* subsp. *Typhoides*, and *M. longifolia* subsp. *Schemperei* **[9] .** *M. longfolia* subsp. *Typhoides* has Leaves to 1.8cm broad –lanceolate, sharply serrate, the base hardly clasping, but *M. longfolia* Subsp. *Schimperi* (briq.)Briq. Has Leaves to 8mm broad, narrowly lanceolate, obscurely serrate to entire the base clasping **[28].**

According to**[28,29],** and Similarity matrix results we can notice that the two studied ecotypes are morphologically similar to subsp. *Schimperi*, not subsp. *Typhoids* so the results of this search concluded that, the two tested samples are *Mentha longfolia* subsp. *Schimperi*, but the slight phenotypic difference mentioned above and 8% dissimilarity due to what?

The answer may be backed to different environmental condition or there is new variety from *M. longfolia* subsp. *Schimperi* are found in Siwa Oasis.

Table (7): Total bands number of bands from used ISSR primers, N. of monomorphic bands and the percentage of polymorphic bands

Primer cod	Total band /primer	N. of polymorphic bands	N. of monomorphic bands	% of polymorpfism
1	17	1	16	0.05
2	21	1	20	0.04
3	26	4	22	0.15
4	25	5	20	0.20
5	16	0	16	0
6	12	0	12	0
7	16	0	16	0
8	22	0	22	0
9	22	2	20	0.09
10	20	0	20	0
11	25	3	22	0.12
12	16	2	14	0.125
total	248	18	230	

Table (8): Similarity Matrix of Dice Coefficient for two *Mentha* ecotypes.

	1	2
1	1.00	0.92
2	0.92	1.00

2. Productivity evaluation studies:

2.1. Herb of yield parameters

Data in Table (9) show that, behavior *Mentha longfolia* ecotypes under Baloza site conditions in two seasons gave the highest values and significantly increase for fresh weight / m^2, fresh weight /fed., dry weight / $m^2$and dry weight / fed in compared to the growing under Siwa Oasis site conditions. These results hold true in the first and second cuts of the two seasons.

Moreover, data tabulated in table (10) indicate that, Siwa ecotype gave the highest values and significantly increased for all yield parameters (fresh weight / m^2, fresh weight /fed., dry weight / m^2 and dry weight / fed) compared to St. Catharine ecotype under this study in the two cuts of the two seasons respectively. While St. Catharine ecotype gave the lowest values in this respect.

The data presented in table (11) show that, fresh weight / m^2, fresh weight /fed., dry weight / m^2 and dry weight / fed were influenced by interaction between locations and ecotypes in the two seasons. Whereas, in most cases Siwa ecotype which cultivated under Baloza location gave significant increase in fresh weight / m^2, fresh weight /feddan., dry weight / m^2 and dry weight / feddan followed by Siwa ecotype which cultivated under Siwa location conditions compared to St. Catharine ecotype that grown at Siwa site and Baloza site, respectively . whereas, there was non-significant differences between Saint Catrien ecotype that grown at Siwa site and Baloza site which gave the lowest values in all yield parameters in the two cuts during the two seasons.

Table (9). Effect of different ecological locations on productivity of *Mentha longfolia* plants in the two cuts during the two seasons 2016and 2017.

Charact. / Treatments	Fresh weight / m^2 (g)		Fresh weight /fed.(kg)		Dry weight / m^2 (g)		Dry weight /fed.(kg)	
	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut
	First season							
Location(Baloza)	633.8a	697.9a	2535.4a	2791.8a	170.0a	198.1a	680.0a	792.5a
Location(Siwa)	360.4b	388.0b	1441.4b	1552.0b	129.9b	174.6a	519.8b	698.2a
	Second season							
Location(Baloza)	625.7a	1032.7a	2502.9a	4130.8a	209.6a	312.9a	838.3a	1251.8a
Location(Siwa)	557.8b	715.9b	2231.4b	2863.6b	202.6b	200.7b	810.3b	802.7b

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

Table (10). Effect of different ecotypes on productivity of *Mentha longfolia* plants in the two cuts during the two seasons 2016and 2017

Charact. / Treatments	Fresh weight / m^2 (g)		Fresh weight /fed.(kg)		dry weight / m^2(g)		dry weight /fed.(kg)	
	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut	1^{th} cut	2^{ed} cut
	First season							
Siwa ecotype	676.1a	725.1a	2704.4a	2900.5a	203.7a	241.6a	814.7a	966.3a
Cathrien ecotype	318.1b	360.8b	1272.4b	1443.2b	96.3b	131.1b	385.1b	524.4b
	Second season							
Siwa ecotype	786.2a	1389.4a	3145.0a	5557.5a	258.7a	380.0a	1035.0a	1520.0a
Cathrien ecotype	397.3b	359.2b	1589.2b	1436.9b	153.4b	133.6b	613.5b	534.4b

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

Table (11). Effect of interaction between different ecological locations and different ecotypes on productivity of *Mentha longfolia* plants in the two cuts during the two seasons 2016and 2017

Charact. / Treatments		Fresh weight / m2 (g)		Fresh weight /fed.**(kg)**		dry weight / m2 **(g)**		dry weight /fed.(kg)	
		1th cut	**2ed cut**	**1th cut**	**2ed cut**	**1th cut**	**2ed cut**	**1th cut**	**2ed cut**
		First season							
Location (Baloza)	Siwa ecotype	954.1a	1035.3a	3816.5a	4141.0a	251.3a	281.2a	1005.0a	1125.0a
	Cathrien ecotype	313.6c	360.6c	1254.3c	1442.5c	88.8c	115.0c	355.0c	460.0c
Location (Siwa)	Siwa ecotype	398.1b	415.0b	1592.3b	1660.1b	156.1b	201.9b	624.3b	807.6b
	Cathrien ecotype	322.7c	361.0c	1290.6c	1443.9c	103.8c	147.2c	415.2c	588.9c
		Second season							
Location (Baloza)	Siwa ecotype	770.0a	1626.3a	3080.0a	6505.0a	230.0b	482.5a	920.0b	1930.0a
	Cathrien ecotype	481.44b	439.1c	1925.8b	1756.6c	189.1c	143.4c	756.5c	573.5c
Location (Siwa)	Siwa ecotype	802.5a	1152.5b	3210.0a	4610.0b	287.5a	277.5b	1150.0a	1110.0b
	Cathrien ecotype	313.2c	279.3d	1252.7c	1117.2d	117.6d	123.84c	470.5d	495.4c

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

These differences in *Mentha longfolia* plants productivity may be due the existence of a wide range of ecological conditions between the different studied locations. At North Sinai Governorate, there was an increase in the parameters of yield accompanied with increasing air temperature and solar radiation in Sinai where the air temperature and solar radiation are increased (Table 6). The significantly increase in yield parameters were observed at Baloza location (31^{o} $3^{\backslash}$ N and 32^{o} $36^{\backslash}$ E) . On the other hand, Siwa Oasis location, which is located in the western desert of Egypt (29^{o} $21^{\backslash}$ N and 25^{o} $40^{\backslash}$ E, 300 km South of the Mediterranean Sea and -19 m below sea level), the extremely high air temperature during summer months (Table 6) led to a heat stress on plants and therefore, the lowest yield parameters were obtained at this location; i.e. fresh and dry weights of herb/m^2 during both seasons.

Differences in the results can be explained by differences in environmental and geographical factors (temperature, rainfall, altitude, hours of sunshine, etc.) of regions under study. These results are in harmony with that obtained by [30]**,** who mentioned that, the rate of plant development is a function of temperature and usually increases linearly from above the species-specific base temperature until the optimum temperature. **[31],** on wild *Salvia fruticosa* plants, reported that the plants were affected by the mean annual temperature and the total annual sunshine at the different growing locations. **[32,33,34]** found that, the interactive effects of light with temperature play an important role on plant growth of *Salvia officinalis*. Also, **[15]** found that, all herb quantity and quality parameters of *Salvia fruticosa* were varied according to location and **[35]** reported that, flowering shoot yield were significantly different between the two populations of *Mentha longifolia* plants.

In general, Geographical differences are among the most significant parameters affecting the growth and the yield of medicinal plants . Accordingly, the effects of a set of different environmental parameters including climate (longitude, latitude, and height above the sea level) and soil (salinity, pH, organic matter and soil texture) were investigated on the morphology and hence the subsequent growth and yield of pennyroyal **[36].**

2.2. Essential oil parameters

Data of the essential oil parameters for the two ecotypes of *Mentha longfolia* under the effect of different ecological locations are shown in tables (12, 13 and 14). The obtained results indicate that, all essential oil parameters (essential oil percentage, essential oil yield /m^2 and essential oil yield /feddan) were affected and varied according to the different ecological locations. Baloza site recorded the highest values and non-significant differences in this regard compared to Siwa site. In both seasons, the significantly highest essential oil percentage, essential oil yield /m^2 or essential oil yield /feddan were recorded in Siwa *M. longfolia* ecotype, while the lowest ones were detected in Cathrien *M. longfolia* ecotype for all cuts.

Data presented in table (14) reveal that, essential oil percentage and yield were influenced by interaction between locations and ecotypes in the two seasons. Whereas, Siwa *M. longfolia* ecotype when

growing under Siwa environment gave the highest values for all studied parameters followed by growing under Baloza conditions in comparison with Cathrien *M. longfolia* ecotype which cultivated under Siwa or Baloza conditions. Furthermore, in most cases, Cathrien *M. longfolia* ecotype which cultivated under Siwa or Baloza conditions recorded the lowest values in essential oil percentage, essential oil yield /m^2 or essential oil yield /feddan. These results are hold true in two cuts of the two seasons.

These results may be attributed to that plants at this location recorded its highest dry weight of herb per plant and/or essential oil percentage. these results are hold true with that reported by **[15]** who found that, volatile oil parameters of *Salvia fruticosa* were varied according to location. Meanwhile, **[37,38]** recorded that**,** the genetic properties of wild populations determine the production of the secondary metabolites, the environmental conditions can also greatly affect on the quantity and quality of such metabolites. Also, various researchers reported that essential oil yield and its components in medicinal and aromatic plants in general is primarily related to their genetic **[39],** climate, edaphic, elevation and topography **[40].**

Table (12).Effect of different ecological locations on essential oil productivity of *Mentha longfolia* ecotypes in the two cuts during the two seasons 2016and 2017

Charact. / Locations	Essential oil(%)		Essential oil yield / m^2 (ml)		Essential oil yield / fed (l)	
	1th cut	**2ed cut**	**1th cut**	**2ed cut**	**1th cut**	**2ed cut**
	First season					
Baloza Location	1.5a	2.4a	3.6a	4.3a	14.6a	17.2a
Siwa Location	1.8a	1.6b	2.6a	4.7a	10.5a	18.9a
	Second season					
Baloza Location	2.3a	1.8b	3.8b	8.4a	15.1b	33.8a
Siwa Location	1.6b	3.2a	5.3a	7.7a	21.3a	30.7a

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

Table (13). Effect of different ecotypes on essential oil productivity of *Mentha longfolia* plant in the two cuts during the two seasons 2016and 2017

Charact. / Ecotypes	Essential oil (%)		Essential oil yield / m^2 (ml)		Essential oil yield / fed (L)	
	1th cut	2ed cut	1th cut	2ed cut	1th cut	2ed cut
	First season					
Siwa ecotype	2.8a	3.6a	5.8a	8.4a	23.1a	33.4a
Cathrien ecotype	0.5b	0.5b	0.5b	0.7b	1.9b	2.7b
	Second season					
Siwa ecotype	3.1a	4.1a	8.1a	15.0a	32.5a	60.1a
Cathrien ecotype	0.8b	0.9b	1.0b	1.1b	3.9b	4.5b

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

Data recorded in table (15) reveal that, the constituents of *M. longfolia* volatile oil had been affected by the different planting locations and different ecotypes. 1,8 Cineole, 1-Menthone and Pulegone were the main dominant chemical constituent in Siwa *M. longfolia* ecotype oil. While, Piperitone oxide**,** Piperitone, Piperitenone and 1,8 Cineole were major compounds in Cathrien *M. longfolia* ecotype oil. These results can be summarized as follows:

Siwa *M. longfolia* ecotype which cultivated in Baloza or Siwa conditions, the most abundant components in volatile oil were 1,8-cineole (29.29 – 29.66),1-menthone (16.94 – 21.69), pulegone (29.99 – 29.41), β-pinene (4.29 – 3.57), Endo-borneol(4.48 -4.08), sabinene(2.39 – 1.66), Piperitone oxide (0 - 0.51) piperitone (0.14 – 0.37), piperitenone (0.53 – 0.09), and α-pinene (2.41 -1.98), respectively . Meanwhile, Cathrien *M. longfolia* ecotype which cultivated under Baloza or Siwa ecological conditions, the dominant

components in volatile oil were **Piperitone oxide** (13.25 – 8.17), piperitone (18.55 – 6.74), piperitenone (23.05 – 32.34), 1,8-cineole (6.13 – 21.38),1-menthone (3.68 – 0.63), pulegone (9.33 - 2.57), β-pinene (2.69 – 3.54), Endo-borneol(2.98 – 3.85), sabinene(1.09 – 1.34) and α-pinene (2.56 – 2.43), respectively.

Table (14). Effect of interaction between different ecological locations and ecotypes on volatile oil productivity of *Mentha longfolia* plant in the two cuts during the two seasons 2016and 2017

Charact. / Treatments		Essential oil (%)		Essential oil yield / m^2 (ml)		Essential oil yield / fed (L)	
		1th cut	2ed cut	1th cut	2ed cut	1th cut	2ed cut
		First season					
Baloza Location	Siwa ecotype	2.9a	3.0b	7.2a	8.4a	28.6a	33.6a
	Cathrien ecotype	0.1c	0.2c	0.1c	0.19b	0.5c	0.7b
Siwa Location	Siwa ecotype	2.8a	4.1a	4.4b	8.3a	17.6b	33.2a
	Cathrien ecotype	0.8b	0.8c	0.8c	1.2b	3.4c	4.8b
		Second season					
Baloza Location	Siwa ecotype	3.1a	3.4b	7.2b	16.7a	29.0b	66.6a
	Cathrien ecotype	1.4b	0.2d	0.3d	0.3c	1.1d	1.0c
Siwa Location	Siwa ecotype	3.1a	4.8a	9.0a	13.4b	35.9a	53.5b
	Cathrien ecotype	0.1c	1.6c	1.7c	1.9c	6.8c	7.9c

Means having the same letter (s) within the same column are not significantly different according to LSD for all-pairwise comparisons test at 5% level of probability

Table (16). Effect of interaction between different ecological locations and ecotypes on essential oil compounds of *Mentha longfolia* plant during the first season 2016.

	components	Baloza location		Siwa location	
		Siwa ecotype	Cathrien ecotype	Siwa ecotype	Cathrien ecotype
1	l-Phellandrene	0.09	-	0.08	0.13
2	**α-pinene**	**2.41**	**2.56**	**1.98**	**2.43**
3	**Camphene**	**1.00**	**1.06**	**0.89**	**0.99**
4	verbenene	0.04	-	0.04	0.05
5	**Sabinene**	**2.39**	**1.09**	**1.66**	**1.34**
6	**β-PINENE**	**4.29**	**2.69**	**3.57**	**3.54**
7	α -Myrcene	0.71	0.45	0.30	0.39
8	α-Phellandrene	0.02	-	0.02	-
9	α-Terpinene	0.08	-	0.07	0.16
10	D-Limonene	0.27	1.38	0.14	2.00
11	**1,8Cineole**	**29.29**	**6.13**	**29.66**	**21.38**
12	Ç-Terpinene	0.10	0.13	0.09	0.27
13	trans Sabinene hydrate	0.12	-	0.10	0.24
14	α-terpinolene	0.08	-	0.05	0.09
15	Iso-amyl2methyl Butyrate	0.02	0.46	0.02	0.05
16	Linalool	0.14	-	0.13	-
17	Cis-Sabinol	0.42	0.20	0.34	0.24
18	Cis-Verbenol	0.21	0.16	0.20	0.18
19	4hydroxy3(1methylethyl) benzaldehyde	-	-	-	0.08
20	**l-Menthone**	**16.94**	**3.68**	**21.69**	**0.63**
21	**Endo-Borneol**	**4.48**	**2.98**	**4.03**	**3.85**
22	Iso-pulegone	0.72	-	0.59	-
23	4-Terpineol	0.31	0.23	0.32	0.58
24	Citronellal	0.02	-	-	-
25	α-Terpineol	3.78	2.26	2.41	1.75
26	3,7Undecanedione,6,6,10 trimethyl	-	-	0.02	-
27	Cyclohexanone	0.07	-	0.19	0.04
28	Phenol,2(2methyl2prope-nyl)(CAS)	-	0.51	-	0.49

29	Bornyl formate	-	-	0.04	-
30	(R)3Methylphthalide	-	0.15	-	-
31	Valeric acid, dodecyl ester	-	0.33	-	0.11
32	**Pulegone**	**29.99**	**9.33**	**29.41**	**2.57**
33	**Piperitone oxide**	**-**	**13.25**	**0.51**	**8.17**
34	**Piperitone**	**0.14**	**18.55**	**0.37**	**6.74**
35	(1S)1Methyl1,3dihydroisobenzot hioPhene	-	-	-	0.29
36	Trimethylphenylsilane	-	0.16	-	-
37	Bornyl acetate	-	0.26	0.03	-
38	Sabinol	-	-	0.02	-
39	α-Terpinyl propionate	-	-	-	0.09
40	Diosphenol	-	-	-	0.09
41	ë Elemene	-	0.13	-	-
42	**Piperitenone**	**0.53**	**23.05**	**0.09**	**32.34**
43	l-Verbenone	-	-	0.07	-
44	Piperitenone oxide	0.09	3.57	0.17	7.07
45	Caryophyllene	0.72	2.24	0.33	0.40
46	Bergamotene	-	0.10	-	-
47	Humulene	0.02	-	-	-
48	Germacrene-D	0.06	0.20	-	-
49	Ar-Curcumene	-	0.16	-	-
50	α-Guaiene	-	0.12	-	-
51	Ç-Elemene	0.02	0.12	-	-
52	Ç-Cadinene	0.07	0.34	0.04	0.12
53	Nerolidol	-	0.50	-	-
54	Caryophyllene oxide	0.08	0.31	0.15	0.49
55	Cubenol	0.02	-	0.02	0.06
56	α-Cadinol	0.24	0.79	0.16	0.56
57	13-Epimanool	-	0.37	-	-
	Total	99.98	100.00	100.00	100.00

IV. Conclusion

This work is the first paper which study the mentha ecotype from Siwa Oasis in Comparison to St. Cathrine ecotype. The results of the molecular systematic studies concluded that the two studied *Mentha* ecotypes collected from South Sinai (St. Catharine) and Siwa Oasis are the same species *Mentha longfolia* L. species and also, the same subspecies *Schimperi* and the slight mentioned difference was may be due to either of different environmental conditions in Siwa Oasis than St. Catherine or a new Varity of subspecies *Schimperi* was founded in Siwa Oasis. These results are useful for taxonomic scientists' of Egyptian flora and need further studies for more confirmation. Mean while, the best locations for cultivation and production of the studied two *Mentha longfolia* L ecotypes as a promising land in the desert was at Baloza location followed by Siwa Oasis location for both of the ecotypes of Habaq plant.

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El-henawy,A.A.A.. " Molecular Systematic And Productivity Evaluation Of Some Ecotypes Of Mentha Longfolia Under Different Ecological Conditions ." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.10 (2018): 57-67.